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Focus on regulated and emerging animal diseases (REDs) – 2014 review

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EDITORIAL

This is the sixth edition of this special annual issue, devoted to a health review of the main regulated and emerging diseases (provisionally classified in this regard as Category 1 health hazards).

As in 2013, the health situation in France in 2014 was generally very good.

For many regulated health hazards, only a few cases are detected annually: equine infectious anaemia, porcine brucellosis, enzootic bovine leukosis, viral diseases in freshwater fish, rabies in bats, atypical BSE, atypical scrapie, etc.

For other health hazards, no cases were detected in 2014: bovine brucellosis, brucellosis in small ruminants, Aujeszky's disease in pigs, classical scrapie, bluetongue in mainland France, nor in Corsica since May 2014.

In both situations, however, it is still essential to maintain vigilance and the quality of surveillance at a high level. We are reminded of this by the unpredictable nature (what, when, where and in which epidemio-clinical form) of the recent epizootics or sporadic cases due to recurrences, reintroductions or emergences: sporadic cases of bovine brucellosis in 2012, Schmallenberg disease in 2012, recurring imported cases of rabies in carnivores, and of course more recently bluetongue serotype 8 and West-Nile fever in 2015.

These changes in the epidemiological situation of regulated diseases require the rapid adaptation of surveillance procedures: easing them if the situation improves (e.g. if disease-free status is regained), reinforcing them if the health situation deteriorates (e.g. for bovine tuberculosis), and reactivating and increasing vigilance in the event of resurgence. Tailoring surveillance procedures to the surveillance objectives and the health situation, and constantly seeking to improve efficiency are among the core missions of the French National Epidemiological Surveillance Platform for Animal Health (ESA Platform).

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The *Bulletin Épidémiologique* is a joint publication of the French Agency for Food, Environmental and Occupational Health & Safety, and the Directorate General for Food of the Ministry of Agriculture, Food and Forestry.

Definitions

Outbreak surveillance

Outbreak surveillance, previously known as passive surveillance, refers to any monitoring activity that relies on spontaneous notification of cases or suspected cases of a monitored disease by source data contributors. Under an outbreak surveillance scheme, it is therefore not possible to predict the quantity, nature and geographic location of data that will be collected. Outbreak systems are particularly suitable in situations where early warning is needed should a disease emerge or re-emerge. This applies to epidemiological surveillance of exotic diseases, which covers the entire population. In this case, all sources of data for notification of suspected cases need to be mobilised to ensure early and rapid transfer of information.

Programmed surveillance

Programmed surveillance, also called planned or active surveillance, involves the acquisition of data through pre-scheduled actions following a methodology that enables extrapolation of the findings to the monitored population. Unlike outbreak surveillance, it is possible to determine in advance the quantity, nature and geographical location of the data that are to be collected by the scheme. Such routine surveillance can be carried out in an exhaustive manner, covering the entire target population, or can focus on a sample of the population. When a specific sample is monitored, it can be considered representative of a group, for example through random selection. The sample surveillance system involves occasional collection of data through surveys or repeated collection using a sentinel population. A risk population may also be chosen as the sample group.

Glossary and references

ACERSA: French Certification Association for Animal Health

AGID: Agar gel immunodiffusion

ANSES-ANMV: French Agency for Food, Environmental and Occupational Health & Safety - French Agency for Veterinary Medicinal Products

ANSES: French Agency for Food, Environmental and Occupational Health & Safety

APDI: Prefectural declaration of infection

APMS: Prefectural monitoring order

ASDA: National health certificate (for cattle)

BDNI: National identification database

BNEVP: National division for veterinary and plant health investigations

CF: Complement fixation test

CRPM: French Rural and Maritime Fishing Code

CSD-ESA: Data center for epidemiological surveillance in animal health

DAAF: French Directorate for Food, Agriculture and Forestry (Overseas Territories)

DDAAF: Departmental Directorate for Food, Agriculture and Forestry

DDecPP: Departmental Directorate for Protection of the Population

DGAL: French Directorate General for Food

DRAAF: Regional Directorate for Food, Agriculture and Forestry

DTL: Departmental testing laboratory

ELISA: Enzyme-linked immunosorbent assay

ESA Platform: French National Epidemiological Surveillance Platform for Animal Health

EU: European Union

FDC: Departmental hunting association

FRGDS: Regional federation of animal health protection farmers' organisations

GDS: Animal health protection farmers' organisation

GTV: Veterinary technical group

IBR: Infectious bovine rhinotracheitis

IFN-gamma: Interferon gamma

ILPT: Inter-laboratory proficiency test

MA: Marketing Authorisation

MAAF: Ministry of Agriculture, Food and Forestry

ND: Notifiable disease

NDCCM: Notifiable disease with compulsory control measures

NRL: National Reference Laboratory

OIE: World Organisation for Animal Health

ONCFS: National Office for Hunting and Wildlife

OVS: Health organisation

OVVT: Veterinary and technical organisation

PCR: Polymerase chain reaction

PCV: Voluntary group programme (for certification)

RBT: Rose Bengal Test

SAGIR: French wildlife disease surveillance network

SICTT: Single Intradermal Comparative Tuberculin Test

SIRE: Equine information database

SITT: Single Intradermal Tuberculin Test

SNGTV: French national society for technical veterinary groups

SRAL: Regional Food Authority

TSE: Transmissible spongiform encephalopathy

Access to legal documentation concerning regulated diseases

- All French regulatory texts can be viewed on the Légifrance website (http://www.legifrance.gouv.fr/) or with restricted access in their consolidated versions on the Galatée website (http://galatee.national.agri/) and the BO-Agri website (https://info.agriculture.gouv.fr/gedei/site/bo-agri)
- Memoranda cited as references can be viewed on the website of the French Prime Minister (http://circulaire.legifrance.gouv.fr/index.php?action=accueil) or with restricted access on the Galatée website (http://galatee.national.agri/), the Nocia website (http://nocia.national.agri/) and the BO-Agri website (https://info.agriculture.gouv.fr/gedei/site/bo-agri)

Bovine Tuberculosis in France in 2014: a stable situation

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Abstract
The overall situation of France regarding bovine tuberculosis remained highly satisfactory: annual incidence was well below 0.1% and in most of the infected herds that have been detected, the number of animals with lesions was very low. Diagnostic slaughtering increased slightly in 2014, proof of growing awareness among stakeholders and improved investigation of suspected cases. Information campaigns on slaughterhouse detection began to yield encouraging results with a rise in suspected cases, although the number of actual confirmations remained stable. The epidemiological situation improved in some areas, while others faced unexpected re-emergence. The persistence of the disease in some areas both in livestock and wildlife requires special attention and long-term efforts in order to achieve eradication.

Keywords
Regulated disease, Bovine tuberculosis, Surveillance, Cattle

Résumé
Tuberculose bovine en France en 2014: une situation stable
La situation sanitaire de la France vis-à-vis de la tuberculose bovine demeure globalement très satisfaisante en 2014 : l'incidence annuelle est restée largement inférieure à 0,1 % et dans la plupart des élevages infectés détectés le nombre d'animaux présentant des lésions est extrêmement limité. Le nombre d'abattages diagnostiques a encore augmenté légèrement en 2014, témoignant d'une mobilisation croissante des acteurs permettant une meilleure investigation des suspicions. La sensibilisation faite sur la détection en abattoir porte également ses fruits avec une augmentation des suspicions tout en conservant un nombre de lésions confirmées stable. Certaines zones voient leur situation s'améliorer, tandis que d'autres ont connu des résurgences inattendues. Enfin, certaines voient la maladie persister en élevage ou au sein de la faune sauvage ce qui impliquera une attention et une implication soutenue et raisonnée dans le temps afin de mener à bien l'éradication.

Mots-clés
Maladie réglementée, tuberculose bovine, surveillance, bovins

The data presented are based on data consolidated by the Departmental Directorates for Protection of the Population (DDecPPs) in the food information system (SIGAL) as well as on data submitted under DDecPP responsibility for the annual report.

Surveillance of tuberculosis

Organisation of programmed screening on farms

An overview of surveillance requirements and health control measures for bovine tuberculosis is presented in Box 1. Wildlife surveillance is also implemented under the Sylvatub scheme through several systems of varying intensity, depending on the situation in each *département* (Box 2).

In most *départements*, screening campaigns for tuberculosis on livestock farms are scheduled for the October to April wintering period, and not based on the calendar year. Therefore, the results for the 2014 calendar year shown here correspond to the end of the 2013/2014 surveillance period and to the beginning of the 2014/2015 campaign, possibly with slight differences in implementation methods.

The rate of screening for 2014 reported by the DDecPP is shown in Figure 1 and Table 1. Most *départements* (n=52) have discontinued systematic tuberculin testing for several years now. An increasing number of *départements* (n=20) have chosen to determine a tuberculin testing schedule for a specific zone ("zoning") that is different from the rest of the département. Zoning is established by the Departmental Prefect and must be submitted for an opinion to the DGAL. This also applies to changes in screening intervals at *département* level.

The geographic distribution of tested farms (Figure 2) is consistent with that of the screening intervals per *département* (Figure 1). Screening is carried out primarily in *départements* that have undertaken zoning but also in herds classified at-risk located in *départements* where programmed screening for tuberculosis through tuberculin tests has been discontinued. This may be the case for instance following identification of an epidemiological link with an outbreak or because of at-risk production, such as raw milk. In all, over the year 2014, 13,714 cattle farms underwent single intradermal tuberculin testing (SITT) or single intradermal comparative tuberculin testing (SICTT),

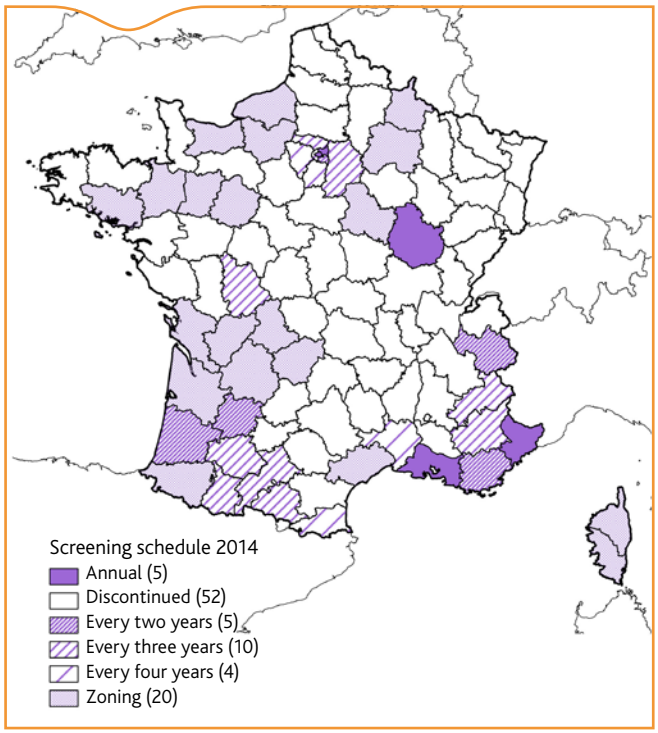

Figure 1. Programmed screening intervals for bovine tuberculosis by *département* in 2014

Box 1. Surveillance and health control measures for bovine tuberculosis

Objectives

The general objective of tuberculosis surveillance is to detect cases in order to eradicate the disease and maintain the officially disease-free status at farm and country levels.

Scope of surveillance programme

Bovine tuberculosis due to *Mycobacterium bovis, Mycobacterium tuberculosis* or *Mycobacterium caprae*.

The population monitored

All cattle farms across France.

Other susceptible populations undergo routine surveillance through *post-mortem* inspection at the slaughterhouse, particularly goats, sheep, and swine, as well as farmed deer.

Monitoring of wildlife such as deer, wild boars and badgers is performed through the Sylvatub specific surveillance scheme.

Definition of a case

The applicable definitions are those described in the regulations. In short:

- animals are considered infected either following detection of one of the mycobacteria referred to in the regulations by cell culture or PCR, or for various combinations of results for *post-mortem* tests (these combinations follow regulatory definitions),
- animals are considered suspect after a non-negative reaction is found in one of the screening tests that can be used when the animal is alive or if lesions suggestive of bovine tuberculosis are observed at the slaughterhouse,
- animals are considered likely to be contaminated when there is an epidemiological link to infected herds.

Surveillance programmes

Screening

Surveillance of bovine tuberculosis involves several complementary systems.

- Systematic surveillance at the slaughterhouse: inspection of all animals slaughtered for human consumption. Only *post-mortem* inspection is truly relevant for tuberculosis. It involves examination of a certain number of organs including the primary tuberculosis sites such as the lungs and retropharyngeal, tracheobronchial and mediastinal lymph nodes. If suspect lesions are detected, the organs are removed along with associated lymph nodes and examined in a laboratory qualified for PCR/bacteriological testing of mycobacteria.
- Programmed surveillance on the farm: testing required to obtain and maintain the officially disease-free status of herds. Depending on the health situation in the *département*, the screening interval can be adapted, ranging from the annual screening of all animals over six weeks of age to discontinuation of programmed screening. In some situations, zoning is performed and testing is reinforced in certain municipalities based on a health risk assessment. Irrespective of the interval in effect in a *département*, programmed screening can be requested annually for a period of three to five years on farms that are classified at-risk due to epidemiological links to an infected farm.
- Alongside programmed surveillance, screening can also be implemented when animals are moved. Given that the health system is considered robust and that France is officially TB-free, screening of animals on introduction may be waived, except in certain cases:
  - > if it takes more than six days for the animals to transit between two establishments,
  - > if the animals leave a farm classified as at-risk due to proximity to a domestic or wildlife outbreak or because of previous infection,
  - > if the animals transit through a farm with a high turnaround and come from a farm located in a *département* where the cumulative 5-year prevalence of bovine tuberculosis is higher than the national average.
- In all cases, screening is performed using either single intradermal tuberculin testing (SITT) or single intradermal comparative tuberculin testing (SICTT) depending on knowledge of the risk of atypical reactions. Tests are read 72 hours post-injection. Under specific conditions, especially for animals whose containment is difficult (fighting bulls), SITT screening can be reinforced using systematic IFN- gamma testing. The sensitivity (Se) and specificity (Sp) of these tests are not perfect and depend on their conditions of use (whether there are intercurrent agents, breed-related or physiological factors, etc.) (Vordermeyer 2006):
  - > SITT: Se ~ [80% – 91%] and Sp ~ [75% – 99.9%]
  - > SICTT: Se ~ [55% – 93%] and Sp ~ [89% – 100%]
  - > Bovigam IFN-gamma: Se ~ [81% – 100%] and Sp ~ [88% – 99%]
  - > Recombinant IFN-gamma: Se ~ [84% – 98%] and Sp ~ [92% – 96%]

Management of suspected cases and health control measures

Control measures aim to confirm or disprove the status of suspect animals and, if necessary, to eliminate infection from the herd. Testing protocols for suspected cases have been harmonised nationally, taking into account the different initial tests (SITT or SICTT). The following principles are applicable in all cases:

- If non-negative results are found for a farm, a risk analysis is carried out by the DDecPP to assess whether the suspicion is low or high. This analysis takes into account epidemiological criteria and, if necessary, further tests are performed to retest all or part of the herd, based on the health control measures. These tests are carried out using SICTT or, when available, by experimental IFN-gamma including specific peptides. In the event of low suspicion, animals are retested six weeks later or are directly slaughtered for diagnostic purposes. In this case, samples are taken to test for mycobacteria by PCR and cell culture, even if there are no macroscopic lesions. If suspicion is high from the outset, or because reactions to tests performed six weeks after low suspicion confirm the suspected cases, reactors are slaughtered diagnostically and the herd is retested after this diagnostic slaughter. An experimental protocol has been used since the 2013/2014 campaign to evaluate whether the IFN-gamma test carried out when reading the intradermal tuberculin result could replace tuberculin retesting carried out six weeks later, in order to assess inconclusive results. When infections are confirmed, herds that are likely to be contaminated, i.e. those with an epidemiological link to the infected herd, are screened with no limitation on the period of contact. This screening can detect links to fattening herds that received cattle many years before. Once all of the cattle from the outbreak or those that have been in contact with the animal from the outbreak have been slaughtered, the DDecPP can stop the investigations based on its assessment of the risk. In other cases, testing is undertaken using SITT, IFN-gamma or SICTT, or there is diagnostic slaughter, either of the reactor animals or systematically in some cases. When necessary, the herds are classified as at-risk, to be monitored through annual screening for three years.
- If an infection is confirmed, the infected farm must be cleansed. This generally involves complete depopulation of the herd with increased inspection at the slaughterhouse, followed by cleaning-disinfection of farm facilities. Until now, in certain specific cases, justified by preservation of local breeds or experimentally in Dordogne and Côte-d'Or, cleansing measures may have involved partial depopulation. Since July 2014, this procedure is available in all *départements* but requires an opinion from the mandated veterinarian, the GDSs, the TB coordinator, and the Directorate General for Food. In this scenario, animals are tested using SICTT or IFN-gamma on several occasions. Reactor animals are slaughtered for diagnostic purposes. The herd is considered to be cleansed after two favourable tests have been performed at a two-month interval, and is considered re-certified after two further favourable controls at two-month intervals.

Regulatory References

Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine

French Rural Code, Book 2, Preliminary Title and Title II

Ministerial Order of 15 September 2003 establishing the technical and administrative framework for collective prophylaxis and control measures for bovine and caprine tuberculosis

Table 1. Data on programmed screening of bovine tuberculosis by tuberculin testing on certified livestock farms in 2014 in France

Cattle herds as of 31/12/2014		212,550
ODF herds as of 31/12/2014 (%)		212,290 (99.88)
Schedule of programmed screening (number of départements)	Discontinued	(52)
	Annual	(5)
	Every two years	(5)
	Every three years	(10)
	Every four years	(4)
	Zoning	(20)
Herds undergoing SITT (%)*		10,990 (5.2)
Herds undergoing SICTT (%)*		2,724 (1.3)
Number of screening SITTs*		475,330
Number of screening SICTTs*		215,424
Number of SITT-positive herds (% of tuberculin tested herds)*		127 (1.2)
Number of SITT-non-negative herds (% of tuberculin tested herds)*		584 (5.3)
Number of SICTT-positive herds (% of tuberculin tested herds)*		115 (4.2)
Number of SICTT-non-negative herds (% of tuberculin tested herds)*		695 (25.5)
Number of non-negative SITTs (% of SITTs performed)*		2,069 (0.4)
Number of postiive SITTs (% of SITTs performed)*		660 (0.1)
Number of non-negative SICTTs (% of SICTTs performed)*		1,863 (0.8)
Number of postiive SICTTs (% of SICTTs performed)*		204 (0.1)
Veterinary practices involved*		909
Veterinary practices reporting non-negative intradermal tuberculin tests (%)*		278 (30.6)
Number of tests on movement		139,429

* as part of programmed screening
ODF: officially disease-free

accounting for about 6.5% of farms (Table 1). The main changes *versus* 2013 are related to adaptation of zones based on cases detected in 2013. For instance, screening was reinforced in Calvados, Sarthe and Marne. The Charente, Côte-d'Or, Dordogne and Pyrénées-Atlantiques *départements* account for approximately 6% of all French herds, but had 38% of the herds tested using SITT and 57% of those tested using SICTT. Special attention is therefore paid to the results of these four *départements*.

Involvement of veterinary professionals

Tuberculin testing (215,424 SICTTs and 475,330 SITTs) was carried out by 909 veterinary professionals (veterinarians or veterinary associations). The number of tuberculin tests was slightly lower *versus* 2013 but the number of professionals was the same.

Of the 753 veterinary professionals carrying out SITTs, the median number of farms tested by professionals was 3 and the mean number of SITTs was 636. Of the 366 veterinary professionals that performed SITTs on at least four different farms, there were 28 farms screened on average and 1,239 SITTs on average.

Of the 311 veterinary practices that carried out SICTTs, the median number of farms tested as part of programmed screening by professionals was 2 and the mean number of SICTTs performed was 698. Of the 166 veterinary professionals that performed SICTTs on at least two different farms, there were 16 farms screened on average and 1,261 SICTTs.

Of note, more than half of the veterinary professionals carried out testing in only one or two farms. It is important that these professionals be included in training and information campaigns to ensure quality screening procedures.

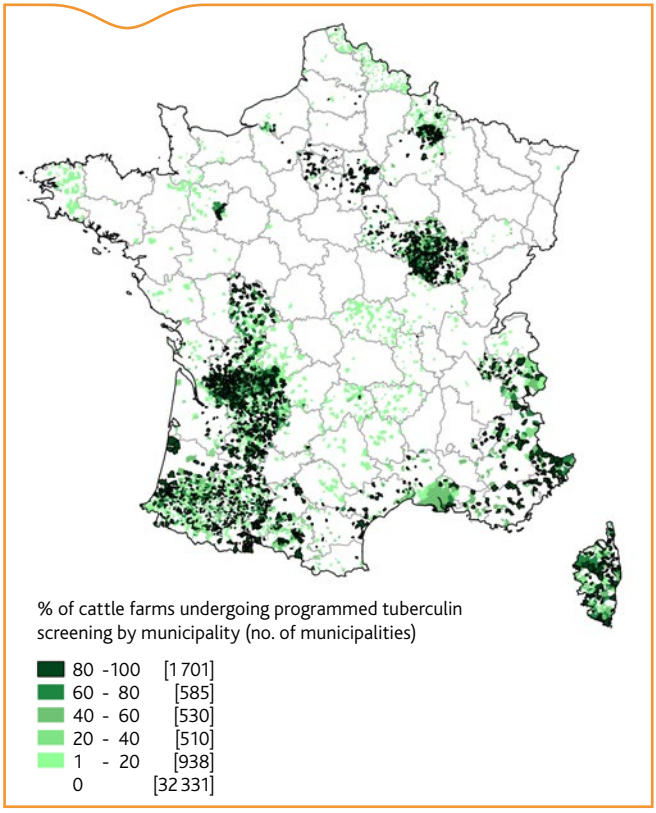

Figure 2. Rate of cattle farms undergoing tuberculin testing by municipality in France in 2014 as part of annual programmed screening campaigns

Use of interferon gamma

In *départements* where cattle are raised for bullfighting and where conditions for carrying out tuberculin testing are particularly difficult, first-line tuberculosis screening using interferon-gamma (IFN-gamma) was scheduled alternating with or in addition to intradermal tuberculin testing. An article presenting the full results of the study in the Camargue is available (Desvaux *et al.* 2015). In the Landes *département* in 2013-2014, 26 herds of ganaderia, in which animals are intended for shows, were screened using IFN-gamma. Dordogne has also implemented a strengthened follow-up protocol using interferon in parallel with intradermal tuberculin testing for screening. This protocol mainly targets farms with epidemiological links (n=68 in 2013-2014).

Surveillance on animal movements

Tuberculin testing on movement of animals was carried out for 139,429 cattle in 20,370 herds. However, data from several *départements* could not be processed due to data entry issues.

Scheme coordination

Training sessions and awareness meetings were again organised in 2014 to maintain the effectiveness of the surveillance system. The general training session on bovine tuberculosis, as part of occupational training for veterinarians to carry out certain public functions under contract with the administration (the "health mandate"), was organised by the Ministry of Agriculture and the National society for technical veterinary groups (SNGTV) in 33 *départements* with the participation of 178 veterinarians. Practical training on tuberculin testing continued to be developed *versus* 2013 and included 36 *départements* in 2014 with 209 veterinarians participating. In 2014, 86 professionals participated in the national training session on bovine tuberculosis intended for staff at the DDecPP. Also in 2014, the number of meetings organised by the DDecPP with bovine tuberculosis on the agenda was 93 with mandated veterinarians (109 in 2013) in 66 *départements* (63 in 2013) and 116 with livestock farmers (149 in 2012) in 40 *départements* (43 in 2013).

Furthermore, 57 meetings (96 in 2013) were organised in 32 départements (49 in 2013) for implementation and follow-up of the Sylvatub surveillance scheme.

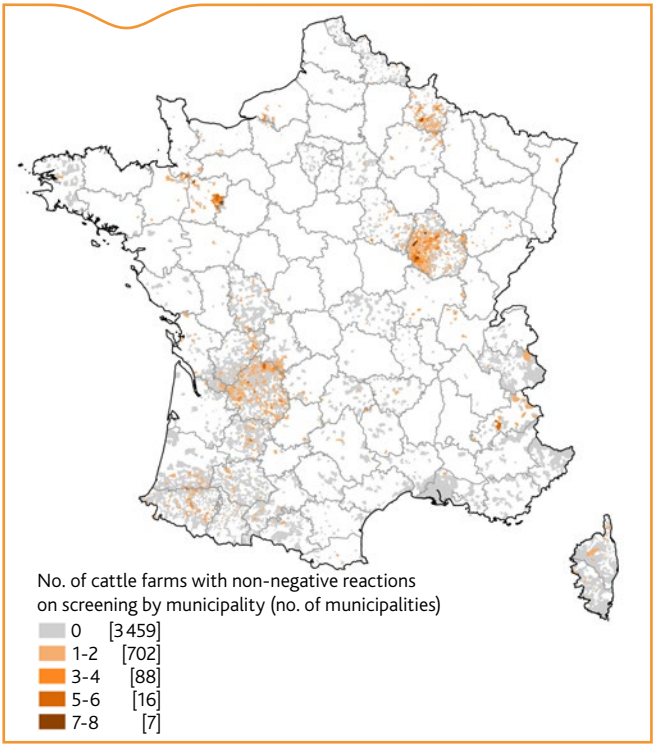

Figure 3. Distribution of the number of farms with a non-negative reaction in 2014 by municipality

Results of programmed screening

Tuberculin tests with non-negative results

Data available for 2014 show relative stability compared to 2013. 3,932 non-negative reactions (i.e. 0.6% of tuberculin tests *versus* 0.7% in 2013) were found on 1,279 farms (i.e. 9.2% *versus* 9.6% in 2013) (Fediaevsky *et al.*, 2013). The proportion of animals with non-negative reactions per herd was 0.47% on average using SITT and 1.6% using SICTT. These findings are surprising given the higher specificity expected with SICTT. The hypotheses and values for a few *départements* are indicated below.

There was an inconsistent geographic distribution of farms that had at least one non-negative reaction to a test (Figure 3). It is difficult to interpret why there were zones with tuberculin testing that had no non-negative results, because this depends on the influence of factors likely to cause reactions in cattle, the size of herds in the zone (and therefore the type of production), local testing conditions, and satisfactory data transmission. On average, 5.3% of herds tested with SITT presented at least one non-negative reaction, *versus* 25.5% of herds tested with SICTT. The difference is slightly smaller than in previous years. The proportion of herds presenting at least one non-negative reaction was 5.1% in Charente (7.8% in 2013), 31.7% in Côte-d'Or (36.7% in 2013), 9.6% in Dordogne (7.5% in 2013), and 3.9% in Pyrénées-Atlantiques (3.5% in 2013). The levels remain consistent, with a very high level of non-negative herds in Côte-d'Or.

The number of reactor cattle was lower *versus* 2013. In 2014, it was 2,069 (04%) for SITT and 1,863 for SICTT (0.8%), *versus* 2,716 (0.6%) and 2,171 (1.0%), respectively in 2013. Of the cattle with a non-negative SITT result, 31.9% presented a positive result. Of the cattle with a non-negative SICTT result, 11.0% presented a positive result, which is consistent with the higher specificity of SICTT. In Charente, the proportion of cattle with at least one non-negative reaction was 0.1% using SITT (0.8% in 2013), in Côte-d'Or 0.7% using SICTT (SITT no longer used), in Dordogne 0.3% using SITT (0.4% in 2013), and 0.7% using SICTT (1.4% in 2013), and lastly in Pyrénées-Atlantiques, it was 0.1% with SITT (1.1% in 2013) and 0.4% with SICTT (0.3% in 2013).

The finding of a lower proportion of non-negative cattle with SITT, although less specific, than with SICTT, across all *départements* in 2014, is surprising. This difference can be explained by various factors, including the use of SICTT in zones with a high prevalence of atypical reactions, greater attention paid by professionals during measurement of skin folds related to the method, and lastly a classification bias due to the risk of results corresponding to retests in herds with SITT reactions having been erroneously attributed to intradermal tuberculin screening results.

The non-negative results were reported by 278 veterinary practices, i.e. an increase of 16% *versus* 2013. The veterinary practices that reported at least one non-negative reaction carried out 72% of tuberculin tests country-wide *versus* 60% in 2013. They accounted for 86.8% of tuberculin tests carried out in Charente (30.3% in 2013), 97.0% in Côte-d'Or (84.8% in 2013), 94.7% in Dordogne (39.6%inn 2013) and 68.8% in Pyrénées-Atlantiques (42.1% in 2013). These increases may be partial indicators of greater sensitivity of detection on the basis of a better rate of reporting in these four *départements*.

In 2014, the number of SITTs and the proportion of non-negative results detected are positively correlated (correlation $\tau = 0.26$ $p<10^{-16}$), this is also true of the number of SICTTs and the proportion of non-negative results detected (correlation $\tau = +0.19$, $p<10^{-6}$). Given that tests are not infallible in terms of specificity, this positive correlation was expected.

Screening with IFN-gamma

Use of this test was reported in a specific article about surveillance in the Camargue (Desvaux 2015). In the Landes *ganaderias*, eight farms presented non-negative results with interferon. As part of reinforced surveillance in Dordogne, 22 farms presented a reaction that was non-negative on intradermal tuberculin testing.

Surveillance on animal movements

Based on collected data, non-negative results were obtained for 186 animals (0.1% of tested animals) in 141 herds, i.e. 0.7% of herds tested

Table 2. Surveillance of bovine tuberculosis at the slaughterhouse in 2014 based on reasons for inspection

		Number	Proportion (%)
Routine surveillance	ODF herds with suspected case at slaughterhouse	>532	100%
	Cattle from an ODF herd with suspected bovine TB lesions	532	
	Cattle from an ODF herd with a lesion confirmed to be bovineTB (rate of confirmation %)	25	4.7%
Diagnostic slaughter	Herds having undergone diagnostic slaughter	841	0.4%
	Herds with confirmation on diagnostic slaughter (rate of confirmation %)	84	10%
	Cattle undergoing diagnostic slaughter	2,203	
	Cattle undergoing diagnostic slaugher confirmed infected (rate of confirmation %)	90	4.1%
Partial depopulation	Herds undergoing partial depopulation*	44	
	Cattle undergoing partial depopulation*	2,926	
	Reactor cattle undergoing partial depopulation*	184	
	Cattle undergoing partial slaughter confirmed infected*	36	1.2%
Complete depopulation	Herds undergoing complete depopulation	61	
	Herds undergoing complete depopulation with lesions	29	47.5%
	Cattle slaughtered under complete depopulation	7,669	
	Cattle slaughtered under complete depopulation with lesions	175	2.28%

* based on data available in SIGAL and reported by the DDecPP in the annual report
ODF: officially disease-free

Table 3. Number of bovine tuberculosis outbreaks in France in 2014, detection circumstances and funding

Incident outbreaks 2014 (herds) (%)	105 (0.05)
Prevalent outbreaks 2014 (herds) (%)	190 (0.089)
Prevalent herds as of 31/12/14 (%)	83 (0.039)
Infected imported cattle	1
Proportion of herds undergoing complete depopulation (%)	58.1
Outbreaks detected at slaughterhouse (%)	20
Outbreaks detected through programmed screening (%)	60
Outbreaks detected through movement surveillance (%)	1
Outbreaks detected by epidemiological survey (%)	18
Outbreaks detected in another way (%)	1
Veterinary fees (%)	11.3
Compensation (%)	63.8
Laboratory fees (%)	19.8
Cleaning-disinfection (%)	0.8
Miscellaneous costs (%)	0.7
State screening subsidy (%)	3.7

in this programme (2% in 2013). These herds were distributed among 44 *départements* of the 78 *départements* that reported results (versus 24 of 60 in 2013).

Slaughterhouse surveillance

Based on collected data, 532 cattle (224 in 2013) from officially disease-free herds in 64 *départements* (45 in 2012) (Table 2) presented suspected tuberculosis lesions at the slaughterhouse. The number of cases confirmed by this system has remained stable and as a result, the confirmation rate of these lesions has dropped to 4.7% (25/532) *versus* 13.5% in 2013. The higher number of suspected cases associated with a reduced rate of confirmation is an encouraging sign of improved sensitivity for this type of screening and of the effectiveness of training set up at slaughterhouses. The stable number of confirmations is also a reassuring indicator of the general veterinary picture for bovine TB. The number of suspected cases at the slaughterhouse is still inconsistent but has increased in three of the four priority *départements*, which is a good sign. This number has changed from zero in Charente, 22 in Côte d'Or and four in Dordogne in 2013 to 7, 46 and 12 respectively for each *département* in 2014. The number of suspected cases (n=11) is stable in Pyrénées-Atlantiques. The number of cattle confirmed as infected in 2014 was one in Charente, two in Côte-d'Or, three in Dordogne and two in Pyrénées-Atlantiques, *versus* zero, one, four and five respectively in 2013. In 2014, 114 slaughterhouse staff had received training on tuberculosis in the previous five years. Given the staff turnover, it is important to pursue this awareness programme.

Surveillance of herds likely to be infected

On the basis of available data, an epidemiological link with an outbreak was identified during the calendar year for 3,655 herds in 73 *départements*. In Charente, data recording was not finalised. 516 herds with a link to an outbreak were found in Côte-d'Or, 1,141 in Dordogne (versus 55 in 2013, but this Figure was recorded very inconsistently in Sigal), and 124 in Pyrénées-Atlantiques. These variable findings may be related to differences in epidemiological situations or different data processing methods. The situation should become more harmonised following publication of a new memorandum enabling greater follow-up of epidemiological surveys with higher quality tools (NS 2015-468).

Tuberculin tests were carried out in nearly three times as many herds with an epidemiological link than in 2013, i.e. 1,483 *versus* 690, there was nonetheless a stable proportion of 41% of investigated links (versus 44% in 2013). Of these, 337 showed non-negative reactions (9% *versus* 22.8% in 2013).

Diagnostic slaughter

Diagnostic slaughter was performed in 8% of herds with an epidemiological link (296/3,655) and in some cases was performed irrespective of the results of intradermal tuberculin tests. These investigations led to confirmation of infection on 33 farms, i.e. a confirmation rate in herds with likely infection and undergoing diagnostic slaughter of around 11% (33/296).

Measures taken in suspect herds

Collected data indicate that 1,301 herds spread across 61 *départements* underwent tuberculin testing as part of health control measures when cases were suspected, and 324 had at least one non-negative reaction (25% compared with 45.3% in 2013). It appears that there was a considerable decrease in the proportion of herds with non-negative reactions. However, data at the herd level were not available for Charente, Dordogne, and Côte-d'Or. In Pyrénées-Atlantiques, there were 96 herds (versus 120 in 2013) with an intradermal tuberculin test as part of control measures, of which nine presented non-negative reactions, i.e. 9.4% *versus* 12.5% in 2013. At the animal level, the number of non-negatives in terms of the number of animals tested was stable in Charente (5.1% in 2014 *versus* 5.0% in 2013), lower in Côte-d'Or (2.1% in 2014 *versus* 2.6% in 2013) and in Dordogne (1.7% in 2014 *versus* 2.1% in 2013), and higher in Pyrénées-Atlantiques (1.33% in 2014 *versus* 0.47% in 2013).

IFN-gamma tests continued to be used for the second consecutive year as part of an experimental diagnostic programme that is being evaluated scientifically. The conclusions are expected in the first half of 2016.

Diagnostic slaughter

One or more diagnostic slaughter orders were issued for 841 farms (976 in 2013). A total of 2,203 cattle were slaughtered for diagnostic

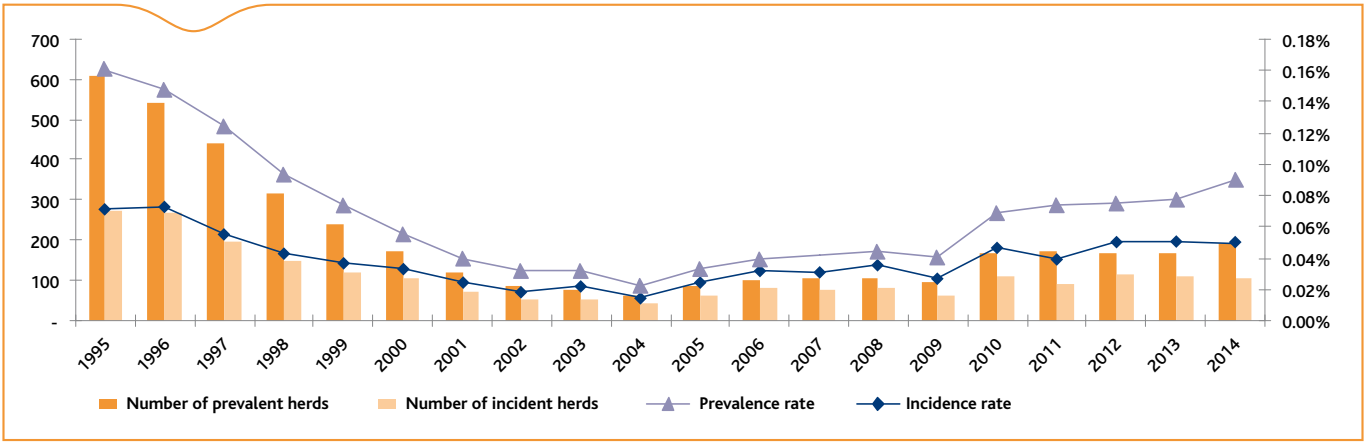

Figure 4. Change in the prevalence and incidence of bovine tuberculosis from 1995 to 2014

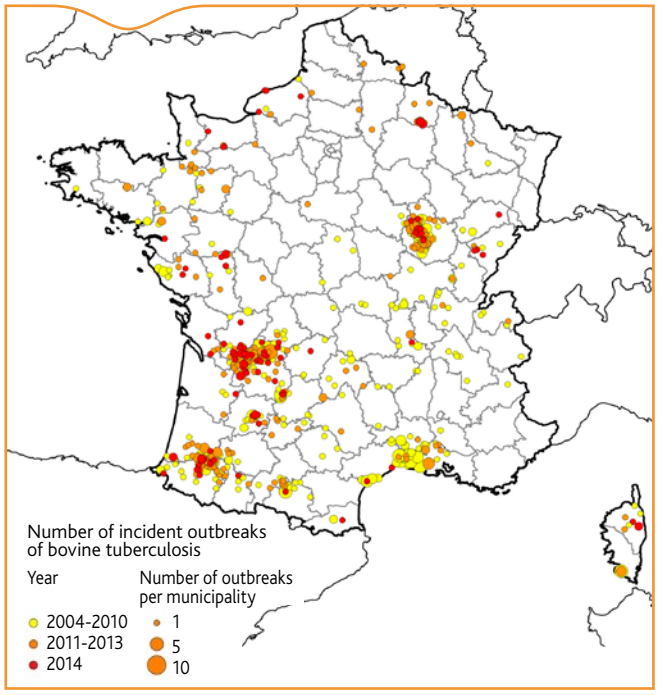

Figure 5. Geographic distribution by municipality of incident outbreaks of bovine tuberculosis in France from 2004 to 2014

purposes (2,004 in 2013), up by 10%. The confirmation rate was 10.0% (84/841) at the farm level (8.0% in 2013) and 4.1% (90/2,203) at the animal level (5.6% in 2013) (Table 3). There were 66 slaughtered cattle in Charente, 381 in Côte-d'Or, 310 in Dordogne, and 235 in Pyrénées-Atlantiques, of which 24.2%, 2.3%, 7.4%, and 2.1% were confirmed as infected, respectively. The situation in Charente (which had a very high confirmation rate among animals slaughtered for diagnostic purposes) contrasted with that of the other *départements*, and with the data for 2013 (2.7% confirmation rate). However, the ratio of the number of cattle slaughtered diagnostically per individual animal with a non-negative reaction on screening by tuberculin test could not be calculated with the available data.

Outbreaks

Incidence, prevalence and geographic location

In 2014, 105 herds were reported as newly infected (112 in 2013), i.e. an incidence of 0.05% (105/212,550) and prevalent cases amounted to 190 infected herds, yielding a prevalence rate of 0.075% (190/212,550). These values have been stable since 2012 (Figure 3) (Fediaevsky *et al.*, 2013).

As for geographic location (Figure 5), 46% of incident outbreaks were detected in Aquitaine with a slight decrease in the number of outbreaks in all of the region's *départements*. Charente however showed a marked increase in the number of outbreaks, with an increase from two in 2013 to 12 in 2014. Following suspicion on necropsy of a goat, an outbreak was identified on a mixed goat and cattle farm in Deux-Sèvres, leading to detection of four outbreaks in all. In the Burgundy region, the number of new outbreaks in Côte-d'Or continued to decrease (-50% in 2014 and -30% in 2013), and no new outbreak was detected in Nièvre or in Yonne. In Ardennes, five secondary outbreaks were detected, still in the same zone, through investigations carried out following the 2012 slaughterhouse cases, and the same BCG strain was involved. In the Camargue, two new outbreaks were detected by programmed surveillance on the farm, confirming the effectiveness of the plan implemented in the zone and the benefits of sustained vigilance. In Ariège, one new outbreak was detected in the at-risk zone identified in 2010. In Mayenne, no new outbreak was identified. In Haute-Corse, outbreaks continued to be detected in zones where they had been identified in the past.

Means of detection

Overall, more than 78% of incident outbreaks in 2014 were detected on farms by skin test screening based either on routine tuberculin screening (60%), (Table 4, Figure 6), or on farms "likely to be infected", i.e. those that have an epidemiological link to an outbreak (18%). The proportion of outbreaks detected through slaughterhouse screening was again lower compared to previous years, which is reassuring.

Control of the disease

Control of infected herds was carried out by partial depopulation in 44 outbreaks in 9 *départements*, and by complete depopulation in 61 outbreaks in 21 *départements*. Since control through partial depopulation is slower, of the 78 prevalent outbreaks as of 31 December 2014, 64% were still undergoing partial depopulation.

According to available data, 2,926 cattle were managed by partial depopulation. Of these, there were 184 reactors (6%) and 36 cattle confirmed as infected (1.2%) spread across five *départements*. On five farms, control initially through partial depopulation was changed to complete depopulation (one in Ardennes, three in Dordogne, and one in Pyrénées-Atlantiques). Complete depopulation led to 7,669 cattle being slaughtered: 175 (2.28%) of them, spread across 29 herds, had lesions suggestive of bovine tuberculosis (Table 3). This means, on the one hand, that in 53% of the outbreaks managed through complete depopulation, no lesions were detected and infection was confirmed positive only through the index case, and on the other, that in 47% of the herds, lesions were found in an average of six cattle. However, this mean Figure masks wide diversity. In Charente, the mean number of cattle with lesions in the herds controlled through complete depopulation and where lesions were detected was 11.3. There were zero cattle with lesions in farms managed by complete depopulation in Côte-d'Or, and a mean of 1.2 in Dordogne, and 8.3 in Pyrénées-Atlantiques, while in these *départements*, there were respectively 11, three, five, and six complete depopulations in 2014, and three, zero, five, and three farms in complete depopulation where lesions were found. These data appear to indicate detection of outbreaks at an advanced stage in Charente, which should prompt greater vigilance in the follow-up of controls and screening the coming years.

Costs

On the basis of cost information provided by the DDecPPs, funding from government for 2014 was €17,537,028 before tax to cover the items listed in Table 3. The mean national expenditure (compensation and disinfection costs) per prevalent outbreak was €107,000 in 2014. This indicator, which hides significant differences, is however stable. The amount was €106,000 in 2013, €114,000 in 2012, and €108,000 in 2011. It will be interesting to follow up this indicator with the opening of partial depopulation to the entire country by the new Memorandum

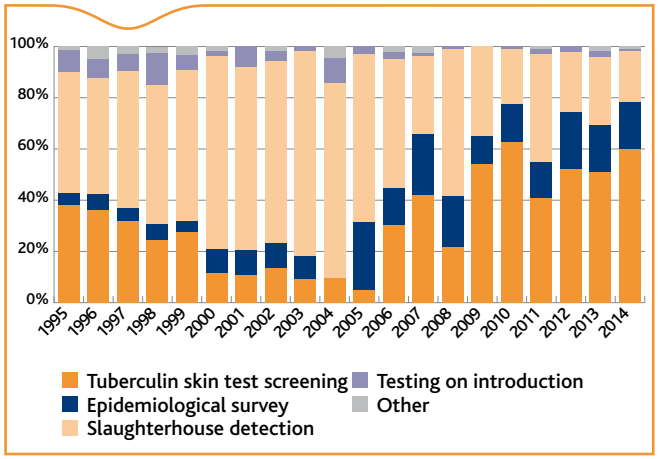

Figure 6. Distribution of detection means (%) for bovine tuberculosis outbreaks between 1995 and 2014

2014-691 of 20 August 2014. Furthermore, these costs must be added to those related to wildlife surveillance estimated at around €1M, with 80% provided by the central administration and 20% by DDecPPs.

Discussion

The completeness and accuracy of collected data could be improved. Tools to simplify the centralisation and extraction of data to coordinate efforts and communicate on the system are being developed. These are structural efforts concerning information systems, their use, and their exploitation, and will help to use data more directly as early as 2015. A significant improvement in data quality is to be expected in the coming years.

In terms of surveillance, on-farm testing has remained stable, measured in particular by the rate of non-negative reactions to skin tests and a decrease in the relative share of outbreaks detected at

Box 2. Sylvatub: Tuberculosis surveillance in wildlife

Since the discovery of the first red deer infected with tuberculosis in Brotonne forest (Seine-Maritime) in 2001, wild infected animals have subsequently been identified in several *départements* across France: Côte-d'Or, Corse-du-Sud, Haute-Corse, Pyrénées-Atlantiques, Dordogne, and Charente, then Ariège (ANSES, 2011; Hars *et al*, 2010). At the end of 2011, on the initiative of the Ministry of Agriculture, a national surveillance program called Sylvatub was established as part of the National Epidemiological Surveillance Platform for Animal Health. It includes outbreak and programmed surveillance protocols with the aim to carry out an integrated assessment of sampling procedures, to harmonise diagnostic methods, and to centralise data from various surveillance systems (Rivière *et al*., 2013).

Badger surveillance

In 2014, 2,727 badgers from 27 *départements* were analysed, including 361 found dead on the roadside or collected by the SAGIR network (outbreak surveillance) in *départements* with reinforced surveillance and 2,366 captured in at-risk areas (programmed surveillance). The number of infected badgers detected was 86 in total in seven *départements*, including 10 from outbreak surveillance (3.2% apparent prevalence) and 76 from programmed surveillance (2.7% apparent prevalence) (Figure 1) *versus* 9/211 (4.3%) and 65/1,508 (4.3%) in 2013. The 60% increase in the number of trapped badgers was associated with a 25% decrease in the proportion of positive badgers. This observation requires critical analysis of the sampling protocol.

Deer

Between 01/08/2013 and 31/07/2014, 347 red deer and 33 roe deer were inspected or analysed from 24 different *départements*. Of these analysed deer, 52 were identified through outbreak surveillance (suspicions on lesions in hunted animals and deer found dead collected by SAGIR network) and 328 through the programmed surveillance plans for hunted animals in at-risk areas. None of the analysed deer were found to be infected (Figure 1), compared to four in 2013.

Wild boar

Between 01/08/2013 and 31/07/2014, 1,372 wild boars were analysed from 30 *départements*. Of these analysed wild boars, 66 were identified through outbreak surveillance (suspicions on lesions in hunted animals and wild boar found dead collected by SAGIR network) and 1,306 through programmed surveillance plans for hunted animals in at-risk areas. In all, tuberculosis infection was detected in 44 wild boars from seven different *départements,* including 10 detected through outbreak surveillance and 34 through programmed surveillance (Figure 1). The proportion of infected animals was therefore slightly higher than in 2013 (6/48 and 20/1270, respectively).

In 2013-2014, infected wildlife was always identified in relation to the presence of the disease in cattle, both in terms of the similarity of implicated strains and the geographic areas. *Départements* with infected wildlife were Ardennes, Charente, Côte-d'Or, Dordogne, Corse-du-Sud, Haute-Corse, Landes, Lot-et-Garonne, Pyrénées-Atlantiques and Seine-Maritime.

The results of the Sylvatub programme should however be interpreted with caution given the wide range of surveillance protocols involved. Detailed reports are available in the the ESA Platform website (www.plateforme-esa.fr).

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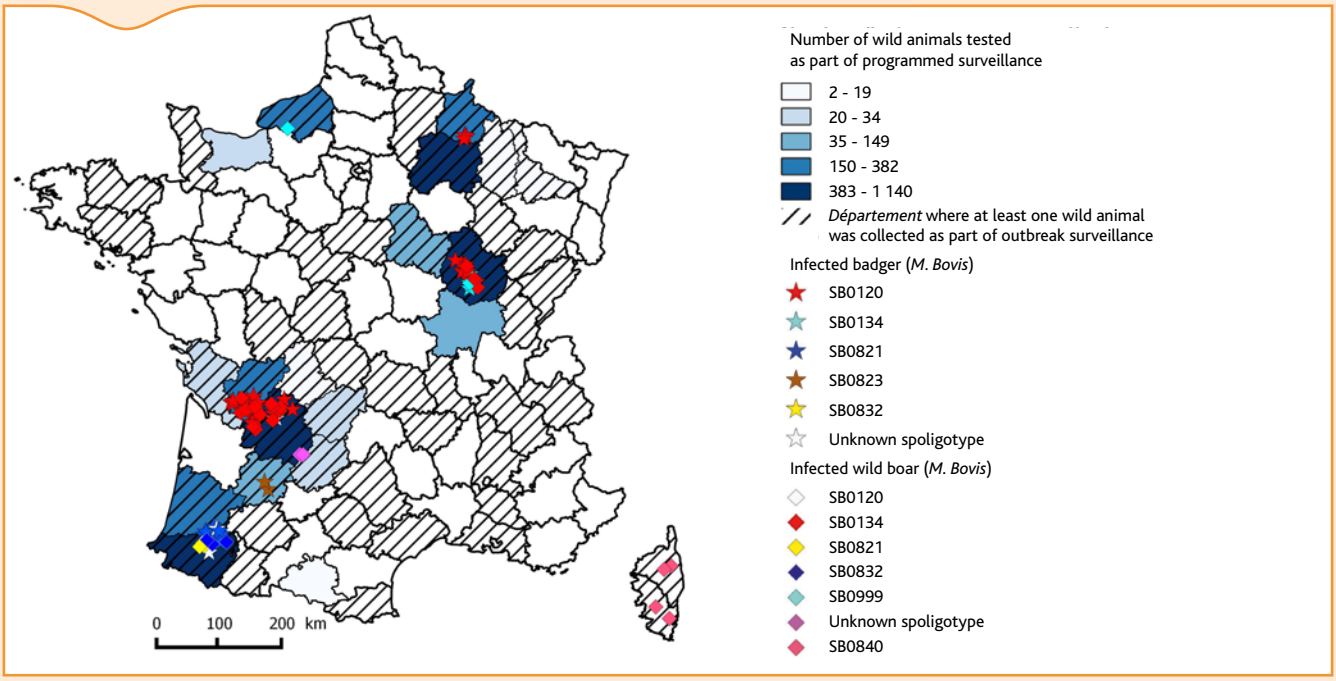

Figure 1. Distribution and results of the analyses undertaken under the Sylvatub program in wild ongulates from August 2013 to August 2014 and in badgers in 2014

the slaughterhouse. This improvement has contributed to detection of outbreaks at earlier stages of infection. The fact that many diagnostic slaughter procedures are carried out with no infection being confirmed should not detract from the fact that some of these negative procedures involve infected non-detected animals (limited sensitivity of diagnostic slaughter), cases that have thankfully been removed (Bekara, 2014). The high increase in the number of suspected cases at the slaughterhouse with no increase in the number of confirmations is a positive sign concerning renewed awareness efforts directed towards slaughterhouse personnel.

The south-west *départements* have an increasingly large proportion of the detected outbreaks. Interlinking of farms through contacts between neighbouring plots and infected wildlife in this zone call for sustained efforts in screening and control measures for farms to guarantee long-term eradication of this disease, without letting a wildlife reservoir develop.

Partial depopulation is a way to improve the social and financial acceptability of control measures for infection generally detected at an early stage. It can now be used not only in certain geographic zones but on the basis of a certain number of criteria aimed at not endangering the effectiveness of the control measures. This will need to be confirmed.

In late 2014, revision of the second version of the national plan for the control of bovine tuberculosis was initiated. The core of this action plan is to put forward proposed actions that take into account the need to control the disease in the long-term and to coordinate efforts, particularly concerning earlier detection. In this context, the role of interferon, the screening strategy in terms of geography and timing, as well as surveillance based on risk (movements in particular) will be re-evaluated through national and regional consultation of the stakeholders through 2015, and in the medium-term through research and modelling projects.

The overall situation in France in 2014 was favourable, with an annual incidence rate below 0.01%, making bovine tuberculosis a rare disease. However, the status of officially disease-free territory does not mean the disease has been eradicated. Therefore, all stakeholders have multiplied their efforts in various areas of the country, with increasing effectiveness, but this should be strengthened in some areas and sustained in others.

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Box 3. Genotype of strains involved in bovine tuberculosis outbreaks in 2014 in France

In 2014, genotypes (spoligotyping + VNTR techniques) of *M. bovis* were determined for 93 of the 105 incident outbreaks in 2014. Twenty different genotypes were found. The phenomenon of regionalisation of strains is still as marked as in previous years. The most commonly represented genotypes among the 93 outbreaks (72%) were "BCG-Ardennes", "BCG-Côte d'Or", "BCG-Dordogne-Charente", F7 in Pyrénées-Atlantiques, and F41 in Lot-et-Garonne.

Less common types such as F1 in Corsica, F61 in Camargue, F15 in Pyrénées-Atlantiques, "GB35-Ariège-Haute Garonne", or "GB54-Sud-Ouest", already observed in these same regions the previous year, were also found in 2014.

Moreover, other locally recurrent types such as "GB54-Doubs", observed in 2011 and 2012, SB0999 regularly found from 2004 up to 2011 in the south of Dordogne, as well as type F5 observed in 2003 and 2010 in Pyrénées-Atlantiques, and F96 observed in Hautes-Pyrénées in 2000 and 2003, reappeared in 2014 in the same regions. Type "GB35-Calvados" observed in 2014 in Calvados, was also found in 2008 in cattle from this area. Concerning the BCG strain in Pyrénées-Orientales, this type was already implicated in outbreaks in this *département* in 2005 and 2011. This re-emergence phenomenon was also found in a more marked manner with the discovery in 2014 of a GB20 type already found in the same mixed caprine/bovine herd in 1990 in Deux-Sèvres. Detecting these uncommon types a long time apart in the same regions highlights the lack of knowledge on the epidemiology of these outbreaks and calls for overall reinforced vigilance in these areas.

This characteristic high level of regionalisation of strains was a helpful guide in determining the origin of outbreaks with F110 type in Dordogne or "GB35 Calvados" type in Corrèze. These two types found in the *départements* in question for the first time were probably introduced to meat production farms that practice milk-fed veal production with dairy cows, by beef cattle cows" from Ille-et-Vilaine for the first and Normandy for the second, where these types had already been found in the past.

Concerning the other strains of spoligotype GB54, the "main" VNTR type was already found in the 1990s in Seine-Maritime but since this is a common type in both France and Spain, it is not possible to establish the origin of the outbreak with certainty *via* strain typing.

Strains of the "GB54 Spain" type belong to strains introduced directly from Spain with imported animals.

In conclusion, local persistence of strains was once again reported in 2014, with some strains being particularly dominant and others being expressed more intermittently. This demonstrates weakness in detection and elimination of the disease in these regions.

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Absence of **bovine brucellosis** confirmed in 2014, but vigilance must be maintained

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Abstract

France has been declared officially free from bovine brucellosis by the European Commission since 2005. Two outbreaks were confirmed in 2012 (the first due to a *Brucella abortus* infection in an imported cow, the second due to a wild reservoir of *Brucella melitensis* in the Bargy Massif in Haute-Savoie), but the implemented control measures made it possible to maintain the country's disease-free status. Reinforced surveillance measures implemented in the Bargy Massif did not detect any outbreaks in 2013 or 2014, in either cattle or small ruminants. While surveillance results have been favourable so far, the vigilance of all those involved in the programmed and outbreak surveillance of brucellosis should be maintained. Furthermore, discussions are under way to improve abortion notification, to coordinate reports as well as possible with the differential diagnosis protocol for abortions, and to better analyse collected data.

Keywords

Category 1 health hazard, Regulated disease, Bovine brucellosis, Surveillance, Control

Résumé

L'absence de brucellose bovine est confirmée en 2014, mais la vigilance reste de mise

La France est reconnue officiellement indemne de brucellose bovine par la Commission européenne depuis 2005. Deux foyers de brucellose bovine ont néanmoins été confirmés en 2012 (le premier lié à l'importation d'un bovin infecté par Brucella abortus, *l'autre lié à un réservoir sauvage de* Brucella melitensis *dans le massif du Bargy en Haute-Savoie) mais leur maîtrise a permis de maintenir le statut indemne. Une surveillance renforcée dans le massif du Bargy se poursuit et aucun foyer n'a été détecté en 2013 et 2014, ni dans le cheptel bovin ni chez les petits ruminants. Ces résultats favorables ne doivent toutefois pas faire diminuer la vigilance des acteurs impliqués dans les dispositifs de surveillance programmée et événementielle de la brucellose. Des démarches ont d'ailleurs été engagées pour faire évoluer le dispositif de déclaration des avortements, l'articuler au mieux avec le protocole de diagnostic différentiel des avortements, et mieux exploiter les données ainsi collectées.*

Mots-clés

Danger sanitaire de 1ère catégorie, maladie réglementée, brucellose bovine, surveillance, police sanitaire

Infection of an animal by any *Brucella* other than *B. ovis* and *B. suis* biovar 2 is classified as a Category 1 health hazard (Ministerial Order of 29 July 2013). Some *Brucella* species are found more specifically in certain animal reservoir species, for instance, *B. abortus* in cattle and *B. melitensis* in small ruminants. Given the risk to public health, the surveillance system in ruminants in France targets these two species of *Brucella*.

France has been officially recognised as bovine brucellosis-free since 2005 (Decision EC/2005/764). Although no cases had been detected since 2003, two cases of bovine brucellosis were confirmed in 2012 (one in the Pas-de-Calais *département* linked to the introduction of a cow from Belgium, the other in the Bargy Massif in the Haute-Savoie *département*, linked to wildlife) (Garin-Bastuji *et al.*, 2013; Rautureau *et al.*, 2013). In 2014, the objectives of bovine brucellosis surveillance were i) to demonstrate that the outbreaks of 2012 had been brought under control, and thus justify maintaining France's disease-free status, and ii) to enable sufficiently rapid detection of any re-emergence of brucellosis.

Surveillance system for bovine brucellosis

Current surveillance and control measures for bovine brucellosis have been in place since 2010 (Box 1). Surveillance is based on the declaration and the investigation of abortions, as well as on annual serological screening (on blood or pooled milk) of all cattle herds (with the exception of exempted fattening herds).

Brucellosis screening campaigns on farms are organised during the winter season, between October and April, and not over the calendar year. In contrast, surveillance data are collected by calendar year for management reasons (annual reports and financial reports). As a result, the results shown in this article cover monitoring carried out from January to December 2014, i.e. including the end of the 2013/2014 farm year and the beginning of the 2014/2015 farm year.

Programmed surveillance: serological surveys

Data for screening performed in 2014 covered 173,326 herds (81.5%) undergoing "prophylaxis"[1], among the 212,550 cattle herds in the country (Table 1). The screening by serological analysis on blood (individual or pooled) concerned 117,194 herds (67.6%) and screening by analysis of pooled milk concerned 56,132 herds (32.4%).

Outbreak surveillance: declaration and investigation of abortions

Concerning surveillance of abortions, a total of 65,743 abortions were recorded in 2014 (compared with 61,021 in 2013) in 36,777 different herds (34,329 in 2013) (Table 1).

Like in previous years, but with slightly higher proportions, the ratio of reporting breeders was higher on dairy farms (37%) or mixed farms (39%) than on beef farms (16%) and very small farms (1%; these farms have less than 10 breeding cows). A single abortion was reported by

1. Herds with at least one animal over 24 months, excluding exempt fattening units.

Box 1. Surveillance and health control measures for bovine brucellosis

Objectives of the surveillance programme

- Early detection of any re-emergence of brucellosis in domestic cattle.
- Provide evidence of the country's officially bovine brucellosis-free status.

The population monitored

All domestic cattle herds in mainland France.

Surveillance procedures

Programmed surveillance

Programmed surveillance consists of annual serological screening either through blood samples from at least 20% of animals over 2 years of age, or on pooled milk from herds to be monitored. An exemption from annual serological screening may be granted by the DDecPP under certain conditions described in the Ministerial Order of 22 April 2008 for fattening herds in which cattle are kept in closed facilities. Blood screening is carried out using the Rose Bengal Test (RBT)[1]. The complement fixation (CF) test, which is more specific than the RBT, is only implemented in the event the RBT proves positive (a negative CF can refute a positive RBT). Milk screening is performed using an ELISA method.

Outbreak surveillance

Reporting all abortions is mandatory. Any cow that aborted must undergo serological screening by RBT and a swab sample from the uterine cervix is taken for bacteriological analysis in the event of positive serology (positive RBT and CF).

Health control measures

Investigation of non-negative results in programmed surveillance

The result of individual screening on blood is considered to be unfavourable when both tests (RBT and then CF) are successively positive. Blood screening leads to a suspected case being declared (i.e. the issuing of a Prefectural Monitoring Order (APMS)) only after two series of controls at a six to eight week interval, both of which were unfavourable. A brucellin test is then carried out.

1. In cattle, the Rose Bengal test can be replaced by an ELISA test on pooled serum from ten animals, along with an individual RBT in the event of a positive result.

If screening on milk produces an unfavourable result, a second control on pooled milk is carried out six to eight weeks later. If the second repeat control is positive, the sample is sent to the NRL, which performs a ring test. If this new test gives a positive result, the herd is placed under APMS and the animals that contributed to the pooled milk undergo individual serological controls (RBT and CF). If some of these serological controls yield unfavourable results, a brucellin test is then carried out.

The brucellin test is performed on a group of animals (10 individuals) including the animals that reacted positively to the previous individual serological tests plus seronegative contact animals. If the brucellin tests (or, in their absence, a renewed individual serological control) are positive, then diagnostic slaughter is performed to detect *Brucella* on the lymph nodes.

The herd is considered infected and placed under APDI if a *Brucella* strain is detected on culture, or if the suspected farm has a direct epidemiological link to an infected farm, through animal movements, for example.

Investigation of non-negative results in outbreak surveillance

If screening of a positive cow having aborted is positive, the farm is placed under APMS and the uterine cervix swab is taken for bacteriological analysis. If the swab is not available or cannot be collected, for example if antibiotics have been administered, diagnostic slaughter of the animal is performed to carry out bacteriological testing of the lymph nodes. The farm is placed under APDI if the bacteriological analysis is positive.

Measures taken in herds under Prefectural declaration of infection (APDI)

The whole herd is slaughtered if *Brucella abortus* or *B. melitensis* is isolated.

Regulations

Council Directive 64/432/EEC of 26 June 1964, as amended, on animal health problems affecting intra-Community trade in bovine animals and swine, establishing requirements for control measures applicable to intra-Community trade and import of animal sperm from the swine species.

Ministerial Order of 22 April 2008 establishing the technical and administrative framework for collective prophylaxis and control measures for bovine brucellosis

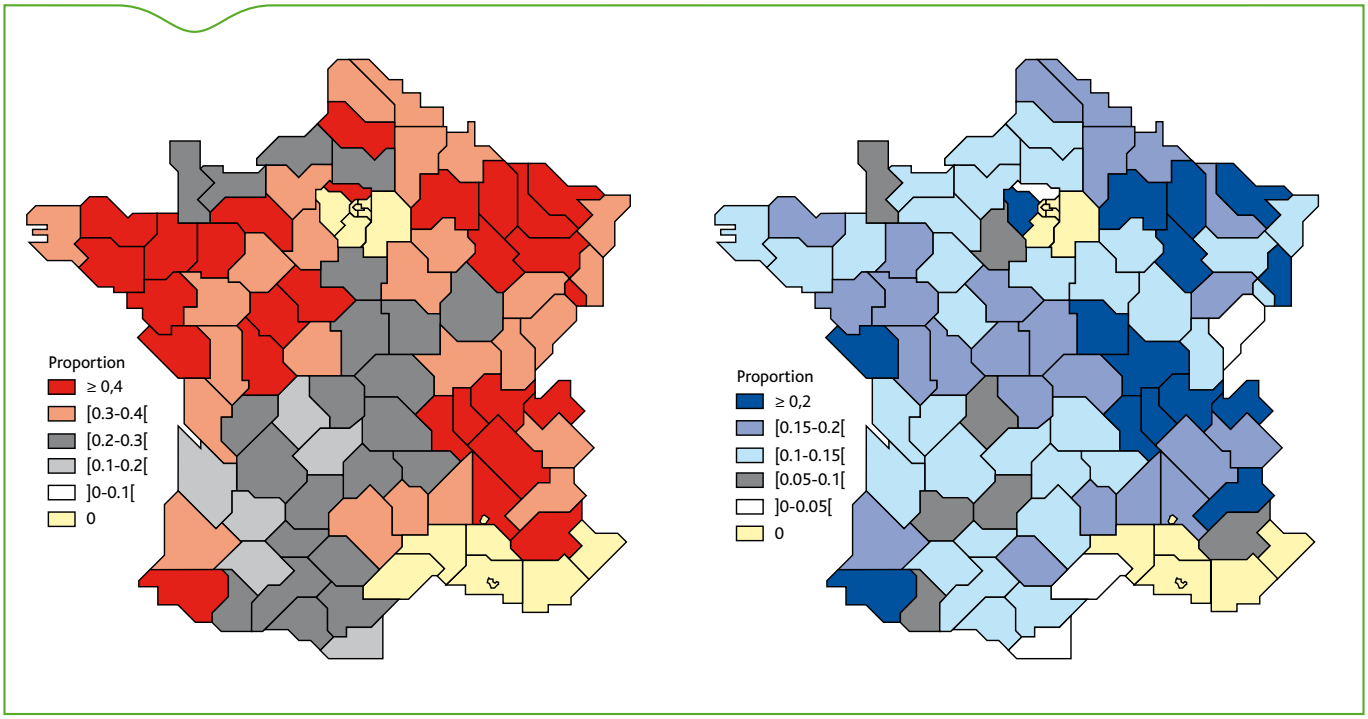

Figure 1. Departmental distribution of the proportion of breeders making declarations in dairy (left, in red) and beef farms (right, in blue)

Table 1. Surveillance and health control measures for bovine brucellosis by region of mainland France for 2014

Region	Population on 31 December 2012		Programmed surveillance						Investigation of suspected cases							
			Serological tests			Test on pooled milk			Abortions			Epidemiological investigation				
	Farms	Animals	Number of farms	Number of animals	Number of non-negative animals at first test	Number of farms	Number of pooled samples	Number of non-negative animals at first test	Number of herds reporting at least one abortion	Number of reported abortions	Number of positive serology results following abortion	Number of animals with serological tests	Seropositive animals	Brucellin tests	Diagnostic slaughter	Farms under Prefectural Monitoring Order
Alsace	2,399	167,796	1,290	10,497	1	724	724	5	368	635	0	0	0	0	0	0
Aquitaine	13,052	695,715	9,425	95,381	2	1,633	1,633	0	1,678	2,763	0	141	1	2	0	4
Auvergne	16,466	1,576,201	10,768	155,391	8	3,654	3,654	30	2,526	3,734	2	108	6	0	4	13
Basse-Normandie	20,245	1,607,551	8,070	71,402	2	6,770	6,794	1	2,745	5,202	0	4	0	10	2	4
Bourgogne	9,525	1,346,635	7,291	138,453	4	558	559	1	1,624	2,625	0	3	1	0	0	3
Bretagne	22,284	2,036,218	7,813	65,851	1	7,137	7,143	11	5,890	11,647	1	12	1	0	0	4
Centre	5,736	610,429	4,108	65,061	5	902	907	0	847	1,564	0	22	3	34	2	6
Champagne-Ardenne	4,840	593,233	2,658	37,755	0	1,607	1,614	3	927	1,768	1	2	1	52	1	6
Corse	1,038	65,000	849	11,291	0	0	0	0	0	0	0	0	0	0	0	0
Franche-Comté	6,268	616,959	1,822	19,516	1	3,917	3,927	10	1,471	2,387	1	45	1	0	2	3
Haute-Normandie	6,282	601,391	3,312	32,982	5	2,151	2,205	2	926	1,649	1	351	1	11	2	5
Île-de-France	503	29,025	294	2,824	0	22	23	0	19	43	0	0	0	0	0	0
Languedoc-Roussillon	3,174	212,333	2,021	23,890	1	357	357	3	361	479	0	0	0	40	0	4
Limousin	9,578	1,070,111	8,225	125,463	12	261	261	3	1,031	1,418	1	5	1	97	2	12
Lorraine	8,452	935,292	4,234	53,477	2	3,069	3,089	15	1,789	3,386	1	3	1	19	0	3
Midi-Pyrénées	17,951	1,202,055	12,983	145,440	0	2,518	2,518	2	2,111	3,158	1	0	1	0	1	6
Nord-Pas-de-Calais	8,890	700,399	3,712	34,690	5	3,848	4,004	1	1,547	2,786	0	5	3	12	2	6
Pays de la Loire	25,471	2,533,936	11,614	152,512	0	7,995	8,000	9	5,330	10,692	1	311	3	30	3	4
Picardie	5,644	529,744	2,712	29,889	12	2,077	2,099	1	874	1,539	0	0	0	0	0	2
Poitou-Charentes	7,295	754,043	5,165	71,199	1	1,255	1,257	0	1,119	2,049	0	1	1	0	1	2
Provence-Alpes-Côte d'Azur	1,304	66,794	908	10,991	4	132	132	3	93	156	0	0	0	80	0	4
Rhône-Alpes	16,153	1,011,152	7,920	76,919	7	5,545	6,095	19	3,501	6,063	2	261	9	76	0	15
Total	**212,550**	**18,962,012**	**117,194**	**1,430,874**	**73**	**56,132**	**56,995**	**119**	**36,777**	**65,743**	**12**	**1,274**	**34**	**463**	**22**	**106**

68% of beef herds making declarations, 5% of dairy farms making declarations, and 51% of mixed production farms making declarations; these proportions differed very little from 2013. The other farms reported between 2 and 24 abortions. Out of the 61,526 visits, 4,163 (or 6.8%) reported multiple abortions.

The proportion of farms making declarations varied greatly by *département* (Figure 1). In dairy farming, it was higher than 40% in 24 *départements*, and zero in 13 *départements*. In beef farming, it was higher than 15% in 39 *départements*, and lower than 5% in 15 *départements*.

The fact that the proportion of farms reporting an abortion varied significantly between *départements* can be explained by differing departmental policies concerning the implementation of a protocol for differential diagnosis of abortions (with partial payment of analytical costs carried out for this purpose), and the level of coordination activities by local stakeholders.

Enhanced surveillance in the Bargy Massif

Following the outbreak of *B. melitensis biovar 3* in cattle in Haute-Savoie in 2012 (Rautureau *et al.*, 2013), reinforced screening was implemented as of 2012. In autumn 2014, the protocol implemented concerned herds with at least one animal grazing in the theoretical habitat of the Mountain ibex and included:

- monthly screening of pooled milk (ELISA) for all dairy herds concerned (n=61);
- screening after summer grazing for beef herds (n=15) (ELISA or RBT).

From 2014, the scheme no longer concerned all adult animals but a fraction of the herd (20% of animals aged more than 24 months with a minimum of 10 animals), focusing on animals that spent time in the Bargy Massif, and specifically gestating cows or those that had given birth since their return from summer grazing.

Between June and December 2014, just one sample of pooled milk proved positive in the ELISA test. This result was refuted by a ring test performed by the NRL.

In autumn 2014, 196 animals were tested using blood samples. No positive results were found.

The overall results of the screening analyses of blood and milk obtained in the framework of the enhanced surveillance in the Bargy Massif have therefore been favourable since 2012.

Suspected and confirmed cases

Overall, results obtained for 2014 concerning suspected and confirmed cases are stable versus 2013. Detection of a case of *Brucella suis* biovar 2 during introduction control of an animal should be noted (Box 2).

Suspect abortions

Only 12 of the 65,743 reported abortions, i.e. 0.018%, were associated with a positive serological result *via* both RBT and CF testing, the regulatory definition of suspect animals.

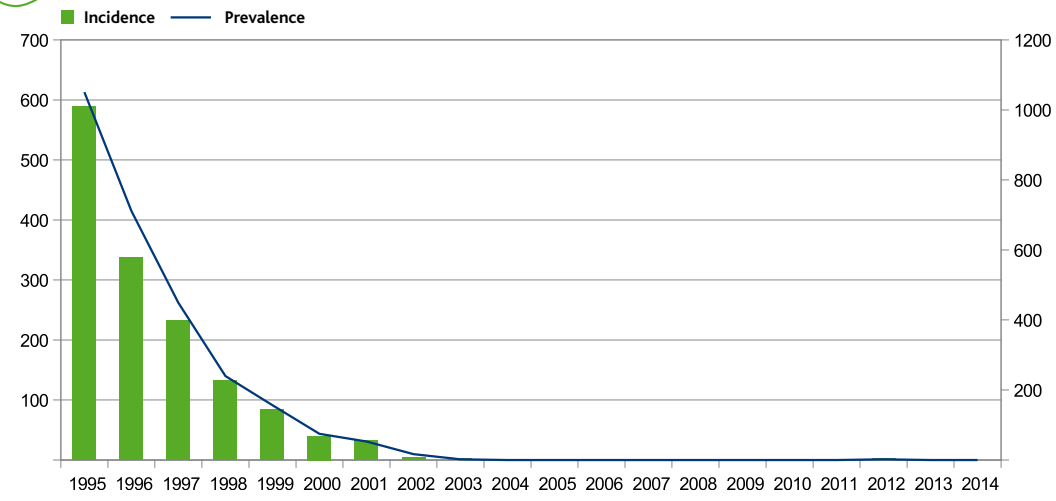

Figure 2. Annual figures for incidence and prevalence of herds infected with bovine brucellosis in France from 1995 to 2014

Suspected cases from programmed serological screening

In the context of screening on blood, 790 animals in 701 herds (or 0.6% of the tested herds) returned positive serological results after screening. Among these, 73 animals in 66 herds again proved positive during the repeat test carried out six to eight weeks later.

Regarding screening on milk, 247 herds presented an unfavourable initial result, and 119 presented an unfavourable result after a second test on milk six to eight weeks later (0.44% and 0.21% of the herds initially tested, respectively).

Investigations under APMS

The investigations carried out as part of health control measures in these herds included serological analyses (n=1,274), brucellin tests (n=463) and/or diagnostic slaughter (n=22), with no subsequent confirmation of brucellosis. Of note, in suspected cases as part of APMS investigations, screening tests, particularly the brucellin test, are carried out and interpreted for a group of animals, and not only on suspected animals.

Box 2. Identification of a case of infection with *Brucella suis* biovar 2 on control of a introduced animal

As part of a control on purchase, a cow of the Limousin cattle breed with no clinical signs was found to have positive serological results (ELISA, RBT and CF) at a two month interval, then a positive reaction on a brucellin skin test (5.3 mm). After slaughter, the NRL confirmed the presence of *Brucella suis* bv. 2 in this animal in late April, on the basis of a sample culture. The bacterium was found only in the udder and the retro-mammary lymph nodes. Investigations on the source farm (Creuse *département*) and destination farm (Vendée *département*) ruled out other cases and the herds were not slaughtered. Analysis of the nodes of the last calf born to this cow showed no infection on slaughter in 2015.

It appears that this infection was isolated and asymptomatic like in the two previous cases of contamination with *B. suis* bv. 2 detected in France in ruminants (a cow in 2000 and a sheep in 2009). The only other cases reported in the world occurred in Belgium (Fretin *et al.*, 2013) and in Poland (Szulowski *et al.*, 2013). Given the size of the wild animal reservoir for *B. suis* bv. 2 (wild boars and hares), these incidental cases appear to point to accidental contaminations of these, very likely atypical hosts. Importantly, *B. suis* bv 2 is considered an opportunistic pathogen with a low zoonotic potential for humans (only three cases described in France in immunodepressed patients). These cases do not appear to represent a public health issue outside specific at-risk populations (Garin-Bastuji *et al.*, 2006). The "officially bovine brucellosis-free status" of France, under the terms of Directive 64/432/EEC, closely related to isolation of *Brucella abortus* or development of evolutive brucellosis, i.e. abortions related to *Brucella* infection or other clinical signs, was not compromised.

The brucellin test, again available since 2013, is especially useful for the differential diagnosis of false-positive serological reactions since it is as sensitive as serological methods (individual sensitivity of about 80%) but presents a much higher specificity (Pouillot *et al.*, 1997). Use of brucellin should therefore be promoted strongly since the test can rule out certain suspected cases found in programmed surveillance without requiring diagnostic slaughter.

During 2014, a total of 106 farms were placed under Prefectural Monitoring Orders (APMS) (herds considered suspect) *versus* 129 in 2013.

Figure 2 shows annual figures for incidence and prevalence of bovine brucellosis in infected herds in France from 1995 to 2014.

Costs

Costs concerning surveillance of brucellosis are presented in a specific article of the *Bulletin Épidémiologique* (Hénaux *et al.*, 2015).

For bovine brucellosis, the authorities cover the following costs:

- all expenses relating to veterinary visits, samples and analyses incurred for the investigation of abortions,
- costs relating to the investigation of suspicions arising as a result of programmed surveillance: veterinary visits, samples and analyses carried out when an APMS is imposed.

Visits and initial screening analyses as part of programmed surveillance are paid for by the owners of the animals, with possible subsidies (especially by the General Councils) which vary between *départements*.

In 2014, the French government allocated approximately €3.4M to surveillance and control of bovine brucellosis (compared with €4M in 2013). Veterinary costs accounted for approximately €2.9M, laboratory costs for €450,000, and compensation and miscellaneous expenses for €54,000.

These sums do not take into account the costs of running and managing the technical and financial aspects of the scheme, particularly in terms of civil servants involved in the scheme and bodies delegated by the administration.

Conclusion

Like in previous years, false-positive serological results were observed in 2014 on screening for bovine brucellosis on blood and milk samples. These results may be related to poor specificity, associated with the intrinsic performance of the tests, or cross-reactions (Box 3). The diagnosis protocol adopted enables investigation of non-negative results before declaring a farm "suspect" and imposing an APMS. As

Box 3. Cross reactions on follow-up of a herd in Corrèze

Non-specific serological reactions persisting well beyond six weeks and/or for a large number of animals have sometimes been observed. The environment, breeding conditions, or age could explain these reactions (Pouillot *et al.*, 1998). However, the effect of these risk factors has not been demonstrated in a reproducible manner.

As an example, as part of serological screening, 14 animals within the same group of 36 individuals presented a positive serological result (RBT and CF). As soon as this result was obtained, brucellosis was ruled out both through a brucellin skin test in this group and by serological controls of all the cattle over 24 months of age (129 animals), which were all negative. Follow-up of the group was suggested in order to assess whether, even in the case of a high incidence of intra-herd false-positive serological reactions (FPSRs), these reactions disappeared with time, as is usually observed when FPSRs only concern one or two animals per herd. Some of these animals had high CF levels (4 above 100 international CF test units (ICFTU)/ml), and were monitored for five months with conventional RBT and CF testing, but also with indirect ELISA tests carried out by the NRL. The serological response persisted for five months, at least in some animals. The group was considered to be an epidemiological unit made up of a homogenous set of heifers grazing in the same area. Further investigations at the grazing area in question did not provide an explanation for this event. In late 2014, programmed surveillance on the herd showed no new cross-reaction.

such, in 2014, by screening blood and retesting six to eight weeks later, it was possible to rule out about 90% of false-positive results obtained in first-line testing.

Concerning milk screening, retesting enabled dismissal of about 50% of false-positive results obtained in first-line testing.

This possibility of ruling out false-positive results obtained in first-line testing through retesting six to eight weeks later is particularly beneficial because it reduces "false alerts", which are an obstacle to commitment from the participants in surveillance schemes. As a result, the specificity of the system is increased, without reducing its speed of response.

Moreover, use of brucellin, once again available as of April 2013, plays a significant role in improving the acceptability of management measures for suspected cases since it enables rapid decisions to be made concerning the status of a suspect farm and reduces the need for diagnostic slaughter.

France is officially free of bovine brucellosis, but the two cases that occurred in 2012 underlined the importance of maintaining a high level of vigilance in order to be able to quickly identify any re-emergence of brucellosis, thus avoiding intra-herd contagion and preventing its possible spread to other farms. This detection ability mainly relies on outbreak surveillance and the system for reporting of abortions. Given the results for the year 2014, the proportion of reporting farms increased *versus* the previous year. However, the level of under-reporting, thought to be high, is probably related to low acceptability of the system by players in the sector. As such, it may be necessary to adjust the scheme to make it more efficient, particularly considering the expenditure of the government to operate it.

Follow-up groups from the ESA platform dealing with topics related to surveillance of abortive diseases are currently working on improving the reporting system for abortions in ruminants.

Discussions revolve around:

- changing the mandatory reporting system for abortions, including surveillance practices (definition of abortion, screening procedures for brucellosis), follow-up of results of surveillance through health status and operational indicators, and feedback to players operating in the sector, specifically through reports from the CSD-ESA (follow-up group for reporting of abortions),
- parallel development of a differential diagnosis process for abortive diseases led by professionals (follow-up group for exploitation of data on differential diagnosis).

These discussions are fuelled in particular by the assessment of the mandatory reporting scheme for abortions in cattle (run by the ANSES Lyon Laboratory), and analysis of results of the cattle health visits (VSB) 2014, which looked at the surveillance of abortions, specifically obstacles and strengths of the reporting process (the results of these VSBs will be presented in a forthcoming article in the *Bulletin Épidémiologique*).

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Brucellosis in small ruminants in 2014: 95 *départements* of metropolitan France are now officially disease-free

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Abstract
No outbreak of sheep or goat brucellosis has been reported in France since 2003. Sixty-four *départements* have been declared officially free of sheep and goat brucellosis by the European Commission since 2006, and 31 new *départements* obtained this status on 9 December 2014. Only one metropolitan *département,* the Pyrénées-Atlantiques, is not officially recognised as disease-free, due to a vaccination programme against ovine epididymitis caused by *Brucella ovis*. Vaccination against the disease was stopped in early 2008 in all other parts of the country. In order to detect any possible reintroductions of the infection, surveillance is based both on repeated serological controls of flocks (programmed surveillance) and on abortion notification (outbreak surveillance). This contributes to maintain disease-free status in the concerned *départements*. No outbreak of small ruminant brucellosis were reported in 2014. While some positive serological reactions were observed, the investigations conducted on these cases all demonstrated that brucellosis was not the cause.

Keywords
Notifiable disease, Regulated disease, Sheep and goat brucellosis, Programmed surveillance, Outbreak surveillance, France, 2014

Résumé
Brucellose des petits ruminants en 2014 : 95 départements de France métropolitaine sont désormais indemnes
La France n'a connu aucun foyer de brucellose ovine ou caprine depuis 2003. Soixante-quatre départements *étaient reconnus officiellement indemnes par la Commission européenne depuis 2006. Le 9 décembre 2014, 31* départements *supplémentaires ont obtenu ce statut. Seul un département métropolitain (Pyrénées-Atlantiques) n'a ainsi pas été reconnu officiellement indemne en raison d'un programme de vaccination contre l'épididymite contagieuse à* Brucella ovis *(la vaccination contre la maladie n'est plus pratiquée sur le reste du territoire depuis début 2008). La surveillance, fondée sur un dépistage sérologique régulier dans les troupeaux (surveillance programmée) et sur la surveillance des avortements (surveillance événementielle), vise à détecter une réintroduction de l'infection, maintenir le statut indemne (pour les* départements *reconnus comme tels). Aucun foyer de brucellose n'a été détecté chez les petits ruminants en 2014. Des réactions sérologiques positives ont été obtenues, mais les investigations menées ont infirmé l'origine brucellique dans chacun des cas.*

Mots-clés
Danger sanitaire de 1ère catégorie, maladie réglementée, brucellose ovine et caprine, surveillance programmée, surveillance événementielle, France, 2014

Infection of any domestic animal by any *Brucella* other than *Brucella ovis* and *Brucella suis* biovar 2 is classified as a Category 1 health hazard (Ministerial Order of 29 July 2013). Small ruminants are the preferred hosts and primary reservoir of *Brucella melitensis*.

Surveillance and control measures for sheep and goat brucellosis are described in Box 1. These are the new measures introduced by the publication of technical and financial ministerial orders on 10 October 2013.

Surveillance system

The data presented below were extracted from the national information system, SIGAL, and also include information collected by Departmental Directorates for Protection of the Population (DDecPPs) as part of the annual survey on animal health. Given the difficulties in consolidating data from SIGAL, certain information concerning herd surveillance is incomplete. Caution should therefore be exercised when interpreting the presented data.

Nation-wide data are given in the text, while Table 1 shows data by region.

Qualification of départements and herds

Since December 2014, 95 of the 101 *départements* in France have been recognised as officially sheep and goat brucellosis-free (Commission Decision 2014/892/EC). All metropolitan *départements*, including the Pyrénées-Atlantiques *département* (due to a vaccination programme against contagious epididymitis), are now recognised as officially free of sheep and goat brucellosis.

According to the data in SIGAL, 123 herds of small ruminants (out of 118,421 registered in SIGAL for all of France) were disqualified for administrative or health reasons[1] on 31 December 2014.

Programmed surveillance: serological surveys

The data recorded in SIGAL and those collected from the *départements* (Table 1) show that in 2014, 36,226 herds and 1,361,339 animals underwent serological screening, out of a total of 118,421 herds registered in SIGAL accounting for 7,001,465 animals more than six months old, according to the annual census, or 30.6% of herds and 19.5% of small ruminants more than six months old.

Note that to maintain an officially disease-free status, a *département* must test at least 5% of all animals over the age of six months (see Box 1).

1. Disqualification is different from placing a herd under Prefectural monitoring order (APMS), even though both cases lead to restrictions on animal movements. In the first case, the conditions have not been satisfied to qualify the herd as "officially brucellosis-free" (e.g. due to failure to undertake mandatory serological testing). In the second case, there is a suspicion of brucellosis in the herd, for example due to a non-negative serological result obtained from an aborting female.

Box 1. Surveillance and health control measures for sheep and goat brucellosis in 2014

The Ministerial Order of 10 October 2013 establishing the technical and administrative framework for collective prophylaxis and control measures for sheep and goat brucellosis amended the provisions of 13 October 1998 and introduced new surveillance methods, which are described in this box.

Objectives of the surveillance programme

- Detect as early as possible the emergence of any new outbreak in domestic sheep and goats.
- Provide evidence on the status of the 95 *départements* considered officially sheep and goat brucellosis-free.

The population monitored

Domestic sheep and goats throughout France.

Surveillance procedures

Programmed surveillance

Programmed surveillance is based on mandatory serological screening performed at a rate that can vary between *départements*.

The maintenance of herd qualification is based on the screening, at a predefined rate, of a representative fraction of animals, defined as follows:

- all non-castrated males over the age of six months,
- all animals introduced (excluding by birth) into the holding since the previous test,
- 25% of females of reproductive age (sexually mature) or in lactation, with no fewer than 50 per farm. On farms where there are fewer than 50, all these females must be tested.

Since the implementation of the new decree, the representative fraction of animals to be screened in herds has been the same for sheep and goats (whereas previously 100% of goats had to be screened), irrespective of the type of production (raw milk products or any other).

By default, the fraction of animals defined above is tested annually. The control interval can, however, be relaxed depending on the *département* where the herd is located (Table 1), except for producers of raw milk, for which the rate is still annual.

In *départements* that are officially brucellosis-free, officially brucellosis-free herds retain their status if the departmental screening programme is carried out correctly.

In addition, the Prefect may impose stricter measures, including the maintenance of annual testing for herds deemed at risk (for example, farms with an epidemiological link to an outbreak, or because of practices related to transhumance).

Before the entry into force of the new provisions for surveillance, the relaxed screening rate could be as infrequent as every ten years. Currently, the maximum applicable attenuation is five-year programmed screening (Memorandum DGAL/SDSPA/2014-157 published on 27-02-2014 relative to sheep and goat brucellosis: programmed and outbreak surveillance).

Table 1. Minimum testing rate for a herd to retain its qualification as officially brucellosis-free, depending on the qualification of the département in which it is located*

Qualification of the *département* in which the officially brucellosis-free herd is located	Testing rate to apply to the herd
Département not officially brucellosis-free, with fewer than 99% of herds officially free	Annual
Département not officially brucellosis-free, with more than 99% of herds officially free	At least every three years
Département officially brucellosis-free	Determined by the *département*'s programmed screening plan. The latter must screen at least 5% of the *département*'s eligible animals every year (which is equivalent to a five-year screening rate: annual screening of 25% of eligible animals in 20% of farms)

* Excluding farms producing raw milk, for which the rate is always annual

Outbreak surveillance

The rules governing the reporting of abortions have been modified, so as to revive the awareness of breeders and veterinarians regarding this procedure and adapt to situations frequently encountered on farms.

All abortions (even isolated cases) must be recorded in the farm register, but now only the reporting of abortive episodes (defined as three or more abortions, over a period of seven days or less) is mandatory. If this threshold is reached, the farm's veterinarian must be informed of the episode, so that investigations may be initiated. However, if the veterinarian considers that an abortion in a herd of small ruminants is suggestive of brucellosis, especially in small herds, then the veterinarian may report the suspicion, which triggers investigations under the same technical and financial conditions (operations financed by the State) as a suspicion based on three successive abortions.

The definition of abortion in small ruminants has also been revised in order to improve the positive predictive value of reports of abortions regarding brucellosis. Abortion is now defined as follows: "An infectious abortion is defined as the expulsion of a foetus or a stillborn animal or one that dies within twelve hours of birth, excluding abortions that are clearly of accidental origin" (Article 2 of the Ministerial Order of 10 October 2013). Therefore, clearly accidental abortions and animals dying after twelve hours of birth are no longer taken into account.

Health control measures

Diagnostic protocols and health control measures are set out in Memorandum DGAL/SDSPA/2014-157 published on 27-02-2014 relative to sheep and goat brucellosis: management of suspicions. Application of the Ministerial Order of 10 October 2013.

Investigation of non-negative results in programmed surveillance

The screening test used for programmed surveillance campaigns is a Rose Bengal Test (RBT). The complement fixation (CF) test is only used in the event the RBT proves positive. A result is considered unfavourable when both tests are positive (a negative CF can refute a positive RBT).

Suspicions (*i.e.* giving rise to an APMS) with programmed surveillance are only issued after two rounds of unfavourable tests (unfavourable initial serological screening, then a repeat test six to eight weeks later again unfavourable for RBT and CF). A brucellin test[1] is then performed for a group of animals (20 individuals) including the animals that reacted positively to the previous individual serological tests and seronegative contact animals (if brucellin testing is not possible, the positive animals are again tested serologically individually).

If the brucellin tests (or, in their absence, a renewed individual serological control) are positive, then diagnostic slaughter is performed to search for *Brucella* on the lymph nodes. The herd is considered infected and placed under Prefectural declaration of infection (APDI) ifa *Brucella* strain is detected on culture, or if the suspected farm has a direct epidemiological link to an infected farm, through animal movements, for example.

Investigation of non-negative results in outbreak surveillance

Abortions are investigated by serological testing. A swab sample from the uterine cervix of aborting females is also taken for bacteriological analysis if the serological analysis proves positive (both RBT and CF positive); failing that, diagnostic slaughter is performed.

A farm is placed under APMS following an abortion if serological testing is unfavourable (RBT and then CF if the RBT is positive). The farm is placed under APDI if the bacteriological analysis of the swab is positive.

Measures taken in herds under APDI

The whole herd is slaughtered if *Brucella abortus* or *melitensis* is isolated.

Regulations

Council Directive 91/68/EEC of 28 January 1991, as amended, on animal health conditions governing intra-Community trade in ovine and caprine animals

Ministerial Order of 10 October 2013 establishing the technical and administrative framework for collective prophylaxis and control measures for ovine and caprine brucellosis

1. Except when the animals on the farm have been vaccinated for brucellosis

Outbreak surveillance: reporting and investigation of abortions

In 2014, 2,541 holdings of small ruminants reported a total of 4,891 abortions, distributed over 67 *départements* (Table 1). It is difficult to compare the number of reports in 2014 with the number of reports in previous years, due to changes in the regulations governing the reporting of abortions (see Box 1). Currently, only abortive episodes (three abortions or more within seven days or less) are subject to mandatory reporting; isolated abortions no longer have to be reported but do need to be recorded in the farm register. Therefore, the slight decrease in the number of reported abortions and reporting herds does not necessarily indicate a decline in the reporting system.

The 2,541 reporting herds account for 2.1% of the 118,421 herds of small ruminants recorded in SIGAL. It should be noted that approximately 30% of the herds of small ruminants recorded in SIGAL are herds with less than five adults, in which there are likely few breeding animals and therefore few animals likely to abort.

There are substantial variations between *départements*. Thus in four *départements* (Lot, Tarn, Indre-et-Loire, Pyrénées-Atlantiques) more than 10% of herds reported an abortive episode, while in 30 others no abortions of small ruminants were reported.

Overall, the proportions of herds reporting abortions remain lower than the expected values, given the frequency of abortion in small ruminants, as has already been pointed out in previous years. This under-declaration could prevent the system from being sufficiently sensitive and responsive, reducing its effectiveness for the early detection of brucellosis in the event of re-emergence of the disease. This is why the reporting procedures have changed, in order to bring them closer into line with the risk of infection for brucellosis. Additional work still in the test phase is currently being undertaken in collaboration with professional managers. Its aim is to assist farmers in identifying causes of abortion, in order to encourage them to more readily report infectious abortions and thus improve the early detection of brucellosis in the event of reintroduction.

Enhanced surveillance in the Bargy Massif, département of Haute-Savoie

In 2012, following an outbreak in cattle of *B. melitensis* biovar *3* (Rautureau *et al.*, 2013), enhanced screening was introduced on the return from summer pasturing in the Bargy Massif. Since the autumn of 2014, the system implemented on the return from summer pasturing has involved only a fraction of each herd (25% of small ruminants over the age of six months with no less than 50 animals), and no longer all animal adults, with priority given to animals that have been kept in the Bargy Massif and in particular gestating females and females that have given birth since their return from summer pasturing. In this context, 1,484 animals from 20 herds were tested (six goat herds and 14 sheep herds), including 14 herds from the Haute-Savoie *département* and the other six herds from the Rhône and Hautes-Alpes *départements*. Special vigilance was applied to dairy goat herds

Table 1. Surveillance and health control measures for sheep and goat brucellosis by region of metropolitan France for 2014

Region	Number of small ruminants		Programmed surveillance			Outbreak surveillance		Investigations of suspected cases				Herd qualification
	Number of herds	Number of animals over the age of six months	Number of tested herds	Number of tested animals	Number of non-negative animals with screening	Number of herds reporting at least one abortion	Number of reported abortions	Number of animals with serological test	Number of animals with positive serological test	Number of animals with bacteriological culture	Number of animals with brucellin skin test	Number of herds placed under APMS
Alsace	1,396	35,729	485	8,508	0	16	22	22	0	0	0	0
Aquitaine	9,624	705,899	4,744	183,693	4	449	1,099	1,103	1	25	1	1
Auvergne	6,224	425,001	1,492	46,492	0	140	208	208	1	1	0	1
Basse-Normandie	8,575	107,409	2,601	27,626	1	15	15	16	0	15	0	1
Bourgogne	4,974	217,155	1,378	40,683	2	155	169	171	1	28	0	3
Bretagne	9,978	110,377	1,371	17,586	0	23	57	57	0	0	0	0
Centre	5,148	263,741	1,408	55,021	0	183	316	316	0	0	0	0
Champagne-Ardenne	1,963	111,281	310	9,343	0	32	56	56	0	0	0	0
Corse	872	113,680	555	42,693	0	8	33	33	1	1	0	1
Franche-Comté	2,414	57,915	219	6,954	0	16	16	16	1	3	0	2
Haute-Normandie	4,844	71,522	542	7,117	0	7	14	14	0	0	0	0
Île-de-France	912	15,946	180	4,866	0	6	7	7	1	0	10	1
Languedoc-Roussillon	3,490	320,312	1,284	63,206	5	61	129	134	5	39	30	5
Limousin	5,443	398,688	1,103	30,880	0	66	95	95	0	0	0	0
Lorraine	2,842	176,922	757	15,755	0	7	8	8	0	0	0	0
Midi-Pyrénées	12,531	1,615,592	7,860	345,554	7	549	836	843	4	4	0	4
Nord-Pas-de-Calais	2,345	47,810	494	7,678	0	21	22	22	0	0	0	0
Pays de la Loire	8,771	251,895	989	21,281	0	96	117	117	0	0	0	0
Picardie	2,493	78,005	751	12,627	0	25	55	55	7	8	0	2
Poitou-Charentes	6,849	771,749	978	54,878	0	203	574	574	2	133	10	2
Provence-Alpes-Côte D'Azur	3,854	643,655	2,342	202,717	2	111	447	449	2	5	60	3
Rhône-Alpes	9,919	445,327	4,350	155,481	3	343	586	589	2	7	42	4
Outre-mer	2,960	15,855	33	700	0	9	10	10	0	0	0	0
Total	**118,421**	**7,001,465**	**36,226**	**1,361,339**	**24**	**2,541**	**4,891**	**4,915**	**28**	**269**	**153**	**30**

by preceding these tests with screening of milk during the summer pasturing period (an experimental protocol followed by the NRL). All of the test results were favourable. Only one sheep had results that were positive for RBT and negative for CF, which gave rise to a repeat test which proved favourable (unlike the general screening scheme, this enhanced surveillance protocol provides for a repeat test whenever an RBT result is positive, despite a negative CF result).

Suspected and confirmed cases

Out of the 4,891 abortions reported in 2014 in small ruminants in France, seven provided a positive serological result (RBT+ and CF+), i.e. 0.13% of seropositive females among those having aborted.

Among the animals undergoing serological screening (1.4 million), 24 still had a non-negative result in the second repeat test (the number of positive animals for the first test could not be estimated due to differences in recording procedures between *départements*), causing a suspicion of brucellosis to be reported.

Overall, 153 brucellin skin tests following positive serological results with programmed surveillance, 250 bacteriological analyses of swabs, and 12 bacteriological analyses after diagnostic slaughter were required to refute these suspect results, which caused 30 herds to be placed under surveillance (APMS). Note that the number of bacteriological swab analyses undertaken was much higher than the number of non-negative serological results after abortion (which is the only case requiring such analyses). The *départements* in which these analyses were undertaken will be contacted to identify the source of this deviation.

A goat herd in the Ardèche *département* had a large number of positive serological results not attributed to brucellosis (Box 2).

Box 2. Particular case of a suspicion in goats in the Ardèche département

In the context of programmed surveillance, 25 goats from a dairy herd of 191 animals (100% of the animals were tested) had a positive RBT result, including 22 that also had a positive CF result. Brucellosis was immediately ruled out by a brucellin skin test in this group.

The epidemiological investigation did not find any evidence in favour of a brucellosis suspicion. However, the drinking water analysis showed non-compliant results (flora and total coliforms); it did not, on the other hand, find any agents such as *Yersinia enterocolitica* O:9, *Salmonella urbana* or *Escherichia coli* O157:H7, which have antigenic cross-reactions with *Brucella*.

Serological follow-up of the herd was proposed so as to ensure that, with such a high intra-holding incidence of false-positive serological reactions (FPSRs), these would disappear over time, as is usually the case when FPSRs involve only one or two animals per herd. Some of the animals had high titres in the CF (six above 100 CFU/ml) and were monitored for four months with the traditional RBT and CF tests as well as with indirect ELISA tests undertaken by the NRL. Far fewer animals had a serological response after two months (n=9); however, this response was still significant for eight animals after four months (including one that became positive again at four months after having shown negative results at two months). For these animals, it was above all the RBT that remained positive. There were far fewer positive results with ELISA, and the CF was negative for all the animals at four months. Such a trend does not at all correspond to what occurs during outbreaks of brucellosis. At the beginning of 2015, programmed surveillance of this holding did not show any positive reactions.

Costs (amounts expressed before VAT)

For brucellosis in small ruminants, the State reimburses the costs of animal health measures, i.e.:

- all expenses relating to outbreak surveillance (veterinary visits, sampling and analyses performed for the investigation of abortions);
- costs relating to the investigation of suspicions arising as a result of programmed surveillance (veterinary visits, sampling and analyses performed when an APMS is issued).

Visits and initial screening analyses as part of programmed surveillance are paid for by the owners of the animals, with possible subsidies (especially by the Departmental Councils) which vary between *départements*. For small ruminants, the State may also participate in the financing of programmed surveillance in herds excluded from relaxed screening (and accordingly subject to annual screening) because they are deemed to be at risk (due to transhumance or other factors).

The French government allocated around €590,000 to surveillance and control of brucellosis in small ruminants in 2014 (compared with €937,000 in 2013). Veterinary costs accounted for approximately €217,230 (37%), laboratories fees for around €186,500 (32%), subsidies for herds kept under annual testing because deemed at risk for €181,000 (31%) (715,608 animals in 6,139 herds benefited from this assistance, in 21 *départements*), and compensation relating to suspicions plus miscellaneous expenses for €5,600.

These sums do not take into account the cost of running and managing the technical and financial aspects of the scheme, particularly in terms of human resources delegated by the administration.

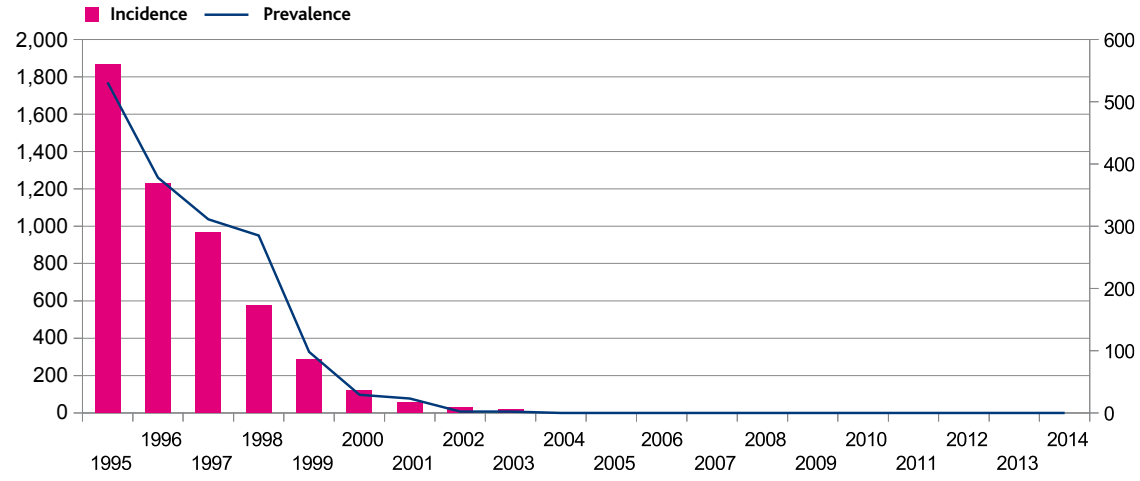

Figure 1. Annual figures for incidence and prevalence of herds infected with sheep and goat brucellosis in France from 1995 to 2014

Discussion

The health status of France concerning sheep and goat brucellosis for 2014 has therefore remained highly satisfactory. No new outbreaks have been detected for over ten years (Figure 1).

However, the two episodes of bovine brucellosis in 2012 are a reminder of the importance of maintaining high levels of vigilance (Rautureau *et al.*, 2013). Like the system in place for cattle farming, brucellosis surveillance in small ruminants is implemented by two complementary systems: periodic large-scale screening, and clinical surveillance based on the reporting of abortions. However, the abortion surveillance system is clearly not yet fully optimal, given the low number of abortions reported.

The new procedures relating to collective screening and health control measures for sheep and goat brucellosis introduced by the ministerial orders published at the end of 2013 (Perrin *et al.*, 2014) take into account the current epidemiological context and are intended to make the system more efficient.

With programmed surveillance, amendments to the decision-making rules applied since 2013 in the event of non-negative results, by combining the various serological tests available, have limited the number of herds placed under surveillance (and restrictions) due to false-positive results.

With outbreak surveillance, the new rules for mandatory reporting (see Box 1), which now require the reporting of abortive episodes only and not isolated abortions, are intended to improve the system's specificity and acceptability. The slight decrease in the number of reporting herds and reported abortions between 2013 and 2014 (respectively 5,186 animals and 3,253 herds in 2013) may be due to these new procedures.

For herds under APMS, the brucellin skin test (available since 2013) is an effective alternative to the diagnostic slaughter of suspect animals.

These new measures are making it possible to refute unfavourable surveillance results more quickly and minimise the constraints for farmers, which is expected to improve the system's acceptance by the various stakeholders and instil it with new momentum.

In parallel with these developments, the gradual introduction, at the request of professionals, of a protocol for differential diagnosis of abortion-related diseases could contribute to strengthening the system for the reporting of abortions. Whenever an abortion is reported, the veterinarian's visit is paid for by the State under the brucellosis scheme. The farmer only pays for samples and analyses unrelated to brucellosis. This protocol is currently being studied in the Midi-Pyrénées region.

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"Erratum in BE 59" Box

There was an error in the data given in the article entitled "*No brucellosis outbreak detected in sheep and goats in France in 2012, but vigilance must be maintained*" in No 59 of the special 2012 *Bulletin Épidémiologique* on regulated and emerging diseases: the total number of reported abortions and the total number of herds reporting at least one abortion in France were incorrect (however, the values given by *département* in Table 1 of the article were correct). The electronic version of this article has been corrected. Likewise, the conclusions regarding the change in the number of reported abortions from 2012 to 2013 published in the article entitled "*Sheep and goat brucellosis in 2013: epidemiological situation and changes to surveillance measures*" in No 64 of the special 2013 *Bulletin Épidémiologique* on regulated and emerging diseases have been modified.

Favourable surveillance results on **enzootic bovine leucosis** in France in 2014: officially disease-free status is maintained

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Abstract
France has been officially disease-free with regard to enzootic bovine leukosis in cattle, sheep and goats since 1999. Annual incidence remains below 0.01%. The aim of surveillance is to maintain the officially disease-free status and to detect any recurrence of enzootic bovine leukosis. All the cases detected in 2014 presented only serological reactions, which is consistent with the disease's pathogenicity, with less than 10% of infected animals developing tumoral forms.

Keywords
Enzootic bovine leucosis, Surveillance, Programmed surveillance

Résumé
Bilan favorable pour la leucose bovine enzootique en France en 2014 : maintien du statut officiellement indemne
La France est reconnue officiellement indemne de leucose bovine enzootique chez les bovins, ovins et caprins depuis 1999. L'incidence annuelle est inférieure à 0,01%. La surveillance a pour objectifs de préserver le statut officiellement indemne et de détecter une éventuelle recrudescence des cas. Les cas détectés en 2014 ne présentaient que des réactions sérologiques ce qui est cohérent avec la pathogénie de la maladie pour laquelle moins de 10 % des animaux infectés développent des formes tumorales.

Mots-clés
Leucose bovine enzootique, surveillance, surveillance programmée

Screening

The surveillance and control system for enzootic bovine leukosis (EBL) was the same as that used in previous years (see Box).

In 2014, 36,141 herds accounting for 16% of French cattle herds underwent serological testing: 70% of these holdings (25,482) were tested using blood tests, and 30% (10,659) by milk testing.

Suspected cases

Of the 10,659 herds screened through milk samples, 69 (0.64%) gave an unfavourable result initially. Of these, 28 (40.5%) again had a positive result on serological retesting of pooled milk.

Of the 25,482 herds screened through blood samples, 20 (0.07%) had at least one positive result in pooled sera analysis. This resulted in individual serological retesting of 62 animals.

For investigation of all suspected cases, 1,547 animals in 31 herds were tested through individual serological samples. These cases were under APMS either due to a second milk test that was unfavourable, or because at least one individual repeat serological control test was unfavourable.

Alongside repeat controls in accredited laboratories, the NRL examined 32 samples by agar gel immune-diffusion (AGID) coming from 29 herds in mainland France following suspected cases on screening.

Outbreak surveillance at the slaughterhouse identified suspicious lesions in two animals from two different herds (in the Calvados and Maine-et-Loire *départements*), but they were not confirmed.

Confirmed cases

Two herds (two animals) from Tarn-et-Garonne were declared infected. Both cases were detected though milk screening.

The positive animals were slaughtered and did not present typical lesions: within the limits of the specificity of the serological reactions, these cases were latent forms of the disease. There was no subsequent confirmation of the tumoral form of the disease.

Nationally, the annual incidence in 2014 at herd level was estimated to be 0.001% (2/218,157). Calculated as a function of the number of tested herds, the incidence was 0.006% (2/36,141). This incidence rate is extremely low and is consistent with that observed in previous years (Figure 1).

A decreasing incidence and relative stabilisation at levels lower than 0.01% have been observed over the past five years. The 2006 peak corresponded to false positives related to an ELISA kit that has since been taken off the market.

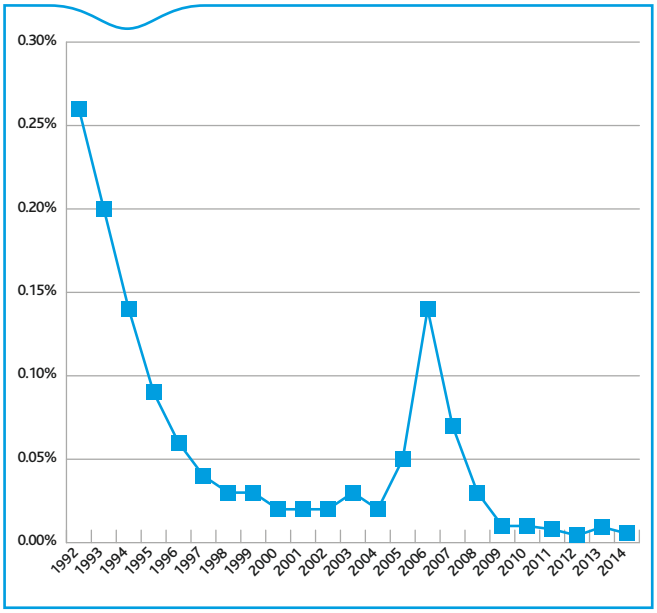

Figure 1. Change in incidence of enzootic bovine leukosis in mainland France from 1995 to 2014 (as a proportion of infected herds)

Box. Surveillance and health control measures for bovine leukosis

Objectives of the surveillance programme

- Verification of the country's officially EBL-free status.
- Detection of any recurrence of cases in domestic cattle.

The population monitored

Domestic cattle across France.

Surveillance procedures

Programmed surveillance

Surveillance by serological screening every five years using blood samples from at least 20% of animals over two years of age, or on pooled milk.

Outbreak surveillance

Surveillance of suspected enzootic bovine leukosis lesions at the slaughterhouse during systematic *post-mortem* examination.

Health control measures

Suspected cases of infection arise either when a positive result is obtained for a test performed on pooled blood samples or on pooled milk, or from suspect lesions identified histologically.

In this case, individual serological testing is performed on all animals over 12 months of age within the herd. If positive animals are detected, the herd is placed under Prefectural declaration of infection (APDI).

Cattle found to be infected are isolated and slaughtered within 30 days.

Disease-free status can only be regained after two rounds of serological testing on all animals over 12 months, with a three to six month interval between rounds.

Regulations

Council Directive 64/432/EEC of 26 June 1964, as amended, on animal health problems affecting intra-Community trade in bovine animals and swine, establishing requirements for control measures applicable to intra-Community trade and import of animal sperm from the swine species.

Ministerial Order of 31 December 1990 establishing the technical and administrative framework for collective prophylaxis and control measures for enzootic bovine leukosis.

Costs

The total amount spent by the State in 2014 for management of LBE, including health control measures and slaughter procedures, is estimated at around €17,000, a stable amount compared to previous years. Most of this budget (€11,630) was allocated to laboratory analyses.

The financial effort remains low and accepTable in view of the objective of maintaining the disease-free status in France.

Discussion

France has been recognised as officially EBL-free since 1999 (Commission Decision 1999/465/EC). The health situation concerning EBL is stable and highly favourable. The disease is well controlled in metropolitan France, despite a few sporadic suspected cases and reports of latent forms.

Some intermediate data in the series of samples following non-negative screening results could not be analysed because of inconsistent quality between *départements*.

However, data concerning first-line screening and incident outbreaks are considered reliable. Outbreak surveillance at the slaughterhouse only led to a very small number of cattle being detected with suspicious lesions. It is not surprising, given the low infection levels and the long course of the disease, that no cases have been detected through this outbreak surveillance.

The sensitivity level of this type of surveillance appears to be rather low and enables only late detection. However, this limited number of suspected cases is consistent with the very low level of incidence found by serological screening, and there are currently no warning signals for potential resurgence of EBL.

Maintenance of this favourable context and the fact that the disease is considered a Category 2 health hazard (Ministerial Order of 29 July 2013) could prompt a revision of the surveillance scheme for EBL in the future. This revision would also allow clarification of certain aspects of the system, particularly concerning procedures and monitoring of health data, in order to provide solutions to the issues mentioned above.

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Bovine spongiform encephalopathy in 2014: continued highly favourable situation leads France to be classified as a country with "negligible BSE risk" in 2015

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Abstract
In 2014, for the third year in a row, no cases of classical BSE (C-BSE) have been identified. The number of cases of atypical BSE detected annually remains stable, with the identification of two cases of type-L BSE (BSE-L) at the slaughterhouse and one case of type H (H-BSE) at rendering. Due to a re-analysis in order to screen for all the atypical cases detected up to now, one atypical H-BSE case was reclassified as BSE-L, which brings the number cases of BSE-L detected in France to 17, and the number of BSE-H cases to 16. With this highly favourable situation regarding the disease, France was in a position to be internationally recognised as having "negligible BSE risk" , which was granted in early 2015.

Keywords
BSE, Epidemiological surveillance, Health control, Cattle, France

Résumé
Encéphalopathie spongiforme bovine en 2014 : une situation toujours très favorable permet l'acquisition du statut « à risque négligeable » en 2015
En 2014, pour la troisième année consécutive, aucun cas d'ESB classique (ESB-C) n'a été identifié. Le nombre de cas d'ESB atypique détectés annuellement se maintient avec l'identification de deux cas d'ESB de type L (ESB-L) à l'abattoir et un cas de type H (ESB-H) à l'équarrissage. A la faveur d'une ré-analyse à des fins de recherche de tous les cas atypiques détectés jusqu'alors, un cas atypique ESB-H a été reclassé ESB-L, ce qui porte à 17 le nombre de cas d'ESB-L et 16 celui des ESB-H détectés en France. Avec cette très bonne situation vis-à-vis de la maladie, la France était en position d'être internationalement reconnue « à risque négligeable », ce qui a été fait début 2015.

Mots-clés
ESB, épidémiosurveillance, police sanitaire, bovins, France

An overview of the BSE surveillance system and health control measures is presented in Box 1.

Trend in the number of cases

In 2014, samples were taken from 857,102 animals at the slaughterhouse (including 227 animals over the age of 48 months from emergency slaughter) and 186,370 animals at the rendering plant.

Of the seven non-negative samples, two samples from the slaughterhouse were confirmed as positive for L-BSE and one from rendering was confirmed as positive for H-BSE (Figure 1). The other four samples were found to be negative by the NRL. For the third year consecutively, no cases of classical BSE (C-BSE) were detected; no clinical suspicions were reported this year.

A total of 1,003 cases of C-BSE have been identified since surveillance was established in 1990. Regarding atypical forms, further to a re-analysis in 2014 in order to screen for all the atypical cases detected up to now, one atypical H-BSE case was reclassified as L-BSE, bringing the number cases of L-BSE detected in France to 17, and the number of H-BSE cases to 16 (Figure 1).

In 2014, 12 cattle were slaughtered as a result of health control measures taken to manage an outbreak of BSE.

Costs (amounts excluding VAT)

Sampling costs

Samples at the slaughterhouse are taken by State employees. This cost in human resources has not been estimated. For samples taken at the rendering plant, the State pays a fixed sum of €7.65 to the rendering plants for the cost of removing heads and placing them at the disposal of veterinarians, and a fixed sum corresponding to one veterinary act (AMV) per sample, which was €13.85 in 2014, to the veterinarians responsible for removing the obex. In total, the State spent approximately €1.4M for removing heads and making them available, and €2.6M for obex samples, for a total of €4M for sample preparation.

Laboratory costs

Analyses of samples taken at rendering plants are fully reimbursed by the State, within the limits of the ceilings determined by the volume of analyses carried out by the laboratories (ranging from €32 if the laboratory performs more than 25,000 analyses per quarter to €40 if the laboratory performs less than 6,500 analyses per quarter). The national average unit cost of the cattle screening test at the rendering plant was €30.40 in 2014. At the slaughterhouse, the State pays a flat-rate contribution of €8 per analysis. In total, the State spent approximately €12.4M for transmissible spongiform encephalopathy (TSE) screening analyses on cattle in 2014: €5.6M for analyses on rendered cattle and €6.8M for analyses on healthy slaughtered cattle.

In total, in 2014 the State spent approximately €16.4M for samples and analyses as part of BSE surveillance at slaughterhouses and rendering plants. These sums do not take into account the costs of taking samples at the slaughterhouse by State officials, nor the costs of coordination or technical and financial management of the scheme, particularly in terms of the state employees involved.

The programme for monitoring and combating TSEs is co-financed by the EU, which in 2014 contributed €5.55 per analysis at the slaughterhouse, €7.40 per analysis at the rendering plant, and 50% of the amount of compensation per bovine animal slaughtered or destroyed, to a maximum of €500.

Box 1. Surveillance and health control measures for BSE in cattle

Objectives

- To determine the prevalence of BSE in cattle.
- To detect, when applicable, any re-emergence of the BSE epizootic.

The population monitored

Programmed surveillance: healthy cattle slaughtered from 72 months of age and at-risk cattle (rendered or culled) from 48 months of age.

Outbreak surveillance: the entire cattle population.

Surveillance procedures

Outbreak surveillance

Carried out through the national BSE epidemiological surveillance network. Based on clinical surveillance of animals on the farm and at the slaughterhouse (suspicious signs detected during ante-mortem inspection). Any suspected case detected on the farm by the attending veterinarian is confirmed or ruled out by the veterinarian coordinating the departmental network.

Programmed surveillance

Since 2001, there have been two surveillance programmes in place:

- Slaughterhouse programme: systematic screening of all cattle intended for human consumption; this screening concerns all cattle over 72 months (48 months between 1 January 2009 and 30 June 2011, 30 months before January 2009 and 24 months between July 2001 and July 2004) , and at-risk cattle over the age of 48 months (24 months until 31 July 2013). As of 1 January 2015, only animals born before 1 January 2002 will be covered by slaughterhouse surveillance.
- Rendering programme: screening of all cattle over 48 months that died on the farm or were euthanised following disease or accidents (24 months from June 2001 to March 2013).

Definition of suspected animals and cases

Any animal with the following characteristics is considered suspect for BSE:

- Living, slaughtered or dead animal presenting or having presented progressive neurological and/or behavioural disorders and/or deterioration of the general state that cannot be attributed to a disease other than BSE,
- Animal with a non-negative or suspect result on a rapid specific BSE test (ELISA, Western Blot or immunochromatographic methods).

Any suspect animal with a positive result for a confirmation test recognised by the Ministry of Agriculture (immunohistochemistry, Western Blot) is considered to be infected with BSE.

Health control measures

In suspected cases of BSE, the farms that held the animal during its first two years of life, and where appropriate the site currently holding the suspect animal, are placed under Prefectural monitoring order (APMS). If the case involves clinical suspicion, the suspect animal is then euthanised and diagnostic samples are taken.

If the case is confirmed, the farm or farms concerned are placed under Prefectural declaration of infection (APDI), and all cattle belonging to the same birth cohort as the confirmed case are slaughtered (animals born up to 12 months before or after birth of the case animal) along with cattle reared with the case animal during the first year of its life, while the case animal was under 12 or 24 months at the site of birth or of rearing, respectively. On these farms, if the affected BSE animal is female, calves born to this case animal in the two years preceding death, or showing clinical signs, or born during the clinical phase, are slaughtered.

Regulatory References

Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

Ministerial Order of 3 December 1990 establishing control measures for bovine spongiform encephalopathy.

Box 2. BSE strains

Until 2003, there was only one known BSE strain. In 2003, two new BSE strains were identified. The atypical biochemical profile of these new strains compared to the 'classical' profile of the BSE strain gave rise to the names used for the three known BSE strains:

- Classical BSE (C-BSE) for the type of BSE responsible for the anazooty due to contamination of animals *via* feed,
- Atypical L-type BSE (L-BSE) for the strain characterised molecularly by a much lower level of the biglycosylated proteinase K-resistant prion protein (PrPres) form and an apparent molecular mass of PrPres that is slightly lower than in C-BSE on Western Blot,
- Atypical H-type BSE (H-BSE) characterised by an apparent molecular mass of PrPres that is higher than in C-BSE on Western Blot.

The two atypical BSE strains also differ from the classical strain in their epidemiological characteristics (Sala *et al.,* 2012):

- A low incidence (1 to 2 cases/million animals tested) that is relatively constant over time and consistent geographically with its presence in countries apparently free from C-BSE, suggesting that these forms are not contagious and not caused by simultaneous exposure of groups of animals, unlike the case of C-BSE,
- A mean age at diagnosis of 12.5 years, which is higher than that of animals with C-BSE (7 years) for the cases detected in France.

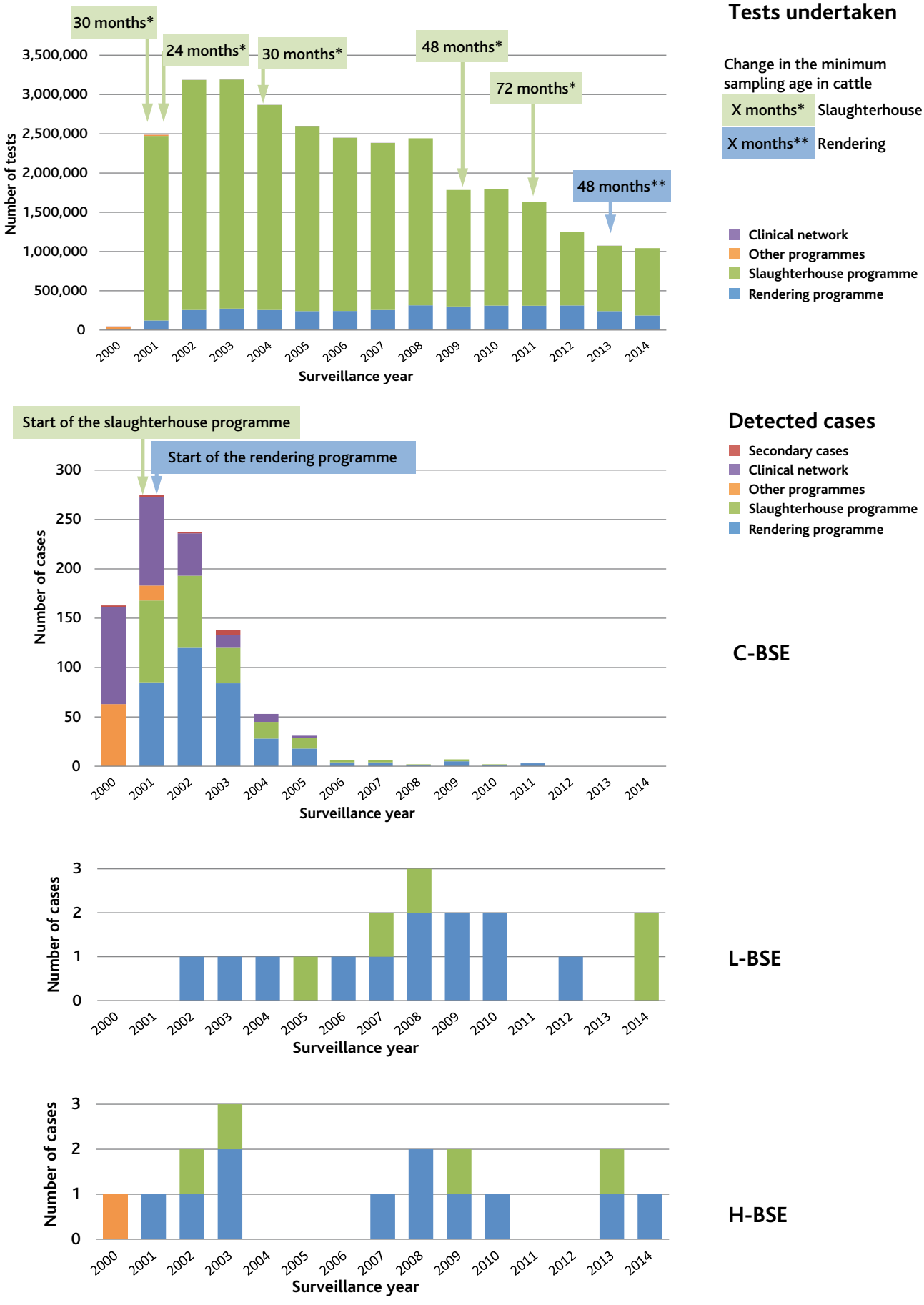

Figure 1. Change in BSE surveillance since 2000: number of tests performed per surveillance programme, and number of detected cases by BSE type and by surveillance programme. From 1991 (start of surveillance) to 1999, 80 cases of classical BSE were detected: 76 by the clinical network and three by 'other' programmes (pilot programmes and supplementary programmes), in addition to one secondary case (an animal found positive after the herd was slaughtered)

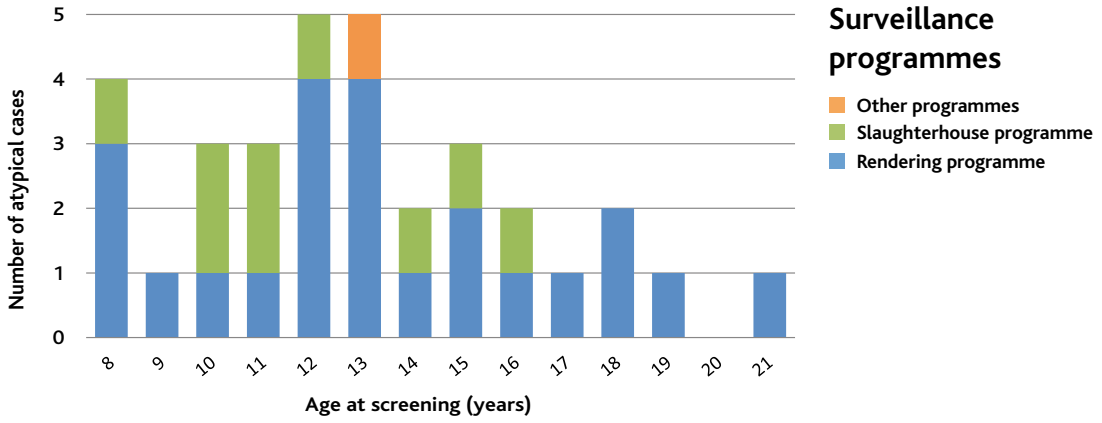

Figure 2. Breakdown of atypical BSE cases since 2000 by age and by surveillance programme. Between 1991 and 1999, no atypical BSE cases were identified

Discussion

The goal of BSE surveillance is to determine the prevalence of the disease and monitor its evolution; surveillance helps ensure that the measures put in place to safeguard human and animal health, including the withdrawal of specified risk materials, are still effective.

However, in order to reduce the cost of surveillance, following a favourable opinion from EFSA, the European Commission allowed Member States to cease tests on healthy animals at the slaughterhouse, considering that the disease is adequately monitored by tests at rendering plants and on at-risk animals at the slaughterhouse (Decision 2009/719/EC). Eighteen Member States stopped these optional tests in 2013. France decided not to stop tests at the slaughterhouse but to limit them to healthy animals born before 1 January 2002. This measure, which took effect on 1 January 2015, is expected to lead to an 80% decrease in the number of tests undertaken at the slaughterhouse (716,671 of 857,102 animals tested at the slaughterhouse in 2014 were born after 2002). On 31 December 2014, there were an estimated 200,000 cattle born before 1 January 2002 still held on French farms.

While relaxing slaughterhouse surveillance will not affect surveillance quality for the classical form of BSE, it is likely that the majority of cases of atypical BSE will no longer be detected through this system. Under this measure, only animals over the age of thirteen years will be tested in 2015, whereas six in nine atypical cases detected at the slaughterhouse up to now have been between the ages of eight and twelve years (Figure 2) and one of the two L-BSE cases detected in 2014 was born in 2004.

The anazooty seems to be under control, with no cases of C-BSE detected for three years now and atypical BSE cases presenting epidemiological characteristics, i.e. animals aged over eight years and mainly reared for meat production, consistent with current knowledge (Sala *et al,* 2012). Thus, with this stable situation in France regarding C-BSE, the application for recognition of the "negligible BSE risk" status from the OIE was favourably received in 2015. The most recent confirmed case of C-BSE concerned an animal born in 2004 and the OIE Terrestrial Code imposes an interval of 11 years between the year of birth of the last case and the recognition of "negligible risk" status (in addition to adequate surveillance, the implementation of risk prevention measures particularly relating to animal feed and imports, and strict health control measures during outbreaks).

No distinction is currently made between classical and atypical BSE in either EU regulations (Regulation (EC) No 999/2001) or international regulations (OIE Terrestrial Code). The same measures are applied in outbreaks of BSE, regardless of the strain identified. Similarly, the rules for obtaining (or losing) territory status as defined in the OIE Terrestrial Code (no status, controlled BSE risk, negligible BSE risk) do not take into account the strains involved in the outbreaks identified. Discussions are currently in progress at the international level to assess the relevance of taking into account the nature of the strains involved for the determination of territory status, and also regarding animal health measures during outbreaks.

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Surveillance of **spongiform encephalopathies in small ruminants** in 2014: no classical scrapie outbreaks detected

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Abstract
In 2014, 60,557 goats and 39,954 sheep were tested at the slaughterhouse and during rendering to screen for transmissible spongiform encephalopathies. Five cases of atypical scrapie were detected in sheep and five in goats. No cases of classical scrapie were detected in any goats or sheep. An overview of surveillance since 2002 shows that classical scrapie prevalence continues to fall in both sheep and goats. A drop in atypical scrapie prevalence was also observed in sheep in 2013 and 2014, most certainly due to a fall in diagnostic test performance.

Keywords
TSE, Small ruminants, Programmed surveillance, Clinical surveillance, Prevalence

Résumé
Surveillance des encéphalopathies spongiformes des petits ruminants en 2014 : aucun foyer de tremblante classique détecté
En 2014, 60 557 caprins et 39 954 ovins ont été testés à l'abattoir et à l'équarrissage pour la recherche d'encéphalopathies spongiformes transmissibles : cinq cas de tremblante atypique chez les ovins et également cinq chez les caprins ont été détectés. Aucun cas de tremblante classique n'a été détecté que ce soit chez les caprins ou chez les ovins. Le bilan de cette surveillance depuis 2002 continue de montrer la diminution de la prévalence de la tremblante classique chez les ovins et les caprins. On constate par ailleurs une diminution de la prévalence de la tremblante atypique chez les ovins en 2013 et 2014, très certainement attribuable à une baisse de performance des tests diagnostiques.

Mots-clés
EST, petits ruminants, surveillance active, surveillance événementielle, prévalence

An overview of the surveillance system, its objectives and implementation methods is presented in Box 1.

Results

Number of tests carried out

A total of 100,511 samples were taken in 2014. The objectives of the surveillance programme were achieved for sheep at the slaughterhouse (10,103 samples). However, a high rate of under-performance was observed at the rendering plant for sheep: the target of 40,000 tested sheep was not achieved; the testing rate was 75% (29,851 tests carried out). The statistics for rendering in 2014 indicate however that testing 10% of the small ruminants that died in 2014 (this sampling value had been established by the DGAL) should have led to the 40,000 expected tests being undertaken.

For goats, the threshold of 10,000 expected samples at the slaughterhouse was not reached (there were 8,681 samples). At the rendering plant, 51,876 samples were taken; the exhaustiveness of this sampling could not be verified in the current conditions of traceability in rendering.

That said, the number of samples taken was compliant with the minimum targets established by the European Commission, i.e. 20,000 samples (including at least 5,000 at the slaughterhouse) for each of the two species (40,000 samples requested *versus* 100,000 taken in France).

Slaughterhouses and rendering plants combined, slightly more than 7,500 goat farms (or about 47% of goat farms counted) and 15,000 sheep farms (or around 32% of sheep farms counted) had at least one animal tested in 2014.

Changes in prevalence for classical and atypical scrapie

The prevalence of atypical and classical scrapie (Figure 1) is calculated as the number of atypical or classical cases relative to the number of tests performed (like in previous years, all the tests used in 2014 were able to detect atypical scrapie).

In 2014, as in the previous year, no cases of classical scrapie were discovered by programmed surveillance in sheep, whether at the slaughterhouse or rendering plant. The prevalence of classical ovine scrapie has been on a downward trend since 2002, both at the slaughterhouse (Mann-Kendall trend test $p=1.6*10^{-3}$) and the rendering plant (Mann-Kendall trend test $p=1.9*10^{-5}$).

Similarly, in goats, for which no cases of classical scrapie have been found at the slaughterhouse since 2008, no cases of classical scrapie were detected at the rendering plant, for the first year. Thus the prevalence of classical caprine scrapie remains low and is on a downward trend, both at the slaughterhouse (Mann-Kendall trend test $p=0.05$) and the rendering plant (Mann-Kendall trend test $p=1.4*10^{-3}$).

In 2014, a total of ten cases of atypical scrapie (sheep and goats combined) were detected by programmed surveillance: there were five goats detected at the rendering plant, four sheep at the rendering plant, and one sheep at the slaughterhouse. All these cases came from farms with different origins.

The apparent prevalence of atypical ovine scrapie has significantly decreased since 2002, both at the slaughterhouse (Mann-Kendall trend test $p=4.1*10^{-3}$) and the rendering plant (Mann-Kendall trend test $p=2.7*10^{-3}$). Trends in this apparent prevalence since 2010 were modelled (Box 2), confirming a significant decrease in 2013 and 2014 compared to 2010.

In 2014, the apparent prevalence of atypical caprine scrapie stabilised at a very low level, both at the slaughterhouse and the rendering plant (non-significant Mann-Kendall trend test).

Genotyping in sheep

There is genetic determinism for sensitivity and resistance to scrapie in small ruminants. Homozygous ARR sheep are almost completely resistant to classical scrapie, while the VRQ, ARQ and AHQ alleles have

Box 1. Surveillance and health control measures for TSEs in small ruminants

Objectives

- To determine estimated prevalence of TSEs in small ruminants.
- To detect, as the case may be, any presence of BSE in small ruminants.

The population monitored

Live sheep and goats, animals at rendering plants or intended for human consumption in mainland France.

Surveillance procedures

Outbreak surveillance

On the basis of clinical signs on farms or on ante-mortem inspection at the slaughterhouse.

If a clinical case is suspected on a production site, the farmer must inform the farm's mandated veterinarian and the suspected case must be reported to the veterinary authorities.

Programmed surveillance

Annual screening was introduced in 2002, providing minimum compliance with the sampling established by European Regulation (EC) No 999/2001.

Slaughterhouse: screening of 10,000 sheep and 10,000 goats aged over 18 months selected at random.

Rendering: screening of 40,000 sheep aged over 18 months selected at random, and systematic screening of all goats aged over 18 months.

Diagnostic procedure

Regardless of the origin of the samples (programmed or outbreak surveillance), brainstem (obex) samples are tested at the competent Departmental Veterinary Laboratory (DVL) corresponding to the sampling site. Each laboratory undertakes rapid diagnostic tests it has selected from those approved at European level (Bio-Rad© or Idexx©). "Non-negative" samples are sent to the NRL (ANSES Lyon Laboratory) for confirmation.

Health control measures

If a case is reported on clinical suspicion or if a non-negative result is obtained on a rapid test, the farms where the suspect animal was born, lived for more than nine months during its first year, or where it gave birth, are considered at risk. These farms are placed under Prefectural monitoring order (APMS), which specifically prohibits the sale of small ruminants, as well as their milk and any derived dairy products.

When screening returns a non-negative result, the sample is sent to the NRL for confirmation by Western blot. The confirmatory analysis serves to i) rule out the presence of a TSE, ii) confirm the presence of atypical scrapie, or iii) confirm the presence of a TSE other than atypical scrapie. A typing analysis is carried out if the confirmatory analysis indicates the presence of a TSE different to atypical scrapie. This typing analysis serves to confirm the presence of classical scrapie, or even BSE.

If the case is confirmed, the herds are subject to health control measures that vary depending on the TSE strain identified:

BSE: total depopulation of the herd of birth and any herds in which the case animal may have given birth;

Classical ovine scrapie: elimination of genetically susceptible animals from the herd of birth. Animals can be sold only to the slaughterhouse and the milk of genetically susceptible animals must be destroyed. These measures are replaced by reinforced follow-up for three years if the affected animal transited through several farms;

Classical caprine scrapie: total depopulation of the herd of birth;

Atypical scrapie: strict monitoring of herds for two years; any animals dying on the farm or slaughtered when aged over 18 months must be screened.

Regulations

Ministerial Order of 2 July 2009 as amended establishing control measures for caprine spongiform encephalopathy.

Ministerial Order of 2 July 2009 establishing control measures for ovine spongiform encephalopathy.

Ministerial Order of 3 December 1990 establishing control measures for bovine spongiform encephalopathy.

Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

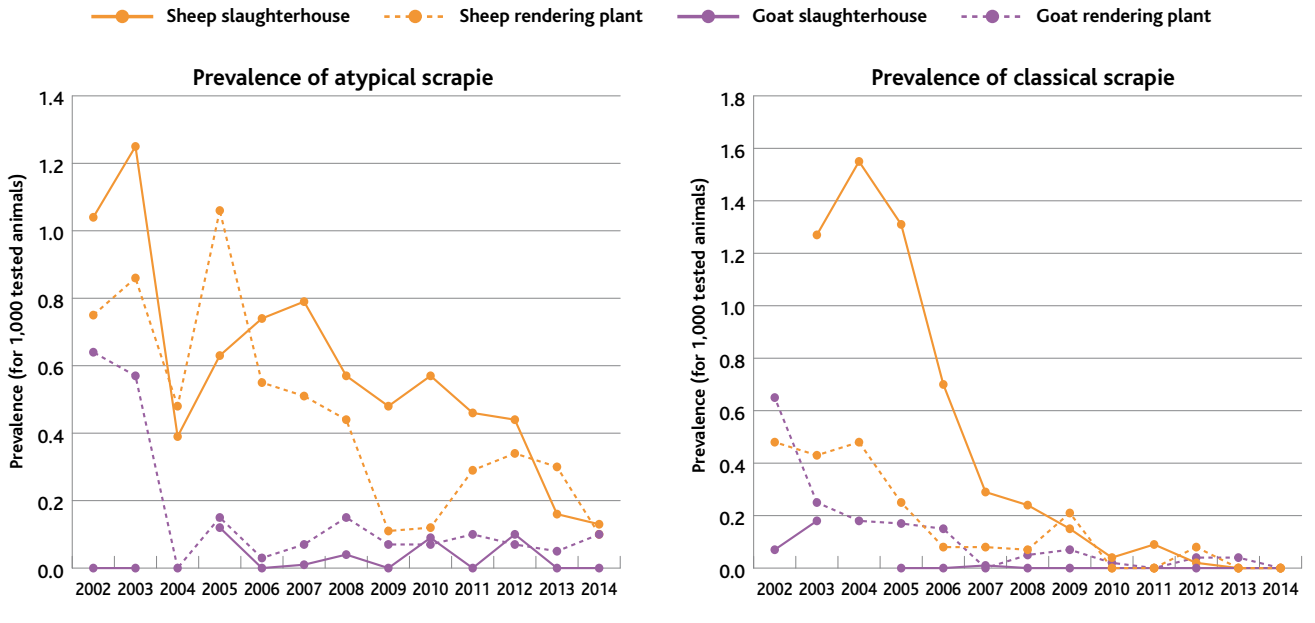

Figure 1. Changes in prevalence of classical and atypical scrapie in sheep and goats at the slaughterhouse and rendering plant

Box 2. Decrease in the prevalence of atypical ovine scrapie from 2010 to 2014

In light of empirical evidence of a decrease in the number of detected atypical scrapie cases in the past few years, trends in the prevalence of atypical scrapie were studied between 2010 (after which only tests capable of detecting atypical scrapie were used) and 2014, using a mathematical model. Given the small number of detected atypical cases in goats, the analysis took into account sheep only, slaughterhouse and rendering programmes combined.

The variable to be explained was the number of detected cases, and since this count variable is proportional to the number of tests undertaken, a decision was made to include this variable in the model. The reference year for the model was 2010. The type of test used (Bio-Rad© or Idexx©) was not taken into account in the model, since for the five years of the study, a single test was used almost exclusively by the laboratories (depending on the year, 85% to 93% of the analyses undertaken were based on only one test).

The results of the model indicate a non-significant decrease in the number of detected cases for 2011 and 2012 compared to 2010 (respectively p=0.58 and p=0.52). However, there was a significant decrease in the number of detected atypical scrapie cases in 2013 and 2014 compared to 2010 at the 5% threshold (respectively $p=7.8*10^{-3}$ and $p=4.3*10^{-3}$); this could not be linked to poor testing rates.

decreasing susceptibilities. Susceptibility to atypical scrapie in sheep is related to the presence of the AHQ and AF141RQ alleles.

Genetics has now been used for more than ten years to combat classical scrapie in sheep. Genotyping takes place on four levels:

- systematic genotyping of sheep that were non-negative when screened at the slaughterhouse or rendering plant (whether or not the presence of a TSE was subsequently confirmed),
- genotyping of congeners in confirmed outbreaks of classical ovine scrapie, in order to identify the animals to be eliminated,
- genotyping on a random sample of sheep that were negative when screened at the slaughterhouse or rendering plant (a target of 600 genotypes per year nationally) to assess the evolution of allele frequencies,
- genotyping conducted as part of the National plan for genetic improvement of resistance to classical scrapie (PNAGRTc) in order to select resistant breeders. The results of the PNAGRTc and the ram census are given in Box 3.

In 2014, of the 706 genotype tests performed in negative sheep at the slaughterhouse or rendering plant, 665 provided interpreTable results. For all breeds combined, the following frequencies were found in the tested animals: ARR allele 60%, ARQ allele 31%, VRQ allele 5%, and AHQ allele 3%. Since 2002, a slight increase in the frequency of the ARR allele in these surveys has been found in all breeds, with a corresponding decrease in the ARQ allele (Figure 2). The proportions of animals harbouring the VRQ and AHQ alleles estimated by this programme appear to be relatively stable (Cazeau *et al.*, 2011).

Costs (amounts excluding VAT)

Sampling costs

Samples at the slaughterhouse are taken by State employees. This cost in human resources has not been estimated. For samples taken at the rendering plant, the State pays a fixed sum of €7.65 to the rendering plants for the cost of removing heads and placing them at the disposal of veterinarians, and a fixed sum corresponding to one veterinary act (AMV) per sample, which was €13.85 in 2014, to the veterinarians responsible for removing the obex. In total, the State spent approximately €650,000 for removing heads and making them available, and €1.2M for obex samples, for a total of €1.85M for sample preparation.

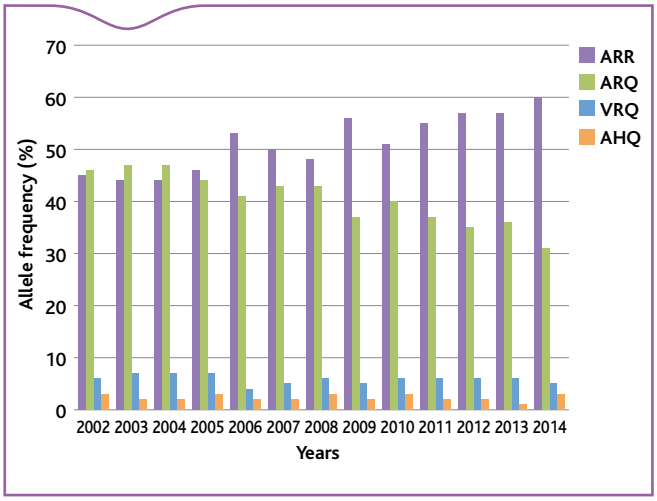

Figure 2. Distribution of allele frequencies per year for negative sheep (slaughterhouse and rendering plant combined)

Laboratory costs

Analyses of samples taken at rendering plants and at the slaughterhouse are fully reimbursed by the State, within the limits of the ceilings determined by the volume of analyses carried out by the laboratories (ranging from €32 if the laboratory performs more than 25,000 analyses per quarter to €40 if the laboratory performs less than 6,500 analyses per quarter). The national average unit cost of the small ruminant screening test at the slaughterhouse and rendering plant was respectively €30.80 and €29.10.

In total, the State spent approximately €3M for TSE screening analyses on small ruminants in 2014 (€2.4M for analyses on rendered animals and 600,000 for those on healthy slaughtered animals).

Total cost for the State

In 2014, the State spent approximately €4.85M for samples and analyses as part of TSE screening at slaughterhouses and rendering plants. These sums do not take into account the costs of taking samples at the slaughterhouse, nor the costs of coordination or technical and financial management of the scheme, particularly in terms of the State employees involved.

In addition, management of the scrapie outbreaks identified in 2014, as well as the compensation for animals and products destroyed, amounted to around €420,000 in 2014. The programme for random genotyping cost €17,800 and that for genotyping carried out during outbreaks cost €5,520. The cost of the genotyping carried out as part of the PNAGRTc was €541,000.

The programme for monitoring and combating TSEs in small ruminants is co-financed by the EU, which in 2014 contributed €7.40 for each screening test at the slaughterhouse and rendering plant, and 50% of the amount of compensation, to a maximum of €50 per destroyed animal.

Discussion

Concerning classical scrapie, a significant decrease in prevalence has been observed since 2002, whether in sheep or goats. No cases of classical scrapie were detected by programmed surveillance in 2014, whether in sheep or goats.

The decrease in prevalence of classical scrapie is possibly explained by the control measures implemented for the disease in affected herds, and by selection of genetically resistant animals. However, given the available data, it is difficult to estimate the evolution of the sheep population's genetic status (that of breeding farms is very well known): the survey programme in rendering plants and slaughterhouses, which

suffers from methodological limitations (number of samples, sampling methods), does not show any major change in genetic structure; in 2013, the ram inventory still included 45% of rams with unknown genotype.

Regarding atypical scrapie, while its apparent prevalence remains stable in goats, it is declining in sheep. The decrease in this form of scrapie considered "sporadic" raises questions as to the survey's ability to actually monitor the prevalence of atypical scrapie. Genetic selection used to reduce classical scrapie has been suggested as a possible cause of the decrease in atypical scrapie, but given the limited impact of genetic selection in the general population, it undoubtedly cannot completely explain this trend.

This decrease may be due to the under-performance of screening tests (for certain batches) and/or a change in tests over time. Since 2010, over 85% of analyses have been undertaken with only one diagnostic kit. The NRL identified several batches of this kit with lower sensitivity for detecting atypical scrapie (A.G. Biacabe, personal communication). To date, batches of diagnostic screening kits are tested only for BSE; this testing is organised by the EURL, which delegates the tests to various European countries. Batches are not tested for scrapie (classical or atypical), whether at European or national level. Furthermore, the test's suppliers do not test their batches for atypical scrapie but only for classical scrapie.

Due to this lack of testing, poor test performance for the detection of atypical scrapie is entirely possible, especially since the molecular characteristics of this type of scrapie are different from those of both classical scrapie and BSE (classical and atypical), making it more difficult to detect.

Overall, both forms of scrapie are rare and remain at very low levels. No suspected cases of BSE were detected in small ruminants in 2014. The system implemented in France exceeds the minimum requirements of the European regulations, which stipulate that 10,000 animals must be tested for each species/plan pair.

ANSES received a formal request in 2014 to examine the possible changes to be made to the surveillance programme for TSEs in small ruminants (Opinion 2014-SA-0032 of 30 September 2014). In light of this opinion, the DGAL decided to keep the current configuration of the surveillance programme in 2015 and 2016. If the number of classical scrapie outbreaks remains as low in these two years as in 2013 (four classical scrapie outbreaks detected) and 2014 (no classical scrapie outbreaks detected), confirming a sustained decrease in the prevalence of this disease, the relevance of scaling down the system will be reconsidered for 2017.

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Box 3. Plan for genetic improvement of resistance to classical scrapie: a few key points

Highly convincing results

The National plan for genetic improvement of resistance to classical scrapie (PNAGRTc) stems from the combined determination of the sheep farming sector and the authorities to use genetics to combat this disease.

This programme, established in October 2001, was specifically set up in dairy- and meat-sheep breeding farms with the following objectives:

- to eliminate the allele with susceptibility to classical scrapie (VRQ) from breeding farms,
- to repopulate scrapie-affected farms with resistant animals,
- to select the allele with resistance to classical scrapie (ARR),
- to disseminate ARR/ARR rams for production farms.

The genotyping carried out as part of PNAGRTc was funded by the Ministry of Agriculture. For several years, this "biological" genotyping has been supplemented by "prediction" genotyping deduced from the genotype of the parents and descendants. Originally intended to run until the end of 2009, the programme was extended to bolster the ability of breeder farms to disseminate resistant breeding males and females.

More than 850,000 genotyping analyses were thus performed between 2002 and 2014. In addition to this, over 7 million informational predictions were carried out.

With respect to the objectives, the programme's results at the end of 2014 can be summarised as follows:

- the VRQ allele has been virtually eliminated from breeding farms (no active ram carriers),
- 98% of active meat rams in breeding farms have the ARR/ARR genotype, compared with only 24% in 2002,
- 98% of AI dairy rams have the ARR/ARR genotype, compared with only 31% in 2002,
- 90% of females from meat breeds and 60% of females from hardy breeds in breeding farms have the ARR/ARR genotype.

These results from breeding farms enable these holdings to supply resistant breeding males and females to all French farmers (including those affected by classical scrapie).

A new tool: the Observatory for Resistance

The genotyping carried out in the framework of the PNAGRTc provides precise knowledge of the frequency of the different genotypes in the selection bases, but cannot provide information on the dissemination of resistance alleles in the rest of the French sheep population.

Since 2012, following an agreement between the authorities and professional sheep organisations, all holders of breeding rams have been invited to provide certain information on these animals (including genotype) during the annual census carried out as part of identification procedures. This inventory of all rams put to use in France responds to two objectives:

- to improve knowledge about the level of resistance to classical scrapie in livestock at national level and in the various production regions,
- to analyse the zootechnical information provided by this census on the origins of the rams used (breed, breeding holding, etc.) to gain a better understanding of the use of the male "pathway", a strategic component in improving herds and for disseminating resistance.

In 2014, the census provided the following results:

- 40,000 sheep farmers declared 168,300 rams. This corresponds to 52% of farmers identified in the BDNI, which contains all known sheep holders, and 80% of farmers identified in the BDNI and owning more than 50 breeders. If rams present on breeding farms are added (which farmers were not required to declare, as their inventory is already managed elsewhere), this makes a total of 168,000 rams analysed.

With regard to resistance, the results are as follows:

- 43% of all rams have the known ARR/ARR genotype,
- 9% have genotypes that are moderately resistant (ARR/AHQ or ARR/ARQ genotypes), susceptible (AHQ/AHQ, AHQ/ARQ or ARQ/ARQ genotypes) or that were incompletely predicted,
- 48% have no known genotypes.

The proportion of unknown rams corresponds to those that were not born on breeding farms (selection bodies). It should be noted that the rams born in selection bodies and disseminated outside the nucleus herd are virtually all resistant rams. This shows that any measure promoting the production and dissemination of resistant animals from breeding farms will help the dissemination of resistance in the remainder of the population and add value to all the efforts of the genetic programme.

A more detailed document containing the 2013 results has been prepared, with data by *département* and by breed (http://idele.fr/no_cache/recherche/publication/IdeleSolr/recommends/recensement-des-beliers-utilises-dans-les-elevages-ovins-francais-2013.html).

Report on regulatory and voluntary surveillance of **infectious bovine rhinotracheitis** in 2013/2014: a stable situation and new opportunities

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Abstract
Infectious bovine rhinotracheitis (IBR) is a viral disease caused by bovine herpesvirus 1 (BoHV-1). The virus mainly manifests respiratory tract and genital tropism. Currently, IBR infection in French holdings is usually asymptomatic and is therefore now mainly a commercial issue both on the national market and abroad. The 2013/2014 surveillance campaign for IBR ended with a national prevalence rate of 9.8%, with the incidence rate reaching 1.9% over this same period. While the national certification rate of IBR-free farms continues to increase slowly (65.9% as of 31 May 2014), it is clear that the current control scheme no longer enables significant improvement in the epidemiological situation. Thus, measures should be taken to improve current analytical tools and to speed up the eradication process, which will also contribute to EU-level recognition of the French control programme.

Keywords
Infectious bovine rhinotracheitis, IBR, Cattle, Category 2 health hazard

Résumé
Bilan de la surveillance réglementée et facultative de l'IBR en France en 2013-2014 : une situation stable et de nouvelles perspectives
La rhinotrachéite infectieuse bovine est une maladie virale, provoquée par l'herpesvirus bovin de type 1 (BHV-1) qui possède un tropisme essentiellement respiratoire et génital. Dans l'élevage français actuellement, l'infection reste le plus souvent asymptomatique et la maladie présente maintenant un enjeu surtout commercial tant sur le marché national qu'à l'étranger. La campagne 2013/2014 de surveillance de la rhinotrachéite infectieuse bovine s'est terminée sur un taux de prévalence national de 9,8 % (le taux d'incidence sur 2013/2014 s'élève à 1,9 %). Si la proportion de cheptels sous appellation « indemne d'IBR » continue lentement d'augmenter (65,9 % au 31 mai 2014), force est de constater que l'actuel dispositif de lutte ne permet plus d'améliorer significativement la situation épidémiologique. Aussi des mesures devraient être prises d'une part pour améliorer les outils analytiques existants et d'autre part pour permettre l'accélération du processus d'éradication, ce qui contribuera également à la reconnaissance européenne du programme de lutte.

Mots-clés
Rhinotrachéite infectieuse bovine, IBR, bovins, danger sanitaire de catégorie 2

Infectious bovine rhinotracheitis (IBR) is a viral disease caused by bovine herpesvirus 1 (BoHV-1). The virus mainly manifests as respiratory tract and genital tropism. However, in French livestock currently, the infection mostly remains asymptomatic and the disease is therefore primarily a trade concern. IBR is included in the Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE) and can therefore be associated with additional guarantees at the European level. This was the background that led to implementation of IBR control measures.

There are currently two complementary surveillance and control schemes for IBR, one mandatory, set up in 2006, and the other voluntary, that leads to certification of farms.

The Box opposite summarises the objectives of the control programme, surveillance procedures, and health control measures for this disease.

This article presents the results obtained for the certification and control systems for the 2013-2014 campaigns (period from 1 June 2013 to 31 May 2014). The results presented below are taken from specific data collection from the GDS using an annual update questionnaire.

Results from the mandatory scheme

Prevalence and incidence

As of 31 May 2014, mandatory IBR screening of herds revealed that 9.8% of tested herds on average had at least one seropositive animal (data from 86 *départements*). This prevalence is stable compared to the previous campaign (prevalence was 9.8% on 31 May 2013) and varies from 0.03% to 89.6% depending on the *département* (lowest prevalence rates are found in the *départements* primarily focussed on milk production) (Figure 1).

The IBR incidence rate for the 2013-2014 campaign was 1.9% (data for 85 *départements*) with values ranging from 0% to 10%, depending on the *département* (Figure 2). Like prevalence rates, incidence was relatively stable compared to the previous campaign (for 2012-2013, it was 1.7%).

For the 2013-2014 campaign, the effective national rate of programmed screening reached 94.1% (data from 86 *départements*). This rate was 94.2% for the 2012-2013 campaign.

Results of testing on introduction of animals to a herd

Data collected for 88 *départements* indicate a proportion of 1.4% seropositive cattle on purchase for all introduced animals, whether certified or not, excluding exempt establishments (i.e. 19,001 cattle out of 1,390,926).

Results from the voluntary scheme

Herd certification level

As of 31 May 2014, 65.9% of herds in mainland France (excluding exempt farms) had an IBR-free or an IBR-controlled status (data from 86 *départements*). Here again, the picture is not consistent country-wide with herd certification percentages varying from 0.4% to 98.2% depending on the *département* (Figure 3).

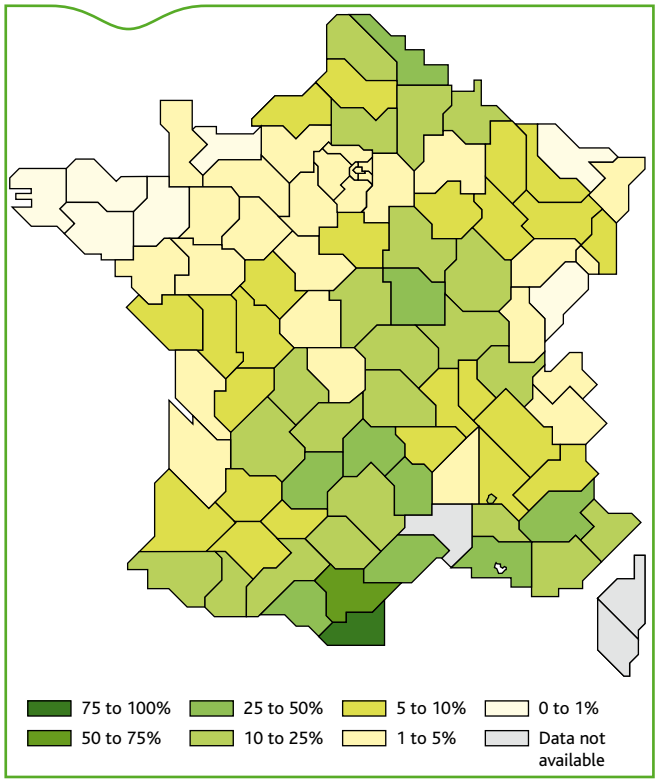

Figure 1. Prevalence (herds) by département as of 31 May 2014 (GDS France data)

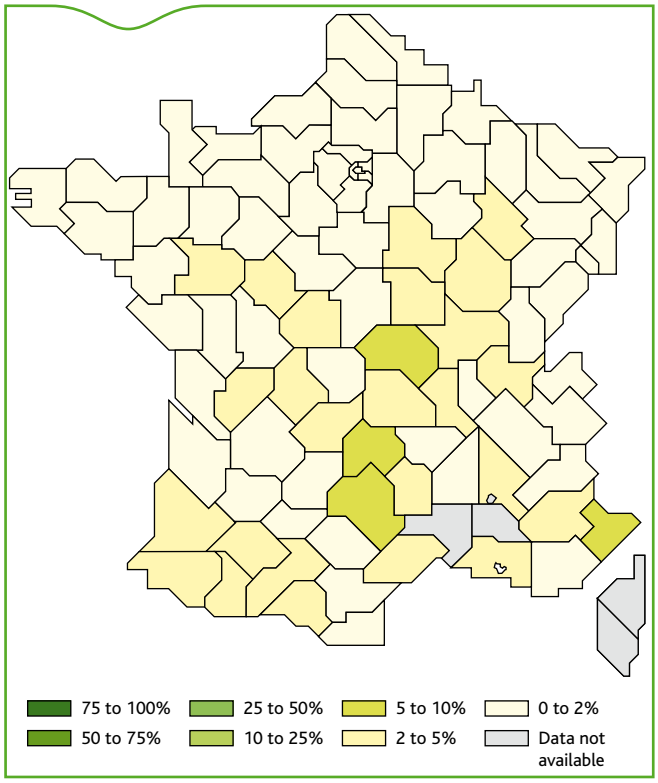

Figure 2. Incidence (herds) by département as of 31 May 2014 (GDS France data)

Box 1. Surveillance and health control measures for infectious bovine rhinotracheitis (IBR)

Objectives

- To determine the estimated prevalence of IBR in cattle.
- To contribute to certification of the health status of herds in France.

The population monitored

Domestic cattle across mainland France.

Surveillance procedures

Mandatory surveillance

This scheme was implemented at the request of farmers, with adoption of a Ministerial Order (Ministerial Order of 27 November 2006) on the basis of the "60% rule", i.e. measures can be imposed if they concern more than 60% of animals or farms in a *département* or a region. This scheme includes:

- Serological screening on introduction of transferred animals not known to be positive and/or vaccinated,
- Serological screening of cattle herds: every 6 months, of bulk tank milk on dairy farms, and annually, through blood sampling of cattle over 24 months of age on beef cattle farms. Exempt fattening herds, as defined in Article 2 of the Order of 22 February 2005, and exclusively housed in closed facilities, are also exempt with regard to this screening.

This scheme is supervised by the GDSs.

Voluntary certification of herds

Since 1996, through officially recognised certification of herds, cattle buyers can be given health guarantees for IBR. The certification system is managed by Acersa. The health requirements underlying herd certification are stipulated in a statement of requirements approved by the Ministry of Agriculture. The certification protocol is based on mandatory screening rules with additional measures for testing on transfer, for contact among animals (summer grazing, competitions, etc.), and if results are not seronegative (positive or doubtful) in the various tests (National Statement of Requirements CC IBR 01, version N, approved by notice appearing in the Official Journal of 20 June 2012). In herds certified "IBR-free", all the animals have IBR-free certification, which is mentioned on the Preliminary health certificate (ASDA). In herds with "IBR-controlled" status, only animals under 48 months of age on the day certification is granted can have this "IBR-controlled" status on their health certificates. Farms are certified by local certification units, called STCs, which bring together the GDS, Veterinary technical groups (GTVs), and analytical laboratories within a *département* or a region. These STCs are authorised by Acersa to issue IBR-free and IBR-controlled certifications, and the accreditation to do so is maintained by an audit procedure.

In both schemes, analysis of pooled sera is used for annual screening, with non-negative pooled samples then giving rise to individual analysis of each serum sample. Controls on introduction are carried out by individual analysis. Any non-negative individual result obtained for an animal with a certification results in a second analysis using a different kit. Quality control of these analyses is ensured by the ANSES Niort Laboratory, designated IBR National Reference Laboratory (NRL) in 2013.

Health control measures

Any animal that is positive must be vaccinated within two months of notification of results, unless the animal is slaughtered.

Regulatory references

Ministerial Order of 27 November 2006 establishing collective control measures for infectious bovine rhinotracheitis.

Order of 22 February 2005 establishing health conditions for possession, movement, and trade of cattle.

Order of 19 August 2011 amending the Order dated 20 November 2001 approving Acersa as an organisation for official certification in animal diseases.

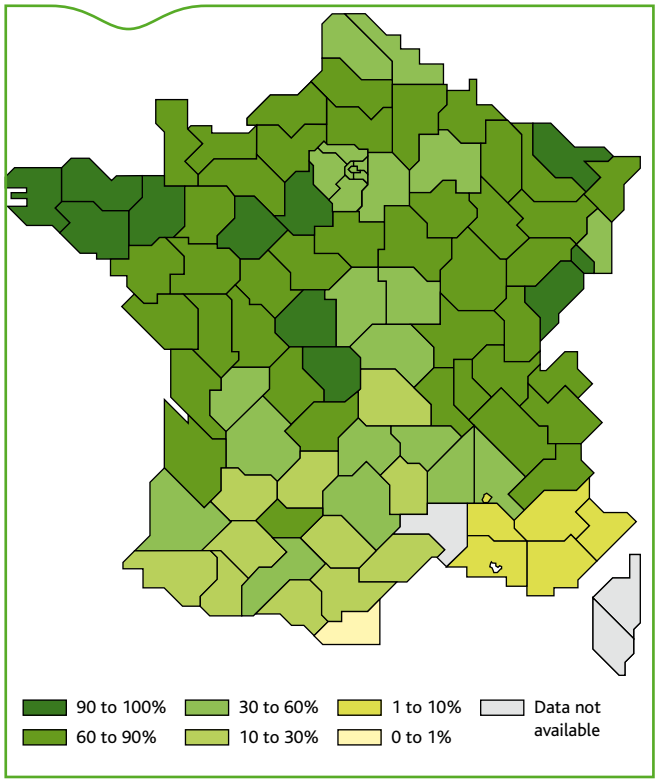

Figure 3. Proportion of certified herds by département as of 31 May 2014 (Acersa data)

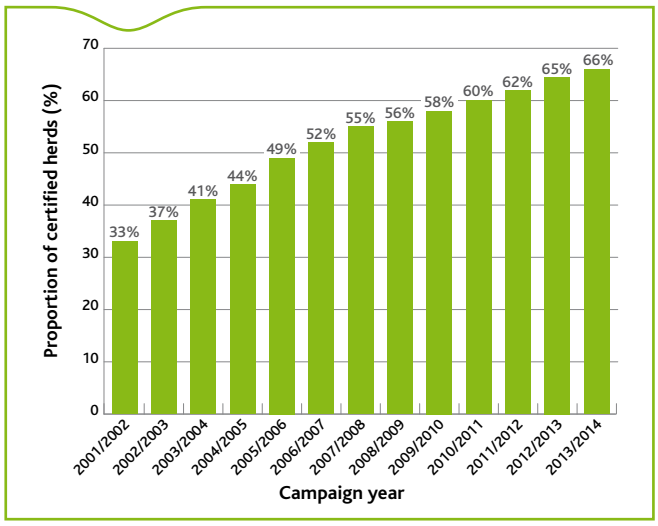

Figure 4. Change in the proportion of IBR certified herds since 2001

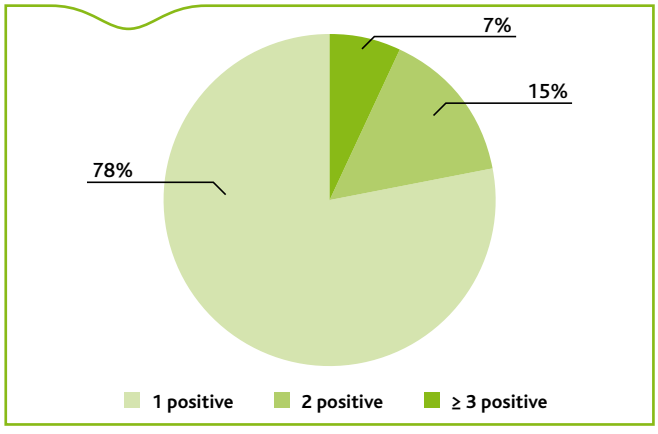

Figure 5. Distribution of IBR-A certified herds in which positive animals were detected during the 2013-2014 campaign, based on the number of positive cases

The number of certified herds has increased steadily since the certification system was introduced as part of Acersa, rapidly between 2001 and 2007, then more slowly in recent years (Figure 4).

Overall, as of 31 May 2014, 123,070 herds were certified. IBR-A certified herds, corresponding to the IBR-free status, were the most common, accounting for 99.4% of certified herds (i.e. 122,330 herds), *versus* only 0.6% with IBR-B certification for IBR-controlled herds (740 herds). This low percentage can be explained by the fact that IBR-controlled status is in general no more than a transitional step for a herd in the process of eradicating the disease.

Incidence of IBR in herds with IBR-A certification

Screened animals were found to be positive during the 2013-2014 campaign in 1,303 herds that had IBR-A certification on 1 June 2013 (representing 1.1% of herds under IBR-A at the start of the campaign) *versus* 1,150 herds for the 2012-2013 campaign (a rate of 0.9% of IBR-A herds as of 1 June 2012).

In 93% of cases, the herds had one or two positive animals (Figure 5), known as "isolated positive". This proportion is increasing compared to that observed for the two previous campaigns (82% of herds with one or two positive animals in the 2011-2012 campaign, 87% for the 2012-2013 campaign).

Discussion of changes in the epidemiological picture and the control scheme

Despite the control measures currently in place, the epidemiological picture has changed little at the national scale and from one campaign to the next. Nonetheless, significant changes have been made or observed concerning the IBR surveillance scheme.

The transfer of the NRL from Sophia Antipolis to the ANSES laboratory located in Niort in May 2013 gave new impetus to projects with various partners.

During the 2013-2014 campaign, some field managers in *départements* with mainly meat production found a significant increase in the number of "positive" analyses of pooled sera that were not confirmed on individual analysis of the sera making up the pooled samples. They also found a higher number of inconsistent results, i.e. individual analyses with positive results using the first kit and negative with the second. Although this ultimately has no impact on the status of the farms involved, an unfortunate effect was a substantial increase in the cost of the scheme because of the increased number of individual analyses.

It was also during this campaign that inconsistencies concerning specificity were found by the manufacturer of a kit used to analyse pooled samples, which could explain some of the findings mentioned. Based on field alerts, the NRL was able to investigate the problem, enabling managers (DGAL, Acersa and GDS France) to react accordingly by providing in particular regular information to laboratories and to scheme managers on the situation, and by issuing recommendations on the measures to adopt. The manufacturers were made aware of the problem and this episode fuelled the initiative already under way by the ANSES Niort Laboratory to work with the stakeholders on the ways of improving the current system for control of reagents and reagent vigilance.

More generally, and independently of this episode, which emphasised the need to consolidate the system for evaluation of reagents, the results obtained as part of follow-up of certification have led to many questions from managers, farmers, and other concerned parties since kits for the analysis of pooled sera were changed during the 2010-2011 campaign. The problem relates to the animals considered isolated positive cases, or "single reactors", that are found more and more often on certified farms. The epidemiological approach to these cases most often leads managers to suspect false-positive reactions. In addition to poor specificity sometimes found for reagent batches,

one of the hypotheses, made specifically by the NRL, is that there may be cross-reactions with other herpesviruses. Several initiatives have been launched by the NRL in collaboration with managers to better understand these cases and put forward more suiTable analytical tools for confirmation and screening. This is why biological material has been collected from this category of animal, i.e. single reactors, from certified herds starting from January 2013. In the interim, until the findings of these initiatives are available, the management rules have been adjusted.

From a broader perspective, managers (DGAL, GDS France and Acersa) share the objective of improving and strengthening harmonisation of the analytical tools used to better meet their goals and enhance effectiveness. This requires a better assessment system. The first step is to build up a new sample bank of sera and milk that is more representative of real conditions, since the current serum bank is now outdated. In parallel, standard sera need to be renewed. This project was started in 2014 by GDS France within its network and will be continued in 2015-2016.

Lastly, notwithstanding these issues, and since the situation has been stable for many years with no particular progression, a decision to accelerate the eradication process was made in 2014. In view of the prevalence and incidence rates observed, a greater effort is required in certain zones, especially those with a focus on meat production. The initial situation and the history of local control strategies can explain this in part, but the differences are above all the result of specific practices. Summer grazing and particularly dense commercial networks, which concern more specifically meat production areas, are at-risk practices. Moreover, the layout of meat production areas is often far more fragmented than in dairy farming, which increases the risks of transmission by multiplying contacts between herds with neighbouring grazing areas. The culling rate is lower in meat production than in dairy production, which slows elimination of possible positive animals. Therefore, exchanges between the various players are continuing to establish the necessary measures to reach this goal, shared by all stakeholders, as soon as possible. At the same time, this will help respond to trade issues by contributing to European recognition of the control programme.

Conclusion

Once the NRL has updated the sample bank, managers will need to work on redefining the performance objectives of screening kits based on the goals of management, concerning both management of certification (problem of single reactors) and in view of the aim of accelerating eradication of IBR. There is a clear need for diagnostic tools that are more reliable and for a confirmation tool that can be used to make simple decisions in the event of suspected cases of false-positive reactions.

Since certain neighbouring countries have achieved recognition of their control programmes, negotiations with European authorities have been reactivated to obtain recognition of the system used in France. The objective of reinforcing measures aimed at eradicating IBR is in line with the goal of achieving recognition.

As a result, the implementation of new measures to eradicate IBR as part of future campaigns will enable farmers to secure, or even improve trade, thus rewarding their efforts in this area.

References

National Statement of Requirements CC IBR 01, version N, approved by notice in the Official Journal of 20 June 2012.

Bovine hypodermosis in France in 2014: no outbreaks detected

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Abstract
During the 2013-2014 campaign, 9,873 herds underwent screening for bovine hypodermosis (serological analyses and visual inspection). Sixty-six percent of surveyed herds were randomly selected and 34% underwent planned checks. With no outbreaks detected, the epidemiological situation in France is therefore considered to be highly satisfactory. While the situation has improved in border areas, such zones remain one of the main risk factors of reintroduction, due to the lack of organised control plans in neighbouring countries, the absence of natural barriers and the proximity of French and foreign herds on summer pasture lands. Therefore, reinforced monitoring in at-risk areas, surveillance of animal introductions and targeted screening continue, so as to avoid undermining the efforts that have been made over the past several years.

Keywords
Bovine hypodermosis, Warble fly, Cattle, Epidemiological surveillance

Résumé
Hypodermose bovine en France en 2014 : aucun foyer détecté
Durant la campagne 2013-2014, les dispositifs de surveillance aléatoire et orientée de l'hypodermose bovine, ou varron, (analyses sérologiques et contrôles visuels) ont porté sur 9 873 cheptels : 66 % des cheptels surveillés provenaient d'un tirage aléatoire et 34 % de contrôles orientés. Aucun foyer n'a été mis en évidence : la situation épidémiologique de la France est donc très favorable. Bien que la situation se soit nettement améliorée dans les zones frontalières, celles-ci restent un des principaux facteurs de risque de réintroduction, du fait de l'absence de plans de lutte collectifs connus dans les pays limitrophes, de l'absence de barrières naturelles, et de la proximité entre troupeaux français et de pays voisins en zone d'estive. Dans ce contexte, la surveillance du varron reste renforcée dans les zones à risque, la surveillance des introductions et les contrôles orientés sont maintenus pour ne pas compromettre les efforts entrepris depuis plusieurs années.

Mots-clés
Hypodermose bovine, varron, bovins, épidémiosurveillance

Hypodermosis (warble) is an internal myiasis in cattle characterised by infestation of subcutaneous connective tissue in the dorsolumbar region by larvae of flies in the *Hypoderma* genus, following a period of larval migration and transformation. The larvae develop in bovine tissue over the winter and emerge in the spring after forming a nodule on the animal's back and perforating the hide.

In the past, this disease had substantial economic consequences: reduced milk production, slowed growth of young animals, immunosuppression caused by larvae, and damage to the hide when the larvae exit through the skin in the spring. For these reasons, farmers came together at the end of the 1980s to implement an organised control plan, region by region. Each regional control scheme had two components: a systematic treatment phase at the beginning of the plan, followed by a testing phase (first visual inspection, then serological analysis) for several years. Serological testing became mandatory for all herds in France in July 1998 and was reinforced by the Ministerial Order of 6 March 2002. A rapid decrease in the country-wide prevalence of hypodermosis was then observed in herds between 1998 and 2001, from 5.7% to 0.4% (Mémeteau *et al.*, 2011). Given the rate of eradication, in February 2006 bovine hypodermosis in its clinical form became a notifiable disease with compulsory control measures (Decree No. 2006-178, 17 February 2006). It is now considered to be a Category 2 health hazard (Ministerial Order of 29 July 2013).

There are currently two surveillance schemes, one voluntary and one mandatory (Box):

- The mandatory scheme is underpinned by the Ministerial Order of 21 January 2009 and relies on:
  - A random surveillance scheme conducted annually to determine whether the prevalence of infestation in a zone is below a certain level (5%). Implementation of this scheme is entrusted to the GDSs.
  - This surveillance scheme entails serological analysis of pooled sera or bulk milk (sampled between 1 December of the previous year and 31 March of the current year for blood samples, and between 1 January and 31 March of the current year for milk samples). Sampling takes place as part of programmed cattle screening procedures for brucellosis and infectious bovine rhinotracheitis (IBR), in a randomly selected group of herds. Animals in herds found to be positive then undergo a sight check in the spring to confirm or rule out the presence of hypodermosis.
  - If necessary, serological surveillance can also be supplemented by random sight checks[1]. These inspections take place during the period when the larvae emerge, between 1 April and 30 June each year.
  - At the end of the random surveillance campaign, and on the basis of an annual report forwarded by the national coordinator (GDS), the DGAL determines which zones are hypodermosis-controlled or hypodermosis-free. An area is considered to be a hypodermosis-controlled zone when the rate of infestation of herds, demonstrated by the random scheme through serology and/or sight checks, has been below 5% for two consecutive years. Hypodermosis-free zones have had an infestation rate, demonstrated by random serological testing, of less than 1% for two consecutive years.
  - A targeted screening campaign is also carried out to detect outbreaks of hypodermosis. This campaign increases the probability that infested herds will be detected, but also aims to raise awareness among breeders for whom the risk of infestation is related to farming methods. Targeted screening focuses on

1. On 31 March each year, if less than 80% of randomly selected herds in an area have been tested serologically, herds that have not been tested are inspected visually to reach the threshold of at least 80% of herds monitored in the area. Serological testing is given preference because sight checks are far less sensitive.

Box. Control measures for bovine hypodermosis

Objectives

Mandatory surveillance

- Confirmation of the controlled or disease-free status of the various regions of mainland France (an infestation rate of below 5% or 1%, respectively).
- Early detection of any outbreak of hypodermosis.

Voluntary certification scheme
Guarantee the status of the herd of origin for animal sales.

Monitored population

Domestic cattle across mainland France.

Surveillance procedures

Outbreak surveillance:
Any cutaneous lesion suggestive of bovine hypodermosis must be notified to the Departmental Directorate for Protection of the Population (DDecPP) in the *département* where the suspect animals are located.

Mandatory Programmed surveillance

- Screening by serological analysis of pooled sera or tank milk in a random sample of herds. Given the qualitative approach, the sample size is determined on the basis of a threshold prevalence level (5% for "controlled" status) and the number of herds present. Any non-negative result for pooled blood samples leads to individual testing. A non-negative result for one or more animals entails loss of the negative status of the herd. A positive result for milk pooled from a number of animals (tank milk) leads to a positive status for the herd. If the result is uncertain, a second sample is taken before 31 March in order to determine the status of the herd. Serological surveillance can also be supplemented by random sight checks seeking to detect any cutaneous lesion.
- Targeted screening of herds or animals considered to be at-risk (epidemiological link with an affected herd, geographic location of the herd in an area at risk of re-infestation, farming practices, non-negative results obtained during serological screening campaigns).
- Monitoring introductions: only animals from at-risk herds (foreign herds or herds reported as being at-risk by the managers) undergo hypodermicid treatment, unless introduced in a finishing herd under exemption with cattle kept entirely in closed facilities, or cattle born after 31 October and introduced before 1 March of the following year (in compliance with the ACERSA statement of requirements for national certification).

Voluntary scheme
This scheme is managed by the French Certification Association for Animal Health (ACERSA) and results in certification of production sites. It is implemented in the field by local certification units (STCs) authorised to grant herds within their area the certification "hypodermosis-controlled" or "hypodermosis-free", guaranteeing the status of the herd of origin when animals are sold. Herds qualify if they are in a controlled or disease-free zone respectively and fulfil the conditions of the national statement of requirements.

Health control measures

Bovine hypodermosis had been a notifiable disease with compulsory control measures in its clinical form since 2006, and is now a Category 2 health hazard.

If a farm is found to have clinical cases of bovine hypodermosis, the clinically affected animal or animals, as well as those suspected of being infested, must be treated.

Regulatory References

Ministerial Order of 29 July 2013 defining Category 1 and 2 animal health hazards.

Ministerial Order of 21 January 2009 establishing collective prophylaxis and control measures for bovine hypodermosis.

potentially at-risk herds, specifically those where there is an epidemiological link to an infested herd, those located in a zone where infestation may recur (particularly border areas, i.e. any municipality located less than 5 km from the border), those subject to certain farming practices (trade, summer grazing) or those where non-negative test results were obtained through random surveillance.

- The second surveillance scheme involves issuing health status certification, to complement the mandatory measures. It serves to guarantee the status of the herd of origin when animals are sold. The scheme is coordinated by the French Certification Association for Animal Health (Acersa) and implemented by local certification units (STCs) authorised to grant the following certifications to herds within their areas: hypodermosis-controlled herd, or hypodermosis-free herd, depending on the zone's status, and guaranteeing the status of the herd of origin when animals are sold. Livestock farmers can apply for either of the certifications if their herds are located in controlled or disease-free zones and fulfil the conditions in the national statement of requirements (ACERSAStatement of requirements - CC VAR 01), and are reported as being in a zone where there is an accredited STC for issuing hypodermosis certifications.

This article presents descriptive results for bovine hypodermosis obtained through the random and targeted surveillance schemes for the 2013-2014 campaign that took place between 1 July 2013 and 30 June 2014. The results presented below are based on data collected specifically from FRGDS groups (Regional federation of animal health protection farmers' organisations) and forwarded by GDS units that implement the bovine hypodermosis surveillance plan.

Results

During the 2013-2014 campaign, 9,873 herds were tested as part of the random and targeted surveillance schemes for bovine hypodermosis through serological analyses and sight checks: 66% of the monitored herds were selected at random, and 34% underwent targeted testing

Random surveillance of herds

Evaluation of the rate of herd infestation is based on a random sampling plan involving computerised random selection from among all herds in a region, excluding finishing herds that are exempt and housed entirely in closed facilities.

Given the qualitative approach, the sample size is determined on the basis of a threshold prevalence level (5% for controlled status) and the number of herds present.

For the 2013-2014 campaign, 7,201 herds were selected at random, and 6,513 of these were tested: 6,391 herds by serological analysis and 122 by visual inspection. Thus 90% of randomly selected herds were controlled, with a completion rate higher than 80% for all regions. The requirement concerning the level of tests to perform, i.e. more than 80% of the random sample, was therefore fulfilled for all regions. This testing level of below 100% is primarily related to the obligation to carry out the serological controls during a given period. A certain number of randomly-selected herds could not be tested, particularly for blood testing, because programmed screening for brucellosis and/or IBR took place before 1 December or after 31 March.

Random serological surveillance

In total, 6,391 herds underwent serological testing: 3,808 were analysed only through blood sampling, 2,001 only through milk, and 582 *via* both blood and milk samples (mixed herds).

During the 2013-2014 campaign, nine herds were identified as seropositive through the random testing scheme (herds providing positive blood samples) as well as one mixed herd in the French regions of Provence-Alpes-Côte d'Azur, Limousin, Languedoc-Roussillon, Franche-Comté and Centre. Sight checks performed on these herds were negative, however. These seropositive herds were therefore not

registered as outbreaks of bovine hypodermosis, but were considered to be the result of residual antibodies or false-positive results. We note that the proportion of positive tests on blood (0.05%) concurs with the test specificity (99.8%, according to the supplier's validation dossier) (Institut Pourquier, 2001). These herds will be included in targeted serological controls next year.

Random visual inspection

In all, 6,382 animals in 122 herds were inspected visually. No outbreak of clinical hypodermosis was detected.

Targeted surveillance of herds

3,360 herds were tested as part of targeted surveillance *via* serological analyses or sight checks.

Targeted serological testing

Serological analyses were carried out in 1,936 herds with blood samples, and with milk samples in 1,101. The majority, 67%, of these targeted serological controls concerned herds in border areas where the risk of reintroduction is highest. These serological analyses detected 29 seropositive herds, located in the French Regions of Rhône-Alpes, Provence-Alpes-Côte-d'Azur, Champagne-Ardenne and Auvergne. Sight checks performed on these herds were negative, however. Again, these seropositive herds were therefore not registered as outbreaks of bovine hypodermosis, but were considered to be the result of residual antibodies or false-positive results.

Targeted visual inspection

In all, visual inspections were carried out in 323 herds. No outbreak of clinical hypodermosis was detected.

Control of introductions and treatment measures

In all, out of a total of 7,158 cattle that should have been screened following introduction in herds in mainland France for the 2013-2014 campaign, 6,191 cattle were actually treated, giving a treatment rate of 86%. If animals are not treated, this results in the implementation of targeted testing of the animal and/or herd of origin.

Tactical treatment (preventive treatment in at-risk herds) was administered for a total of 1,869 cattle in 83 herds. To a very large extent, these treatments were administered in border areas, and were far less numerous. Tactical treatments are no longer systematic, with priority being given to control measures.

Report on implementation of local certification units

The national control scheme covers 21 regions or zones, including six that have borders with Belgium, Luxembourg, Spain or Italy (14 *départements* in all). Most *départements* and some regions are organised into local certification units (STCs) accredited by ACERSAto manage the control plan for bovine hypodermosis (Figure 1).

At this time, only two *départements* on the French mainland have not submitted an application for accreditation of a local hypodermosis STC. These are Nord and Pas-de-Calais. The epidemiological situation close to the border with Belgium has improved (absence of outbreaks and a decrease in the number of positive blood tests). This should facilitate the management of the hypodermosis programme and enable these two departments to engage with it as a result.

Discussion

Results obtained for the 2013-2014 campaign indicate that all regions have an infestation rate below 5% (according to serological testing and/or sight checks) and fulfil requirements concerning the number of tests to perform (more than 80% of the random sample). Therefore, as per the criteria stipulated in the Ministerial Order of 21 January 2009, all the regions of mainland France have controlled-zone status. In addition, the vast majority of *départements* and regions on the mainland have STCs, with regional or departmental organisations accredited by Acersa, and can issue herds with hypodermosis-controlled certification.

During the 2013-2014 campaign, no outbreaks of bovine hypodermosis were detected, despite an outbreak in 2012-2013 following the introduction of an infested animal from Spain in the Midi-Pyrénées region. The epidemiological picture is therefore highly favourable. As a reminder, no outbreaks had been detected during the two previous campaigns, 2010-2011 and 2011-2012.

In view of the very favourable epidemiological situation in France in the past few years, the assumption that the persistence of antibodies can explain the positive serological results must be further investigated. For this purpose, the data concerning the age and origin of the seropositive animals will be collected for future campaigns.

In view of the very low prevalence levels observed over these recent campaigns (Figure 2), some or all of the STCs could work towards obtaining disease-free certification. To achieve this, stricter sampling requirements would be needed and would only be accepTable if groups of neighbouring regions were created. However, recognition as a disease-free zone is not currently planned since there are no economic benefits compared to controlled status.

Costs

The measures taken included awareness-raising initiatives for breeders, administrative and technical follow-up (targeted testing of herds), and tactical treatment of animals, for a total cost of €543,790. In order to carry out these actions, the livestock producers bear a significant share of the costs, even if the aid from the State (€60,000), from regional councils and the general Union of hides and skins are essential to maintain a surveillance adapted to the favourable epidemiological situation in France.

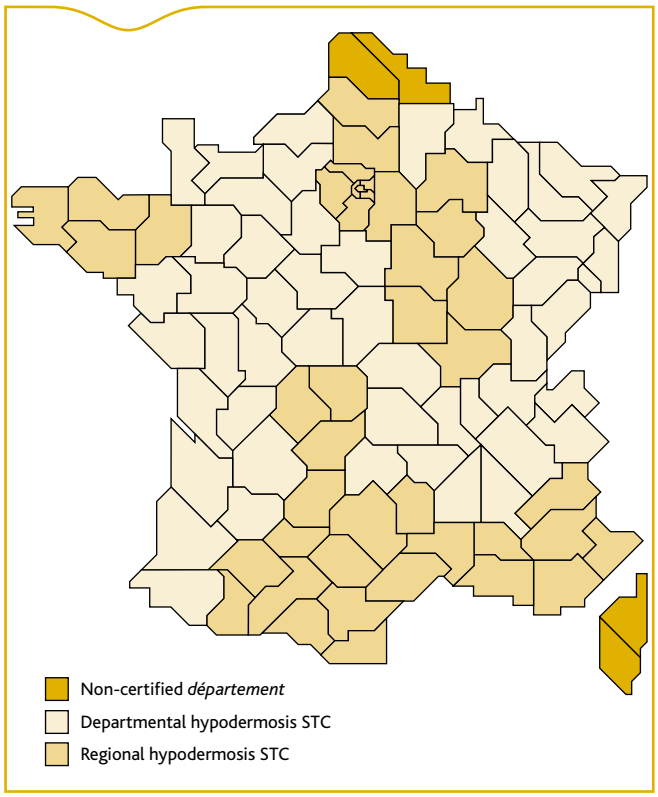

Figure 1. Accredited local certification units (ACERSA data)

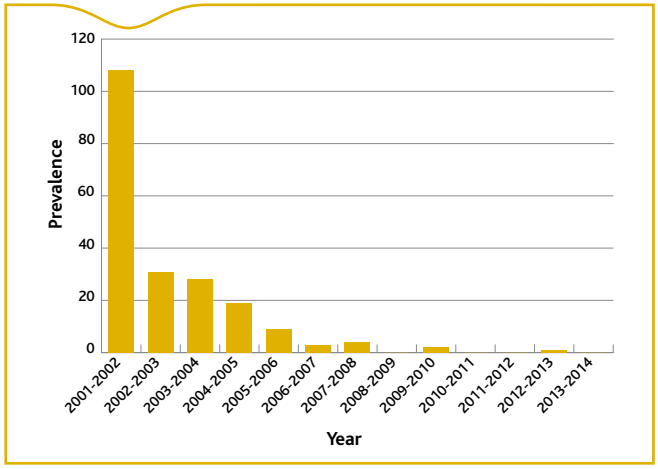

Figure 2. Changes in prevalence of bovine hypodermosis since 2002 in France

The 14 at-risk *départements* bordering Spain, Italy, Belgium or Luxembourg spent €104,180 on control of hypodermosis, or 20% of the national total.

Conclusion

During the 2013-2014 campaign, no outbreaks of bovine hypodermosis were detected. The results obtained in the 2013-2014 campaign mean that "disease-controlled" status can be maintained for all regions concerned, since hypodermosis can be considered absent at the prevalence threshold of 5%.

However, border areas remain vulnerable. The introduction of warble fly and resulting outbreaks is still possible in the absence of acknowledged control plans in neighbouring countries, the absence of natural barriers, and the proximity of French and foreign herds in summer grazing areas (the range of *Hypoderma* warble flies is about 5km).

Given this context, surveillance of at-risk zones, tactical treatment, monitoring of introductions and targeted testing all remain important, with the *départements* most exposed along their borders playing the role of buffer zone.

Acknowledgements

We would like to thank all the accredited laboratories for diagnosis of hypodermosis in serum or milk, all GDS personnel, responsible for implementation of hypodermosis control, and coordinators of local certification units (STCs), who provided the data essential for drafting this article.

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Bluetongue in 2014: mainland France remains free from Bluetongue; BTV-1 epizootic in Corsica is under control

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Abstract
Results of the active and passive surveillance of Bluetongue (BT) in 2014 demonstrated the absence of virus circulation in continental France for the fourth consecutive year. This confirms the official "free from BT" status that continental France recovered on 14 December 2012, after four years of compulsory (2008-2010) and voluntary (2010-2012) vaccination campaigns. The vaccination campaign organized by the official services in Corsica in order to control the BTV-1 epizootic which occurred in September 2013 seems to have been successful since no outbreak has been reported on the Island since May 2014. Clinical vigilance is highly recommended in continental France and Corsica, considering the high risk of introduction through movements of infected animals or through passive dissemination of infected vectors (from Sardinia to Corsica for example).

Keywords
Bluetongue disease, Surveillance, Outbreaks, Ruminants, Category 1 health hazard, Regulated disease

Résumé
Fièvre catarrhale ovine en 2014 : maintien du statut indemne en France continentale, maîtrise de l'épizootie de sérotype 1 en Corse
Les résultats de surveillance événementielle et programmée de la fièvre catarrhale ovine (FCO) en 2014 ont permis de démontrer l'absence de circulation virale en France continentale pour la quatrième année consécutive. Cela permet de confirmer le statut indemne de ce territoire acquis le 14 décembre 2012, après quatre années de vaccination obligatoire (2008-2010) puis volontaire (2010-2012). La campagne de vaccination organisée par l'Etat en Corse pour maîtriser l'épizootie de FCO de sérotype 1 apparue en septembre 2013 semble avoir porté ses fruits puisqu'aucun foyer de FCO n'a été déclaré dans l'île depuis mai 2014. La vigilance s'impose en France continentale comme en Corse, considérant le risque élevé que le virus soit introduit, via les mouvements d'animaux vivants infectés, ou par la diffusion par le vent de vecteurs infectés (depuis la Sardaigne vers la Corse notamment).

Mots-clés
Fièvre catarrhale ovine, surveillance, foyers, ruminants, danger sanitaire de 1ère catégorie, maladie réglementée

This article summarizes Bluetongue health situation in 2014. It has been written before reoccurence of the disease in 2015.

In 2014, there were two schemes in place for surveillance of Bluetongue: outbreak surveillance and programmed surveillance (Box 1).

Results of surveillance for Bluetongue

Outbreak surveillance in mainland France

In 2014, investigations concerning clinical suspicions of BT were conducted in 33 *départements* (Figure 1). In total, 108 cattle from 33 separate farms, 77 sheep from 19 separate farms, one goat and one roe deer were subjected to virological analysis (performed by the NRL or a DTL) following a clinical suspicion of BT. Virological analyses ruled out all suspected cases notified; no case of BT was confirmed in mainland France in 2014.

Three clinically suspect sheep were found in a batch of animals imported from Romania in October 2014. It should be noted that, even though the animals came from a region that was BT-free at the time, the BT virus was already circulating in other regions of the country. This suspicion gave rise to preventive management measures (immediate slaughter of the entire suspect batch). As in the other cases, the suspicion was lifted once negative PCR results were returned for the suspect animals.

The clinical suspicions were mainly reported between the months of July and December (Figure 2), which corresponds to the period during which cases of BT are most likely to emerge. It is possible that veterinarians take the seasonality of risk into account in their differential diagnosis and only declare clinically suspect cases in summer or autumn. Another possibility is that the frequency of occurrence of clinical signs suggestive of BT actually fluctuates seasonally, possibly due to one or more enzootic diseases that themselves fluctuate seasonally. However, this hypothesis cannot be investigated because no information on the real origin of clinical signs is collected when the suspicions of BT are refuted.

In some *départements* with large populations of ruminants, the absence of notifications of clinical suspicions of BT may reflect a decline in the vigilance of farmers and veterinarians. Indeed, the clinical signs of the disease are not especially pathognomonic, and syndromes suggestive of BT should often be encountered in animal husbandry (Box 2, and Zanella *et al.*, 2010). The list of clinical signs suggestive of BT, together with a slideshow presentation including relevant photographs, can be downloaded from the website of the ESA Platform. The DDecPPs were invited to again raise awareness among the network of mandated veterinarians as to the need for clinical vigilance regarding this disease, especially as regards cattle from BT-regulated areas (Spain, Italy, Eastern Europe).

Programmed surveillance in mainland France

Over the course of the year, each *département* (except those with very small ruminant populations) was required to perform serological analyses, preferably on 15 young cattle (otherwise on sheep or goats) from three farms, with a national objective of 1,350 tests. A total of 1,149 serological analyses were finally declared to have been performed by the DDecPPs during 2014 in the annual report of animal health, which is a national rate of 85% (map of the departmental performance rates in Figure 3). It seems necessary to increase awareness among those taking part of the need to achieve a better rate, particularly in the *départements* where no results of programmed surveillance for BT were reported in the SIGAL national information system.

In line with instructions, 100% of these analyses concerned cattle.

As in 2011, 2012 and 2013, no viral circulation was detected on the French mainland through programmed surveillance in 2014 (see previous editions of *Bulletin Épidémiologique* - REDs).

According to the data recorded in Sigal, close to 7% of non-negative results were obtained from among the serological screening tests performed by the DTLs, but none of these analytical suspicions were confirmed following further investigation. This proportion seems to have decreased compared to 2013 (when it was equal to 9%), but remains non-negligible. It is therefore appropriate to continue the effort to respect the criteria for selection of animals (unvaccinated cattle under the age of two years), to ensure that no animal sampled for serological screening was present during the epidemics of 2008-2009 and/or vaccinated during compulsory vaccination campaigns.

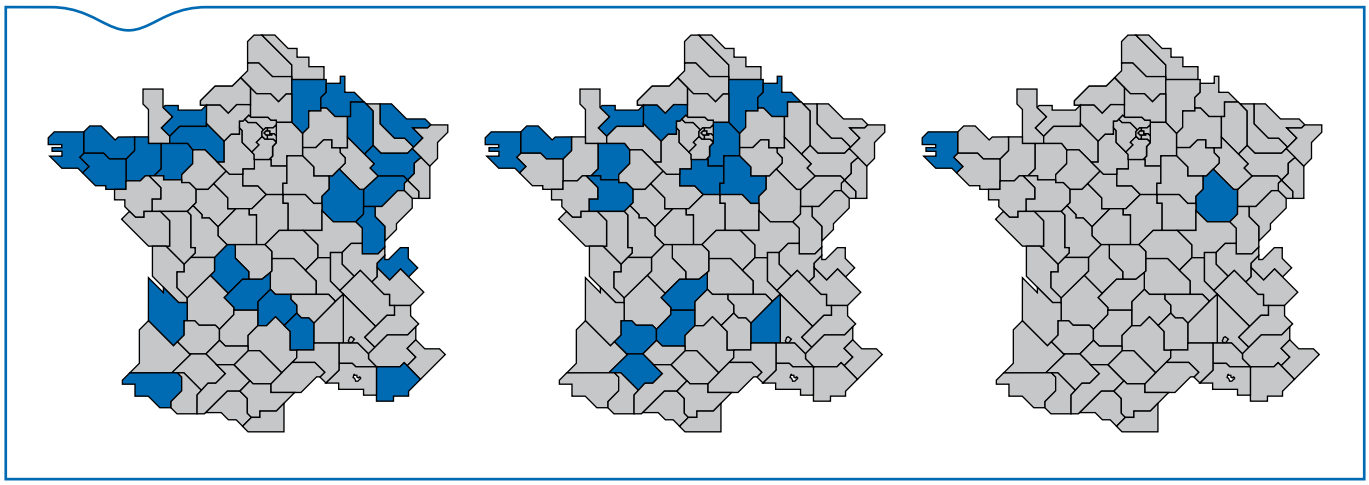

Figure 1. ***Départements*** **in which clinical suspicions of BT in cattle (left), in sheep (centre) or in an unspecified species (right) were reported in 2014 (in blue: at least one clinical suspicion notified, in grey: no clinical suspicion notified)**

Box 1. Surveillance and control measures for Bluetongue in mainland France in 2014

Bluetongue monitoring in 2014 in mainland France was based on two schemes: outbreak and programmed surveillance (entomological surveillance was abandoned on 1 January 2013 following the recovery of disease-free status).

Objectives of the surveillance programme

- To detect the introduction of any exotic serotype (including serotypes 1 and 8).
- To provide evidence for the maintenance of disease-free status for mainland France.

The population monitored

Domestic ruminants.

Outbreak surveillance

Clinical surveillance requires that all holders of animals from susceptible species and all mandated veterinarians notify the administrative authorities of any clinical signs suggestive of BT. Following notification, the suspect farm is placed under surveillance. A description of the clinical signs suggestive of BT is available online on the website of the ESA Platform (www.platform-esa.fr), and in Memorandum 2013-8188 of 20/10/2013 relating to notifications of clinical suspicions of Bluetongue.

Programmed surveillance

Programmed surveillance in 2014 was organised in such a way as to comply with the European regulation (EC No 1266/2007) for the monitoring of BT in disease-free areas: an annual serological survey capable of detecting a prevalence of 20% with a degree of certainty of 95% per geographical unit, which means taking 14 samples per geographical unit per year. In France, the geographical unit chosen was the *département*. Each *département* must take annual samples from fifteen animals from three different herds.

These samples should preferably be taken from cattle of less than two years of age, not having been vaccinated against BT and exposed to the bites of *Culicoides* (i.e. put out to pasture during the summer).

Diagnostic protocol

In 2014, following the recovery of disease-free status for mainland France, the first-line analyses in cases of clinical suspicion were performed by the NRL, ANSES Maisons-Alfort. Diagnosis was carried out by group- RT-PCR analysis (meaning that it was not specific for a particular serotype).

For programmed surveillance, serological analyses consisted of ELISA tests carried out by the accredited departmental testing laboratories (DTLs). If non-negative results were obtained by a DTL, the suspect animals were re-sampled for virological analysis (RT-PCR) carried out by the NRL. This re-sampling was put in place because BT virus can be detected in blood samples several months after infection.

If the RT-PCR is positive (which did not occur in 2014 for animals in mainland France), viral isolation, which is the reference analysis for confirmation of an outbreak, must be performed.

Health control measures in place for 2014

In the event of clinical or analytical suspicion, the farm of origin is placed under Prefectural monitoring order (APMS) pending the results of investigations performed by the NRL.

If an outbreak of BT is confirmed in a disease-free area, the national emergency health intervention plan is implemented by the Prefect.

Regulatory References

Council Directive 2000/75/EC laying down specific provisions for the control and eradication of bluetongue

Commission Regulation (EC) No 1266/2007 on implementing rules for Council Directive 2000/75/EC as regards the control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue

Ministerial Order of 22 July 2011 (amended) establishing the technical and administrative framework applicable to Bluetongue control in mainland France

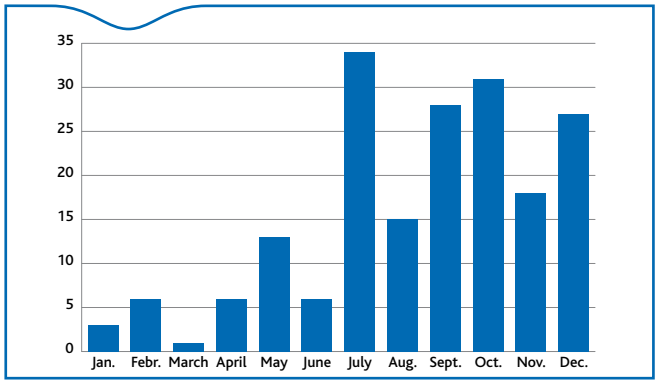

Figure 2. Number of clinical suspicions declared by month

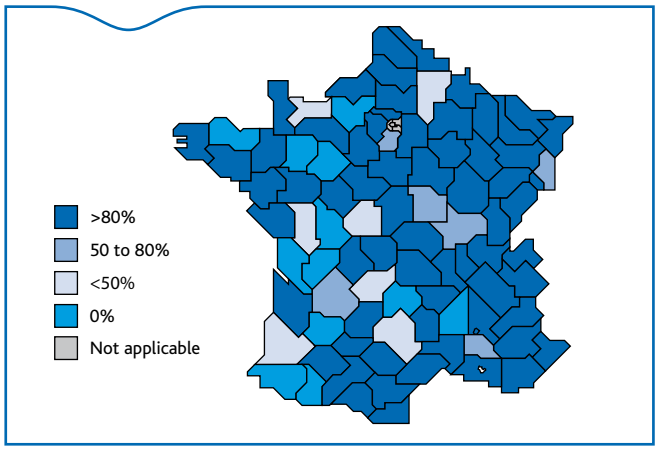

Figure 3. Rate of BT sampling by département based on the number of samples declared by the DDecPPs in 2014

Box 2. List of clinical signs suggestive of BT

Attention, when examining an animal with a clinical picture suggestive of BT, a differential diagnosis must be performed to exclude foot-and-mouth disease with certainty (in particular, it is necessary to verify the absence of vesicles).

General signs
- Listlessness, depression
- Decrease in milk production
- Reduced appetite, anorexia
- Prostration, inability to stand
- Weight loss/muscle loss
- Tachypnea, dyspnea, noisy breathing
- Fever

Limbs
- Stiffness
- Limping
- Oedema and/or congestion of coronary bands
- Oedema on pasterns, fetlock, shin, knee/hock

Head
- Congestion of the muzzle
- Erosions/ulcers/crusting on the muzzle or nasal mucosa
- Congestion of the mucosa of the mouth
- Erosions/ulcers of the mucosa of the mouth
- Oedema of the tongue
- Nasal discharge
- Salivation
- Cyanosis of the tongue (bluetongue)
- Oedema of the inter-mandibular surface and/or of the muzzle
- Conjunctivitis, lacrimation

Udder/vulva
- Congestion of the teats or udder
- Erosions/ulcers/crusting on the teats or udder
- Erosions/ulcers of the vulva

Costs

In 2014, the State spent approximately €45,500 on monitoring BT in mainland France, €32,000 for programmed surveillance and €13,500 for targeted surveillance.

The cost of programmed surveillance corresponds to the cost of ELISA screening analyses (€13,500), of PCRs for refuting non-negative results (€6,500) and veterinary procedures (€12,000).

The cost of targeted surveillance includes the cost of PCR analyses (€11,000) and veterinary procedures (€2,500).

These sums do not take into account the cost of running and managing the technical and financial aspects of the scheme, particularly in terms of human resources delegated by the administration.

The costs of control measures for the BT epizooty in Corsica are given in Box 3.

Discussion and outlook

Following two mandatory vaccination campaigns (2008-2010) and two voluntary vaccination campaigns (2010-2012), the two serotypes of BT introduced into mainland France (BTV8 and BTV1) are no longer detected in mainland France. No outbreak of BT has been identified in mainland France since June 2010 (Table 1), which enabled it to be declared a disease-free territory on 14 December 2012. Disease-free status was maintained in 2014, but continued vigilance is required, considering the epidemiological situation in certain neighbouring countries (Spain and Italy) or Eastern Europe (because of the frequent introduction of ruminants from these zones).

Table 1. Change in the number of BT outbreaks between 2006 and 2014

	2006	2007	2008	2009	2010	2011	2012	2013	2014
Number of serotype 1 outbreaks	0	3	4,932	9	1	0	0	0	0
Number of serotype 8 outbreaks	6	15,257	27,510	77	0	0	0	0	0

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Box 3. Surveillance of the bluetongue epizooty in Corsica in 2014

The results for 2013-2014 of the epizootic episode in Corsica were presented in a short item published in Issue No. 67 of the *Bulletin Epidémiologique* (Desvaux S. *et al.*, 2014).

Background and context

Serotypes 2, 4 and 16 of bluetongue emerged on the island in 2000, 2003 and 2004 respectively, with a peak in the epizooty in 2001 when 326 outbreaks of serotype 2 were confirmed. No outbreaks were confirmed between March 2005 and September 2013.

The programmed surveillance scheme for Bluetongue in Corsica (serological screening of calves at the slaughterhouse) has historically followed different procedures from those in mainland France (virological analysis). In 2013, a new protocol for programmed surveillance, based on RT-PCR analysis of calves at the slaughterhouse, was put in place so as to include Corsica in a programme that meets the requirements for regaining BT-free status, in accordance with the regulatory EU requirements. This approach, initiated in July 2013, was interrupted in September by the emergence of clinical outbreaks of BT in the south of the island.

The emergence of serotype 1 in Corsica, very probably introduced from Sardinia, and its rapid spread in the island, was the subject of an article in the *Bulletin Épidémiologique* of December 2013 (Perrin *et al.*, 2013).

Since the purpose of programmed surveillance is to demonstrate the absence of viral circulation, it was not revived during 2014 because the virus was still circulating.

Outbreak surveillance

Outbreak surveillance follows the same procedures as in mainland France. In 2014, only 31 outbreaks were confirmed (for 107 suspicions) throughout the island despite a surveillance system continuously on alert. Suspicions continued to be reported, although they were less often confirmed (29% of confirmations in 2014 against 79% in 2013). From mid-May 2014, no outbreaks were confirmed, although 33 suspicions were declared and investigated between June and December 2014.

Control measures

Corsica remains a regulated zone for serotypes 1, 2, 4, 8 and 16. Suspected outbreaks of BT therefore do not trigger implementation of a contingency plan. The farms where the virus is identified are placed under APDI, prohibiting the movement of ruminants to and from these farms (except where derogation is granted by the Prefect). This APDI is lifted sixty days after the vaccination of all ruminants present on the holding.

Two compulsory vaccination campaigns were organised, fully financed by the State (covering both the doses and the vaccinations). The first campaign took place from 26 November 2013 to 31 May 2014 (Ministerial Order of 26 November 2013). The second started in July 2014 and ended on 30 June 2015 (Ministerial Order of 4 July 2014). For the first campaign, about 70% of domestic ruminants were vaccinated by mandated veterinarians: approximately 85% of sheep, 30% of goats and 65% of cattle. Most ruminants were vaccinated between January and March 2014.

This quite satisfactory rate of vaccination, in particular in the case of sheep, probably played an important role in the control of the epizooty, explaining the absence of outbreaks reported since June 2014.

Estimated costs (excluding VAT)

In 2014, 1,365 PCR analyses were performed in Corsica, for a total amount of approximately €50,000 in analysis costs and €40,000 in veterinary fees. The vaccine doses administered in 2014 cost €360,000, plus €390,000 in veterinary fees.

Culicoides population activity in Corsica in 2014

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Abstract
We present the diversity and population dynamics of *Culicoides* in 2014 in Corsica, the only remaining area of Metropolitan France that is not Bluetongue-free and which is monitored for *Culicoides*.

Keywords
Entomological monitoring, *Culicoide*, Corsica, Bluetongue

Résumé
L'activité des populations de* Culicoides *en Corse en 2014
***Nous présentons la diversité et la dynamique des populations de* Culicoides *observées en 2014 en Corse, seule partie du territoire métropolitain non-indemne de fièvre catarrhale ovine et concernée par la surveillance entomologique des populations de* Culicoides.**

Mots-clés
Surveillance entomologique*, Culicoides, *Corse*, *fièvre catarrhale ovine

Mainland France regained its Bluetongue-free status in 2012. Since 2013, monitoring of *Culicoides* populations has only concerned Corsica, using two traps per *département*. Trapping is carried out every two weeks, under the responsibility of the DDecPPs, respectively by the technicians of the DDecPP of Corse-du-Sud and of the GDS of Haute-Corse. The samples caught are sent to the Cirad in Montpellier, for identification and enumeration of the *Culicoides* collected.

In all, 88 trapping operations were carried out in 2014, i.e. 88% of the number planned. About 50,000 *Culicoides* belonging to at least 37 species were captured, the majority in Corse-du-Sud (30,000 individuals compared with 20,000 for Haute-Corse). The main species caught were *Culicoides newsteadi* (34.0% of individuals captured), *Culicoides obsoletus/Culicoides scoticus* (29.3%), and *Culicoides imicola* (27.7%). Besides these main species, only *Culicoides pulicaris* and *Culicoides punctatus* were relatively abundant, with more than 1%, i.e. 2.0% and 1.9% respectively. All of these species accounted for 94.9% of catches. In the south-east of the island (site 2APL5 in Figure 1), *C. imicola* (41.0%) and *C. newsteadi* (52.5%) largely dominated catches, while *C. obsoletus/C. scoticus* remained rare (1.4% of catches). In traps near the coast in the centre and the north of the island (sites 2APL7 and 2BPL2), the *C. obsoletus/C. scoticus* species became as abundant (48.0 and 33.5%) as *C. newsteadi* (40.4 and 27.0%) and as *C. imicola* at the site 2BPL2 (30.0%) – this last species was rare at site 2APL7 (2.6%). It is not possible to interpret the diversity observed in the north-centre of the island (2BPL5), because only 975 *Culicoides* specimens were captured. These results, both globally and per site, were quite similar to those observed in 2013, demonstrating broad stability in the diversity of the most abundant species from one year to the next.

In January, the number of *Culicoides* captured remained low, with five to eight parous females/trap-night, but was still above the regulatory "vector activity" threshold. From mid-February, the number of captured *Culicoides* became significant with a maximum of around 200 parous females/trap-night. The abundance of *C. newsteadi* and *C. obsoletus/C. scoticus* species was at its highest in the first part of the year, it then decreased during the summer, before reaching a second peak in the autumn. The populations of *C. newsteadi* seemed to remain more abundant during the summer than those of the *C. obsoletus/C. scoticus* species. Conversely, the populations of *C. imicola* gradually increased in abundance over the year, to reach a peak in September. The populations remained highly abundant at the end of the year: as many as 1,156 parous females/trap-night in early December in the eastern part of the island.

Acknowledgements

We warmly thank all those involved in trapping activities (DDecPP and GDS personnel, as well as livestock farmers).

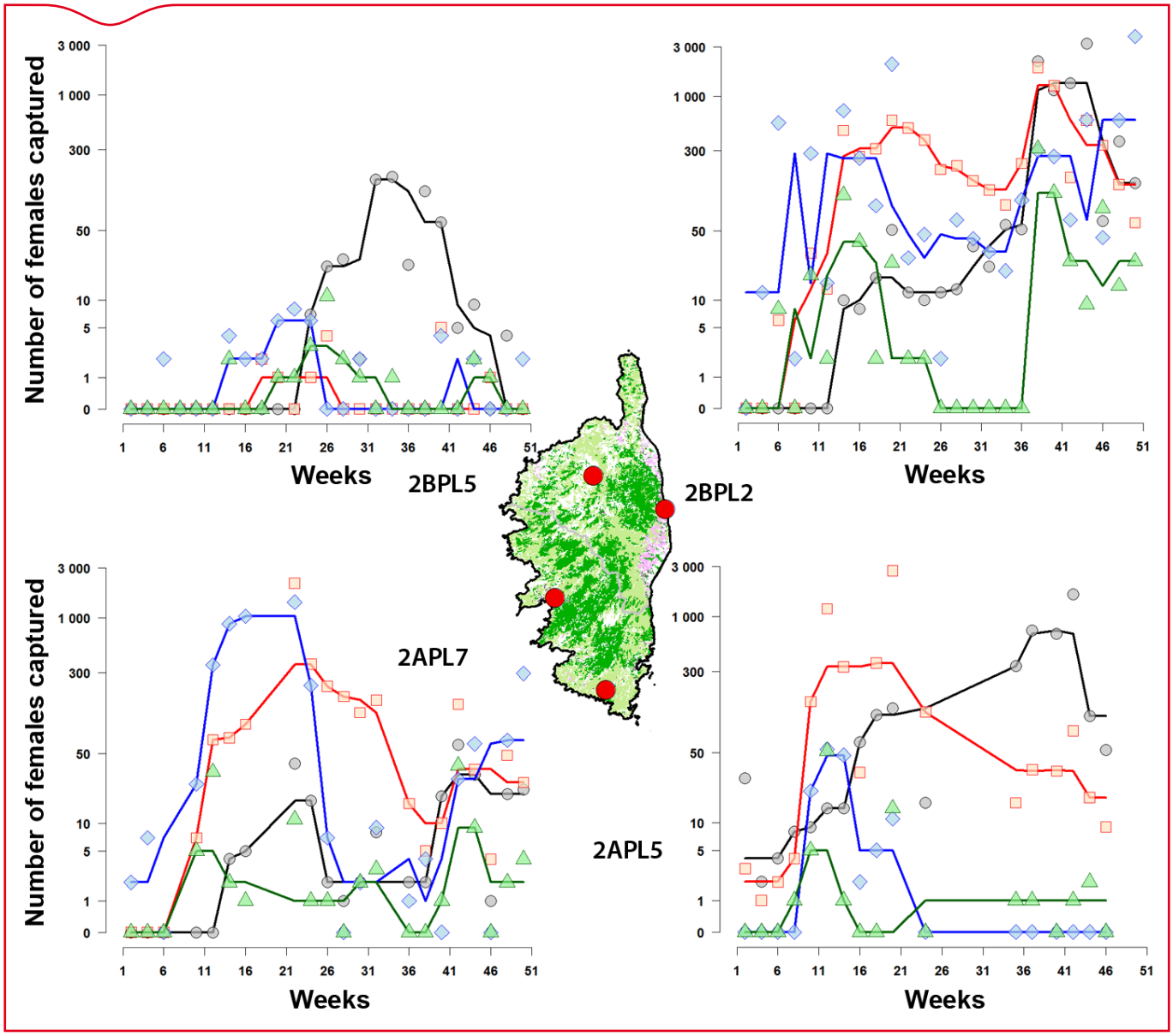

Figure 1. Population dynamics of *Culicoides imicola* (black), *Culicoides* newsteadi (red), *Culicoides* obsoletus/*Culicoides* scoticus (blue) and *Culicoides* pulicaris (green) in Corsica in 2014
The symbols (circle, square, diamond or triangle) correspond to the values actually observed; the curves to an extrapolation (calculation based on the mobile medians) of these specific data. In the interests of readability, only the four most abundant taxa are shown.

Porcine brucellosis in France in 2014: seven outbreaks, including four in local breeds

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Abstract

As in previous years, surveillance of porcine brucellosis in 2014 was based primarily on outbreak surveillance. Nineteen suspicions were reported in 2014, mainly in outdoor holdings, including five based on positive serological results, six on clinical signs and eight due to an epidemiological link with a confirmed outbreak. Two of these suspicions were reported in intensive pig farms (one clinical suspicion and one epidemiological link). Seven outbreaks were confirmed. Five were primary outbreaks, including one in an intensive pig farm. The other two outbreaks were secondary outbreaks due to a boar being introduced from a Gascon-breed outdoor pig farm with a confirmed outbreak. While the outbreaks discovered in 2010 had shown for the first time since 1993 that local breed holdings could also be affected by brucellosis, in the same way as other outdoor holdings, this trend was confirmed in the following years with three outbreaks affecting pigs from local breeds in 2013 and four in 2014.

Keywords

Regulated disease, Porcine brucellosis, Notifiable disease, Category 2 health hazard, Epidemiological surveillance, Swine

Résumé

Brucellose porcine en France en 2014 : sept foyers dont quatre en race locale

Comme pour les années précédentes, la surveillance de la brucellose porcine en 2014 a reposé principalement sur une surveillance événementielle. Dix-neuf suspicions ont été rapportées en 2014, majoritairement en élevage plein-air, dont cinq suite à des contrôles sérologiques, six sur la base de signes cliniques et huit en raison d'un lien épidémiologique avec un foyer. Parmi ces suspicions, deux sont survenues en élevages hors-sol (une suspicion clinique et une en lien épidémiologique). Sept foyers ont été confirmés : cinq étaient des foyers primaires, dont un en élevage hors-sol ; les deux autres étant des foyers en élevage plein-air secondaires d'un foyer en race Gasconne et consécutifs à l'introduction de reproducteurs. Alors que les foyers découverts en 2010 avaient révélé pour la première fois depuis 1993 que les élevages de races locales pouvaient également être concernés par la brucellose, au même titre que les autres élevages plein-air, cette tendance s'est confirmée au cours des années suivantes avec trois foyers portant sur des porcs de races locales en 2013 et quatre en 2014.

Mots-clés

Maladie réglementée, danger sanitaire de catégorie 2, brucellose porcine, épidémiosurveillance, suidés

Here, we present the results of the porcine brucellosis surveillance programme in 2014. Surveillance procedures are detailed in the Box.

Results

There were 5,936 analyses carried out in the quarantine stations and AI collection centres, from a total of 85 holdings; 36 of these analyses, or 0.6%, proved positive. The positive results concerned 12 holdings, with between one and four positive tests during the year for 11 of the holdings, and 13 positive tests for the twelfth. The proportion of positive results per holding varied between 0.4% (2 positive results out of 483) and 9% (2 positive results out of 22). All these reactions were confirmed as being false-positive reactions due to a common antigen between *Brucella spp.* (*suis, abortus, melitensis*) and *Yersinia enterocolitica* O:9. As a reminder, boars undergo individual controls (clinical examination, tests to screen for Aujeszky's disease, classical swine fever and brucellosis) 30 days before entering quarantine, and a new series of individual examinations at least fifteen days after the start of the 30-day quarantine period. For boars presenting a positive result with regard to brucellosis at the first control, a second sample is taken at least seven days and at most three weeks after the initial sampling. In the event that two tests performed on samples taken at least seven days apart are negative, the suspicion of brucellosis is not retained. Any positive serological results are then considered to be false positives. Otherwise, the suspicion of porcine brucellosis is retained, and specific measures are applied. As all the above results were false positives, the prevalences reported only relate to the animals tested in quarantine stations and AI centres.

Nineteen suspicious cases were declared in farms in 2014, including one in a wild boar holding: six were based on clinical signs (abortions/infertility), five followed serological testing, and eight were in holdings with an epidemiological link to an infected farm. Periodic serological tests were set up in 2011 for certain local breeds in which outbreaks had previously been observed, particularly in pig breeds shown at the Paris International Agricultural Show (Bronner *et al.*, 2011). Twelve of these nineteen suspicions were overturned (including the one in the wild boar holding), while seven were confirmed.

Two suspicions were raised in intensive (indoor) holdings in 2014. One was ruled out, the other confirmed.

In 2014, seven outbreaks of porcine brucellosis, including six in outdoor holdings, were declared in seven *départements* (Pyrénées-Atlantiques, Hautes-Pyrénées, Gers, Tarn-et-Garonne, Loir-et-Cher, Mayenne and Yonne, Figure 1). Five of the outbreaks were confirmed after identification of *Brucella suis* biovar 2 by the NRL. Two of the outbreaks were confirmed by serological tests, with epidemiological links to the 2014 outbreak in the Hautes-Pyrénées. An outbreak that was suspected in late 2014 and confirmed on 31 December 2014 has been included in this 2014 annual review even though the management measures mainly spilled over into 2015 (the Yonne outbreak).

For the seven outbreaks mentioned above, 271 pigs were serologically tested, of which 91 were found to be seropositive (BAT+ and CF+) and 54 underwent bacteriological testing, with isolation of *Brucella* for nine of them. The proportion of seropositive pigs per outbreak varied between 5% (n=5 out of 110 pigs) and 70% (n=7 out of 10 pigs tested), with a mean of 50% per outbreak.

Four outbreaks concerned traditional pig farms with the Gascon breed (a relatively rare breed, in which artificial insemination is not practised, and in which individuals are frequently transferred between different holdings). The other three concerned conventional pig holdings, farrow-to-grower or farrow-to-finish, with numbers of sows varying between 40 and 160. The three outbreaks in conventional holdings were discovered *via* outbreak surveillance, based on notification of suspicious clinical signs (abortions, early return to oestrus). Two of the outbreaks in local breeds were detected on the basis of serological testing and the other two (in the *départements* of Gers and Tarn-et-Garonne) were detected in the framework of surveillance of herds having an epidemiological link with one of the outbreaks in local breeds (*département* of the Hautes-Pyrénées).

Costs

In 2014, in the 94 *départements* for which data were provided, the French government invested €22,025 for surveillance and control of porcine brucellosis. Laboratory costs amounted to €16,592 for health control measures and veterinary costs were €5,433. These figures do not include the compensation that is paid out in cases of confirmed porcine brucellosis outbreaks.

Discussion

The profile of holdings affected by porcine brucellosis outbreaks in France has changed since 2010, with outbreaks detected in local breeds and a higher proportion of secondary outbreaks per primary outbreak.

In 2014, as for the previous four years (Bronner *et al.*, 2011; Marcé *et al.*, 2012; Marcé *et al.*, 2013, Marcé *et al.*, 2014), the infection by brucellosis in herds of local breeds was confirmed, with the presence of secondary outbreaks in these types of herds (two of the four cases in local breeds were suspected to be secondary outbreaks). Wildlife remains the primary identified or suspected source of infection.

Although for the past 20 years most outbreaks have occurred in western France, where outdoor holdings are the most frequent, in 2012 for the first time, an outbreak was detected in south-east

Box. Porcine brucellosis surveillance and health control measures

Objectives of the surveillance programme

The aim of porcine brucellosis surveillance is to detect outbreak events rapidly, in order to prevent the spread of the disease to other holdings and, depending on the strains involved, to prevent the risk of zoonosis. For quarantine and artificial insemination (AI) centres (Directive 90/429/EEC), the goal is to ensure that only disease-free boars are used for artificial insemination purposes.

The population monitored

Domestic swine and farmed wild boars throughout mainland France.

Scope of surveillance programme

Brucella suis biovars 1, 2 and 3, *Brucella melitensis* and *Brucella abortus*.

Surveillance procedures

Porcine brucellosis is monitored by outbreak surveillance (testing after observation of clinical signs) in all holdings, and programmed surveillance (routine serological testing) in quarantine stations and AI centres. Programmed surveillance was set up (professional initiative) in late 2010 for holdings of the Noir de Bigorre (Gascon) breed and for local breeds shown at the Paris International Agricultural Show.

Outbreak surveillance

Outbreak surveillance is based on the surveillance of clinical signs typical of brucellosis infection: early abortion with early return to oestrus (abortion or embryonic resorption can affect up to 50% of breeding sows in a holding, while 95% of breeding sows may be infertile), acute orchitis or any other reproductive disorder of an enzootic nature. Arthritis and paresis arising from bone and joint injury can also indicate brucellosis.

Programmed surveillance

Programmed surveillance targets boars used for AI (which are also tested for Aujeszky's disease and classical swine fever), due to the potential role of semen in the spread of brucellosis (the combination of antimicrobials added to collected semen does not eliminate *Brucella*). This serological surveillance is not generalised to other types of holdings that may nonetheless run the risk of the spread or introduction of *Brucella* because serological tests are known to have low specificity and frequent false positives.

A herd becomes suspect in one of the following three circumstances:

- observation of epi- or enzootic clinical signs associated with positive serological tests,
- herds with an epidemiological connection to an infected holding,
- in accredited AI centres or quarantine stations, positive serological reactions as defined in Memorandum 2004/8134 of 12 May 2004.

Epidemiological investigation during an outbreak (trace-back/trace-forward surveys)

For suspected outbreaks, samples are taken by mandated veterinarians for serological testing (blood samples in vacutainer collection tubes) from all breeding pigs or bacteriological analyses (peri- or endocervical swabs or samples of vaginal secretions in sows having aborted or those that show reproductive disorders and/or, after diagnostic slaughter, samples of lymph nodes and/or uterus tissue in sows having aborted, of affected testes for boars with orchitis, of joint fluid from any arthritic pig).

Health control measures

Given the low specificity of clinical signs, any suspected holdings are only placed under prefectural monitoring order (APMS) if the clinical suspicion is confirmed by positive serological results. However, for quarantine stations or AI collection centres, due to the impact that any delay would have for the notification of brucellosis, and given the type of surveillance (clinical and serological), these centres are placed under APMS as soon as positive serological test results are obtained.

Definition of an outbreak

An outbreak of porcine brucellosis is confirmed:

- if the *Brucella* bacterium has been isolated,
- if at least 10% of breeding pigs are seropositive,
- in accredited quarantine stations and AI centres, if the suspected pig(s) originated from an infected holding.

Except for quarantine stations and AI centres, confirmation is thus based on isolation of the pathogen (high specificity, but low sensitivity), or positive serological results (low specificity, but high sensitivity, particularly due to cross-reactions with *Yersinia enterocolitica* O:9). In the absence of any suggestive clinical signs, therefore, isolated positive serological reactions do not in any way constitute a suspicion of brucellosis according to the Ministerial Order of 14 November 2005.

Measures taken in the event of confirmed outbreaks

When an outbreak is confirmed, the prefectural monitoring order is replaced with a prefectural declaration of (brucellosis) infection (APDI). Depending on whether the bacteria could be typed and on the *Brucella suis* biovar isolated, the fate of breeding pigs and growing-finishing pigs differs in terms of whether the meat is subject to mandatory seizure (condemned) or heat treatment. When an outbreak has been confirmed, the entire herd is culled. Ruminants and dogs on the premises are also tested. Epidemiological trace-back and trace-forward surveys are conducted for the six months preceding the first suspicion of outbreak. Depopulation is followed by cleaning and disinfection.

Regulatory References

Directive 90/429/EEC laying down the animal health requirements applicable to intra-Community trade in and imports of semen of domestic animals of the porcine species

Ministerial Order of 14 November 2005 laying down the animal health measures regarding brucellosis in captive swine

Ministerial Order of 7 November 2000 laying down the animal health conditions required for disseminating swine semen

France. A second outbreak was also identified in 2013 in this sector (Figure 1). In 2014, all the outbreaks were identified in *départements* already infected previously, with four outbreaks discovered in Gascon breed herds present mainly in the south-west. Generally speaking, outbreaks detected within a *département* involve isolated cases, which raises questions as to whether some areas may face a higher risk, or concerning the degree of awareness on the part of farmers and veterinarian service staff who detect the clinical cases, or the coverage of epidemiological investigations in the case of primary outbreaks. However, the relative importance of these three possibilities is not known.

Although only three outbreaks were reported in each of the years 2012 and 2013, the detection of seven outbreaks in 2014 does not necessarily reflect an increase in incidence. Outbreaks in outdoor holdings arise sporadically, based on random intrusions by infected wild boar, and two of the 2014 outbreaks had an epidemiological link with a primary outbreak (Table 1). Thus, from 1993 to 2014, the annual number of outbreaks varied between zero and 12 for a total of 94 outbreaks reported over this period.

As in 2013, the majority of outdoor pig holdings for which outbreaks were reported in 2014 had proper fencing for the categories of animals subject to regulatory requirements (sows in the first 4 weeks of gestation). Although other contamination routes are possible (hunting equipment or boots used by the farmer and not cleaned properly, introduction of new animals, for example), this is a reminder that the risk of introduction *via* wildlife is very real and current regulations on fencing are not always sufficient to prevent contact between wild boar and the most exposed animals, in particular sows likely to be in oestrus. Indeed, fencing is currently not mandatory for gilts, gestating sows after the fourth week following mating or artificial insemination, lactating sows and non-pubescent gilts. Thus, some female pigs in oestrus may still be at risk of contamination. Although this is not a regulatory requirement, all pig pens in outdoor holdings should be fenced according to the standards indicated in the Circular DPEI/SDEPA/2005-4073 of 20 December 2005, and not just those containing certain categories of animals.

The epidemiological investigation carried out during the outbreak detected in an intensive pig farm in 2014 revealed that the feed store had not been closed properly and confirmed the observation of wild boar in the vicinity of this feed store for pigs.

Between 2012 and 2014, the proportion of positive serological reactions for the tests carried out in quarantine stations and AI collection centres dropped from 4% in 2012 (235 positive results out of 5,303 analyses) to 1.6% in 2013 (87 positive results out of 5,308 analyses), and then to 0.6% in 2014 (36 positive results out of 5,936 analyses). Memorandum DGAL/SDSPA/N2012-8268 of 18 December 2012, amending the provisions for health control measures concerning brucellosis, authorises the use of ELISA tests on boars, as part of health surveillance for artificial insemination. Considering the serious limitations of the ELISA kits currently available (specificity), in 2011 the Bacterial Zoonoses Unit (the NRL for Brucellosis) of the ANSES Maisons-Alfort Laboratory for Animal Health developed a dual-well ELISA prototype (ANSES test) consisting of the LPS-S and LPS-R *Brucella* antigens (in phases S and R respectively). This test seems to have better specificity with regard to antibodies directed against *Yersinia enterocolitica* O:9. Its use in addition to the recognised tests, despite being strictly limited to regulatory controls of breeding animals and future breeding stock, helped to rule out 269 false-positive serological reactions in quarantine stations and AI collection centres.

As in the preceding years, the results of porcine brucellosis surveillance obtained in 2014 highlight the importance of encouraging professionals to implement biosafety measures (concerning all females likely to be in oestrus), to declare abortions and to implement differential diagnosis. The 2014 cases are also a call to encourage professionals keeping local breeds to strengthen biosafety measures through collective mobilisation and the establishment of preventive measures (control of introduced animals, quarantine, etc.). Programmed surveillance cannot be generalised or extended due to the limited specificity of the serological tests and the very low incidence of porcine brucellosis in France, making it cost-ineffective. However, programmed surveillance can occasionally compensate for the limitations of outbreak surveillance, which has very low sensitivity, although it requires close and intensive monitoring of holdings, due to the high risk of false positives.

Table 1. Distribution of suspicions and outbreaks of porcine brucellosis in France in 2014 according to the type of holding (outdoor or intensive) and the methods that led to the suspicion

	Number of suspicions		Number of confirmations	
	Outdoor holding	Intensive holding	Outdoor holding	Intensive holding
Following clinical signs	5	1	2	1
Following serological testing	5	0	2	0
Epidemiological link with an outbreak	7	1	2	0
Total	**17**	**2**	**6**	**1**

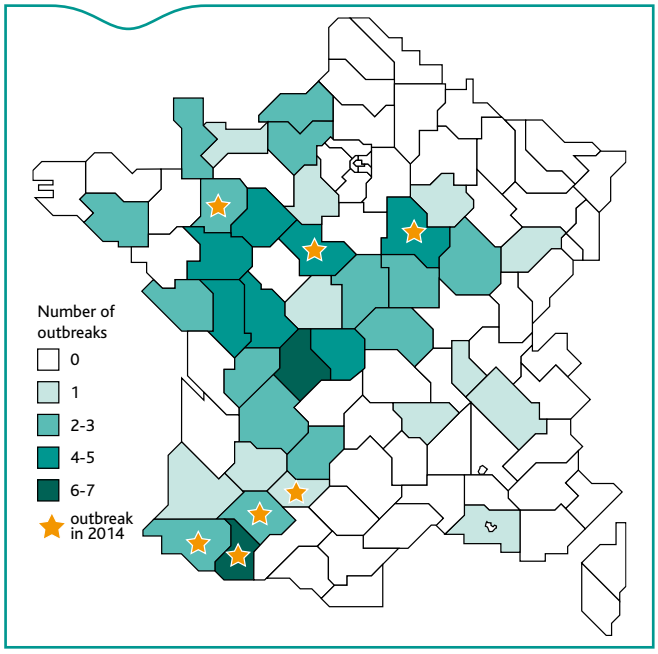

Figure 1. Geographic distribution of confirmed brucellosis outbreaks in pig holdings in France from 1993 to 2014 and location of confirmed outbreaks in 2014

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Upholding of **Aujeszky's disease**-free status in 2014: improvement of detection in high-risk pig herds but decrease in field player vigilance

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Abstract
This article presents the results of surveillance of Aujeszky's disease in mainland France and Reunion Island in 2014. The results show an increase in the number of open air pigs tested, especially among farrow-to-finish farms. However, the proportion of pig farms screened seems to have decreased, especially among grow-to-finish farms. On the other hand, the number of pigs screened in nucleus and breeder-multiplier farms was similar in 2014 to previous years. A decrease in the number of suspicions, serological or clinical, has also been noticed. Despite the fact that no cases of Aujeszky's disease were detected in 2014, the priority for all stakeholders is to remain vigilant. It is especially important that veterinarians include Aujeszky's disease in their differential diagnosis when encountering symptoms (*influenza*-like illness, spontaneous abortions) that cannot be attributed with certainty to another disease.

Keywords
Regulated disease, Category 1 health hazard, Aujeszky's disease, Epidemiological surveillance, France, Official control, Swine

Résumé
Maintien du statut indemne de maladie d'Aujeszky en 2014 : amélioration du dépistage dans les élevages à risque mais baisse de la vigilance des acteurs de la filière
Cet article présente les résultats de la surveillance de la maladie d'Aujeszky en France continentale et dans l'Ile de la Réunion en 2014. Ces résultats rapportent une augmentation du nombre de porcs d'élevages plein-air dépistés, notamment en élevages naisseurs-engraisseurs. La proportion d'élevages dépistés apparaît en baisse, notamment chez les engraisseurs. Le nombre de porcs dépistés en élevages de sélection-multiplication reste stable. Une diminution du nombre de suspicions a été observée, qu'elles soient sérologiques ou cliniques. Bien qu'aucun cas de maladie d'Aujeszky n'ait été confirmé en 2014, le maintien de la vigilance de l'ensemble des acteurs reste la priorité. Il est notamment important que les vétérinaires incluent la maladie d'Aujeszky dans leur diagnostic différentiel lors de signes cliniques (syndrome grippal, avortements) ne pouvant être rattachés avec certitude à une autre maladie.

Mots clés
Maladie réglementée, danger sanitaire de 1ère catégorie, maladie d'Aujeszky, épidémiosurveillance, France, police sanitaire, suidés

Here, we present the results of the Aujeszky's disease (AD) surveillance programme (Box) in mainland France and Reunion Island for 2014.

Population counts used in this report come from holding registration forms filed by pig farmers on or before 31 December 2014 (compiled in the BDPORC, the national pig identification database and transmitted to the DGAL's information system, SIGAL). All pig keepers are required to make this declaration (Ministerial Order of 20 October 2010 amending the Ministerial Order of 24 November 2005). All new pig holdings must be registered and records must be updated if there are any changes to the initial information provided. Since the Aujeszky's disease surveillance programme is not implemented in Corsica (which does not have disease-free status), the pig numbers in this article do not include its two *départements*.

Sampling

Surveillance in nucleus and multiplier herds

Surveillance was conducted in 384 of the 505 nucleus and multiplier holdings identified in the pig holding registry (i.e. a 76% coverage rate).

On average, 45 samples were taken per holding and per year, i.e. 14 samples per quarter, for a total of 20,967 samples. This was a slight increase on the average of 12 samples per holding and per quarter for 2013 (to a level equivalent to 2012) (Marcé *et al.*, 2013; Marcé *et al,* 2014). As a guideline, 12 samples per holding and per quarter are sufficient to detect a minimum intra-holding prevalence in the region of 25%, with a confidence level of 95%.

Overall, assuming that samples were only taken on breeding stock, and according to the pig population count recorded in the BDPORC database, 25% of breeding stock were tested in 2014, or 6% per quarter, i.e. the same levels as in previous years.

Surveillance in outdoor pig production holdings (farrow-to-grower, farrow-to-finish, wean-to-grow and grow-to-finish farms)

In all, 1,691 outdoor holdings (domestic swine or farmed wild boar) were listed as having been tested out of 2,659 holdings in the database (2,438 outdoor domestic pig holdings and 221 holdings with wild boars), i.e. a 64% coverage rate, with 15,669 samples taken.

The rate of implementation of programmed surveillance varied with the type of domestic swine holding, from 66% in farrow-to-finish holdings to more than 100% in wean-to-grow holdings (Table 1).

Of a total of 2,215 outdoor domestic pig holdings whose type (activity, production level) is known in SIGAL, the surveillance programme effectively covered 1,691 (76% surveillance coverage rate), for a total of 15,352 samples.

As a guideline, a mean of nine samples taken per holding and per quarter are sufficient to detect a minimum intra-holding prevalence in the region of 30%, with a confidence level of 95%.

Surveillance in indoor pig production holdings

Despite the lack of mandatory programmed screening, 138 farms underwent screening for Aujeszky's disease (5,359 samples).

Box. Aujeszky's disease surveillance and health control measures

Objectives of the surveillance programme

For mainland France and Reunion Island:

- To confirm France's status as Aujeszky's disease-free (AD-free).
- To detect as early as possible any new appearance of the virus in domestic swine.

The population monitored

Domestic swine and farmed wild boars (categories A and B) throughout mainland France and Reunion Island.

Surveillance procedures

Outbreak surveillance

Two levels of suspicion have been defined based on clinical criteria developed in association with the SNGTV: "high" clinical suspicion corresponding to a diagnosis of inclusion and "low" clinical suspicion corresponding to a diagnosis of exclusion (definitions can be found in Memorandum DGAL/SDSPA/N2013-8011 of 15 January 2013). Regardless of the suspicion, the DDecPP must be notified and sampling must be carried out for serological and virological diagnosis.

Programmed surveillance (DGAL/SDSPA/N2013-8010)

Less intense, but targeted serological surveillance in the most at-risk holdings (risk of introduction in outdoor holdings or risk of spread in nucleus and multiplier herds).

For all outdoor holdings, including grow-to-finish holdings: annual serological testing (15 samples from breeding stock and/or 20 samples from slaughter pigs).

In nucleus and multiplier herds: quarterly serological surveillance (15 samples).

Holdings for which AD-free status was revoked or suspended for administrative reasons (in particular due to absence of programmed screening for more than one year) must request and undergo a requalification procedure. Obtaining AD-free status requires two series of negative serological tests performed at a two-month interval, on at least 15 breeding pigs and 30 slaughter pigs.

Health control measures (DGAL/SDSPA/N2013-8011)

In the case of clinical suspicion, regulations stipulate that samples should be taken for serological and virological (PCR) tests. No APMS is issued in the case of low clinical suspicion. An APMS is issued only in the case of high clinical suspicion, low clinical suspicion associated with positive first-line test results (serology or virology), or low clinical suspicion associated with unfavourable epidemiological investigation results.

Serological suspicion is based on non-negative serological results. An animal considered seropositive for AD is one for which two series of tests have been performed at least 15 days apart and show positive results, with each test including two serological analyses using two different assays (gB and gE), because these two methods can rule out the possibility of non-specific reactions.

In the case of positive serological tests, the farm is visited for clinical examination of the animals and to take more samples for additional serological tests (at least 15 days apart). The holding is placed under APMS if an accredited laboratory produces a positive or ambiguous result in any individual test. If only one or two samples are positive or ambiguous, the health control measures can be "relaxed": movements of pigs to slaughter or to authorised terminal holdings are authorised, providing that the clinical and epidemiological investigation of the holding under serological suspicion has given favourable results, that the destination holding or slaughterhouse has agreed in writing that these pigs can be introduced, and that the destination holding is also placed under APMS.

An animal is considered infected by AD when, even in the absence of any suggestive clinical signs of the disease, the results of serological or virological tests confirm the infection.

A site is considered infected when a pig infected with AD is held there or originates from there.

When an outbreak is confirmed, the prefecture declares the pig farm as infected (APDI), which entails depopulation as quickly as possible and cleaning-disinfection operations. Trace-back and trace-forward epidemiological surveys are implemented to determine the source and the conditions under which the infection spread to the holding, and to identify other holdings that are likely to have been infected.

Regulatory References

Council Directive 90/429/EEC of 26 June 1990 (amended) laying down the animal health requirements applicable to intra-Community trade in and imports of semen of domestic animals of the porcine species

Commission Decision 2008/185/EC of 21 February 2008 (amended) on additional guarantees in intra-Community trade of pigs relating to Aujeszky's disease and criteria to provide information on this disease

Ministerial Order of 28 January 2009 laying down the technical and administrative measures in regard to collective prophylactic measures and animal health rules for Aujeszky's disease in départements with Aujeszky's disease-free status

Ministerial Order of 14 August 2001 on the animal health rules required for intra-Community trade of cattle and swine

Ministerial Order of 7 November 2000 laying down the animal health rules required for disseminating swine semen

Ministerial Order of 9 June 1994 on the rules that apply to trade of live animals, semen and embryos and to the organisation of veterinary inspections

Table 1. Testing for Aujeszky's disease in outdoor holdings in 2014 (domestic swine only, holdings having filed a declaration)

Type of outdoor holding	Number of holdings registered*	Number of holdings tested	Proportion of holdings tested (%)	Number of samples	Average number of samples per holding
Farrow-to-grower	198	152	77	1,536	10
Wean-to-grow	7	15	214**	203	14
Grow-to-finish	1,205	794	66	7,609	10
Farrow-to-finish	805	695	86	6,004	9
Total	**2,215**	**1,691**	**76**	**15,352**	**9**

* Taken from the BDPORC database in the first quarter of 2015 for mainland France. All *départements* were included, although five *départements* did not provide all information on Aujeszky's disease surveillance and *départements* were not requested to validate their pig population counts, which were taken directly from the SIGAL database. The farrow-to-grower category includes farrowing and –wean-to-grow farms; wean-to-finish holdings were included in the grow-to-finish category.
** A failure to update certain holding declarations in BDPORC combined with the lack of corrections by the DDecPPs of pig counts extracted from SIGAL explains why the proportion of wean-to-grow farms tested was greater than 100%.

In all, including all the holdings mentioned previously, 41,995 samples were taken for serological screening of Aujeszky's disease.

Non-negative results

In outdoor production systems, 15 pig holdings presented at least one non-negative result for the first-line ELISA gB test (127 samples). Following these results, 10 sites were placed under APMS. Four sites had to be visited a second time to collect enough serum for confirmatory diagnostics (gE in particular).

In total, regardless of the type of pig holding, 38 sera of pigs or wild boars (relating to 11 suspected cases) underwent second-line testing by the NRL, 15 of them in ELISA gB (a single site) and 23 in ELISA gE.

None of the suspicions in pig holdings were confirmed. Animals from the two wild boar holdings that had been the subject of strong suspicion, following the annual screening, were slaughtered pre-emptively (two wild boars in a holding in the Cher *département* and 61 wild boars in a holding in the Dordogne *département*).

Clinical suspicions

For the entire disease-free territory (mainland France and Reunion Island), one outdoor holding was the subject of a clinical suspicion (Loire-Atlantique *département*): four pigs were tested and returned negative results. Two wild boars (wildlife) were also the subject of a clinical suspicion and underwent testing in the Côtes d'Armor *département*. All these suspicions were overturned.

In the context of clinical suspicions, the NRL received thirteen samples in 2014, from four dogs (from the *départements* of Aisne, Ardennes, Marne and Essonne, all positive), one cow (Haute-Saône, negative), six pigs (two from Corsica and four from Loire-Atlantique, all negative) and two wild boars (Côtes d'Armor, both negative).

The number of clinical suspicions reported by the DDecPPs may be underestimated due to a request for first-line testing made to a laboratory from the network of accredited laboratories in the context of a very low suspicion (exclusion diagnosis).

Costs

In 2014, in the 95 *départements* for which there was usable data, the French government invested around €25,000 for surveillance and control of Aujeszky's disease. Laboratory costs amounted to €11,050 for programmed screening and €380 for health control measures. Veterinary costs were €12,220 for programmed surveillance and €1,210 for enforcing health control measures. In addition, State participation in programmed surveillance in nucleus and multiplier holdings belonging to the French pig breeding agency amounted to approximately €30,400 for sampling and serological analyses (data not consolidated at the date this article was submitted, probably underestimated).

Discussion

No outbreaks of Aujeszky's disease were identified in 2014 in domestic pig holdings in mainland France or Reunion Island. One pig holding showed suspicious clinical signs in mainland France, and one in Corsica. Two wild boar holdings were the subject of serological suspicions. PCR analyses carried out by the network of accredited departmental testing laboratories are not systematically recorded in the central database. Therefore, some differential diagnoses may not have been recorded and the number of tests may be underestimated. Because such data are important for estimating the level of surveillance, this situation needs to change to ensure that the analyses carried out by the network of accredited laboratories can be compiled. The debate around the management of programmed surveillance in pig farming and the computerisation of the results of analyses carried out in laboratories for the swine sector should help, from 2016, better assess the frequency of these differential diagnoses. And even from 2015, it should be possible to communicate the first results of the programmed surveillance campaign in pig farming, *via* computerised exchanges, to the DDecPPs. However, this work still needs to be consolidated in order to integrate the results of the differential diagnoses in these exchanges of computerised data and to connect the NRL to this scheme.

In contrast, the detection *via* programmed surveillance of two seropositive wild boar holdings, which did not however reveal any active viral circulation, recalls the episode that occurred in 2010 (Rose *et al.*, 2010). These results are a reminder that there is a real risk of recurrence of the disease in domestic pigs, in outdoor holdings in particular. Outdoor holdings are particularly exposed to the disease due to the possible contact with wildlife (Rossi *et al.*, 2008), to the fact that they are less closely monitored compared to indoor holdings and also that clinical signs of infection can be attenuated, especially respiratory symptoms due to generally lower viral shedding at lower pig densities. Combining outbreak and serological surveillance in outdoor holdings, whether of domestic pigs or farmed wild boars (for which outbreak surveillance is limited), is therefore essential (Pol and Le Potier, 2011).

It is difficult to compare the results of the serological surveillance conducted in 2014 with those of 2013 (Marcé *et al.*, 2014), even though the same method (based on the declaration of pig holdings) was used to identify the number of pig holdings in France. While the number of herds tested and the number of samples taken are both on the increase, the proportion of tested herds appears to be in decline. The number of farms registered, whether outdoor holdings alone or all pig farms, increased by 4 to 5% in 2014 compared to 2013. As in 2013, the DDecPPs were not given the opportunity to rectify the results of the extraction regarding the number of holdings (data from the holding declarations and data entered in the BDPORC database) and thus to correct the data for holdings that had not yet filed their declaration. The figures used in 2014 relate to the raw extraction from BDPORC data. Farms that had not yet updated their declarations of activity could not therefore be reclassified *a posteriori* by the DDecPPs. It is also possible that data was updated before the extraction, following the implementation of the dataflow between BDPORC and SIGAL to facilitate consistency checking and enable the DDecPPs to remind farmers who have not yet fulfilled their obligations to declare their activity or notify a change. It should also be noted that the number of registered farmed wild boar holdings was stable between 2013 and 2014. The holding declaration is not yet in effect for farmed wild boar holdings or outdoor holdings, even though these are subject to programmed surveillance for Aujeszky's disease. The standard form for the holding declaration was adapted in 2014, whereas the dataflow between BDPORC and SIGAL was only implemented in 2015. The numbers of farmed wild boars are thus very probably underestimated in the SIGAL database, which was the source of the numbers used for this report. It was not possible for the DDecPPs to correct these population data, whether for domestic pigs or wild boars.

The response rate of the *départements* for the questions on Aujeszky's disease was similar in 2014 to that for 2013 (95 *départements* in 2014, compared with 97 in 2013 and 88 in 2012).

The combination of the lack of any correction to the numbers of pigs on outdoor holdings, which rose between 2013 and 2014, and the stable rate of completion by DDecPPs of the questionnaire on Aujeszky's disease, could explain the observed increase in 2014 in the number of farms tested, and the decrease in the proportion of farms tested. Further encouragement is needed to achieve an increase in the proportion of farms tested. The failure to correct the numbers of pigs also explains why the proportion of wean-to-grow holdings screened is more than 100%.

For all of the outdoor farms registered (domestic pigs), the rate of implementation of programmed surveillance was 76%, lower than 2013, despite the increase in the number of samples and holdings included. Nevertheless, the limitations mentioned in the previous paragraph should be borne in mind. Annual serological surveillance

in outdoor holdings, particularly in farrow-to-grower holdings, should help compensate for the limitations of outbreak surveillance. It is now necessary to ensure that screening is effectively and fully carried out, considering that the nine serological tests performed on average only ensure the detection of a seroprevalence level of 30%, which is too high considering the seroprevalence levels that can occur in outdoor holdings (routine tests involving 15 samples can target a prevalence of 20%, with a 5% error rate).

Stagnation in the number of nucleus and multiplier herds analysed was also observed, which may be related to the stagnation in population counts in this type of holding. For this type of holding, the number of samples per holding and per year rose slightly compared to 2013, as did the average number of samples per holding and per quarter. It appears important to maintain this pressure of analysis at the nucleus-multiplier level in order to maintain the sensitivity of the detection system. If the number of samples per holding decreases, detection will only be effective when intra-herd prevalence is higher than 30%, which is too high a threshold compared to those that may be encountered.

It should also be noted that indoor holdings in some *départements* are still subjected to testing even though this type of holding is not targeted by mandatory screening (because these holdings are considered to be at a lower risk of introduction or spread of the virus). These analyses may nevertheless be appropriate for wean-to-grow holdings, which are the farms that disseminate the animals, even if it is on a lesser scale compared to nucleus and multiplier holdings.

Of the 15 outdoor holdings that showed positive first-line serological results, four required a second round of samples on very short order to obtain sufficient serum to carry out the confirmatory tests. This highlights the importance of taking blood samples (not blots) for serological testing on farms, particularly when an outbreak is suspected, to rapidly confirm or refute the presence of an outbreak of Aujeszky's disease. Repeat tests nevertheless remain infrequent and blots can still be useful, especially when containment is difficult. It would seem appropriate to implement practical training on taking blood samples and on containment of animals for mandated veterinarians who do not work frequently in the swine sector.

In conclusion, all players in the pig sector must remain vigilant in order to ensure early detection of any outbreak. On this subject, two clinical suspicions, including one in wildlife (a decrease compared to the previous year), were notified in 2014 in mainland France and on Reunion Island, both recognised as Aujeszky's disease-free territories. The two suspicions were dealt with by the NRL. To increase vigilance, the approach involving exclusion tests for suspected cases should be pursued, and all veterinarians should be encouraged to include Aujeszky's disease in their differential diagnoses when flu-like symptoms and abortions cannot be attributed with certainty to any other disease. The exclusion test makes it easier to report suspicions while reducing the consequences for the holding. The current lack of a reliable system for recording these exclusion diagnoses (record of laboratory analyses performed) means that clinical surveillance activities are not clearly or fully described. It is therefore necessary to improve the tools used for monitoring epidemiological information. It is also important to stress that outdoor holdings are the farms most at risk. It is fundamental therefore that programmed surveillance be carried out on all outdoor holdings, and on at least fifteen pigs per holding, as recommended, to detect any infection as close to the source as possible.

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Review of vigilance with respect to **Classical and African Swine Fevers** in France in 2014

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Abstract

In an epidemiological context in which African swine fever (ASF) has reached member states of the East of Europe (Poland, Lithuania, Latvia, Estonia), and classical swine fever (CSF) is still present in Hungary and Latvia, the confirmation of disease-free status of France and the early detection are still the main objectives of the surveillance performed.

As in previous years, vigilance with respect to CSF has been based on serological and virological surveillance at the slaughterhouse and in breeder-multiplier farms, as well as on event-based surveillance. Surveillance of wild boars in the Eastern part of France has been maintained in 2014, based on serological and virological analyses of hunted boars and virological analysis of boars found dead in the wild, with a reduction of the surveillance for the hunting season 2014-2015. Furthermore, a capture-recapture study on young wild boars was implemented in 2013-2014 in municipalities where seroprevalence was above 10% in young wild pigs in 2012-2013. Vigilance with respect to African Swine Fever (ASF) was based on event-based surveillance and was completed by a serological survey on pigs slaughtered in Corsica at the beginning of 2014.

In 2014, event-based surveillance led to one clinical suspicion being reported in domestic pigs and two in wildlife, while the programmed surveillance in CSF led to several serological suspicions. None of the suspicions were confirmed.

Keywords

Regulated disease, Category 1 health hazard, CSF, ASF, Epidemiological surveillance, Swine, France

Résumé

Bilan de la vigilance à l'égard des pestes porcines classique et africaine en France métropolitaine et Outre-mer en 2014

Dans un contexte épidémiologique où la peste porcine africaine a atteint certains Etats membres de l'Est de l'Union européenne (Pologne, Lituanie, Lettonie, Estonie) et où la peste porcine classique (PPC) est toujours présente en Hongrie et Lettonie, la démonstration du statut indemne de la France vis-à-vis de ces deux maladies, et la détection précoce d'une émergence restent les principaux objectifs de la surveillance menée.

Comme les années précédentes, la vigilance à l'égard de la PPC a reposé sur une surveillance programmée et sur une surveillance événementielle. La surveillance programmée est réalisée par sérologie en élevage de sélection-multiplication, et par sérologie et virologie à l'abattoir. La surveillance de l'ancienne zone infectée par la PPC chez les sangliers dans l'Est de la France, s'est poursuivie en 2014, basée sur l'analyse sérologique et virologique des sangliers tués à la chasse et l'analyse virologique des sangliers trouvés morts en nature, avec un allègement de la surveillance pour la saison de chasse 2014-2015. Par ailleurs, une étude par capture-marquage-recapture de marcassins a été conduite en 2013 et 2014 dans les communes pour lesquelles la séroprévalence était supérieure à 10% chez les jeunes sangliers en 2012-2013. La vigilance à l'égard de la PPA repose sur une surveillance événementielle et a été complétée par une enquête ponctuelle de séroprévalence PPA sur les porcs à l'abattoir en Corse début 2014.

En 2014, la surveillance événementielle a conduit à la notification d'une suspicion clinique de peste porcine en élevage de porcs et à deux suspicions cliniques chez des sangliers sauvages, tandis que la surveillance programmée vis-à-vis de la PPC a conduit à plusieurs suspicions sérologiques. Aucune suspicion a été confirmée.

Mots-clés

Maladie réglementée, danger sanitaire de 1ère catégorie, PPC, PPA, épidémiosurveillance, suidés, France

This article presents the results of the classical and African swine fever surveillance programme in 2014 (Box). Of the 101 *départements* in mainland France and overseas, 90 *départements* answered, at least in part, the questionnaires that were sent out to them, on the number of swine covered by surveillance, suspicions and the results of analyses carried out.

Programmed surveillance

Slaughterhouse surveillance

Classical swine fever (CSF)

The results of the slaughterhouse surveillance programme for CSF are as follows (Table 1):

- Of the 10,210 pigs to be tested using a serological assay (ELISA) across the entire country, 8,039 breeding pigs and 750 slaughter pigs were sampled (86% coverage rate). Screening involved 1,187 pig holdings[1]. On average, seven samples were taken per holding.
- Of the 3,000 samples targeted for virological (PCR) tests nationwide, 1,861 blood samples (from 1,481 breeding pigs and 380 slaughter pigs) were actually taken (62% coverage rate), from 285 pig holdings. On average, seven samples were taken per holding.

Overall, 2.7% (8,039 serological tests and 1,481 virological tests) of culled breeding pigs[2] were serologically or virologically tested at the slaughterhouse, a rate comparable to that observed in previous years (i.e. 2.7% of culled breeding pigs tested in 2013, 2.5% in 2012).

1. One pig farm can comprise several pig holdings and is considered as such if the herds of these holdings are raised separately in independent facilities, at least 500m apart. The holding is the epidemiological unit used for surveillance purposes.
2. The slaughter data are provided by the Statistics and forward studies service (SSP) and processed by the DGAL's Office of slaughterhouses and cutting plants (BEAD).

African swine fever (ASF)

Because of the proximity of infected territories, two geographical zones are subject to programmed surveillance for ASF at the slaughterhouse. On Reunion Island, due to its proximity to Madagascar in particular, a serological surveillance programme has been in place for more than 15 years, based on 250 samples taken at the slaughterhouse in the framework of programmed surveillance for CSF. This scheme was continued in 2014.

In Corsica in 2014, due to the proximity with Sardinia where ASF is enzootic, ASF was the subject of a one-time serological study at the slaughterhouse, in addition to the outbreak surveillance.

Surveillance in nucleus and multiplier herds

Regarding surveillance in nucleus and multiplier herds in 2014, 5,410 samples were taken from 311 of the 505 nucleus and multiplier holdings registered in 2014 (62% of holdings sampled).

On average, 17 samples per holding were taken in 2014, compared with 16 in 2013.

To give a very general idea of the pressure of serological surveillance of CSF at the national level (at the slaughterhouse and in holdings), approximately 2.5% of all breeding pigs (production level, nucleus and multiplier herds and artificial insemination centres) underwent sampling (8,039 samples were taken in the slaughterhouse and 5,410 in holdings).

Results of Programmed surveillance

Overall, out of the 14,199 serological samples taken for CSF testing, 24 produced a non-negative ELISA result, of which 19 resulted from screening in nucleus and multiplier herds, and five from screening at the slaughterhouse. In total, regardless of the type of farm, 24 pig sera were tested by the NRL with the CSF/ruminant pestiviruses differential virus neutralisation assay, in order to rule out any possible serological cross-reaction with ruminant pestiviruses. None of these serological suspicions were confirmed. These 24 non-negative, first-line serological test results represent a false positive rate of 0.2%, compared with a rate of 0.3% observed in 2013.

Regarding CSF virological testing at the slaughterhouse, no positive reactions were detected, in line with expectations and highlighting the very high specificity of the PCR test.

As part of the programmed serological surveillance for ASF on Reunion Island, the NRL received 29 sera from 29 pigs. Due to the unavailability of the ELISA kit at the Reunion laboratory, 26 sera underwent first-line testing by the NRL: eight giving a non-negative result were retested using the immunofluorescence monolayer assay (IFMA). Three other sera were received for retesting following a non-negative ELISA result at the Reunion departmental veterinary laboratory; these were all ruled out by IFMA.

As part of the one-time survey of ASF seroprevalence conducted at the slaughterhouse in Corsica in early 2014, the 401 samples taken were all found to be negative, demonstrating the current absence of circulation of the disease in the population of domestic pigs reared outdoors and sent to the slaughterhouse (Desvaux *et al.*, 2014). All of the favourable results on these 400 sera helped to ensure that the prevalence during the sampling campaign did not exceed 0.74% with a 95% confidence level, for a population of around 8,000 pigs.

Table 1. Results from the classical swine fever surveillance programme at the slaughterhouse in 2014

	Serological testing (ELISA)	Virological testing (PCR)
Target number of samples	10,210	3,000
Actual number of samples	8,789	1,861
Coverage rate (%)	86	62
Number of pig holdings sampled	1,187	285
Average number of pigs tested per holding	7	7
Proportion of culled breeding pigs tested	2.5%	0.5%

Outbreak surveillance

Clinical suspicions

One pig holding (Finistère *département*) was the subject of a clinical suspicion of swine fever in 2014, with four pigs tested at the NRL. Two wild boars were also tested by the NRL in the framework of a clinical suspicion in wildlife reported by the SAGIR[3] network (Lozère and Corse du Sud *départements*). All of these suspicions were ruled out for CSF and ASF.

Surveillance of CSF in wildlife

Wildlife surveillance in eastern France (Moselle and Bas-Rhin *départements*) (Rossi *et al.*, 2011) investigated 3,827 wild boars in 2014. Due to the favourable epidemiological situation (no new cases and continued decrease in seroprevalence) (Rossi *et al.*, 2011; Marcé *et al.*, 2014), programmed surveillance was relaxed in October 2013 for the area that had previously been infected and vaccinated, called the high observation zone (ZOR) since 2012. Spleen and blood samples were taken only from wild boars less than one year old, shot during hunting, with routine serological analysis and virological (PCR) analysis only for seropositive wild boars. Animals found dead continue to be systematically tested using serological and virological analyses. Two wild boars were thus analysed in this framework *via* the SAGIR network in 2014. In total, throughout 2014, 51 virological analyses and 3,904 serological analyses (3,827 ELISA and 77 virus neutralisation assays) were performed in the programmed surveillance scheme. In all, 26 serological analyses proved positive (including 18 in animals of less than one year), 21 were ambiguous and 67 could not be interpreted or did not produce a result. All the virological analyses were negative. In general, seroprevalence continues to decline in the monitored zone, in line with the results of previous years (Rossi *et al.*, 2015a). In 2014, seroprevalence was thus below 1% in young animals of less than one year and below 3% in adults.

Since October 2013, following implementation of the relaxed programmed surveillance scheme, the sera from young wild boars in the ZOR found positive after first-line ELISA assays have undergone confirmatory analysis in order to determine the maternal (vaccine) or post-infectious origin of these antibodies. Thus, 56 sera found positive by ELISA were tested at the NRL for the 2013-2014 hunting season with the differential virus neutralisation assay using the "Alfort" CSF strain (genotype 1, equivalent to the C strain in the vaccine used from 2004 to 2010) and the "Bas-Rhin" CSF strain, (genotype 2.3, strain responsible for the outbreak in the Northern Vosges). Of these 56 sera, 25 were positive for antibodies neutralising the CSF virus, although it was not possible to demonstrate any clear difference between the two Alfort and Bas-Rhin strains. Since September 2014, these sera have only been tested by differential virus neutralisation using the Alfort strain of CSF and the "Aveyron" strain of the border disease virus, in order to check the specificity of the serological result with regard to CSF. Of the 22 sera analysed, four were confirmed as carriers of CSF virus neutralising antibodies.

To supplement this outbreak surveillance, the capture-mark-recapture (CMR) study carried out in municipalities in which seroprevalence in yearling wild boars was greater than 10% in 2012, was continued, with systematic serological and virological analyses in wild boar marked and recaptured, or shot during hunting, in order to determine the origin of the antibodies (infectious or maternal) in this age class. Among the 134 individuals recaptured, twelve were seropositive, including an adult sow. A single positive PCR result was reported during the study, and was ruled out by the NRL. Repeated capture of young wild boars

3. Epidemiological surveillance network for wildlife (ONCFS - National and departmental hunting associations).

Box. Classical swine fever (CSF) and African swine fever (ASF) surveillance and health control measures

Objectives of the surveillance programme

- Early detection of outbreaks in domestic pigs (CSF and ASF).
- Provide evidence that France is free of CSF.

This surveillance also makes it possible to maintain the operational capacity of the network of serological and virological laboratories accredited for CSF diagnosis, to ensure that they can respond effectively to the needs that would arise in the event of an epizootic.

The population monitored

- Domestic pigs and farmed wild boars throughout mainland France and its overseas départements.
- Wild boars in north-eastern France.

Surveillance procedures

Outbreak surveillance

Outbreak surveillance targets both CSF and ASF and is based on the principle that any person (veterinarian, farmer, animal trader, hunter, SAGIR network, etc.) suspecting a case of CSF or ASF must notify the DDecPP.

Programmed surveillance

Programmed surveillance is carried out in slaughterhouses and in holdings (only in nucleus and multiplier holdings).

In slaughterhouses, random serological and virological tests for CSF are carried out on slaughtered breeding pigs throughout France:

- for serological tests, 10,210 samples should be tested annually to detect a prevalence rate of 0.05% (at a confidence level higher than 99%, providing that sampling is random), and thus attest to the disease-free status of mainland France.
- for virological tests, 3,000 samples should be tested to detect a prevalence rate of at least 0.1% (at a 95% confidence level); given that viraemia is short-lived (2-3 weeks at most), the probability of detecting viral circulation in the population is low, so these tests are used first and foremost to maintain the technical skills in the network of accredited CSF PCR laboratories.

In nucleus and multiplier holdings (in which the spread of CSF/ASF is potentially high), annual testing is carried out in each holding: 15 samples for serological tests (for *a minima*l within-holding prevalence rate of 20% at a 95% confidence level).

CSF surveillance in wild boars in north-eastern France

France regained its disease-free status for wild boars on 14 November 2011; surveillance has thus been restricted to a smaller area since 1 January 2012 (DGAL/SDPSA/N2011-8283). The perimeter of this zone was further reduced in October 2013, and was restricted in 2014 to the former infected zone, now called the high observation zone (ZOR). In this zone, and on a voluntary basis, any wild boar hunted or found dead must have its spleen removed for virological (PCR) analysis and a blood sample for ELISA analysis must be taken in a vacutainer blood collection tube. A sample must be taken for virological (PCR) analysis from any wild boar found dead, and for all hunted young wild boars (less than 1 year old), a blood sample for ELISA analysis must be taken in a vacutainer blood collection tube; if the ELISA results are positive, a virus neutralisation assay and virological (PCR) analysis must also be performed.

Definition of suspected cases and confirmed cases

"Suspected to be infected with swine fever": any swine showing symptoms and/or *post mortem* lesions suggestive of swine fever (CSF or ASF) that cannot be attributed with certainty to any other disease or showing non-negative first-line test results.

"Suspected to be contaminated": any swine likely, according to epidemiological information, to have been exposed directly or indirectly to a swine fever virus.

A holding is suspect when it holds at least one suspect animal or when it has an epidemiological connection with a confirmed outbreak.

An outbreak of swine fever may be notified when a holding meets one or more of the following criteria:

1. CSF or ASF virus isolated in an animal or in any derived product thereof.
2. Clinical signs suggestive of swine fever observed in an animal, and viral antigen or genome for CSF (RNA) or ASF (DNA) detected and identified in samples taken from the animal or cohort.
3. Clinical signs suggestive of swine fever observed in an animal of a susceptible species and the animal or members of its cohort show specific antibodies against CSF or ASF viral proteins.
4. CSF or ASF viral antigen or genome observed and identified in samples taken from swine AND the animals show specific antibodies against CSF or ASF viral proteins.
5. Clear epidemiological connection with the appearance of a confirmed swine fever outbreak and at least one of the following conditions is met:

- at least one animal shows specific antibodies against CSF or ASF viral proteins,
- the CSF or ASF viral antigen or genome is detected and identified in samples taken from at least one individual of a susceptible species.

Health control measures

CSF and ASF are Category 1 health hazards, notifiable diseases, and subject to emergency response plans.

Distinction between low serological suspicion and high serological suspicion

When an accredited laboratory announces that one or more individual serological tests resulted in positive or ambiguous results, the holding is placed under APMS surveillance. There are two levels of suspicion, defined since February 2012.

If only one or two samples are positive or ambiguous and there are no suspicious clinical signs or unfavourable epidemiological conditions, suspicion is low and the APMS is adapted to this less threatening situation: movements to a slaughterhouse or a terminal holding are allowed providing that the holding with serological suspicion has been clinically and epidemiologically sanctioned and that the slaughterhouse or destination holding has agreed in writing that animals can be introduced from this holding, and that the destination holding is also placed under APMS. Culled animals are consigned until there are results disproving the suspicion.

In the case of high CSF or ASF suspicion based on clinical signs or epidemiological conditions, an APMS is ordered and no exceptions are possible for the movement of animals. If infection is confirmed, the holding is placed under APDI. All swine are culled immediately, the carcasses are disposed of, the farm is disinfected, and all animal products and by-products are disposed of. Repopulation cannot take place for at least 30 days. This period is longer in the case of ASF infection if the intermediate host (*Ornithodoros* ticks) is likely to have been involved.

In holdings with an epidemiological connection with an outbreak (contact holdings), conservative measures are taken under APMS and call for enhanced surveillance.

In the vicinity of the outbreak, a protection zone with a radius of 3 km is established as well as a surveillance zone with a radius of 10 km within which surveillance, movements and possible exceptions are not as strict as within the protection zone. The measures specific to these regulated zones are available in the Memorandum DGAL/SDSPA/N2006-8194 as amended on the swine fever emergency response plan.

Regulatory References

Directive 2001/89/EC on Community measures for the control of classical swine fever

Directive 2002/60/EC laying down specific provisions for the control of African swine fever

Decision 2008/855/EC concerning animal health control measures relating to classical swine fever in certain Member States

Decision 2004/832/EC approving the plans for the eradication of classical swine fever in feral pigs and the emergency vaccination of such pigs in the Northern Vosges, France

Decision 2002/106/EC approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever

Ministerial Order of 23 June 2003 laying down the measures for the control of classical swine fever

Ministerial Order of 11 September laying down the measures for the control of African swine fever

has made it possible to confirm the disappearance of the neutralising antibodies in ten of the eleven young wild boars initially captured (the last young animal was not recaptured). Titration of neutralising antibodies revealed a strong differential in titres between the adult sow and the young animals. Overall, these results support the hypothesis of the presence of maternal antibodies in young wild boars born three years after the discontinuation of oral vaccination (Rossi *et al.*, 2015a).

Costs

In 2014, the French government invested €145,700 for the surveillance and control of CSF and ASF. Laboratory costs amounted to €135,100 for programmed screening and €9,980 for health control measures. Veterinary costs incurred for health control measures were €620. These figures do not include government funds used for carrying out programmed surveillance in nucleus and multiplier herds that belong to the Breeding Pig Agency, which amounted to €27,610 for the serological analyses (non-consolidated data when this article was submitted, the given Figure is probably an underestimate). French government funds of over €300,000 were spent for wildlife surveillance.

Discussion

As in previous years, the results from the CSF and ASF surveillance programme in 2014 demonstrate that France has maintained its disease-free status.

For serological testing, the number of holdings covered by surveillance in the slaughterhouse in 2014 was comparable to that of 2013, but for virological testing, the decline already observed in 2013 was confirmed in 2014 (Marcé *et al.*, 2014), with the average number of samples per site remaining relatively stable. Overall, the coverage rates have gone from 92% and 98% respectively for serological and virological surveillance in 2012, to 86% and 73% in 2013, and to 86% and 62% in 2014. Regarding serological surveillance of CSF, the number of samples remained stable among breeding pigs and increased slightly among slaughter pigs. As a reminder, blood samples taken at the slaughterhouse aim to meet two objectives: 1) to provide information fundamental to confirming France's disease-free status and to provide proof to the European Union and international authorities that France is free of CSF and ASF, and 2) to maintain the operational capacity of the network of serological and virological laboratories accredited for CSF diagnosis (16 laboratories accredited for serological CSF tests of which eight are also accredited for virological ASF tests), so as to be able to respond effectively in the event of an epizootic. In 2014, an inter-laboratory proficiency test (ILPT) was organised for the CSF virological assay (PCR) and an ILPT for the CSF serological assay (ELISA technique and virus neutralisation technique) with satisfactory results for all the accredited laboratories.

Ideally, breeding pigs reflect the health status of the entire herd due to their long presence in the holding — much longer than that of slaughter pigs. This makes them a target of choice for meeting the first surveillance objective. The age of the pig is not a limiting factor for the second goal. Nonetheless, due to the difficulties in sampling breeding pigs at the slaughterhouse, mainly because some slaughterhouses that processed this type of pig have closed down or refocused their activity in certain *départements* on slaughter pigs, exceptions were allowed, such as those defined in the Memorandum DGAL/SDSPA/N2006-8033 of 7 February 2006 as amended, when samples could not be taken from breeding pigs. In these cases, samples were taken from slaughter pigs. Regarding sampling for virological tests, the total number of samples taken decreased again in 2014 and is now much lower than the target number (1,861 samples out of the targeted 3,000). There was a slight increase in the number of samples taken from breeding pigs, but also a drastic decrease in the number of samples taken from slaughter pigs. This overall decrease can be attributed to slaughterhouses that have ceased to process breeding pigs, with no compensatory sampling on slaughter pigs. In addition, the allocation of samples to be taken by *département* was only updated in September 2014, due to these closures. Depending on the *départements*, this new allocation was not taken into consideration before 2015, which could explain the overall decrease in sampling for 2014.

In nucleus and multiplier holdings, serological surveillance involved an equivalent number of breeding pigs in 2014 compared with 2013, but the average number of samples per holding was slightly higher. This serological surveillance guarantees the disease-free status of the population of breeding pigs in nucleus and multiplier herds in France. At the herd level, the 311 herds that tested negative attest to the disease-free status of the nucleus and multiplier holdings at a prevalence threshold of 1% at a 99% confidence level. However, the actual number of breeding pigs tested per herd (average of 17 pigs) can only detect a minimum within-herd seroprevalence rate of between 15 and 20% at a 95% confidence level.

Of all the holdings that fell under serological suspicions of CSF (n=8, but the precise Figure is not available, in particular for suspicious cases at the slaughterhouse), an APMS was issued for only six of them. As a reminder, any case of serological suspicion must be placed under APMS, though the constraints imposed vary in terms of restrictions on animal movements (Box). Nevertheless, it is useful to be able to adjust management measures implemented in "suspicious" holdings in light of the favourable disease status in France and the risk of introduction. In 2012, regulations introduced the concepts of "high" and "low" serological suspicion.

In parallel, outbreak surveillance led to the reporting of two clinical suspicions in wildlife and just one in livestock, which was placed under APMS (three had been notified in 2013, one in 2012, two in 2011, four in 2010 and none in 2009). This may reflect a low level of vigilance, in spite of the current international health situation. Over the past few years, the low number of reports of suspected swine fever may be related, at least in part, to reluctance to accept the consequences of a suspicion. Yet, the NRL can issue a first series of results within 48 hours of receipt of the samples if accepTable delivery lead times to the NRL are respected, which enables the lifting of restrictions placed on movements following a suspicion. One of the main hurdles may also be the number of samples that need to be taken in holdings (a large number of blood and organ samples).

This vigilance is even more important given the existence of low-virulence CSF strains of the virus that can lead to the onset of attenuated clinical signs, while CSF is still present in Europe. CSF outbreaks in pig holdings were reported in Hungary, Latvia and the Russian Federation in 2013, and seropositive cases were also detected in 2012 and 2013 in wild boar in Croatia. Other cases were reported in wildlife in Hungary, Latvia and the Russian Federation in 2014. An outbreak was reported in a backyard herd in Latvia in June 2014, the previous case dating back to November 2012. An outbreak of CSF in wildlife was also notified in Ukraine in early 2015.

In addition, ASF, which has been present in Sardinia for 35 years, crossed the eastern borders of the European Union in 2014, with 256 cases recorded in the four countries affected (Arsevska *et al.*, 2014, Le Potier *et al.*, 2015; short articles from the international health watch in the Resources Centre of the ESA Platform: http://www.plateforme-esa.fr/). ASF has become established in the Caucasus where it is now enzootic in domestic pigs and in wildlife. ASF also appeared in wild boar in Poland (February 2014), Lithuania (February 2014), Latvia (June 2014) and Estonia (September 2014), and in domestic pig holdings in Poland, Lithuania and Latvia during 2014. ASF was also reported in Ukraine in 2014. These outbreaks continued into 2015 (Le Potier *et al.*, 2015).

The serological study at the slaughterhouse carried out in Corsica was an opportunity to raise awareness yet again among stakeholders about the risk of ASF in Corsica (Desvaux *et al.*, 2014). Two clinical suspicions in livestock had subsequently been notified in 2015 in Corsica at the time of writing this article (May 2015). An evaluation of the ASF surveillance scheme in mainland France and Corsica was

also carried out in 2014 in the framework of the ESA Platform by the OASIS flash method (Dominguez *et al.*, 2014, http://www.plateforme-esa.fr/images/documents/oasis/procodure_oasis_flash_v3.pdf). This evaluation helped identify the strengths of the scheme, such as the reinforcing of its central structure or the revitalising of surveillance in wildlife, and pinpoint cross-cutting and common areas to be strengthened in terms of surveillance capabilities for exotic Category 1 health hazards. Nevertheless, it is apparent that some farmers are reluctant to accept the outbreak surveillance scheme, resulting in substantial gaps in coverage. It would also seem wise to break down the early detection objectives to match each of the possible means of introduction. A plan of action for ASF is being prepared on the basis of the results of this OASIS flash evaluation, the ANSES Opinion No. 2014-SA-0049 on the situation and the risk of emergence of various swine fevers in France, and the recommendations of the Food and Veterinary Office (FVO) on the emergency response plans.

In 2014, CSF surveillance measures in wildlife were still being enforced in the former infected zone in the Northern Vosges (which has now become a high observation zone (ZOR)), following detection of seropositive juvenile wild boars. Due to the favourable change in the situation in wildlife in this zone (absence of new cases and decrease in seroprevalence), the surveillance scheme has been modified with systematic virological analyses being discontinued and voluntary sampling pursued in the zone concerned. In this context, hunters continue to take samples and, although there have been changes in sampling distribution, samples have been taken from all municipalities in the ZOR as part of hunting activities. Since vaccination was discontinued, seroprevalence in the ZOR has decreased, although the presence of antibodies in juvenile wild boar raises questions about the potential persistence of the CSF virus in this zone (Rossi *et al.*, 2013). The results observed so far, i.e. absence of seroconversion observed in 134 captured, marked and recaptured individuals between July 2013 and June 2015, seem to lend support to the presence of maternal antibodies (Rossi *et al.*, 2015a; Rossi *et al.*, 2015b).

All of these data therefore suggest a favourable health situation in the ZOR. These observations provide further support for relaxing the programmed surveillance scheme, in conjunction with enhanced outbreak surveillance that should be advocated and re-initiated in the two *départements* concerned (i.e. Bas-Rhin and Moselle). To this end, the ONCFS decided to begin by meeting with the players in the SAGIR network in order to identify the logistical and human constraints to the collection of dead wild boar, and to find solutions, and then subsequently to coordinate with its partners (SAGIR contacts, Diagnostic test laboratories, DDecPPs) to define the information and alert circuits. In the Northern Vosges, a first meeting was therefore held in each *département* in January 2015. Initial difficulties were reported concerning the collection of wild boar and the sending of samples to the accredited laboratory. Solutions to the logistical problems (in particular the use of temporary storage places) and means of communication *via* the local departmental hunting associations (FDCs) were considered collectively by the participants present at the meeting. Different actions are still to be implemented before the strengthening of outbreak surveillance can become operational: provision of freezers, publication of articles in communication journals distributed by the FDCs, use of sampling kits to be distributed to hunters who collect wild boar carcasses from the roadside for their own consumption, definition of circuits of information between all the players concerned by this wildlife surveillance.

One of the medium-term goals of the CSF/ASF surveillance programme in domestic pigs is to redefine the surveillance plan in slaughterhouses, to take into account the expected levels of prevalence in pig holdings for low-virulence strains of the CSF virus (which are not easily detected clinically), estimated using a mathematical model developed by the ANSES Ploufragan Laboratory. Meanwhile, the entire pig and pork industry is encouraged to maintain its vigilance with respect to swine fevers and promote effective outbreak surveillance, thereby guarding against the spread of classical or African swine fever through the implementation of suiTable health control measures as soon as they are detected.

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Update on the surveillance of avian *Influenza* and Newcastle disease in France in 2014

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Abstract
In 2014, France maintained its status as "free from high and low pathogenic avian *Influenza*" and "free from Newcastle disease", as defined by the OIE Animal Health Code. The end of the year was marked by the circulation of high pathogenic avian *Influenza* H5N8 in northern Europe and an outbreak in Italy. The health situation in neighbouring countries and the communication required improved the vigilance of the different stakeholders, resulting in a slight increase in programmed surveillance activity and in wild bird mortality monitoring. As in previous years, programmed surveillance of avian *influenza* in farms revealed batches of H5-seropositive birds in waterfowl farms, although the virus remained undetected. The surveillance protocols were amended at the end of the year in order to increase their efficiency, with the introduction of a graded system for suspicions.

Keywords
Category 1 health hazard, Regulated disease, Avian *Influenza*, Newcastle disease, Pigeon paramyxovirosis, Poultry, Birds, France

Résumé
Bilan de la surveillance de l'Influenza aviaire et de la maladie de Newcastle en France en 2014
*La France a conservé en 2014 son statut indemne vis-à-vis de l'*Influenza *aviaire hautement et faiblement pathogène et de la maladie de Newcastle au sens du code sanitaire de l'OIE. La fin d'année 2014 a été marquée par un contexte de circulation d'*Influenza *aviaire hautement pathogène (IAHP) à H5N8 dans le nord de l'Europe et l'apparition d'un foyer en Italie. Ce contexte sanitaire dans les pays voisins, et la nécessaire communication, ont permis d'accroître la vigilance des différents acteurs qui s'est traduite par une légère augmentation de l'activité de la surveillance événementielle et du suivi des mortalités chez les oiseaux sauvages. Comme les années précédentes, la surveillance programmée de l'*Influenza *aviaire en élevage a mis en évidence des lots d'animaux séropositifs pour le sous-type H5 au sein d'élevages de palmipèdes, sans pour autant mettre en évidence de virus. Les protocoles de surveillance ont fait l'objet de travaux en fin d'année, pour en augmenter l'efficience, notamment par la gradation des suspicions.*

Mots-clés
Danger sanitaire de 1ère catégorie, maladie réglementée,* Influenza *aviaire, maladie de Newcastle, paramyxovirose du pigeon, volailles, oiseaux, France

The aim of this article is to present the results of the surveillance scheme for avian influenza *(AI)* and Newcastle disease (ND) in France in 2014. The end of 2014 was marked by circulation of the HPAI H5N8 virus in the north of Europe, and the emergence of an outbreak in Italy (EFSA, 2014; OIE 2014b).

In France, following the ANSES Opinion (ANSES, 2014) and the confirmation of a case in birds in Germany (Harder *et al.*, 2014; OIE, 2014a), the level of risk of highly pathogenic avian influenza was increased from "negligible" to "moderate" on 27 November, which led to biosafety measures being enhanced and prohibition measures being taken, concerning for instance certain gatherings of birds.

Following the measures taken and the surveillance put in place, no cases of avian influenza or Newcastle disease were identified in 2014, enabling France to retain its disease-free status with regard to these two diseases.

This article details the results of surveillance in 2014: outbreak and programmed surveillance, and monitoring of wild bird mortality.

Outbreak surveillance of avian *influenza* and Newcastle disease in farmed and captive birds

Procedures

Outbreak surveillance in holdings involves the notification of clinical suspicions of AI or ND in accordance with the Ministerial Orders of 18 January 2008 for avian influenza (Box 1) and 8 June 1994 for Newcastle Disease (Box 2). It is based on the detection and characterisation of AI viruses or avian Type 1 paramyxoviruses in samples from suspect poultry.

Results

The change in the epidemiological context in France was reflected in a slight increase in the number of suspicions in domestic birds and avian influenza screening in birds found dead (Table 1), without any cases of HPAI being confirmed.

Fifteen suspicions of avian influenza were reported in poultry farms, and another five among amateur breeders of pigeons and doves, making a total of twenty suspicions investigated (sometimes a combination of HPAI and Newcastle disease). Laboratory tests ruled out infection by a regulated highly pathogenic virus (HPAI) of subtype H5 or H7.

Subtype H7 was not detected in France in 2014. In contrast, a low pathogenic AI virus of subtype H5 was identified following a non-negative test result from avian influenza screening. It was an LPAI virus of subtype H5N1, detected in the corpses of greylag geese in the framework of checks carried out by the aviation industry.

The additional tests conducted by the NRL on the suspicions that had been reported to it led to the detection of other non-regulated influenza viruses and thereby contributed to a better understanding of the viruses circulating in France. As a result, in 2014, the NRL identified the 2009 pandemic AI virus (H1N1) in two breeder turkey farms where a drop in egg laying had been observed.

With regard to Newcastle disease, viruses were detected in the birds of three private owners. Two of the cases concerned owners of

Table 1. Number of suspected cases of avian *influenza* in France in wild birds and poultry between 2012 and 2014

	2012	2013	2014
Wild birds	49	61	79
Poultry	2	14	15

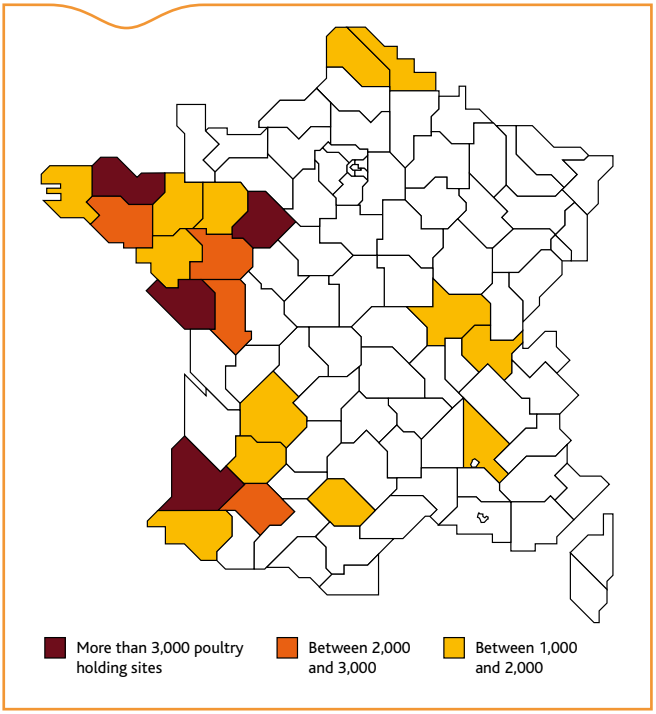

Figure 1. Distribution of poultry holdings (all species) in France registered in the national database in 2014 (source: SIGAL)

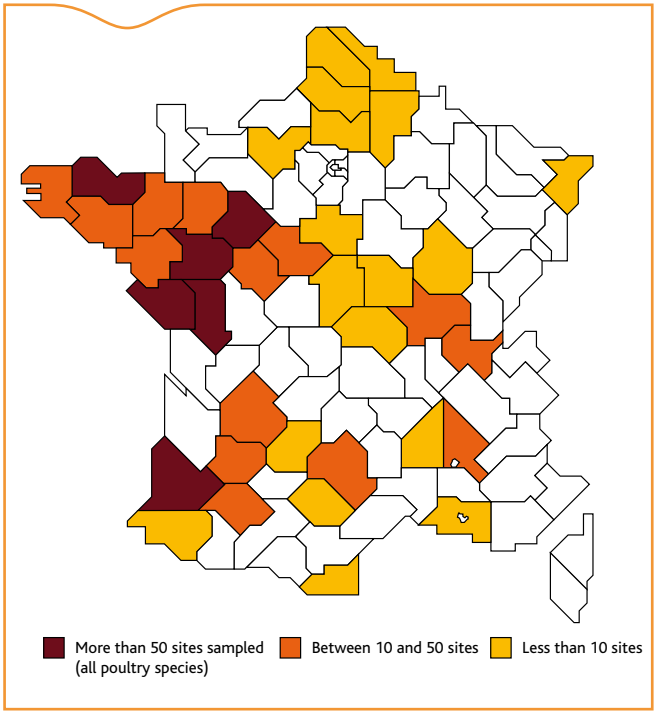

Figure 2. Distribution of poultry holdings in France sampled for annual serological testing in 2014 (source: Memorandum DGAL/SDSPA/N2014-433)

captive pigeons (birds not included in the poultry category according to the guidelines of the OIE and the European Commission) and detection of type 1 paramyxovirus (PPMV1), the pigeon variant of Newcastle disease. The third concerned the owner of a backyard flock comprising Galliformes and water fowl. The type 1 paramyxovirus (PPMV1) detected in samples taken from chickens (*Gallus gallus*) was an avirulent strain that may be a vaccine strain.

Discussion

Due to the fact that no regulated IA or ND viruses were found in poultry, the health status of the country has not been called into question. However, because of the circulation of highly pathogenic avian influenza in Europe in November 2014, the need for vigilance was reiterated by Memorandum DGAL/SDSPA/2014-902 of 19 November.

On 27 November 2014, following the identification of an HPAI virus in a wild bird in Germany, the risk level was increased from "negligible" to "moderate". This increase in the risk level led to enhanced biosafety measures, a ban on certain events, an increase in the level of vigilance and the sensitivity of surveillance, and greater efforts to monitor mortalities in wild birds, although the number of birds actually tested remains low. No cases of HPAI were detected in 2014.

Programmed surveillance on farms

As it has done every year since 2002, France participated in the European surveillance programme for avian influenza both in farms and among wild birds.

Surveillance procedures for 2014 are detailed in Box 1.

As in previous years, the farms and the poultry species identified in the national database (Figure 1) do not correspond to the definition of farms given by Commission Decision 2010/367/EC. Consequently, the sampling plan announced is not always suited to the farms in the various *départements*.

In 2014, the categories of farms to be sampled were taken into account to more closely correspond to Decision 2010/367/EC, mainly by grouping together holdings previously classified in two categories. Thus, only one category of "fattening turkeys" has been retained, limited to free-range turkeys; "fattening ducks" includes both ready-for-gavage and broiler ducks; and pheasant and partridge holdings have been grouped together in the "gallinaceous game birds" category, equating to 140 fewer farms (22%) compared to the 2013 sampling objectives for these production holdings (Memorandum DGAL/SSDPA/N2014-433 of 5 June 2014). In contrast, the sampling plan provided for an increase in the number of goose farms to be sampled. Lastly, the "other" category, which cannot be sorted for the current European-level survey, includes flocks of guinea fowl, which are more easily found in the open air, to the detriment of quail, which are systematically kept indoors and are difficult to sample because of their small size.

Results of programmed surveillance on farms

The survey was implemented between 17 June and 10 December 2014 in 721 poultry farms according to the distribution shown in Figure 2.

In total, 17 water fowl farms (breeder ducks and geese, ready-for-gavage and broiler ducks) were thus confirmed as H5 seropositive, while one breeder duck farm obtained an ambiguous H5 result. Of these 18 holdings, nine underwent additional virological sampling in the same batches as the ones that had yielded the positive or ambiguous results. All the results were negative. The nine remaining farms could not be sampled for virology, because the batches concerned had been slaughtered before receipt of the screening results.

In a context of circulation of the H5N8 virus (at least from November 2014 in Europe) and identification by the EURL of major antigenic differences of the H5N8 virus compared to the antigens recommended until 2014 for the European serological surveys ("2014 recommended antigens"), sera collected at the end of autumn 2014 were analysed retrospectively with an H5N8 antigen provided by the EURL. Thus, the sera of five H5 seropositive domestic water fowl flocks with the "2014 recommended antigens" (three from ready-for-gavage ducks and two from breeder geese) as well as one H5 seronegative breeder duck flock with these same antigens, all collected between 22 October and 17 November 2014, were also selected for analysis with the H5N8 antigen. No increase was observed in antibody titres or number of sera reacting to the H5N8 antigen. Consequently, no traces of infection by an H5N8 virus were detected in these holdings.

Box 1. Avian *influenza* surveillance and health control measures

Objectives of the surveillance programme

- To confirm and maintain France's disease-free status (as defined by the OIE Health Code).
- To provide early warning of any introduction or circulation of a strain of avian *influenza*.
- To ensure the reporting and investigation of suspected cases of avian *influenza*.
- To detect the circulation of strains of low pathogenic avian *influenza* (LPAI) subtypes H5 and H7 in domestic poultry in order to prevent the spread of these low pathogenic strains and avoid the risk of mutation into highly pathogenic strains.
- To ensure programmed surveillance of avian *influenza* in poultry and wild birds.

The population monitored

Poultry, captive birds and wild birds found in France.

Surveillance procedures

Outbreak surveillance

- **In poultry holdings:** notification to the DDecPP of clinical suspicion based on alert criteria (Ministerial Order of 18/01/2008).
- **Wild birds:** notification of mortality and collection of dead wild birds according to instructions dependent on the level of epizootic risk of highly pathogenic avian *influenza* (HPAI). With a negligible level of risk, the definition of abnormal mortality is one swan carcass or five dead birds on a given site within a period of seven days or less (Memorandum DGAL/SDSPA/N2007-8056 of 28 February 2007), while with a moderate level of risk, collection takes place from two Anatidae instead of five (Memorandum DGAL/SDSPA/2014-964 of 4 December 2014).
- **Decoy ducks:** obligation for any holder of decoy ducks for hunting waterfowl to declare, either to their veterinarian or to their local departmental hunting association (FDC), all cases of clustered deaths of decoy ducks or grouped cases of symptoms affecting the nervous system (lack of coordination, tremor, twisted neck, etc.) except for cases of flaccid paralysis (possibility of botulism) (Memorandum DGAL/SDSPA/N2011-8007 of 4 January 2011).

Programmed surveillance

- **In poultry holdings**

European Union measures stress the importance of detecting and controlling outbreaks of low pathogenic avian *influenza* (LPAI) caused by subtypes H5 and H7 in farms, in order to prevent the spread of these low pathogenic strains and to prevent the risk of mutation to highly pathogenic strains.

The method adopted for farms in France is surveillance based on the risk of exposure to infection by AI. It focuses on areas near wetlands and where wild birds congregate, and also *départements* with a high density of poultry holdings (Figures 1 and 2).

Programmed surveillance in livestock holdings is specified in Memorandum DGAL/SDSPA/2014-433 of 6 June 2014 and is based on: (i) the detection of antibodies to AI viruses of subtypes H5 and H7 in a sample of sera from the flocks of poultry concerned, and then ii) in the event of positive results, the detection and characterisation of the corresponding viruses in oro-pharyngeal and cloacal swabs taken from birds in the same flocks, if they have not already been slaughtered. The sampling advocated in Commission Decision 2010/367/EC is intended to detect with a probability of 95% (99% for duck and goose farms) at least one infected poultry holding, when the prevalence of infected poultry holdings is at least 5%.

- **Wild birds and decoy ducks**

Active surveillance ended for these categories in 2012 and 2011, respectively. Programmed surveillance of decoys, with swabs being taken, which is considered from a moderate level of epizootic HPAI risk, was not implemented in 2014.

Vaccination

Vaccination is prohibited in France except for any vaccination programme approved by the European Commission.

Definitions (Ministerial Order of 18/01/2008)

HPAI: Infection caused by an avian *influenza* virus:

- belonging to subtypes H5 or H7 with genomic sequences coding for multiple basic amino acids at the haemagglutinin cleavage site, similar to those observed for other HPAI viruses, indicating that haemagglutinin can undergo cleavage by a ubiquitous host protease,
- or showing, in six-week old chickens, an intravenous pathogenicity index greater than 1.2.

LPAI: infection caused by avian *influenza* virus subtype H5 or H7 that does not fit the previous definition.

Suspicion of avian influenza (highly or low pathogenic): based on:

- epidemiological or clinical evidence or lesions. Depending on the evidence, suspicion can be oriented towards either LPAI or HPAI, and/or
- non-negative results in laboratory tests leading to suspicion of infection by an AI virus (positive H5 or H7 serology or positive PCR for the M or H5 or H7 gene in an accredited laboratory).

Confirmation of avian influenza: confirmation of infection by an LPAI or HPAI virus by the NRL.

Health control measures

- In the case of (clinical or analytical) suspicion:
  - > Holding is placed under an APMS order,
  - > Samples are taken for virological PCR analyses in an accredited laboratory or sent to the NRL for confirmation of a positive PCR obtained in an accredited laboratory and determination of LPAI and HPAI strains.
- In the case of analytical suspicion from a waterfowl holding without clinical symptoms (positive serological tests for H5 or H7 confirmed by the NRL), additional samples are taken for virological screening if the original flock is still present in the holding (Memorandum DGAL/SDSPA/N2008-8287 of 18 November 2008).

A trace-back/trace-forward epidemiological survey is conducted whose objective is to:

- > date the infection event and identify the source of infection,
- > estimate the risk of the virus spreading and thus take control measures according to this risk,
- > determine which holdings are at risk, i.e. holdings with epidemiological connections with a suspect holding, as well as poultry farms located near the suspect holding.

- In the case of a confirmed outbreak, the holding is placed under an APDI order, animals are slaughtered (or sent to a slaughterhouse if infection with LPAI), cleansing and disinfection operations are undertaken, protection and surveillance zones are set up for HPAI (3 and 10 km, respectively) and for LPAI (1 km).

Regulations

Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian *influenza* and repealing Directive 92/40/EEC

Commission Decision 2010/367/EU of 25 June 2010 on the implementation by Member States of surveillance programmes for avian *influenza* in poultry and wild birds

Ministerial Order of 18 January 2008 laying down the technical and administrative measures for the control of avian *influenza*

Ministerial Order of 24 January 2008 regarding the level of epizootic risk due to infection of birds by a highly pathogenic avian *influenza* virus and the surveillance system and control measures for captive birds

Operational indicators of Programmed surveillance

Time to results

In 2014, 38 batches of poultry were received at the NRL for confirmatory analyses by H5 and/or H7 haemagglutination inhibition (HI) assay.

As shown in Table 2, the cumulative time frames for sending samples and conducting sampling and analyses may explain why, when further investigations were needed, the incriminated batch was no longer present in the holding. For this reason, only half of the seropositive flocks were available for additional sampling for detection of the virus.

As in previous years, the longest intervals corresponded to the period between sampling in the farms and receipt by the NRL for confirmation, with an average of 50.9 days and a maximum of 126 calendar days (storage of samples in nearby laboratories was for an average of 10.4 days and a maximum of 73 days, and conducting the screening tests in accredited laboratories and then shipping the batches of sera presumed positive from these laboratories to the NRL took an average of 40.5 days and a maximum of 102 days). These results are worse than those from the previous year and fail to meet the original objectives of the 2014 campaign.

Other data on this interval between conducting sampling in the holdings and receipt of samples at the NRL for confirmation were provided by the SIGAL national database for the 38 batches sent to the NRL:

- the average storage time of blood samples until receipt by the accredited laboratory concerned varies according to the *départements*, ranging from 2.7 days to 20.3 days,
- the average time for receiving the results varies greatly depending on the screening laboratories, ranging from 6.4 days to 57.3 days on average (with a maximum of 11 to 91 days),
- the time taken to send samples screened as positive candidates to the NRL ranges from five to 35 days.

The time between receipt by the NRL of the samples for confirmation, and sending of the corresponding test reports was 6.7 calendar days on average (an improvement compared to 2013), which is fast considering the non-urgent nature of these analyses.

The time between sending test reports for seropositive cases and their return to the source holding was on average 10.1 calendar days, which is quick, and slightly shorter than the average time frame estimated in 2013, proving the high level of responsiveness among the different services involved.

Coverage rate

Table 3 shows the number of samples taken by category of farm as well as the testing rates compared to the objectives for the year. In 2014, the overall sampling rate in the different poultry production holdings (excluding ratites) was 90.1%.

The coverage rate by species varied from 44% to 138%, without taking ratites into account, for which samples were only taken at two farms.

The testing rate in breeder and pre-adult breeder geese – for which the sampling plan had been modified in 2014, from 20 farms to be sampled to 80 – was the lowest in this campaign with only 44% of samples taken. It can be explained by the lack of corresponding holdings for this category. In ostrich farms, samples were taken at the slaughterhouse for safety reasons, and their *ad hoc* slaughter required repeated trips to obtain the necessary samples.

The other species had coverage rates higher than 70%.

It should be noted that results are lacking for ten batches of sera, which were obtained within the deadlines, but sent for analysis after the completion of the survey.

Comparison with previous years

Over the past three serological survey campaigns in holdings, the seropositivity rates were calculated for H5 by production type and by year, as well as the 95% confidence interval obtained by following either the normal or binomial (in the event of small sample sizes) distribution (Table 4).

For breeder geese, ready-for-gavage duck and broiler duck holdings, the confidence intervals show overlapping values for the three years surveyed. There is therefore no significant difference in seropositivity rates between the last three serological surveys.

Lastly, a difference was highlighted between 2013 and 2014 for the production of breeder ducks: only 10.4% of farms were detected H5 seropositive in 2014, whereas 30.8% had been detected in 2013 with non-overlapping confidence intervals. However, in this type of production, the proportion of positive flocks in 2014 was not significantly different from that observed in 2012. Initial analyses have not highlighted any factor concerning the age of the ducks or the sampling date that might explain this change. Various assumptions can be made, in particular, an effect related to the year, a lack of representativeness of the antigens used, or a change in farming practice. A similar phenomenon has been observed at European level (Breed *et al.*, 2015), although no additional explanatory information has been provided to date.

Surveillance of mortality in wild birds

Objectives and design of the surveillance programme

The goal of the surveillance programme for wild birds is the early detection of the highly pathogenic H5N1 subtype in order to protect poultry in farms and public health. It is based on the search for the virus by PCR from oro-pharyngeal and cloacal swabs taken from birds following clustered fatalities (at least five dead birds on the same site in less than a week) or for any swan carcass, as specified in Memorandum

Table 2. Intervals expressed as calendar days during programmed serological surveillance of avian *Influenza* in 2014. In order to compare, number in brackets refers to the 2013 intervals.

BS ❶ → received at screening lab. ❷ → received at NRL ❸ → test report sent (NRL) ❹ → return to holding

	Period 1	Period 2	Period 1+2	Period 3	Period 4
	Blood sampling → received at screening lab.	Received at screening lab. → received at NRL for confirmation, after screening	Blood sampling → received at NRL for confirmation, after screening (comparative 2013 data)	Received at NRL → NRL test report sent (comparative 2013 data)	NRL test report sent → return to holding (comparative 2013 data)
Mean	**10.4**	**40.5**	**50.9 (43.5)**	**6.7 (11.4)**	**10.1 (12.2)**
Minimum	0	6	10 (11)	3 (5)	4 (2)
Maximum	73	102	126 (85)	10 (19)	26 (21)

Box 2. Newcastle disease (ND) surveillance and health control measures

Objectives of the surveillance programme

- To ensure France's ND-free status (as defined by the OIE Health Code).
- To detect as early as possible any evidence of type 1 paramyxovirus virus circulation in poultry and captive birds.
- To ensure the reporting and investigation of suspected cases of Newcastle disease.

The population monitored

Poultry species and captive birds throughout France.

Surveillance procedures

- Outbreak surveillance: notification of clinical suspicions in poultry and captive birds to the DDecPP.
- Programmed surveillance: none.

Vaccination

Mandatory vaccination in pigeons (Memorandum DGAL/SDSPA/N2012-8145 of 9 July 2012).

Definitions

- *Newcastle disease:* infection caused by any strain of Type 1 avian paramyxovirus in day-old chicks with an intracerebral pathogenicity index (ICPI) greater than 0.7.
- *Poultry*: chickens, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants, partridges and flightless birds (ratites), raised or kept in captivity for the purposes of reproduction, production of meat or table eggs or restocking game supplies.
- *Confirmed case of Newcastle disease*: confirmation by the NRL of the presence of a type 1 avian paramyxovirus showing the characteristics of a virulent strain.

Health control measures

In the case of suspicion:

- The holding is placed under an APMS surveillance order, samples (organs) are taken for virological analyses that entail inoculation on embryonated eggs, and are sent to one of the two laboratories accredited for virus isolation.
- Trace-back/trace-forward epidemiological survey: traceability of animals introduced to or leaving the holding during the risk period (21 days before the onset of clinical signs). The objective of this investigation is to:
  - > date the infection event and identify the source of infection,
  - > estimate the risk of the virus spreading and thus take control measures according to this risk,
  - > determine which holdings are at risk, i.e. holdings with epidemiological connections with a suspect holding, as well as poultry farms located near the suspect holding.

When an outbreak is confirmed:

- The holding is placed under an APDI order.
- Birds are slaughtered, cleansing and disinfection measures are implemented, along with protection and surveillance zones of 3 and 10 km, respectively.
- Waiver possible for ornamental birds with a 60-day containment period.

Regulatory References

Ministerial Order of 8 June 1994 laying down the control measures for Newcastle disease

Table 3. Statement of the 2014 avian *Influenza* surveillance in farms

Production	Data extracted from SIGAL on 07-01-2015				Data from the NRL				Additional analyses: molecular analyses according to results reported to the NRL	
	No. batches sampled	No. holdings in which batches were sampled	Target no. Holdings (see Memorandum DGAL/SDSPA/N2014-433)	Coverage rate (farms sampled compared to target - in %)	No. batches sent to the NRL	No. AI positive holdings (IDG)	No. H5 seropositive holdings	No. H7 seropositive holdings	No. retested batches[a]	No. positive batches[a]
Broiler duck	35	35	40	88	2	/	2	0	0/2	/
Mallard duck	14	14	15	93	0	/	0	0	/	/
Breeder and pre-adult breeder duck	79	77	80	96	14	/	7 + 1 ambiguous	0	3/8	0/3
incl. Pre-adult breeder Muscovy (≤24 weeks)	*13*	*13*			*1*	*/*	*0*	*0*	*/*	*/*
incl. Muscovy breeder	*23*	*22*			*2*	*/*	*0*	*0*	*/*	*/*
incl. Pre-adult Peking breeder (≤18 weeks)	*6*	*6*			*/*	*/*	*0*	*0*	*/*	*/*
incl. Peking breeder	*37*	*36*			*11*	*/*	*7 + 1 ambiguous*	*0*	*3/8*	*0/3*
Ready-for-gavage duck	60	59	50	118	6	/	3	0	1/3	0/1
Free-range turkey	53	53	60	88	0	0	/	/	/	/
Breeder turkey	50	46	53	87	1	1	0	0	/	/
Pheasant	19	19	20	95	1	/	0	0	/	/
Breeder and pre-adult breeder goose	36	35	80	44	7	/	5	0	5/5[b]	0/5
incl. pre-adult breeder goose (≤24 weeks)					*0*	*/*	*0*	*0*	*/*	*/*
incl. breeder goose					*7*	*/*	*5*	*0*	*5/5[b]*	*0/5*
Partridge	30	30	40	75	1	/	0[c]	0	/	/
Guinea fowl	65	65	60	108	6	/	0	0	/	/
Caged laying hen	44	44	60	73	0	0	/	/	/	/
Free-range laying hen	63	63	60	105	0	0	/	/	/	/
Breeder hen	57	53	60	88	0	0	/	/	/	/
Free-range broiler	83	83	60	138	0	0	/	/	/	/
Slaughterhouse[d]	43	43	60	72	0	0	/	/	/	/
Ratite	2	2	exhaustif		0	/	0	0	/	/
TOTAL	**733**	**721**	**-**	**90,1[e]**	**38**	**1**	**17 + 1 ambiguous**	**0**	**9/18**	**0/9**

/ : not applicable
a: tested with rRT-PCR for the H5 gene
b: 1 batch tested with rRT-PCR for the M gene
c: 1 batch could not be interpreted
d: samples were only taken from Gallus gallus
e: excluding ratites, the total coverage rate was 719 / 798 = 90.1%

DGAL/SDSPA/N2007-8056 of 28 February 2007. It is carried out in collaboration with the agents of the ONCFS, hunting associations, organisations responsible for the observation, study or protection of wild birds, and also all those who frequent natural environments and the managers of public spaces.

Given the likely role of wild birds in the introduction of the highly pathogenic H5N8 virus in Europe (ANSES, 2014; EFSA, 2014), surveillance of HPAI H5N1 has been extended to detection of the H5N8 subtype. In addition, in priority areas of particular risk (as defined in the Ministerial Order of 24 January 2008), the above-mentioned virological analyses are triggered any time that two dead Anatidae species or one dead swan are discovered, to compensate for the reduction in mortality linked to the low virulence of the HPAI H5N8 virus in Anatidae (Memorandum DGAL/SDSPA/2014-964 of 4 December 2014 on the measures applicable to the moderate level of risk of HPAI).

In addition, AI viruses detected by the accredited laboratories in the framework of research programmes involving wildlife can be sent as necessary to the NRL for typing.

Results of surveillance of wild birds

In 2014, the DGAL was informed of 79 wild birds found dead (Table 1). All were screened for Avian Influenza H5/H7 by PCR, with negative results.

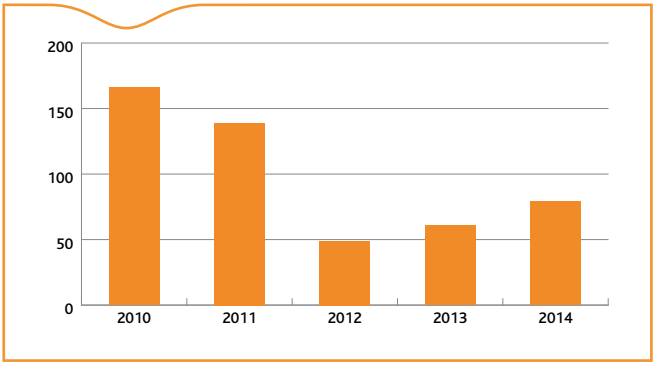

Figure 3. Annual numbers of birds found dead and screened for the AI virus using PCR

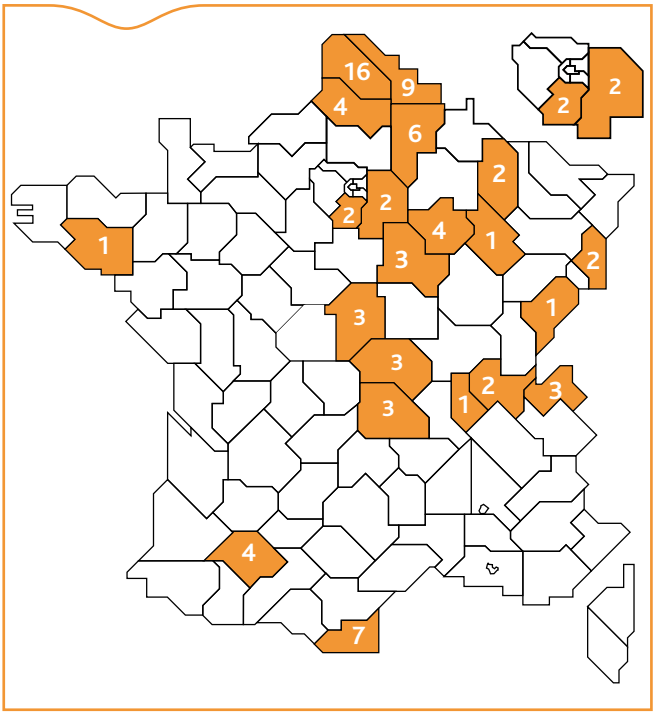

Figure 4. Distribution of dead wild birds analysed by *département* in 2014

Nevertheless, AI viruses not belonging to subtypes H5/H7 were detected. Firstly, subtypes H11N2 and H3N8 were found in mallard ducks in the Pas-de-Calais *département*, respectively in August and October, and secondly, subtype H1N1 was found in Seine-et-Marne in November, in a mallard duck and a swan. In addition, in Columbiformes, type 1 avian paramyxoviruses belonging to three subgroups of the genotype VI were identified.

The number of mortalities reported in the framework of wild bird surveillance rose slightly compared to the 61 birds tested in 2013, with 79 dead birds being analysed in 2014, including 23 in November and December (Figure 3).

Figure 4 shows the distribution of dead birds analysed per *département* in 2014.

Conclusions and outlook

Since the last HPAI outbreak in holdings in 2006, and the summer outbreaks involving wild birds in Moselle in 2007, no HPAI viruses have been detected in France.

Memorandum DGAL/SDSPA/2014-902 of 19 November 2014 reported on the circulation of HPAI H5N8 in Europe and called for vigilance. This memorandum was issued just before the increase in the risk level set by the Decree of 27 November 2014 and for which the applicable measures were specified by Memorandum DGAL/SDSPA/2014-964 of 4 December 2014.

Biosafety measures, such as the containment of farms in priority areas of particular risk, and prohibition measures, including bans on gatherings of birds in areas through which migratory birds pass, have helped reduce the risk of the HPAI virus being introduced in farms from wildlife. However, the Ministerial Order of 24 January 2008 has shown limitations in terms of the clarity and grading of measures, in situations that can involve low-zoonotic or non-zoonotic strains, and a revision of this text is planned.

Due to both: i) the significant antigenic differences of the H5N8 virus compared to the antigens recommended in 2014 for the serological surveys in holdings and ii) the low virulence of this virus in Anseriformes, the European Commission has asked Member States to use the H5N8 antigen as a supplement for the serological tests in ducks and geese during the 2015 survey (Van Goethem, 2015).

In the framework of the Epidemiological Surveillance Platform for Animal Health (ESA Platform), the assessment of HPAI surveillance by the Oasis method recommended standardising and clarifying certain procedures. The development of new surveillance protocols progressed in 2014, both for domestic birds and wildlife, in particular with the description of new forms of HPAI outbreak surveillance in domestic birds in Memorandum DGAL/SDSPA/2015-127 of 12 February 2015.

As regards Newcastle disease and pigeon paramyxovirosis, as in previous years the results show that virulent PPMV1 continues to circulate in enzootic mode, especially in wildlife, which concurs with the observations of the other European countries and confirms the need to vaccinate captive pigeons.

Acknowledgements

The authors wish to thank all those who took part in the serological surveys conducted in holdings and in the surveillance of wild birds and live decoy ducks: farmers, mandated veterinarians, DDecPP staff, ONCFS staff, departmental and national hunting associations, departmental veterinary services and the NRL.

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Table 4. Comparison of results obtained during the 2012, 2013 and 2014 campaigns

	2014			2013			2012		
	No. holdings sampled	No. H5 seropositive holdings	Proportion of H5 positive holdings (in %, [95 % CI])	No. holdings sampled	No. H5 seropositive holdings	Proportion of H5 positive holdings (in %, [95 % CI])	No. holdings sampled	No. H5 seropositive holdings	Proportion of H5 positive holdings (in %, [95 % CI])
Breeder quail[c]	/	/	/	15	0	0 [0.0-21.8]	15	0	0 [0.0-21.8]
Broiler duck	35	2	5.7 [0.7-19.2]	82	0	0 [0.0-4.4]	76	0	0 [0.0-4.7]
Mallard duck	14	0	0 [0.0-23.2]	20	0	0 [0.0-16.8]	18	0	0 [0.0-18.5]
Breeder and pre-adult breeder duck	77	7 + 1 ambiguous	10.4 [4.6-19.5]	78	22 + 2 ambiguous	30.8 [20.8-42.2]	72	13[a] + 1 ambiguous	19.4 [10.3-28.6]
RFG duck[d]	59	3	5.1 [1.1-14.2]	93	5	5.4 [1.8-12.1]	93	3 + 2 ambiguous	5.4 [1.8-12.1]
Caged turkey[c]	/	/	/	66	0	0 [0.0-5.4]	69	0	0 [0.0-5.2]
Free-range turkey	53	0	0 [0.0-6.7]	59	0	0 [0.0-6.1]	58	0	0 [0.0-6.2]
Breeder turkey	46	0	0 [0.0-7.7]	64	0	0 [0.0-5.6]	49	0	0 [0.0-7.3]
Pheasant	19	0	0 [0.0-17.7]	34	0	0 [0.0-10.3]	37	0	0 [0.0-9.5]
Breeder and pre-adult breeder goose	35	5	14.3 [4.8-30.3]	16	4	25.0 [7.3-52.4]	16	2	12.5 [1.6-38.6]
Partridge	30	0[b]	0 [0.0-11.6]	33	0	0 [0.0-10.6]	28	0	0 [0.0-12.3]
Guinea fowl	65	0	0 [0.0-5.5]	49	0	0 [0.0-7.3]	56	0	0 [0.0-6.4]
Caged laying hen	44	0	0 [0.0-8.0]	46	0	0 [0.0-7.7]	47	0	0 [0.0-7.6]
Free-range laying hen	63	0	0 [0.0-5.7]	79	0	0 [0.0-4.6]	67	0	0 [0.0-5.4]
Breeder hen	53	0	0 [0.0-6.7]	59	0	0 [0.0-6.1]	60	0[b]	0 [0.0-6.0]
Free-range broiler	83	0	0 [0.0-4.4]	87	0	0 [0.0-4.2]	91	0	0 [0.0-4.0]
Slaughterhouse	43	0	0 [0.0-8.2]	53	0	0 [0.0-6.7]	46	0	0 [0.0-7.7]
Ratite	2	0	0 [0.0-84.2]	2	0	0 [0.0-84.2]	4	0	0 [0.0-60.2]
TOTAL	**721**	**17 + 1 ambiguous**		**935**	**31 + 2 ambiguous**		**902**	**18a + 3 ambiguous**	

a: 1 flock both H5 seropositive and H7 ambiguous
b: with 1 batch that could not be interpreted
c: quails and caged fattening turkeys were sampled and analysed until 2013. These two production types were not targeted in 2014.
d: ready-for-gavage
The 95% confidence intervals were calculated for a binomial distribution, according to the statistical test applied (i.e. depending on sample size).
The ambiguous flocks are regarded as positive.

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An overview of implementation of the programme for *Salmonella* control in *Gallus gallus* and *Meleagris gallopavo* flocks in 2014

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Abstract

The mandatory programme to control *Salmonella* covers all *Gallus gallus* and *Meleagris gallopavo* flocks. The infection rate in flocks of breeders and future breeders of the species *Gallus gallus* rose in 2014 as compared to 2013. The infection rate for *Salmonella* Enteritidis and Typhimurium in units for laying hens producing eggs for human consumption also rose sharply. In turkey sector breeder units, the number of cases of infection remained stable. And last, the number of broiler chickens and fattening turkeys in which *Salmonella* Enteritidis and *Salmonella* Typhimurium were detected rose slightly. When looking at all the sectors except breeder turkeys, this rise was mainly due to increased presence of *Salmonella* Enteritidis. Overall control programme costs rose in proportion with the rise in the number of cases.

Keywords

Salmonella, Epidemiological surveillance, Health rules, *Gallus gallus*, *Meleagris gallopavo*

Résumé

Bilan d'exécution du programme de lutte contre* Salmonella *dans les troupeaux des espèces* Gallus gallus *et* Meleagris gallopavo *en 2014

Le programme de lutte obligatoire contre les salmonelles concerne tous les troupeaux de Gallus gallus *et de* Meleagris gallopavo. *Le taux d'infection dans les troupeaux de futurs reproducteurs et reproducteurs de l'espèce* Gallus gallus *a augmenté en 2014, comparé à 2013. Le taux d'infection vis-à-vis de* Salmonella *Enteritidis et Typhimurium à l'étage poules pondeuses d'œufs de consommation a également fortement augmenté. À l'étage reproducteur de la filière dinde, le nombre de cas d'infection est resté stable. Enfin, le nombre de troupeaux de poulets de chair et de dindes d'engraissement, dans lesquels* Salmonella *Enteritidis et* Salmonella *Typhimurium ont été détectées, a légèrement augmenté. Pour l'ensemble des filières hors dindes de reproduction, l'augmentation est largement due à la présence accrue de* Salmonella *Enteritidis. Le coût global du programme de lutte est en augmentation, en proportion avec l'augmentation du nombre de cas.*

Mots-clés

Salmonella, *épidémiosurveillance, police sanitaire,* Gallus gallus, Meleagris gallopavo

The presentation of the annual review aims to estimate prevalence in *Gallus* in the broiler and layer sectors, and in turkeys, in the different breeding and production levels. With regard to the objectives set by the European regulations for each of these compartments, apart from pre-adult periods, these prevalences are analysed over time. The changes observed can then be compared with different parameters.

Testing programme

In 2014, 83,459 flocks were tested (Table 1), of which 1.2% were primary breeder flocks, 4.8% multiplier flocks, 8.7% table egg-layer flocks and 85.3% turkey or chicken meat-producing flocks.

At the primary breeding level, *Gallus gallus* broilers accounted for 78.6% of the tested flocks, *Gallus gallus* layers, 9.8% and turkeys, 11.6%. At the multiplier level, *Gallus gallus* broiler flocks accounted for 65.6% of the tested flocks, *Gallus gallus* layer flocks, 5.7% and turkey flocks, 28.9%.

The positive cases involving Category 1 Salmonella health hazards for all *Gallus gallus* and *Meleagris gallopavo* sectors are given in Table 2.

Turkey breeder flocks

In 2014, the prevalence was 0.41% in turkey breeder flocks, which is much lower than the European target of 1% set by Regulation (EU) No 1190/2012. Since 2010, the prevalence rate has been consistently low, oscillating between 0 and 0.42%, which is equivalent to between zero and three cases per year.

As in previous years, serotype Typhimurium (both *sensu stricto* and its variants) was present in this sector (two cases out of three).

Gallus gallus *breeder flocks (multiplier level of the broiler and layer sectors)*

No cases were identified in the breeding level of the table-egg laying sector.

Table 1. Number of flocks tested and number of birds covered by the programme in 2014

Sector and stage	Number of flocks tested	Total number of animals covered by the programme
***Meleagris gallopavo* - breeding level**		
Pre-adult primary breeder	57	154,014
Adult primary breeder	59	111,215
Pre-adult multiplier	493	2,175,116
Adult multiplier	670	1,796,940
***Gallus gallus* - broiler sector - breeding level**		
Pre-adult primary breeder	410	2,896,240
Adult primary breeder	374	2,024,088
Pre-adult multiplier	1,199	11,804,155
Adult multiplier	1,442	13,022,702
***Gallus gallus* - layer sector - breeding level**		
Pre-adult primary breeder	45	368,100
Adult primary breeder	53	535,936
Pre-adult multiplier	93	1,232,994
Adult multiplier	135	1,488,645
***Gallus gallus* - layer sector - production level**		
Pre-adult table egg layers (pullets)	2,387	57,288,000
Adult table egg layers	4,928	76,093,248
***Gallus gallus* and *Meleagris gallopavo* - production level**		
Meat production (broilers and fattening turkeys)	71,414	830,901,890
Total	**83,759**	**1,001,893,283**

Objectives of the surveillance programme

The ultimate purpose of *Salmonella* surveillance in poultry flocks is to prevent the occurrence of food-borne diseases. For this, the overall objective of the surveillance is to detect the presence of any infection by *Salmonella* in the targeted poultry sectors for the purpose of enabling appropriate control measures to be established. *Salmonella* bacteria are generally transmitted vertically through the different levels of the breeding scheme; surveillance therefore involve not only poultry raised for food production (eggs, meat), but also poultry reared for breeding purposes. The specific objectives of the surveillance programme are as follows:

- to detect, control and eradicate infections by Category 1 health hazard *Salmonella* serotypes, as defined by Decree No 2012-845 of 30 June 2012, with the aim of reducing their prevalence and the risk that they present to public health,
- to assess the progress made in light of the obtained results,
- to monitor the emergence of any *Salmonella* serotypes.

The population monitored

For *Salmonella* serotypes classified as Category 1 health hazards, French regulations include the following variants in their definition of *Salmonella* Typhimurium: 1,4,[5],12,i:-, 1,4,[5],12,-:1,2 and 1,4,[5],12,-:-: . (Table 1)

Table 1. Poultry populations monitored with regard to *Salmonella*

	Salmonella Enteritidis	*Salmonella* Hadar	*Salmonella* Infantis	*Salmonella* Typhimurium	*Salmonella* Virchow
Breeder flocks *Gallus gallus*	x	x	x	x	x
Breeder flocks *Meleagris gallopavo*	x			x	
Layer flocks *Gallus gallus*	x			x	
Broiler flocks *Gallus gallus* et *Meleagris gallopavo*	x			x	

Surveillance covers flocks of *Gallus gallus* (hens and chickens) and *Meleagris gallopavo* (turkeys), irrespective of their level in the poultry breeding scheme, their geographic location or their epidemiological situation (Table 1), with the exception of small flocks (less than 250 birds).

Surveillance procedures

Samples are taken by a mandated veterinarian, by a technician designated and trained in veterinary sampling techniques by a mandated veterinarian, or by DDecPP/DAAF staff technicians:

- In poultry farms and hatcheries, the minimum frequencies and the basic sampling programme are set by European regulations; French regulations voluntarily extended these regulations;
- For other *Salmonella* serotypes (Category 2 health hazards): epidemiological surveillance based on a systematic sampling programme carried out before moving or culling any poultry flock.

It should be noted that since 2013, all farms with more than 250 adult breeding turkeys have been subject to official controls, whereas previously the European regulations only required sampling of 10%.

Health control measures (for Category 1 health hazard Salmonella *serotypes)*

Control measures remain unchanged since 2009; they were extended to turkey flocks in 2010.

- Suspicions are based on any positive result from samples taken in the environment of a poultry flock. The suspected flock is then placed under a prefectural monitoring order (APMS) that imposes restrictions on the sale of poultry from these flocks. The DDecPP/DAAF orders a series of official samples to confirm or disprove infection. Suspicion is disconfirmed if two successive series of samples test negative; infection is confirmed if one of the samples tests positive. However, for broilers and fattening turkeys, no systematic confirmatory tests have been performed since the abolition of confirmatory sampling on muscles by the Ministerial Order of 24 April 2013; the APMS issued after a first positive test is sufficient for health control measures to be implemented.
- Confirmation: In the event of a confirmed infection, the poultry farm is declared infected by the prefecture (APDI) and health control measures vary with production type. In all cases, cleaning and disinfection operations must precede repopulation with a new batch.
  - > For breeders or pullets (future table egg-laying hens), mandatory preventive elimination of poultry and waste;
  - > For table egg-laying hens, preventive elimination is strongly encouraged by offering compensation to the farm operator, but is not mandatory; however, eggs from an infected flock can only be sold to the food-processing industry where they undergo heat treatment;
  - > The cleaning and disinfection operations are of utmost importance; the effectiveness of these operations must be officially validated before repopulation, and compensation is contingent upon this inspection.

For broilers, the new Ministerial Order of 24 April 2013 included the following amendments:

- > confirmatory sampling is limited to special cases that will be described in detail in a forthcoming ministerial memorandum, in case of risk of spread to layer or breeder holdings,
- > if confirmatory samples are also positive (i.e. APDI declaration), the entire flock can be culled shortly thereafter (depending on the risk of contamination for exposed holdings),
- > implementation of Regulations (EU) No 200/2012 (regarding broilers) and No 1190/2012 (regarding fattening turkeys) extending the validity of test results to 6 weeks before culling for long fattening periods (i.e. 81 days for broilers, 100 days for turkeys) or in organic poultry production.

The strains isolated for testing are held at the ANSES Ploufragan-Plouzané Laboratory, the National Reference Laboratory (NRL) for *Salmonella*. This strain collection can be used for retrospective typing studies or antimicrobial resistance profiles.

Regulatory References

European Regulation (EC) No 2160/2003 lays down the general framework for controlling *Salmonella* infections in the poultry sector in Member States (MSs). Specific regulations for implementing EU legislation define the prevalence targets and the details of the testing programme (sampling protocol, duties of the farm operators and competent authorities, laboratory analyses):

- Regulation (EU) No 200/2010 for adult breeding flocks of *Gallus gallus*,
- Regulation (EU) No 517/2011 for laying hens of *Gallus gallus*,
- Regulation (EU) No 200/2012 for flocks of broilers,
- Regulation (EU) No 1190/2012 for fattening and breeding flocks of turkeys.

The French national control programme was progressively aligned with European regulations as it was being developed:

- Ministerial Orders of 26 February 2008 regarding flocks of *Gallus gallus* breeding hens and table egg-laying hens,
- Ministerial Order of 4 December 2009 as amended regarding breeding turkeys;
- Ministerial Order of 24 April 2013 as amended regarding broilers and fattening turkeys.

Table 2. Number of flocks infected with regulated *Salmonella* strains in France in 2014

Sector	Stage	SE	SE associated with ST	ST	ST i:- variant	ST -:1,2 variant	ST -:- variant	SH	SV	SI	TOTAL
Turkey breeder	Pre-adult primary breeder							NA	NA	NA	0
	Adult primary breeder							NA	NA	NA	0
	Pre-adult multiplier				1			NA	NA	NA	1
	Adult multiplier	1		1	1			NA	NA	NA	3
Gallus gallus breeders - broiler sector	Pre-adult primary breeder										0
	Adult primary breeder										0
	Pre-adult multiplier	2		0				1	1	0	4
	Adult multiplier	5		5				0	0	1	11
Gallus gallus breeders - layer sector	Pre-adult primary breeder										0
	Adult primary breeder										0
	Pre-adult multiplier										0
	Adult multiplier										0
	Pre-adult table egg layer (pullet)	2		2	5	1		NA	NA	NA	10
	Table egg layer	37	1	16	3	0		NA	NA	NA	57
Broiler and fattening turkey	Meat production	135		265	57	8	24	NA	NA	NA	489
TOTAL		**182**	**1**	**289**	**67**	**9**	**24**	**1**	**1**	**1**	**575**

Caption: SE: *Salmonella* Enteritidis, SH: *Salmonella* Hadar, SI: *Salmonella* Infantis, ST: *Salmonella* Typhimurium, SV: *Salmonella* Virchow, NA: Not applicable

Since 2011, serotype Enteritidis had been absent or had a low presence in both sectors, broiler and layer.

In 2014, it reappeared in the broiler sector with two cases (out of four) at the pre-adult stage and five cases (out of 11) at the adult stage.

Regarding the remaining cases in the broiler sector, five flocks tested positive for Typhimurium. The other three positive flocks relate to serotypes Hadar and Virchow in pre-adult, and Infantis in the adult stage.

The overall infection rate, for all breeding and production levels, was 0.23% for pre-adult breeders (0.36% in 2013) and 0.55% for adult breeders (0.11% in 2013), which is lower than the European target of 1% for adult breeders as set by Regulation (EU) No 200/2010. Due to the cases detected in the broiler sector at the adult stage, the total number of positive flocks has therefore increased significantly at the multiplier level compared to 2013 (Chasset *et al.*, 2014).

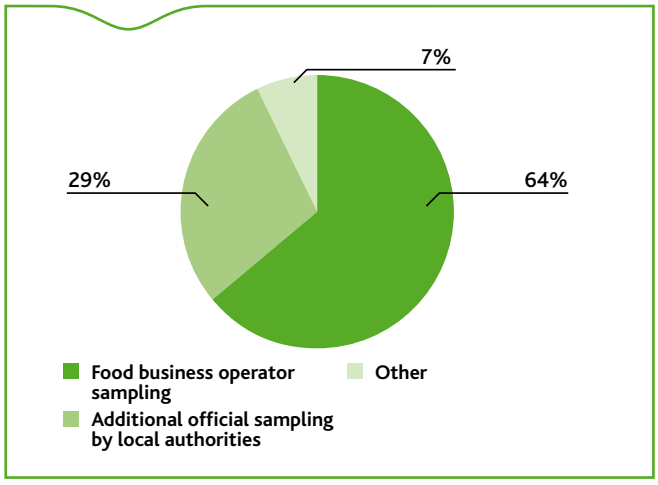

Figure 1. Detection of suspected cases of infection with *Salmonella* Enteritidis and *Salmonella* Typhimurium at the table egg-layer level in 2014

Laying hen flocks

At the production level in the French layer sector, European Regulation (EU) No 517/2011 targets a 10% reduction in the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium every year, or a stable rate of less than 2%. The targeted percentage of reduction in prevalence was set on the basis of an EU-wide baseline survey in 2005 (8% observed in France).

In 2014, as has been the case since 2010 (Chasset *et al.*, 2014), the targeted prevalence rate of less than 2% in layer flocks was met, with a value of 1.16% compared with 1.42% in 2012, and 0.58% in 2013 (Table 2). Thus, in 2014, a renewed increase in the number of cases was observed compared with 2013. This increase is mainly due to a significant number of flocks testing positive for the serotype Enteritidis, representing two-thirds of positive cases in this year. In pullets (future egg-laying hens), ten cases were detected, double the number for 2013.

The rate of unconfirmed results in laying hens was similar to that of 2013, at approximately 50%.

For the table egg-layer production level in 2014, the distribution of suspected cases of infection with *Salmonella* Enteritidis and *Salmonella* Typhimurium is shown in Figure 1. Two-thirds of the suspected cases were detected through mandatory screening carried out by poultry farm operators.

The number of screening operations performed by operators was on average six times higher than those carried out by the State services. Nevertheless, as can be seen in Figure 1, the operators only detected twice as many cases of *Salmonella* as the State services. Screening carried out by the State services is therefore three times more effective than that carried out by the professionals.

Flocks of broiler chickens and fattening turkeys

The results obtained in 2014 (489 cases) were higher compared to 2013 (455 cases) and represent a net increase compared to 2012 (364 cases). However, they remain within the European target set by Regulations (EU) No 200/2012 and (EU) No 1190/2012 for the end of 2012: i.e. less than 1%. With 19% of the national share of broiler flocks testing positive, Reunion Island contributes, as in the previous year, to this increase with regard to serotype ST. With 38 cases in 2014,

Table 3. Changes in prevalence since 2007 and comparison with the European targets since 2010 for all the poultry sectors involved in the control programme for *Salmonella*

Sector	Stage	2007	2008	2009	European target	2010	2011	2012	2013	2014
***Meleagris gallopavo* - breeding level**	Pre-adult breeder	NA	NA	NA	NA	0.22%	0.70%	0.36%	0.00%	0.18%
	Adult breeder	NA	NA	NA	1.00%	0.00%	0.30%	0.11%	0.42%	0.41%
***Gallus gallus* - breeding level**	Pre-adult breeder	0.57%	0.45%	0.26%	NA	0.00%	0.07%	0.47%	0.36%	0.23%
	Adult breeder	0.69%	0.54%	0.26%	1.00%	0.47%	0.30%	0.13%	0.11%	0.55%
***Gallus gallus* - Table egg production level**	Pullet	0.66%	0.48%	0.54%	NA	0.13%	0.15%	0.10%	0.16%	0.38%
	Layer	3.85%	3.16%	2.56%	2.00%	1.62%	1.45%	1.42%	0.58%	1.16%
Broiler and fattening turkey	Meat production	NA	NA	0.52%	1.00%	0.49%	0.54%	0.50%	0.58%	0.64%

Table 4. Changes in prevalence for breeder flocks of *Gallus gallus* in the broiler and table egg-layer sectors since 2004 (expressed as a %)

Breeding level	Stage	*SE*											*ST* including its variants									
		04	05	06	07	08	09	10	11	12	13	14	04	05	06	07	08	09	10	11	12	13	14
***Gallus gallus* breeders - layer sector**																							
Primary breeders	pre-adult	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	adult	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Multipliers	pre-adult	0	0	0	0	0	1.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.44	0
	adult	0	0	0	0.88	0	0	0.77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Gallus gallus* breeders - broiler sector**																							
Primary breeders	pre-adult	0	0	0	1.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.66	0	0
	adult	0	0	1.4	0	0	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0.52	0.71	0	0
Multipliers	pre-adult	0	0	0.1	0.12	0.6	0.2	0	0	0	0.09	0.17	0	0	0.1	0.2	0	0	0	0	0.47	0.09	0
	adult	0.2	0.6	0.2	0.33	0.1	0.1	0.3	0	0	0.08	0.35	0.1	0.1	0.1	0	0.6	0	0.23	0.3	0.05	0.08	0.35

Table 5. Impact of health control measures on production in 2014

Sector and stage	Number of flocks positive for Category 1 health hazard *Salmonella* in 2014	Number of flocks slaughtered or culled	Number of animals slaughtered or culled	Number of destroyed or heat-treated eggs
***Meleagris gallopavo* - breeding level**				
Pre-adult primary breeder	0	0	0	NA
Adult primary breeder	0	0	0	0
Pre-adult multiplier	1	1	4,964	NA
Adult multiplier	3	3	12,182	94,900
***Gallus gallus* - broiler sector - breeding level**				
Pre-adult primary breeder	0	0	0	NA
Adult primary breeder	0	0	0	0
Pre-adult multiplier	4	4	46,912	NA
Adult multiplier	11	11	78,658	614,740
***Gallus gallus* - layer sector - breeding level**				
Pre-adult primary breeder	0	0	0	NA
Adult primary breeder	0	0	0	0
Pre-adult multiplier	0	0	0	NA
Adult multiplier	0	0	0	0
***Gallus gallus* - layer sector - production level**				
Pre-adult table egg layers (pullets)	10	10	201,940	NA
Adult table egg layers	57	56	417,151	6,698,648
***Gallus gallus* - broiler sector and *Meleagris gallopavo* - production level**				
Meat production (broilers and fattening turkeys)	489	489	3,856,589	NA
TOTAL	**575**	**574**	**4,618,396**	**7,408,288**

or almost 8% of the total, compared to 15 cases in 2013, the Drôme is the second *département* making a significant contribution to this increase in prevalence.

Variants of *Salmonella* serotype Typhimurium were present in flocks of broiler chickens and fattening turkeys, particularly the monophasic variant 1,4,[5],12,i:-. For the first time, the variant 1,4,[5],12,-:- was frequent, with 24 cases (Table 2).

Changes in prevalence

A favourable change in prevalence has been observed since control programmes for *Salmonella* were set up in the various poultry sectors, with the exception of the broiler sector (Table 3). However, this year prevalence was significantly higher than in the previous year for all the sectors. Regarding breeder turkeys, the number of annual cases remains low, because the number of flocks and thus the number tested is itself low.

European regulations set targets for prevalence, as mentioned above, for each sector, which are then only calculated on adults for the regulated *Salmonella*, including the 1,4,[5],12,i:-, variant and to the exclusion of other variants. European targets have thus always been met in France in all four poultry sectors.

Regarding breeder flocks, detailed results for the breeding and multiplier levels are given for the layer and broiler sectors (Table 4). It appears that the layer sector, with a few exceptions, most often has zero annual prevalence, whereas the broiler sector, with a higher frequency and number of infections, must be monitored carefully, given the significant increase in 2014.

Control measures

Application of health control measures in breeders and layer hens continued to have a significant impact in 2014, with the elimination of 87 flocks (including 57 layer flocks), 761,807 birds and the destruction or heat treatment of around seven million table eggs (Table 5). It should be noted that positive broiler poultry flocks are slaughtered at the end of their fattening period, with nevertheless specific measures at the slaughterhouse, such as slaughter at the end of the line, and removal of offal for appropriate heat treatment. They are therefore not included in the numbers of flocks destroyed for health reasons.

Changes in the costs of control measures and official analyses

The budget for the control programme for *Salmonella* allotted by the French government to health measures continually decreased until 2012 in line with the fall in the number of infected flocks. However, in 2013, and even more so in 2014, expenditure on the control programme increased, due to the rise in the number of cases (Figure 2). Costs cover the confirmatory analyses and the analyses undertaken upon inspection of cleaning and disinfection operations, financial compensation for animals slaughtered or culled following an administrative order, the destruction or heat treatment of eggs, cleaning and disinfection operations, involvement of mandated veterinarians and various other fees related to the control programme. Compensation accounts for the majority of expenditure and its annual amount varies greatly depending on the type, age and size of the contaminated flocks.

The overall cost of the official analyses is stable with a budget of approximately €450,000 per year, with the same number of analyses and the cost of the analyses increasing only moderately.

For all the sums spent by the French government, there is a European 50% co-funding scheme for the compensation of slaughtered or culled animals and destroyed eggs and for official analyses. For 2014, the upper limit granted to France was reached (€1,360,000).

Discussion

The mandatory *Salmonella* control programme that covers all *Gallus gallus* and *Meleagris gallopavo* flocks was assessed by the EU's Food and Veterinary Office (FVO) during an audit conducted from 19 to 29 November 2013. The FVO particularly focused on compliance with the quality and frequency of screening, the control of the competent authority over screening carried out by the professionals, and the levels of sampling required for the official screening.

The report concluded that the programme is implemented correctly throughout the whole of France. However, it made a series of recommendations. It can be consulted on the internet (http://ec.europa.eu/food/fvo/audit_reports/details.cfm?rep_id=3280).

Overall, the results from 2014 were less favourable than those of 2013 (Chasset *et al.*, 2013).

The national control programme for *Salmonella* implemented since 1998 in *Gallus gallus* breeder flocks and layer flocks, since extended to broiler and turkey flocks, provides satisfactory results and the overall cost of the programme had been decreasing gradually, before increasing again from 2013, on a like-for-like basis.

For the *Gallus gallus* breeding level, the infection rate rose in 2014. Although the number of positive flocks at this level is still relatively low (around 15 per year, with APMSs or suspicions nevertheless totalling 44 cases), the public health and economic consequences of these infections are potentially high. In the layer sector at the production level, the number of infections also increased in 2014. At the breeding level in the turkey sector, the number of cases of infection has remained stable. As noted for *Gallus gallus* breeder flocks, the public health and economic consequences of these infections are potentially high. In the broiler sector, the infection rate increased slightly, with *Salmonella* Enteritidis being especially implicated.

Implementing biosafety measures to avoid introducing and spreading pathogens in poultry farms, particularly in holdings with breeders or laying hens that for the most part have adopted a disease control charter, have continued to prove useful in the control programme for *Salmonella* in *Gallus gallus* and turkey flocks. Meanwhile, given the increase in prevalence, greater vigilance is needed

The number of infections increased significantly in 2014, in large part due to the increase in the serotype *Salmonella* Enteritidis, all sectors combined. Some of the detected cases that tested positive for *Salmonella* Enteritidis in broiler flocks could be due to vertical contamination: two large broiler hatcheries were contaminated by this serotype in 2014, as a result of trade (exchanges between hatcheries, often within the EU) in hatching eggs.

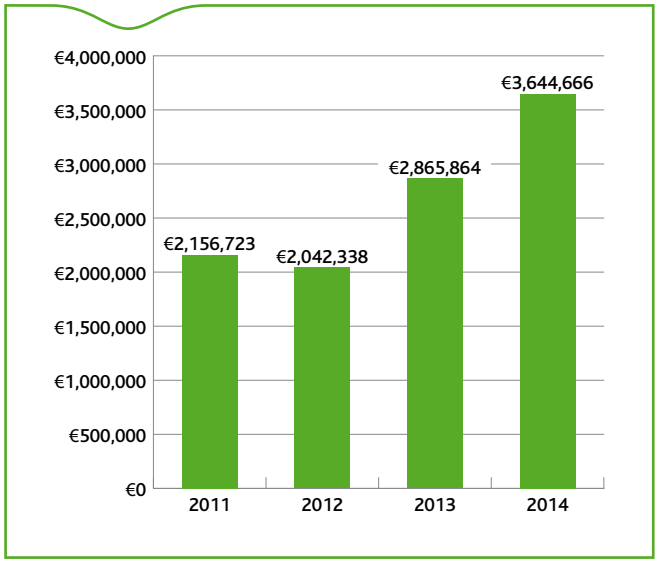

Figure 2. Annual government funds spent for the control programme for *Salmonella* in poultry holdings from 2011 to 2014

The results for 2015 will help determine whether the increase in recorded cases in 2014 was transient or a trend. In any event, a good level of surveillance needs to be ensured, along with the effectiveness of the control programme.

As mentioned in more detail above for laying hens, suspicions were reported primarily from routine mandatory testing carried out by farm operators. However, for an equal number of food business operator sampling and official sampling, *Salmonella* has always been detected more frequently by competent authority inspectors. The effort to raise awareness among professional organisations should be pursued in order to increase the level of detection by farmers.

In addition, these results should be qualified, with the rate of unconfirmed results growing constantly since 2008. Since 2013, the rate of unconfirmed results for *Salmonella* cases has been of the order of 50% for suspicions in layer hen flocks, and even higher in breeder flocks (only one case in three confirmed).

The reasons for the constant increase in the rate of unconfirmed results need to be identified. They may have multiple origins, which can be complex to analyse. For this reason, a formal request has been made to ANSES. The fact that fewer and fewer cases are being confirmed may have the effect of maintaining a minimum level of contamination, or even maintaining the dynamics of contamination, which could explain the threshold effect or the possible resurgence of prevalence that is currently being observed.

When ANSES issues its opinion, a debate will be held on the scope of the criteria for confirmation. Initially, to follow up one of the recommendations of the FVO, confirmations will no longer be systematic, but will require a request to be made by the farmers.

Regarding Reunion Island, a specific plan of action is to be launched, including in certain specific cases the use of an attenuated live vaccine, to reduce the number of holdings contaminated following a resurgence of *Salmonella* Typhimurium. The ultimate objective is to reduce the prevalence of *Salmonella* Typhimurium, which remains high in this overseas *département*.

Regarding the Drôme, the *département* in mainland France concerned by high prevalence of *Salmonella* Enteritidis since the implementation of the control programmes, the action plan implemented since 2010 aimed at reducing these prevalences in both the layer and broiler sectors has not yielded the expected results, despite the efforts undertaken by the State services. A mission scheduled in 2015 is seeking to assess the implementation of the action plan, to adapt it if necessary by prioritising the actions to be taken according to the priorities chosen and to verify compliance with the regulatory control measures for *Salmonella* and biosecurity.

It should not be forgotten that the ultimate objective of *Salmonella* surveillance in poultry flocks is to prevent the occurrence of foodborne illnesses (FBIs). Indeed, for the years 2009 to 2013, eggs, egg products and poultry meat were responsible for nearly 37% of all *Salmonella* FBIs, with *Salmonella* Typhimurium, Enteritidis and the monophasic variant 1,4,[5],12,i :- of Typhimurium accounting for more than 70% of the serotypes reported. Since 2012, this variant has been at the origin of more FBIs than serotype Enteritidis.

In the farms monitored, *Salmonella* Typhimurium variants represent one-quarter of the cases of *Salmonella* Typhimurium (*sensu lato*) infections. The variant 1,4,[5],12,i :- has been increasing constantly for the last few years, accounting for two-thirds of variants of Typhimurium. With nine cases, the variant 1,4,[5],12,-:1,2 has represented a non-negligible proportion of infections since 2013. Unlike in previous years, the non-motile variant 1,4,[5],12,-:- was present in 2014 with 24 cases in broiler poultry. Fully consistent with the development of *Salmonella* FBIs, the control measures are therefore justified in the first place for *Salmonella* Typhimurium, Enteritidis and the monophasic variant 1,4,[5],12,i:- of Typhimurium. For the other two variants, not included so far in the surveillance and control programmes at the European level, the trends should be monitored in the coming years.

The analyses carried out at the end of a flock production cycle before slaughter help to monitor all non-regulated *Salmonella* serotypes. The control programme, focused on regulated diseases, is thus also useful for monitoring non-regulated serotypes that, once emergent, can become high zoonotic risks, such as *Salmonella* Kentucky CIPR which shows multiple drug resistance (Guillon *et al.*, 2013).

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Surveillance of equine infectious anaemia: two outbreaks detected in the South of France in 2014

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Abstract

In 2014, the French network of 12 laboratories approved by the Ministry of Agriculture to perform the serological diagnosis of equine infectious anaemia (EIA), completed over 15,500 tests using Agar Gel Immuno-Diffusion (AGID). Twelve of these tests were found positive for EIA and involved two horses kept in the Gard *département*, in two towns approximately 3 kilometres away from each other. The surveillance plan implemented following the declaration of these cases led to the testing of 205 horses in the Gard, in addition to the two positive equids. Serological analyses of the 205 horses all gave negative results. These data indicate that no transmission of the virus was detected neither between horses in the stables where the two infected horses were found, nor to horses that had more or less prolonged contact with them, nor to horses that were kept in the vicinity. On the other hand, phylogenetic analysis of the isolates collected from the two infected horses shows that there was no connection between the two cases reported in the Gard in 2014. Even though these two cases were found only a few kilometres from each other, molecular characterization of their viral isolates showed that they were different and had no common origins. These data confirmed the information collected during field surveys that showed no epidemiological link between the two cases. The second case of EIA declared in 2014 in the Gard was detected because the holding where it occurred was inside the 4 km surveillance zone set up following the discovery of the first case.

Keywords

Category 1 health hazard, Regulated disease, Equine infectious anaemia virus, Surveillance, Equids

Résumé

Surveillance de l'anémie infectieuse des équidés : deux foyers détectés dans le Sud de la France en 2014

En 2014, l'ensemble des laboratoires agréés par le ministère de l'Agriculture pour le diagnostic sérologique de l'anémie infectieuse des équidés (AIE), au nombre de douze, ont réalisé plus de 15 500 tests d'immuno-diffusion en gélose (IDG). Parmi ces analyses, douze ont été trouvées positives pour l'AIE et concernaient deux équidés stationnés dans le département du Gard, sur deux communes distantes de 3 kilomètres environ. La surveillance mise en place suite à la déclaration de ces foyers a conduit au dépistage de 205 équidés, en plus des deux équidés trouvés positifs. Les analyses sérologiques réalisées à partir de ces 205 équidés ont toutes présentés un résultat négatif. Ces données indiquent donc qu'il n'y a pas eu de transmission virale détectée aux équidés des structures où étaient hébergés les chevaux infectés ni à ceux identifiés comme ayant eu des contacts plus ou moins prolongés avec eux ou stationnant dans une zone géographique proche. D'autre part, l'analyse phylogénétique des isolats prélevés sur les deux équidés infectés montre que les deux foyers déclarés dans le département du Gard en 2014 sont indépendants. En effet, même si ces deux foyers ne sont distants que de quelques kilomètres, la caractérisation moléculaire des isolats viraux montre qu'ils sont différents et ne présentent donc aucune origine commune. Ces données confirment les informations recueillies au cours des enquêtes de terrain qui ne montraient aucun lien épidémiologique entre les deux foyers. Le second foyer d'AIE, déclaré en 2014 dans le département du Gard, a été trouvé car la structure se trouvait à l'intérieur du périmètre de surveillance de 4 kilomètres mis en place suite à la découverte du premier foyer.

Mots-clés

Danger sanitaire de 1ère catégorie, maladie réglementée, virus de l'anémie infectieuse des équidés, surveillance, équidés

Equine infectious anaemia (EIA) is caused by the Equine Infectious Anaemia virus (EIAV) belonging to the *Retroviridae* family, genus *Lentivirus*, which also includes Human Immunodeficiency Virus (HIV), Bovine and Feline Immunodeficiency Viruses (BIV and FIV) and the visna-maedi virus.

EIAV infects only Equidae (horses, donkeys, mules and zebras). Following infection, Equidae remain infected for life and are contagious for other Equidae even when there are no clinical signs (Issel *et al.*, 1982). The bloodborne virus is transmitted from one animal to another mainly by biting insects or iatrogenically through contaminated needles or surgical equipment (Foil *et al.*, 1983; Hawkins *et al.*, 1973). Insects – primarily horse flies and stable flies – are mechanical vectors; although the virus does not multiply within the insect, the infectious virus can remain in its mouthparts for several hours after a bite. The virus is disseminated most effectively by this type of mechanical vector-borne transmission when horses are gathered for equestrian events, since horse flies and stable flies often stop feeding to finish their meal on another host.

In France, EIA is currently classified as a Category 1 health hazard (Ministerial Order of 29 July 2013). The economic and health consequences of this disease can severely impact the horse industry since all positive Equidae must be slaughtered and exports of Equidae to certain third countries can be called into question.

An overview of the current EIA surveillance and control system is presented in the Box provided.

The serological test for EIAV recommended by the World Organisation for Animal Health (OIE) is the agar gel immunodiffusion assay (AGID) known as the Coggins test (Coggins and Norcross, 1970) according to French standard NF U47-002 (AFNOR, 2010). This test is required only for certain imports and exports of Equidae and, in the case of a stallion, as one of a battery of tests prior to reproduction (IFCE, 2010); it can also be requested by a buyer during vetting. This is why EIA outbreaks are often detected by a practising veterinarian following suggestive clinical signs in a client's horse. This initial suspicion may lead to the screening of other seropositive Equidae nearby or with an epidemiological link, whether ill or asymptomatic.

Objectives of the surveillance programme

To detect EIA in Equidae throughout France.

The population monitored

Domestic Equidae (horses, donkeys, mules and hinnies) nationwide.

Surveillance procedures

Outbreak surveillance

Clinical surveillance relies on owners, veterinarians and the network of laboratories accredited to perform serological analyses to detect EIA. It also relies on the results of autopsies. The Ministerial Order of 23 September 1992 defines a suspicious case as any Equidae showing marked signs of listlessness (typhoid-like state), anaemia or weight loss accompanied by fever. Any Equidae testing positive with an agar gel immunodiffusion assay (AGID, better known as the Coggins test) is considered to be infected.

RESPE, the French network for epidemiological surveillance of equine diseases, supported by a "sentinel" veterinary network, set up a "Piro-like" sub-network on 1 May 2014. Any sentinel veterinarian detecting an Equidae with fever associated with at least one other clinical sign on a predefined list (including loss of appetite, listlessness, loss of condition, oedema, petechiae, etc.) must take a blood sample to screen for four pathogens, including EIA virus.

Programmed surveillance

Programmed surveillance includes several different measures:

- Breeding stallions are mainly monitored systematically.
  - > All stallions used in artificial insemination programmes are tested on a regular basis. If the semen is to be sent to another European Union country, a negative Coggins result must be produced in the two weeks preceding the first collection. If it is for the national market, a negative Coggins result must be produced in the three months before the first collection during the first breeding season, then every three years before the breeding season.
  - > Stallions used naturally in certain breeds must also be tested in accordance with their stud book recommendations. A negative Coggins result must be produced in the three months prior to the first service then every three years. In 2014, this screening was mandatory for the following breeds: Thoroughbred, AQPS, French Trotter, Arab and DSA, Anglo-Arab and DSAA, French Saddle, Corsican horse, French Saddle Pony, New Forest, Haflinger, Welsh, Connemara, Merens and Shagya. This surveillance is coordinated by the French horse and riding institute, IFCE.
- All exported Equidae must be tested in accordance with the health requirements of the importing country. Imported Equidae must also be screened for EIA according to the exporting country, the type of importation (temporary, permanent or readmission after temporary export) and the type of use (slaughter or other). Screening is not mandatory for Equidae transported within the EU except for Equidae from Romania. This measure was introduced in 2010 (2010/346/EU) following several cases of EIA in the United Kingdom, Belgium and France in 2009 and 2010 among Equidae imported directly from Romania.

"Voluntary" surveillance

It is recommended to test for EIA whenever there is a change of ownership, particularly as the disease is considered a redhibitory defect. Tests of this kind can detect asymptomatic carriers which play an important role in spreading the disease because they act as reservoirs for the virus. A diagnosis must be established and actions to cancel the sale, when necessary, must be undertaken within 30 days of delivery. Several auction houses require any Equidae on sale to have had a negative Coggins result in the weeks preceding the sales.

Animal health rules

Any clinical suspicion or confirmation by the results of analysis by an accredited laboratory must be declared to the Departmental Directorate for Protection of the Population (DDecPP) and the Directorate General for Food (DGAL). Any clinical suspicion or positive test by an accredited laboratory must be sent to the National Reference Laboratory (NRL) for confirmation, which for EIA is ANSES's Dozulé Laboratory for Equine Diseases.

Should EIA be suspected, the veterinarian must isolate the animal and check its identity. The veterinarian immediately informs the DDecPP and takes a sample of serum, which is sent to an accredited laboratory for analysis along with comprehensive contextual data.

When EIA is confirmed, a declaration of infection (APDI) is issued, thus initiating health control measures. An epidemiological survey is led by the DDecPP, supported by the NRL. All the Equidae on the site of the outbreak are screened, as are all those considered at risk, i.e. animals generally within two kilometres of the outbreak and/or having been in direct contact with the infected Equidae. The site is visited by a mandated veterinarian who must list and identify all the Equidae present as needed. All equine movements in or out are prohibited. Buildings must be treated to eradicate all insects and thoroughly disinfected. All the Equidae on site must have a Coggins test and any positive animals are isolated and euthanised within 15 days. An epidemiological survey is conducted to find and test all the Equidae having been in contact with the infected animals. The Equidae present on the site of the outbreak are regularly tested (Coggins test). The health control measures are only lifted when all the Equidae have shown a negative result on two Coggins tests performed on two serum samples taken three months apart. The State financially contributes to veterinary visits in the event of a suspected or confirmed infection and to disinfection and insect control operations. It assumes EIA diagnostic costs and provides compensation to owners of horses slaughtered as part of an APDI.

Regulations

Outbreak surveillance and programmed surveillance in the event of an outbreak with health control measures

Ministerial Order of 23 September 1992 describing the sanitary measures for equine infectious anaemia.

Ministerial Order of 23 September 1992 determining the financial measures related to the health control measures for equine infectious anaemia.

Programmed surveillance of breeding stock

Ministerial Order of 4 November 2010 determining the conditions for health approval of Equidae semen collection centres and the animal health conditions for semen trade within the Community.

Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(I) to Directive 90/425/EEC.

Stud book regulations available on the IFCE website: http://www.haras-nationaux.fr/information/reglementation/races-et-stud-books.html.

Programmed surveillance of EU trade, imports and exports

Council Directive 2009/156/EC of 30 November 2009 on animal health conditions governing the movement and importations from third countries of Equidae.

Commission Decision of 6 January 2004 establishing the list of third countries and parts of territory thereof from which Member States authorise imports of live Equidae and semen, ova and embryos of the equine species and amending Decisions 93/195/EEC and 94/63/EC.

Commission Decision 92/260/EEC of 10 April 1992 on animal health conditions and veterinary certification for temporary admission of registered horses.

Commission Decision 93/195/EEC of 2 February 1993 on animal health conditions and veterinary certification for the re-entry of registered horses for racing, competition and cultural events.

Commission Decision 93/196/EEC of 5 February 1993 on animal health conditions and veterinary certification for imports of Equidae for slaughter.

Commission Decision 93/197/EEC of 5 February 1993 on animal health conditions and veterinary certification for imports of registered Equidae and Equidae for breeding and production.

Commission Decision of 18 June 2010 on protective measures with regard to equine infectious anaemia in Romania.

The animal health requirements of third countries are available at: https://teleprocedures.franceagrimer.fr/Expadon/.

Other

List of accredited laboratories for the detection of EIA: http://agriculture.gouv.fr/maladies-animales.

Health overview in 2014

In 2014, the network of twelve diagnostic laboratories accredited to perform serological analyses for EIA carried out 15,585 serological analyses; the breakdown of the reasons for undertaking these analysis (breeding tests (stallions), animal imports and exports, sales) is not known. Of these analyses, there were 5,021 involving mares, 4,516 involving stallions and 704 involving geldings. The sex of the Equidae for the 5,344 remaining analyses was not specified in the analysis request.

In 2014, three analyses undertaken by two different accredited laboratories were sent to the NRL (ANSES – Dozulé Laboratory for Equine Diseases) for confirmation. One of the three samples was declared negative by the NRL. The other two were confirmed as positive for EIA. These two samples tested positive in twelve analyses (they were tested several times by the accredited laboratory and the NRL) and came from two Equidae in the Gard *département*. The number of analyses carried out in 2014 was stable in relation to 2013, when 15,274 AGID analyses had been undertaken by the network of accredited laboratories. In 2013, two Equidae (donkeys) kept on Réunion Island (Hans *et al.*, 2014) were also found to be positive for EIA.

Reported outbreaks in the Gard *département*

The first EIA outbreak reported in October 2014 in the Gard *département* involved a stable of fifteen horses with different origins (including France, Netherlands, Spain and Portugal). The index case was a six-year-old Friesian stallion. This stallion was tested for EIA in the context of official mating controls. Its owner wanted to use it for mating to start breeding Friesian horses. This stallion was confirmed as positive for EIA by the NRL on 3 October 2014 and was euthanised on 14 October. In accordance with the regulations, this outbreak was placed under prefectural declaration of infection (APDI) and the fourteen remaining Equidae in the stable were tested for EIA. None were found positive. The investigation showed that the index case had come from the Netherlands with a mare of the same breed, found negative for EIA, and had been purchased at the age of around six months. Over the last five years, these two Equidae had been used for some rides, but according to the owner they had never left the Gard *département* and never showed any clinical signs.

Epidemiological investigations identified stables in the Gard with Equidae within four kilometres from this first outbreak; these stables were placed under prefectural monitoring order (APMS). The index case had been hosted in various pastures located within one kilometre of its town of residence. All of these pastures were considered part of the outbreak. A three-kilometre radius was thus established around this one-kilometre area. After the index case was euthanised and field investigations were undertaken, 205 Equidae were tested for EIA in this four-kilometre radius and another Equidae was found positive for EIA in a neighbouring town.

This second outbreak was detected thanks to the sero-epidemiological investigation launched further to the detection of the first outbreak located approximately four kilometres away. The second Equidae positive for EIA was a 21-year-old mare of unknown origin that had been kept in the town for twenty years. This Equidae was confirmed as positive for EIA by the NRL on 24 October 2014. None of the other seventeen Equidae belonging to the same stable were found positive for EIA.

In addition to identifying equestrian stables and Equidae with an epidemiological link to the reported outbreaks in order to establish appropriate surveillance measures, the aim of the epidemiological investigations was to determine whether there was an epidemiological link between the two 2014 outbreaks, and between these two 2014 outbreaks and those that had occurred in the Vaucluse and Gard *départements* in 2012 (these outbreaks were geographically close: they were located approximately 25 and 50 kilometres from the town of the first 2014 outbreak) and/or those occurring in the Var in 2009.

The information collected further to the field investigations did not indicate any epidemiological link between the two cases of EIA. No contact between these Equidae and/or these two stables could be demonstrated. Likewise, it was not possible to link these two outbreaks to those reported previously in the same region in 2009 in the Var or in 2012 in the Gard and Vaucluse.

Molecular epidemiology

To genotype the EIA strains isolated from the two Equidae testing positive for infection in 2014, the *gag* gene – about 1,400 nucleotides long – was sequenced. The two viral isolates were characterised from tissue samples (spleen, liver, mesenteric lymph nodes) taken from the two Equidae after euthanasia.

A phylogenetic analysis was undertaken for comparison and classification of the viral isolates in relation to those previously encountered in France and those described in the literature (Figure 1). This phylogenetic analysis, undertaken with MEGA 5.1 software, showed that the isolates characterised from the Friesian stallion and the mare of unknown origin in the Gard *département* in 2014 were different.

However, the viral isolate characterised from a sample from the index case was similar to those isolated in the Vaucluse in 2012. That said, the field investigations were unable to establish an epidemiological link between this Equidae and those tested in 2012 in the Vaucluse, in a town located approximately 25 kilometres north of that of the 2014 index case.

Likewise, the viral isolate characterised from the mare of unknown origin was similar to those isolated in 2009 in the Var *département* where sixteen horses were found to be infected. Once again, field investigations and visits found no epidemiological link between these two outbreaks. According to the owner, the mare of unknown origin was purchased approximately twenty years previously from a horse dealer based in the Vaucluse, who had indicated that she came from Romania. An analysis of the phylogenetic tree seems to support the assumption of a local infection of the Equidae, on French soil, and not an infection "imported" from Romania twenty years prior.

Financial overview

In 2014, the DDecPP of the Gard spent approximately €22,000 on the control of EIA. This amount does not take into account the time spent by staff involved in the implementation and monitoring of epidemiological investigations. While non-negligible, it remains limited compared to levels of spending for diseases of other species (tuberculosis, bluetongue, etc.). The low incidence of EIA, euthanasia of infected Equidae only, and the capping of compensation for the owners of euthanised animals explain this limited financial cost.

However, the person-time devoted to the management of EIA, including field interventions and the administrative management of records, is far from negligible.

Field work is particularly complicated due to a lack of reliable information regarding the location and identification of horses, the need to have up-to-date contact details for owners, and the fact that multiple parties are likely to be involved, since each owner is free to choose a veterinary practitioner. The administrative management of EIA is extremely cumbersome because investigations often involve several individuals (each one owning a small number of Equidae). As such, various prefectural monitoring orders (APMS) have to be written and then lifted. Furthermore, EIA management is a long-term task, since it consists in establishing and monitoring the results of two series of consecutive analyses undertaken three months apart.

In this case, the investigations and analyses involved 205 Equidae, half of which were held in three stables and the other half of which were spread out across 35 stables. The DDPP's staff spent over thirty full working days managing these two outbreaks.

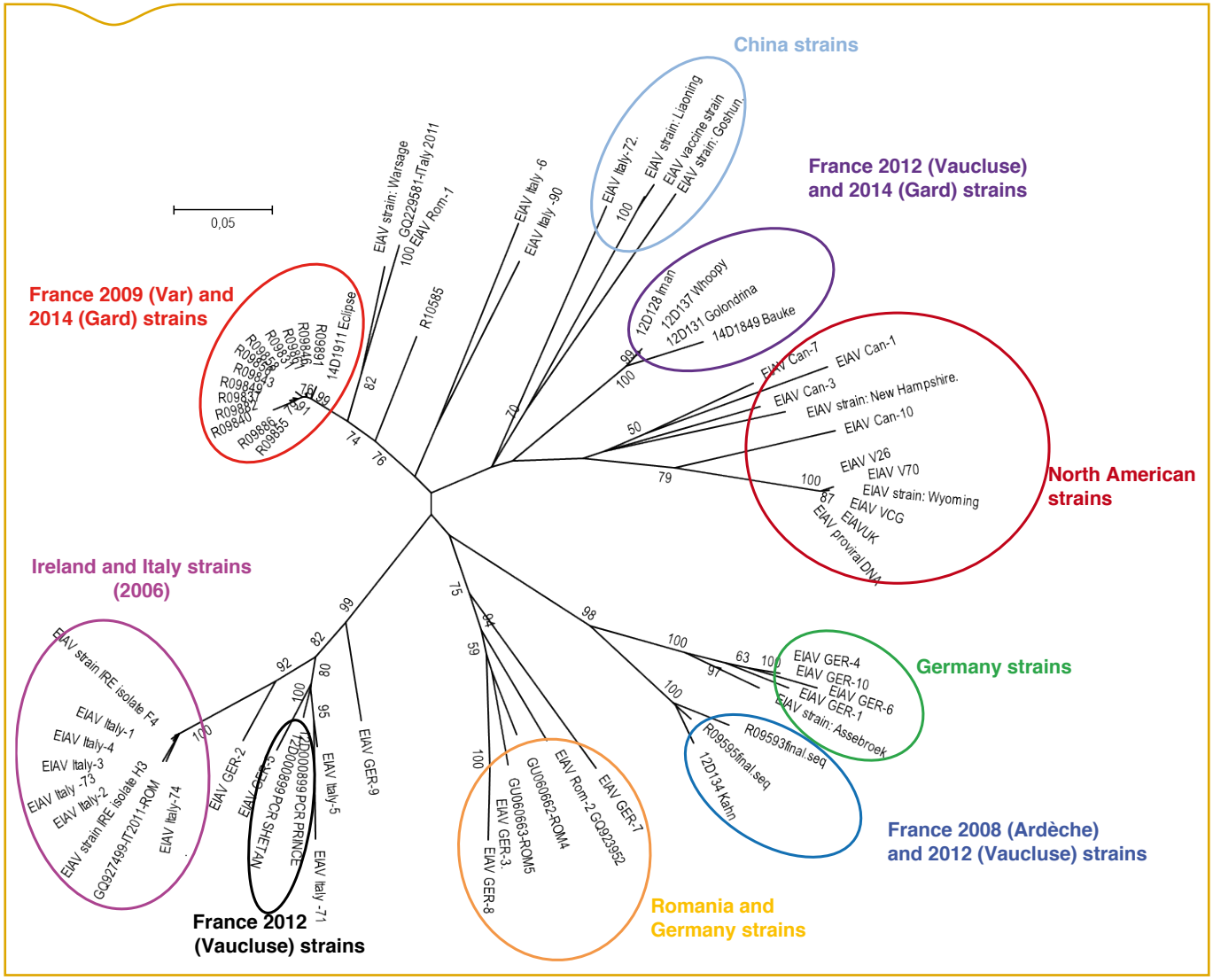

Figure 1. Phylogenetic analysis of nucleotide sequences of the *gag* gene in 69 Equine Infectious Anaemia virus isolates (1,400 nucleotides), including 24 isolated in France between 2007 and 2014

Discussion and conclusion

EIA is transmitted through the transfer of contaminated blood, either by biting insects (horse flies and stable flies) or iatrogenically (use of dirty syringes/needles, etc.). Epidemiological investigations show that, most of the time, spread of the virus within an equine population from an asymptomatic Equidae is low. Health risks and the risk of disease transmission are greater when there are infected Equidae showing clinical signs (together with high viraemia leading to maximum risk of viral transmission) and when a stable with hundreds of horses is involved, even though the virus's natural rate of transmission between animals fortunately remains fairly low.

Currently, only stallions used for artificial insemination have to be tested for EIA every three years, in addition to those used for natural mating in several breeds. There is no available information on the health status of the national equine population with regards to EIA. Information is obtained only when sporadic outbreaks occur. In this case, field investigations and mandatory testing shed light on the seroprevalence of EIA in one or more towns. Such investigations provide a small-scale snapshot of the situation but do not give an overview of the status of the French equine population, particularly in geographic areas with poor reporting and little or no identification of horses, and for sub-populations of Equidae that are very rarely monitored in the context of active surveillance programmes (mating, international trade, sales) such as recreational horses, heavier breeds, the meat industry, donkeys, etc.

Moreover, this is the first time since 2007 that viral isolates responsible for EIA outbreaks in France (Figure 1), separated in time, have very likely been epidemiologically related. The viruses characterised in 2014 in the Gard were very similar to those isolated in the Vaucluse and Var in 2012 and 2009 respectively. The data collected up to 2012 had supported unrelated sporadic outbreaks. Each new outbreak highlighted a new viral isolate, different from those of outbreaks in previous years. An analysis of the phylogenetic data obtained in 2014 shows that there are at least two separate viral isolates circulating in the equine population in the South of France. It should be noted that since 2008, reported EIA outbreaks had always been located in separate *départements*. The outbreak reported in 2008 was located in the Ardèche *département* (Rème *et al.*, 2009). The 2009 outbreak occurred in the Var (Hans *et al.*, 2010). In 2010, Dordogne, Gironde and Lot-et-Garonne were affected in addition to the North of France (cases "imported" from Romania) (Ponçon *et al.*, 2011). In 2012, outbreaks were discovered in the Vaucluse and Gard (Hans *et al.*, 2013). And in 2013, the only reported outbreak was located on Réunion Island in the Indian Ocean (Hans *et al.*, 2014). In 2014, this was the first time since 2008 that a second EIA outbreak was reported in the same *département* (the Gard), even though the two 2012 and 2014 outbreaks occurred approximately 50 kilometres from one another. In addition, the first 2014 outbreak was located only 25 kilometres from the one reported in the Vaucluse in 2012. This geographic proximity could explain the characterisation of two very similar isolates with the same origin. This seems to indicate that the infected Equidae detected in 2012 and 2014

may have had direct contacts resulting in viral transmission. However, the epidemiological investigations undertaken in the field did not find any direct contacts. These Equidae also could have been held in the same stables, causing them to become contaminated through contact with an infected animal. Likewise, the epidemiological investigations did not find any such stables. Furthermore, the 2014 index case was a five-and-a-half-year-old Equidae and the owner, who had had it for five years, indicated that this Equidae, purchased in the Netherlands, had never left the town. One assumption that could connect the 2012 outbreak reported in the Vaucluse to the first outbreak reported in the Gard in 2014 is that there was a common parking area in the South of France before the index case arrived in the Gard at the end of 2008. This possibility could not be confirmed due to a lack of reliable testimonials for a period covering the last five years.

No links were found between the second outbreak in the Gard in 2014 and the outbreak reported in the Var in 2009. However, the two stables in question were recreational stables whose horses are mainly purchased from horse traders in the South of France selling horses whose origin often cannot be verified. These horse dealers generally do not keep records showing their purchases and sales or the origin of the horses purchased/sold.

In conclusion, the EIA outbreaks reported over the last few years in France have primarily been located in the south-east of France and the infected Equidae have mainly been recreational horses. This population is not subject to any regulatory surveillance for EIA, unlike populations of breeding and sport Equidae. It is thus extremely difficult to assess the prevalence of the disease within this population. Nonetheless, it would seem that the EIA virus is circulating, albeit discreetly, in the equine population intended for recreational activities in the south-east of France.

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Monitoring of **Category 1 health hazards for fish** in 2014: a stable situation

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Abstract
The concomitant intensification of single-species breeding in the aquaculture industry and the trade of fish and eggs have complicated fish farm health management in production areas by encouraging the emergence and spread of pathogens such as rhabdoviruses, responsible of Viral haemorrhagic septicemia (VHS) and Infectious haematopoietic necrosis (IHN). Appropriate monitoring was set up starting in the 1990s in order to more effectively assess the health situation. Surveillance applies to salmonid farms (which rank first in French fish production), as well as to pond-based fish farming, due mainly to the presence of pike, a typical species for this biotope and a carrier of VHSV. In 2014, monitoring results confirmed the maintenance of a stable health situation in France with regard to VHS. Two silent outbreaks of IHN were detected and eradicated. Two outbreaks of Koi herpes virus (KHV) were also detected, confirming the contamination of our national carp population by this disease which has been detected regularly for over a decade.

Keywords
Fish, Viral diseases, Category 1 health hazards, VHS, IHN, KHVD, ISA

Résumé
Surveillance des dangers sanitaires de première catégorie pour les poissons : une situation stable pour l'année 2014
La généralisation de l'élevage mono-spécifique intensif dans la filière piscicole et l'intensification des échanges de poissons et semences a complexifié la gestion sanitaire des élevages dans les bassins de production en favorisant l'apparition et la diffusion d'agents pathogènes tels que les rhabdovirus, responsables de la septicémie hémorragique virale (SHV) ou de la nécrose hématopoïétique infectieuse (NHI). Une surveillance appropriée a été mise en place à partir des années 1990 pour tenter de mieux appréhender cette situation sanitaire. Cette surveillance concerne en premier lieu les élevages de salmonidés (qui occupent la première place dans la production piscicole française), mais également la pisciculture d'étang, en raison de la présence d'une espèce typique de ce biotope, sensible au virus de la SHV : le brochet. Les résultats de la surveillance en 2014 confirment le maintien d'une situation sanitaire stable sur le territoire vis-à-vis de la SHV. Deux foyers silencieux de NHI ont été détectés et éradiqués. Deux foyers d'herpèsvirose de la carpe (HVC) ont été détectés, confirmant la contamination de notre cheptel national de carpes par cette maladie détectée régulièrement depuis plus d'une décennie.

Mots-clés
Poissons, maladies virales, dangers sanitaires de 1ère catégorie, SHV, NHI, HVC, AIS

In fish, four non-exotic viral diseases, previously classified as notifiable diseases with compulsory control measures (NDCCM), are henceforth defined as Category 1 health hazards, under the terms of Decree 2012-845 of 30 June 2012 (Table 1). Three of these diseases are endemic in France. Among them, viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) are currently the most important. The third, Koi herpes virus (KHV), has been detected sporadically in France since 2001, and the number of reports seems to be on the rise since 2011 (Papin *et al.*, 2012). France is officially free of infectious salmon anaemia (ISA).

These regulated diseases have been subject to surveillance since Directive 2006/88/EC came into effect, in response to the health requirements set by European regulations to protect fish farms and to facilitate trade. Note that the list of diseases (Annex IV, Part 2 of this Directive) was amended by an Implementing Directive (2014/22/EU) during 2014, with the introduction of a distinction between genotypes of the ISA virus. For ISA, this list now targets only the pathogenic strains with a deletion in the highly polymorphic region (HPR) of the viral genome.

Table 1. Classification of regulated fish diseases, their pathogens and the health situation in France on 31 December 2014

Disease	Pathogen	Regulations	Health situation on 31/12/2014
Viral haemorrhagic septicaemia (VHS)	Rhabdovirus	Category 1 health hazard (formerly NDDCM)	Present
Infectious haematopoietic necrosis (IHN)			Present
Koi herpes virus disease (KHVD)	Herpesvirus		Present
Infectious salmon anaemia (ISA) HPR-deleted genotype	Orthomyxovirus		Absent

Surveillance of these diseases is based on a dual system: mandatory surveillance (outbreak and programmed surveillance) and a voluntary scheme (targeted surveillance through programmes to achieve disease-free status) (see Box).

Results of surveillance in 2014

In the framework of outbreak and programmed surveillance, a total of 2,058 analyses (1979 by cell culture and 79 by PCR) were performed by the accredited laboratories and the NRL in 2014 (a rise of 2.1% compared with 2013 and of 17.4% compared with 2012; source NRL).

Surveillance of VHS

Three outbreaks of VHS in rainbow trout were reported in 2014 in the framework of outbreak surveillance, one in the Meuse *département* and the two others in Moselle. The last two outbreaks, which occurred in neighbouring farms, were epidemiologically related, as the two viral strains isolated were genetically identical. The phylogenetic analysis of viral strains (Figure 1) also shows that the two viruses isolated in the Meuse and the Moselle are almost identical and very probably have a common origin. The epidemiological investigation conducted by the DDecPPs of the Meuse and the Moselle as well as by the aquaculture resource person for the region based at the DDecPP of the Meuse, with

assistance from the DDecPP of the Territory of Belfort, revealed that pike are strongly suspected to be at the origin of the contamination in the Meuse. This investigation was not able to identify the precise origin of the original outbreak, however.

Comparisons of the sequences of the gene coding for the viral glycoprotein (Figure 1) show that the same strain is probably at the origin of the three outbreaks: 99.8% similarity between the strain isolated in the Meuse and those from the Moselle outbreaks (as these last two sequences are 100% identical, they are represented by the single taxon TAC\FR-57\2014) was observed. They also confirmed strong similarity (98.4%) with a sequence isolated in 2004 from pike (Br.\Fr-63\2004) and a viral sequence isolated in the Vienne *département* in 2013 (TAC\Fr-86\2013).

Finally, the viral sequences isolated in 2014 are very close to a viral sequence isolated in Germany in 2002 (TAC\DE\2002) (Figure 1).

Surveillance of IHN

Two outbreaks of IHN detected through targeted surveillance were reported in 2014, without any clinical suspicion having been raised in the two farms affected. One outbreak was detected in the Doubs *département* following self-inspection and the other was detected in the Manche *département* following an analysis for the purpose of disease-free certification. Since the hatcheries of these farms benefited from effective isolation measures and the juvenile stages are thus protected from infection, the viral infection was latent and silent in the sub-adult and adult stages in both cases.

Surveillance of KHVD

Two outbreaks of KHVD were reported in 2014, following the observation of abnormal mortality in carp. One outbreak occurred in a pond in the Pas-de-Calais and the other in a private basin of Koi carp in Saône-et-Loire. The epidemiological investigation carried out by the relevant *départements* of the DDecPPs of Pas-de-Calais and Saône-et-Loire, and the aquaculture resource person for the region, based at the DDecPP of Pas-de-Calais, concluded that contamination may have originated with recently-imported Koi (ornamental) carp.

Disease-free certification of fish farms regarding VHS and IHN

Four additional fish farm units or areas (i.e. a total of nine fish farms) were declared free of VHS and IHN in 2014. On 31 December 2014, 408 fish farms were certified free of VHS and IHN out of a total of 621 freshwater aquaculture sites identified in 2008 (Agreste, 2011). To these sites can be added an unknown number of ponds, estimated to be in the tens of thousands.

Costs

With data available this year for all 101 *départements*, the sum of €4,452 was spent in 2014 under the surveillance programme to finance outbreak-related visits (veterinary fees and analysis costs) and €13,067 for visits to evaluate the disease-free status of fish farms, including €12,475 in analysis costs. The cost of these health control measures was €38,457 (compensation for slaughter or disinfection). All these operations cost the state a total of €68,715 in 2014, excluding rendering costs.

Discussion

The number of outbreaks of VHS in 2014 returned to a level comparable to previous years (Figure 2), after the peak of 2013 when an infected farm had transmitted the virus to many other sites, which each became secondary outbreaks. (Roman *et al.*, 2014). The epidemiological investigation following the outbreak in the Meuse in 2014 incriminated pike as a vector, a species found in association with viral sequences isolated in 2014 that segregate together in the phylogenetic analysis (Figure 1). The role of pike as a reservoir of the VHS virus, discussed recently (Roman *et al.*, 2013), is here again a topical issue. It is therefore recommended that salmon farming be rigorously separated from the pond-based fish-farming sector in order to reduce the risk of contamination. The strong similarity between the viral sequences analysed in strains in 2014 and a sequence from a viral strain isolated in Germany suggest epidemiological links between the farms of the two countries.

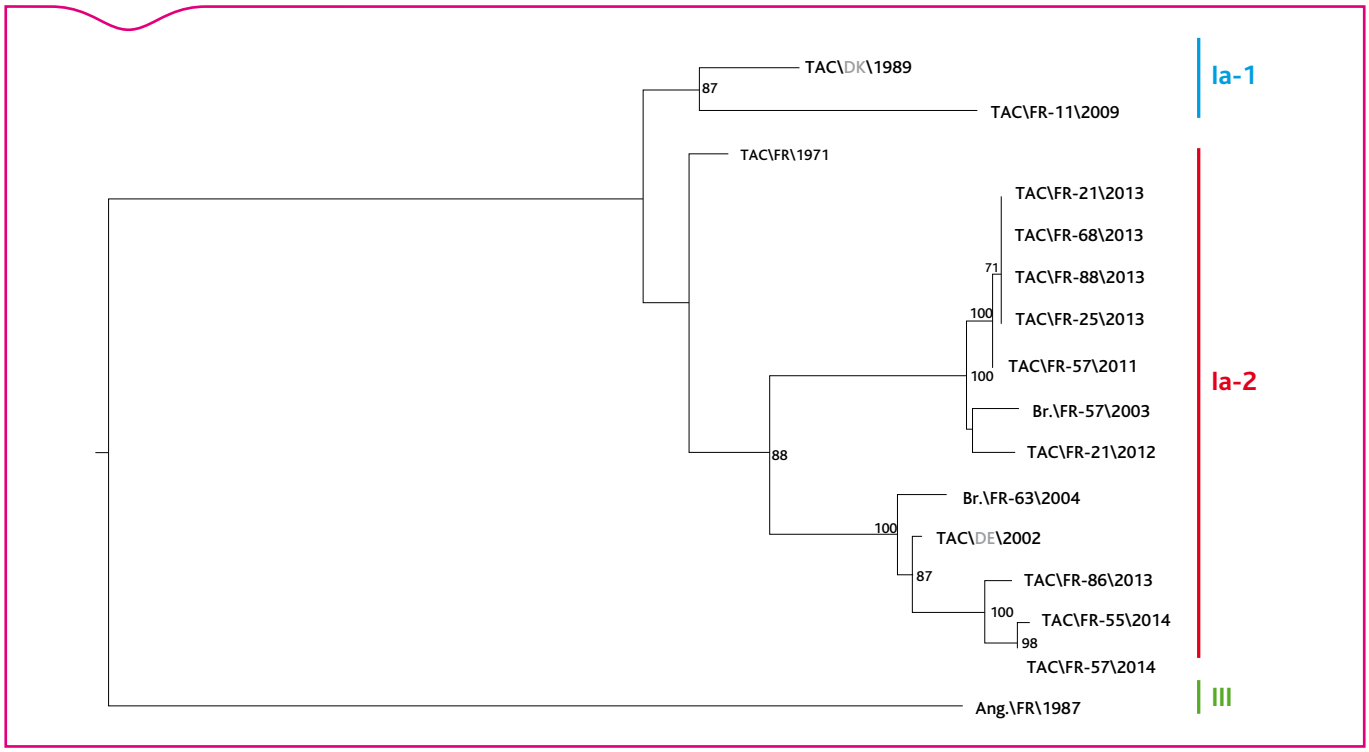

Figure 1. Groups of sequence similarity for the envelope glycoprotein (1524 nt) of the VHS virus.
Each isolate is identified by the following code: Host species (in French)/country-*département*/year. Br: Pike; TAC: rainbow trout; Ang: eel. Phylogenetic analysis created with SeeView in PhyML with 100 bootstrap replicates, using the GTR model. Only bootstrap scores greater than 70 are shown. The classification into genogroups (I, II or III) is based on Kahns *et al.* (2012).

The suspected under-declaration of IHN suggested in 2014 (Roman *et al.*, 2014) is confirmed (Figure 2). This disease can go unnoticed during clinical examination if the more susceptible juvenile stages are protected by careful containment. The factors that tend to foster under-reporting of outbreaks include the low number of veterinarians specialised in aquaculture, the lack of awareness of some fish farmers and fish keepers, professionals and amateurs alike, and the lack of compensation for the value of fish lost to disease when the fish farmer was not involved in a programme to achieve fish disease-free status.

The generalisation of animal health certification and the multiplication of inspections for disease-free certification should progressively improve the detection of regulated diseases, in particular IHN, as a consequence of the resulting surveillance schemes.

KHV was sporadically detected in France in 2001 and 2002, but recurrent outbreaks have been reported since 2008, suggesting that either the virus has become established in France or infected fish are repeatedly being introduced. This disease should henceforth be monitored more closely.

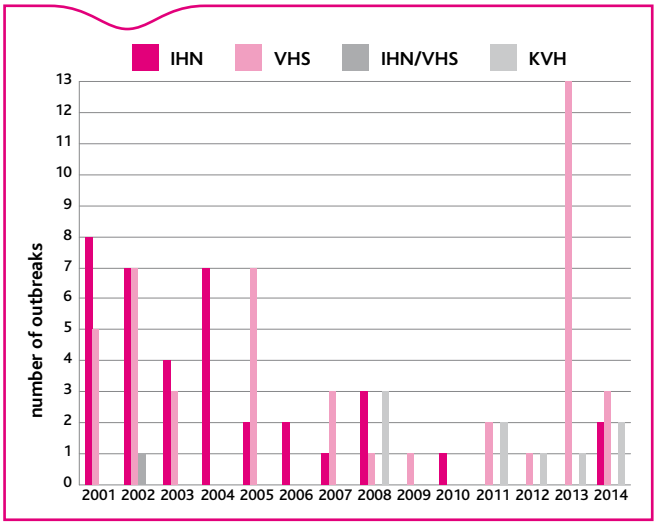

Figure 2. Changes in the number of outbreaks of regulated fish diseases reported since 2001

Box. Surveillance and health control measures for regulated fish diseases

Objectives of the surveillance programme

- To early detect any outbreak of a regulated disease.
- To confirm France's official disease-free status for infectious salmon anaemia (ISA).
- To grant "disease-free" status to aquaculture areas and farms (fish farms, pond-based aquaculture) in order to protect farms (from VHS, IHN and KHV) and facilitate trade.

Monitored population

Farmed and ornamental fish.

Surveillance procedures

Outbreak surveillance

Reporting of any suspected or confirmed cases to the DDecPP (or the DDAAF in French overseas *départements*), in the event of abnormal mortality or observation of clinical signs suggestive of a regulated disease. If any suspicion is declared, samples are taken for first-line analysis by one of the seven accredited laboratories and, if necessary, results are confirmed by the NRL at the ANSES Ploufragan-Plouzané laboratory (by identification of the virus using cellular and/or molecular methods).

Programmed surveillance

Since 2011, outbreak surveillance has been supplemented by the implementation of an animal health certification programme for aquaculture farms. This certification, which is mandatory, is issued by the relevant local authority (DDecPP or DDAAF). It requires that the person responsible for the aquaculture farm carry out a risk analysis and draw up a corresponding health surveillance scheme that includes regulated diseases. Clinical inspections by an accredited veterinarian and audits by the relevant authority are scheduled at a frequency depending on the level of risk determined for the fish farm (from one per year to every 4 years in the framework of a procedure to maintain disease-free status for zones or compartments where the risk level is high or low, respectively). Samples are analysed in the event of suspicion.

Targeted surveillance: (voluntary) disease-free certification programmes of fish-farming zones and compartments

Professionals may set up voluntary programmes for acquiring "disease-free" status focused on a single farm or a larger area including several farms and natural aquatic areas, as stipulated in EU regulations. The farmer may choose either a short programme with extensive sampling (two clinical inspections and two samples of 150 individuals each, once a year for two years), or a longer programme with less intense sampling (two clinical inspections of 30 individuals each, once a year for four years). In France, these programmes currently only involve VHS and IHN. The list of aquaculture zones and compartments certified free of VHS and/or IHN can be consulted on the MAAF website, at the following address: http//agriculture.gouv.fr/maladies-des-animaux-aquatiques.

Genetic monitoring

All strains of VHS and IHN virus isolated in France are collected by the NRL. The gene encoding the envelope glycoprotein of the VHS virus is systematically sequenced. More recently, the same procedure has been introduced for the IHN virus. A comparison of these sequences sometimes reveals multiple similarities that can be traced back to a common ancestor strain. These genetic studies are often useful in epidemiological investigations.

Health control measures

If an outbreak of a regulated disease is detected, health control measures are implemented (in compliance with Directive 2006/88/EC, transposed into French law by the decree of 4 November 2008). In the event of suspicion, the DDecPP or the DDAAF issues an APMS (prefectural monitoring order). If the infection is confirmed by an accredited laboratory and/or the NRL, the infected fish farm is placed under an APDI (prefectural declaration of infection), with measures for eliminating dead fish or those showing clinical symptoms and for draining, cleaning and disinfecting ponds. An epidemiological investigation is also carried out.

Regulatory References

Council Directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals. Please note that the rules for the application of this Directive have been adopted recently by the EU, detailing the procedures for surveillance and certification and proposing an update of the analytical methods used. These rules should enter into application in the course of 2016.

Order of 4 November 2008, on the health control measures applicable to animals and products in the aquaculture sector and the prevention of certain diseases in aquatic animals and the measures for combating these diseases.

Order of 8 June 2006 amended, on the animal health qualification or authorisation of primary production units, or businesses placing on the market products of animal origin or foodstuffs containing products of animal origin.

At European level, the review of surveillance data for 2013 (EURL data: http://www.eurl-fish.eu/) shows that cases of VHS and IHN are probably under-declared in a number of countries, with respective totals of 52 and 54 establishments (out of an overall number estimated at 8,896) considered infected. Same situation for KHV, with 50 fish hatcheries (out of 11,831) listed as infected on 31 December 2013.

For the countries of Northern Europe, the most problematic and common pathologies for 2014 were pancreatic disease (alphaviruses), Amoebic gill disease or AGD (pathogen = *Paramoeba perurans*) and Winter Ulcer Disease (*Moritella viscosa*). Sea lice continue to be a major problem. Continental Europe has been particularly affected by the aeromonases (*Aeromonas salmonicida, hydrophila*, etc.), Enteric Redmouth Disease (*Yersinia ruckeri*), flavobacteriosis (rainbow trout fry syndrome: *Flavobacterium psychrophilum*), and AGD, pathologies shared with the Mediterranean countries, which have also reported a number of cases of lactococcosis (*Lactococcus garvieae)* and nodavirosis (a virus causing encephalopathy and retinopathy) in marine fish farms.

Acknowledgements

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Surveillance report on **honeybee** (*Apis mellifera*) **diseases and disorders** in 2014

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Abstract
The surveillance of notifiable bee diseases includes diseases found in France such as American foulbrood, varroasis, nosemosis caused by *Nosema apis*, the Asian hornet, and two exotic pathogens, *Tropilaelaps spp.* and *Aethina tumida*. Several surveillance systems described in this article contribute to the surveillance of honeybee diseases and colony losses. The European Epilobee/Resabeille programme has studied some of these diseases, and is closely linked to the surveillance scheme for bee disorders set up in 2002 to handle cases of acute bee mortality where intoxication by plant protection products was suspected. This scheme was renewed in October 2014. Results confirmed previous trends regarding the enzootic circulation of the first two diseases and showed that *Tropilaelaps spp.* and *Aethina tumida*, recently discovered in southern Italy, were not found in France. Massive acute mortality cases are also described.

Keywords
American foulbrood, Nosemosis, *Tropilaelaps*, Asian hornet, *Aethina*, Mortality, Depopulation, Bees, Surveillance, Intoxication

Résumé
Bilan de la surveillance des maladies réglementées et troubles des abeilles domestiques* Apis mellifera *pour l'année 2014
La surveillance des maladies réglementées des abeilles concerne des maladies présentes en France telles que la loque américaine, la varroose, la nosémose à Nosema apis*, le frelon asiatique ainsi que les deux agents pathogènes exotiques que sont* Tropilaelaps *spp. et* Aethina tumida*. Plusieurs dispositifs décrits dans cet article contribuent à la surveillance des maladies et des mortalités d'abeilles. Le programme européen Epilobee/Résabeille s'est notamment intéressé à certaines de ces maladies. Le dispositif de surveillance des troubles des abeilles mis en place en 2002 traite les cas de mortalités aiguës d'abeilles avec suspicion d'intoxication par des produits phytosanitaires ; ce dernier a été rénové en octobre 2014. Les résultats confortent ceux des années précédentes concernant la circulation sous forme enzootique des deux premières maladies, et confirment l'absence de* Tropilaelaps *spp. et d'*Aethina tumida *sur le territoire dans un contexte d'introduction d'*A. tumida *dans le Sud de l'Italie. Les mortalités massives aiguës sont également décrites.*

Mots-clés
Loque américaine, nosémose,* Tropilaelaps*, frelon asiatique,* Aethina*, mortalité, dépopulation, abeilles, surveillance, intoxication

Surveillance scheme for bee diseases and mortality

Surveillance of diseases and mortality of honeybees, *Apis mellifera,* is unusual in that it covers both biological and chemical risks.

Some of the biological risks are subject to regulations and are monitored particularly closely. Four health hazards have been classified as Category 1 in France: *Paenibacillus larvae* (American foulbrood), *Nosema apis* (nosemosis), *Aethina tumida* (small hive beetle) and the *Tropilaelaps clareae* mite; two others have been classified in Category 2*: Varroa destructor* (varroasis) *and Vespa velutina* (Asian hornet) (Decree 2012-845 of 30 June 2012 and Ministerial Order of 29 July 2013). *Paenibacillus larvae*, *Varroa destructor* and the two exotic pathogens (*A. tumida* and *Tropilaelaps spp.*) are also regulated at European level by Regulation (EU) no. 206/2010 and the Directives 92/65/EEC and 82/894/EEC, and at international level by the Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE) (Table 1).

Each monitoring scheme for bee diseases and mortality funded or subsidised by the State in 2014 has a specific range of actions, described in Box 1.

A key event for 2014 was the arrival of the small hive beetle, *Aethina tumida,* in Italy. A total of 61 outbreaks were discovered during the last four months of 2014 in Sicily and Calabria. The reinforced surveillance schemes implemented are described in Box 2.

Health inspections

Health inspections are carried out jointly, and depending on the nature of the missions, by staff of the departmental directorates for protection of the population (DDecPPs) or of the regional food authorities (SRALs), by specialist veterinarians and bee health inspectors (ASAs). On 15 October 2014, the organisation of the ASAs was dissolved and a new player was defined, the bee health technician (TSA) (Article L. 243-3 of the French Rural Code).

DDecPP staff carried out 437 visits in 2014: 101 random inspections, 126 as the result of an alert by a beekeeper and 159 targeted visits, mainly concerning the systematic inspections needed for imports (ten for the establishment of a health certificate).

In total, 1,131 active ASAs are listed in those *départements* that responded, an average of ten per *département*, with significant disparity between *départements* (from 0 to 80 ASAs). An important mission for these ASAs is to visit apiaries. In 2014, 2,781 visits were made, with an average of 28 visits per *département*: 2,223 (80%) of these visits were conducted by ASAs at the request of the DDecPPs.

Lastly, we are witnessing a growing interest on the part of veterinary practitioners, who are becoming more specialised in bee diseases (acquiring the inter-institution diploma in "Beekeeping and bee diseases"). Forty one visits were carried out by veterinarians in the framework of the monitoring of regulated diseases.

Table 1. List of regulated health hazards to bees in France

Hazard	Common name	Nature of the hazard	Regulations	Health status in mainland France
Paenibacillus larvae	American foulbrood	Bacterium	- Category 1 health hazard - Directive 92/65/EEC (Annex A) - Regulation (EC) no. 206/2010 - OIE	Present
Nosema apis	Nosemosis	Fungus	- Category 1 health hazard	Present
Aethina tumida	Infestation by the small hive beetle	Insect	- Category 1 health hazard - Directive 92/65/EEC (Annex A) - EU Directive 82/894/EEC - Regulation (EC) no. 206/2010 - OIE	Absent
Tropilaelaps spp.	Infestation by the *Tropilaelaps* mite	Mite	- Category 1 health hazard (for *Tropilaelaps clareae*) - Directive 92/65/EEC (Annex A) - EU Directive 82/894/EEC - Regulation (EC) no. 206/2010 - OIE	Absent
Varroa destructor	Varroasis	Mite	- Category 2 health hazard - Directive 92/65/EEC (Annex B) - OIE	Present
Vespa velutina	Asian hornet	Insect	- Category 2 health hazard	Present

Results

Results from surveillance of Paenibacillus larvae, the agent of American foulbrood

The DDecPPs recorded 241 clinical suspicions of American foulbrood in SIGAL, the official database maintained by DDecPP staff. Eleven APMS orders were issued for the apiaries concerned (5 % of the cases). Among these suspect cases, 208 new outbreaks of American foulbrood (or 86 %) were confirmed (Table 2). Seventy-nine outbreaks were the subject of an APDI.

Table 2. Annual number of suspected cases and confirmed outbreaks of American foulbrood between 2010 and 2014

	2010	2011	2012	2013	2014
Clinical suspicions	348	290	232	354	241
Confirmed Outbreaks	95	121	97	209	208

Results from surveillance of Nosema apis

The DDecPPs recorded 20 clinical suspicions of nosemosis (caused by *N. apis*). No AMPS or APDI were recorded for 2014 (Table 3).

Table 3. Annual number of suspected cases and confirmed outbreaks of nosemosis between 2010 and 2014

	2010	2011	2012	2013	2014
Clinical suspicions	64	43	25	98	20
Confirmed Outbreaks	7	5	2	5	0

Results from surveillance of Aethina tumida

Four suspected cases recorded by the DDecPPs led to the issuing of an APMS. The identifications conducted by the NRL for bee diseases helped to rule out these suspicions. No suspicions were reported as a result of the monitoring scheme implemented for queen bee imports.

Results from surveillance of Tropilaelaps clareae

One suspicion, which did not lead to the issuing of an APMS, was recorded in 2014. Identification by the NRL helped to eliminate the suspicion. No suspicions were reported as a result of the monitoring scheme implemented for queen bee imports.

Results from surveillance of Varroa destructor

Varroa destructor is endemic in France (apart from a few island territories such as the *Île* d'Ouessant).

The visits carried out in the framework of the Résabeilles surveillance network showed that 4.70% and 12.35% of the apiaries presented clinical signs suggestive of varroasis, in spring and summer respectively (Hendrikx *et al.*, 2015).

Results from surveillance of Vespa velutina

Between April 2014 and April 2015, three new *départements* were colonised by the Asian hornet: the Aube, the Seine et Marne and the Val de Marne (Figure 1).

Results from surveillance of bee colony mortality

One hundred and fifteen alerts of disorders from 42 different *départements* were reported to the DDecPPs in 2014. The investigations carried out by the State services (DDecPP and SRAL) indicated a pathological origin in 20% of the cases, and a toxicological origin in 3.5% of the cases; it was not possible to reach any conclusion in the other cases.

Toxicological analyses were positive (above the limit of detection) in 32 cases. In total, 32 different chemical compounds were identified, including four whose use is not authorised in France: coumaphos, endosulfan, carbaryl and chlorfenvinphos. In four of these cases (3.5% of all the alerts declared), seven chemicals were identified in sufficient concentration (> LD_{50}) to confirm a toxic origin for the mortality observed. They were the following substances: Chlorpyrifos-ethyl, fluazifop, tebuconazole, prothioconazole, permethrin, tetramethrin and carbaryl (Table 4).

In addition, at least six analyses carried out in 2014 also revealed associations of chemicals likely to be responsible for mortality: tau-fluvalinate, coumaphos, chlorpyrifos-ethyl, endosulfan and spirotetramat (Table 4).

Table 4. List of substances involved / potentially involved in mortality in 2014

Residues	Use	Plant health	Veterinary	Biocides
Carbaryl	I			
Chlorpyrifos-ethyl	I			
Fluazifop	H			
Tebuconazone	F			
Prothioconazole	F			
Permethrin	I			
Tetramethrin	I			
Coumaphos	I			
Tau-fluvalinate	I			
Endosulfan	I			
Spirotetramat	I			
Chlorpyrifos-ethyl	I			

Authorised product - Prohibited product - Substance detected in association likely to be responsible for mortality
F: Fungicide, I: Insecticide, H: Herbicide

Box 1. Surveillance and health control measures for honeybee (*Apis mellifera*) diseases and disorders

Objectives of the surveillance programme

- To ensure early detection:
  - > of any introduction of the exotic pathogens, *Aethina tumida* and *Tropilaelaps* spp., in France and guarantee the country's pest-free status for trade and export purposes.
  - > of outbreaks of American foulbrood and *Nosema apis* nosemosis to prevent the spread of these two pathogens in France.
- To determine the status of zones (parasite-free or not).
- To determine the prevalence of bee health hazards and disorders (e.g. mortality) and detect any possible resurgence.
- To collect alerts concerning the mortality observed in bee colonies in order to implement investigations taking the toxic risk into account.

The population monitored

Every beekeeper is required to complete an annual declaration of the location of apiaries and the number of hives (Act No. 229-967 and the Ministerial Order of 11 August 1980) (Table 1). In 2014, 38,748 beekeepers made a declaration, for a total of 1,043,444 hives. Because of the under-reporting, the real French bee population is estimated to be 1,600,000 hives.

Table 1. Annual number of declarations by beekeepers between 2011 and 2014

	2011	2012	2013	2014
Beekeepers	30,416	30,542	32,352	38,748
Hives	814,750	899,886	949,660	1,043,444

Surveillance procedures

Outbreak surveillance

- Network for annual surveillance of bee disorders (Memorandum DGAL/SDSPA/SDQPV/N2012-8113), which was replaced on 14 November 2014 by the surveillance scheme for mass acute mortality and diseases, classified as Category 1 health hazards in bees (Memorandum DGAL/SDQPV/2014-899), which enabled investigations to be extended to mass acute winter mortality and the exploration of toxic causes.
- Mandatory declaration of all suspicions of Category 1 and 2 health hazards affecting the bee *Apis mellifera* (Article L201-9).
- Updating of a map of the distribution of *Vespa velutina* by the National Museum of Natural History (MNHN) (Memorandum DGAL/SDSPA/N2013-8082).

Programmed surveillance

- Epilobee epidemiological surveillance network, with its French components Résabeilles and Ecotox. This network was established in six French *départements* (Cantal, Drôme, Haut-Rhin, Bouches du Rhône, Indre et Loire and Finistère) in 2012 and the programme came to an end on 31 December 2014. Sixty-six apiaries per *département*, chosen at random, were visited three times (in autumn, spring and summer) over two successive annual campaigns during which an in-depth clinical examination of the colonies took place, aimed particularly at estimating mortality. Samples were also taken in cases of suspicion of disease and systematically on some visits in order to determine the prevalence of *Varroa destructor*, and of *N. ceranae and N. apis* in the spring. Bee bread and honey were also screened for plant protection substances. The results of this study will be the subject of a specific publication.
- Random surveillance. It is based on the implementation of random inspections planned at departmental level by each DDecPP without a coordinated national framework. The number and frequency of these "random" visits therefore vary from *département* to *département*.
- For queens, bees and drones imported from non-EU countries, targeted surveillance involves systematic laboratory examination of transport cages and the bees they contain to detect the *A. tumida* hive beetle and *Tropilaelaps* spp. mites in accordance with Regulation (EU) No 206/2010.

Laboratories

- National Reference Laboratory: ANSES Sophia-Antipolis Laboratory.
- A network of eight departmental laboratories accredited to diagnose American foulbrood and nosemosis (Memorandum DGAL/SDPRAT/N2012-8199 of 10 October 2012).
- A network of laboratories accredited for detecting the risk of introducing the small hive beetle and *Tropilaelaps* mites *via* imported queen bees or drones from non-EU countries (Memorandum DGAL/SDPRAT/N2011-8128 of 8 June 2011).
- Six laboratories specifically accredited for analysis in the framework of the Résabeilles scheme.

Health control measures

The Ministerial Order of 23 December 2009 lays down the animal health measures applicable to Category 1 health hazards.

- In the event of suspicion of a Category 1 health hazard, the apiary is placed under APMS, which leads to investigations and possibly the establishment of precautionary measures.
- In the event of laboratory confirmation, the apiary is placed under APDI surveillance in compliance with the Ministerial Order of 11 August 1980 on combating contagious bee diseases amended by the Order of 23 December 2009 with, according to the case, implementation of containment measures, destruction of infected colonies, destruction or disinfection of equipment, and an epidemiological investigation to identify cases linked to the first outbreak, along with compensation for affected beekeepers.
- Epidemiological investigation. The various field visits to apiaries as part of the surveillance programme or in compliance with health control measures are carried out by DDecPP staff or bee health inspectors appointed by the Prefect and authorised to carry out specific surveillance missions on behalf of the State. In the future, veterinarians mandated in beekeeping will be directly involved in the framework of health control measures.

Regulatory References

Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements

Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(I) to Directive 90/425/EEC

Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases in the Community.

Ministerial Order of 11 August 1980 regarding the control of contagious bee diseases amended by Ministerial Order of 23 December 2009

Ministerial Order of 29 July 2013 defining Category one and two animal health hazards.

Commission Implementing Decision of 4 July 2012 concerning a financial contribution by the Union to certain Member States to support voluntary surveillance studies on honeybee colony losses.

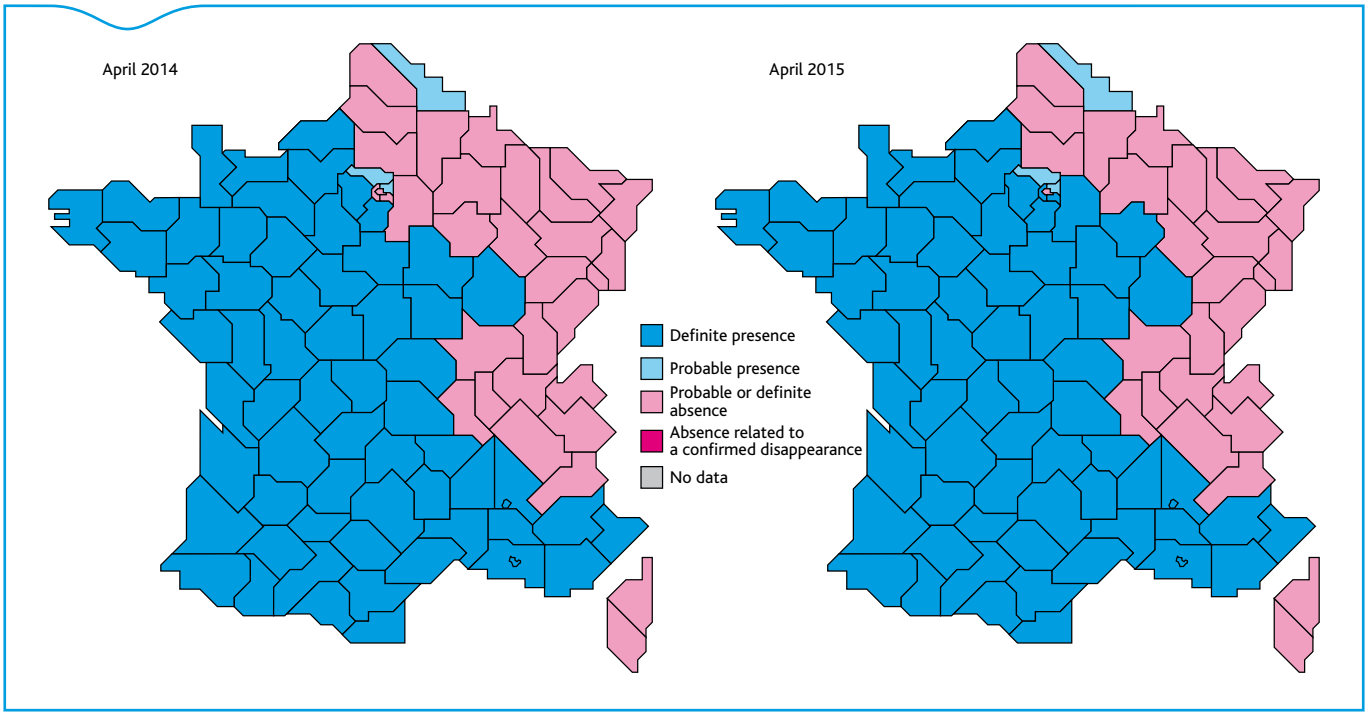

Figure 1. Change in the range of *Vespa velutina* between April 2014 and April 2015 (source: French Natural History Museum)

The Résabeilles surveillance network helped estimate the bee colony mortality rate at 13.7% during winter 2013/2014 and 11.1% during the 2014 beekeeping season (Chauzat *et al.*, 2015; Hendrikx *et al.*, 2015).

No funds were available for monitoring the colony mortality rate in France for the winter of 2014/2015.

Costs

The review of costs incurred by the various State services for implementing the bee surveillance schemes is not exhaustive, so the results presented below are only indicative (amounts are in euros excluding tax):

- random or targeted health visits were estimated by the DDecPPs at €45,383 in 39 *départements*,
- visits for issuing health certificates were estimated to cost €515 by the ten *départements* responding,
- visits carried out following suspicions of a disease by beekeepers amounted to €11,647 in 27 *départements*,
- thirty-four *départements* incurred costs for laboratory analyses for pathogen screening estimated at €8,337.

In addition, analyses for toxicological screening cost €38,258.

Total expenditure in 2014 (visits, health control measures, analyses, etc.) for the *départements* that provided information amounted to about €104,140.

It should be noted that the Résabeilles surveillance scheme cost €767,948 over the duration of the project (two years), with European funding covering 70% of the total cost of the programme.

Discussion

It should be remembered that each surveillance scheme has its own limitations and peculiarities (Lee *et al.*, 2015), which are not specifically detailed in this article.

No surveillance scheme is currently able to make a thorough assessment of the health situation of the French bee population, for various reasons, including:

- a partial knowledge of the bee population because of under-reporting,
- subclinical carriage of certain health hazards that has not been precisely evaluated (e.g. *Paenibacillus larvae*, the agent of American foulbrood),
- the probable limited sensitivity of outbreak surveillance, based on reporting by beekeepers or beekeeping stakeholders,
- poor knowledge of the clinical signs suggestive of the diseases that should be subject to mandatory declaration,
- programmed surveillance schemes that are not suitably representative of the French bee population,
- no harmonised definition of bee diseases and disorders,
- technical limitations in the screening of chemical residues.

Paenibacillus larvae, *the agent of American foulbrood*

For American foulbrood, France only practices clinical surveillance, unlike other European countries, which screen for the presence of *P. larvae* spores in honey or debris collected from hive bottom boards. In France, the management of outbreaks is not very precise and it is impossible to determine the situation (prevalence, incidence, geographic distribution) of this health hazard from the number of APDIs issued for *P. larvae*, mainly because of under-reporting. The Résabeilles study showed that during the first visit that took place in autumn 2012, more than 10% of apiaries visited were clinically affected by American foulbrood (Chauzat *et al.*, 2015).

This observation prompted an analysis of the likely causes of these under-declarations:

- poor knowledge among beekeepers of the regulatory control measures, and fear of the consequences of their implementation (e.g. restriction measures),
- the difficulties encountered by some DDecPPs in mobilising and sustaining the resources,
- the low levels of compensation awarded to beekeepers for outbreaks,
- poor knowledge by some beekeepers of the clinical signs suggestive of American foulbrood,
- the low level of health monitoring by some beekeepers and unauthorised control practices (use of antibiotics).

These findings raise questions about the efficacy and relevance of the management measures in force for American foulbrood.

Box 2.

The discovery of *Aethina tumida* in the south of Italy in September 2014 led the DGAL to strengthen vigilance with regard to this Category 1 health hazard (Ministerial Order of 29 July 2013):

- a first instruction dated 23 September 2014 (DGAL/SDSPA/2014-770) informed the State services of this discovery. They were asked to raise awareness among the stakeholders of the beekeeping sector as quickly as possible of the risk posed by this health hazard and the beekeepers' obligation to declare any suspicion to the DDecPP,
- a second instruction dated 20 November 2014 (DGAL/SDSPA/2014-842) called on the State services to strengthen vigilance, particularly with regard to the trade and import of bees governed by Directive 92/65/EEC and Regulation (EC) No 206/2010, specified the measures to be taken to raise awareness among the stakeholders of the beekeeping sector, and planned inspections to be carried out at wholesalers, distributors and beekeepers,
- a third instruction dated 6 February 2015 (DGAL/SDSPA/2015-113) asked the State services for a summary of the actions carried out and information collected,
- a fourth instruction dated 28 April 2015 (DGAL/SDSPA/2015-406), drawn up in the light of the information gathered by means of these summaries, with the support of the Epidemiological Surveillance Platform for Animal Health (ESA Platform) and the recommendations of the NRL for bee diseases, specified the enhanced surveillance procedures to address this health hazard. The aim is the early detection of any emergence of *Aethina tumida* in France in order to ensure its eradication. The enhanced surveillance scheme includes:
  - > outbreak surveillance *via* declarations by beekeepers of all suspect cases,
  - > programmed surveillance based on the risk. This consists of systematic visits to apiaries identified as presenting a particular risk of being infested, following the investigation by the National division for veterinary and plant health investigations. The risk factors targeted are the bees' zone of origin, the date of introduction in France, the presence of a health certificate, the type of biological material (swarms on frames, swarms alone, packages of bees, queens). Two hundred and ninety-one beekeepers have been identified. These visits are still in progress and no outbreak has so far been identified by this scheme.

Nosema apis, *agent of nosemosis*

Until 1996, *Nosema apis* was the only known species of microsporidia in the honeybee, *A. mellifera*. The clinical expression of the nosemosis caused by *N. apis* includes digestive disorders (mainly diarrhoea), nervous disorders (bees unable to fly, crawling bees, paralysed bees) and population losses, with a predominance of cases in the spring and their virtual disappearance during the summer. This form of nosemosis is called type A nosemosis.

For the last few years, the clinical prevalence of *N. apis* nosemosis seems to have been falling from year to year. The official notifications leading to APDIs have followed this same trend: 46 APDIs were issued in 2007 (Memorandum DGAL/SDSPA/N20009-8061), seven in 2010, two in 2012 (Bendali *et al.*, 2013) and none in 2014.

This phenomenon is probably the result of the crossing of the species barrier of another microsporidia, *N. ceranae*, a parasite of the bee *A. cerana* which now infests the honeybee, *A. mellifera,* and currently predominates in France. Because the two species of microsporidia occupy the same ecological niche - the epithelial cells of the bee ventricle - competition has been introduced. *N. ceranae* seems to have adaptive advantages over *N. apis* (lower infective dose, spores more resistant to high temperatures, more spores produced, greater number of epithelial cells infected at D4 and D7).

The nosemosis caused by *N. ceranae* is qualified as type C or "dry nosemosis" due to an attenuated clinical picture (population loss, mortality, colony weakening, with an absence of diarrhoea and crawling bees) and silent carriage, despite the sometimes high infection rates.

Recent studies carried out in different European countries, including France, show that the *N. ceranae* species is ubiquitous and largely predominates (Chauzat *et al.*, 2015), which explains the low number of clinical suspicions of *N. apis* nosemosis in France, and the absence of APDIs issued for 2014.

The current surveillance scheme appears able to detect any clinical resurgence of *N. apis* nosemosis. Nevertheless, the procedures for monitoring *N. ceranae* should be examined, even though this agent is not currently regulated. Unlike *N. apis*, clinical surveillance is not possible because of the attenuated clinical signs associated with its presence. However, it was recently demonstrated, in the framework of co-exposure, that interactions with chemical agents or other pathogens may cause disorders in bee colonies (Vidau *et al.*, 2011). In the event that bee colony disorders are reported in the framework of Memorandum DGAL/SDQPV/2014-899 or as part of a future surveillance scheme, screening and quantification of *N. ceranae* spores could be carried out systematically in order to better assess these phenomena.

Aethina tumida, *small hive beetle*

Despite the increased risk of introduction of the small hive beetle in France since it was discovered in Italy, the number of suspicions recorded by the State services remained low in 2014.

In view of the massive campaign to raise awareness among beekeepers and their representatives, the low number of suspicions may suggest that *Aethina tumida* is not established in France. This low number may also be indicative of under-reporting by beekeepers, especially since *Galleria mellonella* (greater wax moth) and *Achroia grisella* (lesser wax moth), which are frequently observed, develop larvae similar to those of *A. tumida*, and other beetles may be identified in the hives.

In order to enhance the sensitivity of the surveillance schemes currently in place (outbreak surveillance and surveillance by systematic examination of cages of queens imported from non-EU countries), other programmed surveillance schemes have been set up and are presented in Box 2. A first review of these schemes will take place at the end of the 2015 beekeeping season.

Tropilaelaps clareae

Only *Tropilaelaps clareae* is regulated in France (Ministerial Order of 29 July 2013) whereas since 2007 and the advances made in molecular biology tools, this species has been separated into two distinct species, the first, which has kept the name of *Tropilaelaps clareae*, and a second, which was named *Tropilaelaps mercedesae*. Both are likely to cause severe damage to colonies of *Apis mellifera* bees and warrant monitoring.

Just like *Aethina tumida*, the low number of suspicions recorded by the State services should be examined.

Awareness campaigns among beekeepers and beekeeping managers, as well as programmed surveillance schemes to complement the current schemes (outbreak surveillance and surveillance by systematic examination of cages of queens imported from non-EU countries) are possible ways to improve the sensitivity of surveillance.

Varroa destructor, *agent of varroasis*

The current regulations making it mandatory to report infestation of colonies by *V. destructor* (Ministerial Order of 29 July 2013) do not seem to be suited to the epidemiological situation of the parasite in France. Moreover, no notifications were registered by the State services in 2014. In contrast, French island territories such as the *Île* d'Ouessant remain free of the parasite. The plan to have the parasite-free status of this territory recognised by the European Union could enable trade to be regulated to prevent introduction of the parasite. Obtaining and maintaining this recognition is dependent on the establishment of a surveillance scheme to guarantee the parasite-free status (Article 15 of the European Directive 92/65/EEC).

The classification of *V. destructor* as a Category 2 health hazard means that its management is the responsibility of the professionals. This was the context that led to the implementation of regional programmes to combat *Varroa*, managed by recognised regional animal health

organisations (OVS-A). The DGAL provides financial support, half of which is supplemented by European funds managed by France Agrimer, to pay the salaries of the people responsible for implementing the control plan. The OVS-As of the Bretagne and Centre regions were eligible for the 2013/2014 season, and for the 2014/2015 season eligibility was granted to the OVS-As of Aquitaine, Bretagne, Centre, Corse, Provence-Alpes-Côte d'Azur and Rhône-Alpes. One of the objectives of these plans, which are intended to be introduced throughout the country, is the monitoring of *Varroa destructor*. Indeed, the implementation of rational management of *Varroa destructor* infestation requires in particular monitoring of the parasite population within the bee colony, with the beekeeper being required to intervene before this parasite population exceeds a threshold threatening the survival of the colony. An initial assessment of these plans will be carried out in the last quarter of 2015.

In the event of health disorders being observed in bee colonies in the framework of Memorandum DGAL/SDQPV/2014-899 or as part of a future surveillance scheme, the level of parasitism by *Varroa destructor* should be estimated systematically even in the absence of clinical signs characteristic of varroasis. This estimate can be performed *post-mortem* if possible and/or by studying colonies from unaffected apiaries. Indeed, *Varroa destructor* is a factor weakening bee immunity and can increase the colony's sensitivity to other stress factors.

Vespa velutina, *Asian hornet*

The scheme provided for by Memorandum DGAL/SDSPA/N2013-8082 (Box 1) is helping to measure the inexorable spread of this predator. The expansion front is estimated to advance by 60 km a year (Rome *et al.*, 2015). Beekeeping stakeholders indicate that the impact of the Asian hornet seems to vary, depending on the areas that have been colonised and from one year to the next. It might be wise to develop an indicator for determining the pressure of predation depending on the geographical areas and periods of the year in order to assess this phenomenon. Implementation of the Ministerial Order of 29 July 2013 making it mandatory to report the discovery of any *Vespa velutina* specimen or nest to the prefect could help monitor the density of hornet nests and changes over time.

Monitoring of mass mortality of bee colonies

The results from this scheme should be analysed with caution, given that reporting is not mandatory. Moreover, the number of notifications recorded by the State services is low compared to the health difficulties regularly reported by beekeeping stakeholders. In addition, the investigations, especially toxicological ones, are impeded by late notifications, which make it impossible to conduct a full investigation into the toxic risk. This is part of the reason why many cases remain unexplained following the investigations.

In order to increase this scheme's effectiveness, information campaigns have been conducted among representatives of the beekeeping profession at the national level. Others could be targeted at beekeepers, to make them more aware of the scheme.

A large number of the investigations carried out showed the concomitant presence of chemical contaminants and pathogens, although it is not possible to conclude, in the current state of knowledge, as to a cause-and-effect relationship between these various stress factors. In the light of the results obtained in the field, only experimental studies could investigate the mechanisms involved, in order to identify the relative share of each of the risk factors identified.

In Europe, a normal winter mortality rate of bee colonies has been estimated empirically at less than 10%. The average winter mortality rate in France during the 2013/2014 winter was estimated at about 14%. France is situated in a middle range between countries with a very low mortality rate (< 5%) and countries where the rates are very high (> 20%). The mortality rate observed in France in the beekeeping season is particularly high compared to other European countries. This trend had already been observed during the 2013 season. Efforts should now be made to explain this French specificity.

Outlook

In order to improve the efficiency of the health initiatives, including the surveillance actions in the beekeeping sector, the DGAL is continuing to implement the new bee health organisation launched in 2013:

- at the national level: a committee of beekeeping experts reporting to the national advisory council for animal and plant health policy (CNOPSAV) is currently being set up,
- at the regional level, in terms of health governance, the creation of a beekeeping section within each regional animal health organisation (OVS) is planned. The animal OVS is a member of the regional health association (ASR) and participates in the regional advisory council for animal and plant health policy (CROPSAV),
- regarding players in the field, the Minister of Agriculture has decided to call on mandated veterinarians with competence in beekeeping for the health control missions. In addition, the bee health inspectors (ASAs) have become bee health technicians (TSA) and work under the responsibility of a veterinarian.

Moreover, the surveillance schemes are set to improve through a revision of the methodological, technical and regulatory aspects, with the support of the French Epidemiological Surveillance Platform for Animal Health (ESA Platform) and by involving, as far as possible, all those contributing to health in the beekeeping sector.

With the end of the European surveillance programme, Epilobee, the current surveillance system will be supplemented by a new scheme called the Observatory of mortality and beekeeping alerts (OMAA), which will collect and exploit data on mortality and disorders affecting honeybee colonies. This scheme is in preparation.

Lastly, the national surveillance and control strategy will be adapted with regard to the health hazards. Two ANSES reports will shortly be made public, one on the prioritisation of biological pathogens in bees, and the other on ANSES's expert appraisal of co-exposure of bees to stress factors. These reports will provide a basis for a working group led by the DGAL and made up of members of the beekeeping expert committee. The aim will be to define a new categorisation of bee health hazards and ultimately to prioritise health actions in the beekeeping sector.

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Report on **animal rabies** surveillance in France: 3 serotine bat cases detected in 2014

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Abstract
Since France was officially declared rabies-free in 2001, the disease continues to be reported in mainland France in illegally imported pets (dogs and cats) incubating rabies when entering the country, as well as in bats. Currently, the rabies surveillance network mainly concentrates on pets and bats. In 2014, no positive pets were reported. However, three new rabies cases were identified in serotine bats, bringing the total number of rabies cases detected in Chiroptera to 57 since 2001. The discovery of novel species of lyssavirus and the regular detection of rabid bats each year highlight the need to maintain and reinforce rabies surveillance in France.

Keywords
Surveillance, Rabies, Pets, Bats

Résumé
Bilan de la surveillance de la rage animale en France : 3 cas détectés sur des sérotines communes en 2014
Depuis que la France métropolitaine a été officiellement déclarée indemne de rage en 2001, les cas rapportés sont limités aux seules chauves-souris autochtones et aux carnivores domestiques illégalement importés sur le territoire. Comme les années précédentes, le réseau d'épidémiosurveillance de la rage est principalement tourné vers la surveillance de la rage des carnivores domestiques et des chiroptères. En 2014, aucun cas de rage n'a été détecté sur des carnivores domestiques. Cependant, trois cas ont été identifiés sur des chauves-souris (sérotine commune). Ces trois nouveaux diagnostics positifs portent à 57 le nombre de cas de rage identifiés chez des chiroptères. La détection annuelle sur le territoire métropolitain de chauves-souris infectées et la découverte de nouvelles espèces de lyssavirus soulignent la nécessité de maintenir et de renforcer la surveillance épidémiologique dans toutes les régions françaises.

Mots-clés
Surveillance, rage, carnivores domestiques, chauves-souris

Rabies is a viral zoonosis causing acute encephalomyelitis. It is caused by a virus of the *Rhabdoviridae* family, *Lyssavirus* genus, which is currently thought to include fourteen species (ICTV, 2012). Found in the saliva of infected animals in the final phases of the disease, the virus is generally transmitted to another animal or to humans through biting. Rabies causes more than 55,000 human deaths each year around the world, according to estimates by the WHO (WHO, 2013). Different species of pets (mainly dogs, especially in Africa and Asia) or wild animals (for example foxes and bats) can maintain and transmit the lyssaviruses responsible for the disease. In France, rabies is a notifiable disease that must be reported to the OIE (OIE, 2012). It is recognised as a Category 1 health hazard (Ministerial Order of 29 July 2009). Metropolitan France has been officially recognised as rabies-free since 2001 (Ministerial Order of 30 April 2001), except for the period from February 2008 to February 2010 following the import of a rabid dog which led to secondary cases (Dacheux *et al.*, 2008). The outbreak surveillance of rabies remains a topical issue in France, because of regular imports of pets incubating rabies and of cases diagnosed each year among bats.

Results from Outbreak surveillance

In 2014, 1,839 animals were sent to the two laboratories for rabies diagnosis (Box). Of them, 29% (n=495) had no known history of human contamination and were sent to the ANSES NRL in Nancy. The other samples, i.e. 73% (n=1,344), were sent to the National Reference Centre for Rabies (NRC) at the Institut Pasteur in Paris (IPP). As every year, dogs and cats made up the majority of animal species diagnosed, with respectively 34% and 36% of the total (Table 1). Foxes accounted for only 2.3% (n=42) of the samples received by the two laboratories in 2014. The epidemiological surveillance network for rabies in bats, which was extended in 2000, continues to prove its worth, with Chiroptera representing a significant share (25%) of the animal species received for diagnosis of rabies and constituting, at nearly 88%, a large majority of the wild species investigated.

The geographical distribution (Figure 1) of animals received for diagnosis of rabies remains fairly homogeneous in metropolitan France, and also in the overseas *départements* (French Guiana, Reunion Island, Guadeloupe and Martinique).

Ninety-eight percent of the samples received (n=1,803) were analysed: One thousand eight hundred were diagnosed as negative and three were diagnosed as positive for rabies. These three cases of rabies were all detected in serotine bats in the Cher, Loir-et-Cher and Haute-Vienne *départements*.

Case of indigenous rabies in a bat in the Cher *département*

On 4 June 2014, a bat identified as a serotine bat was diagnosed by the NRL as positive for rabies using immunofluorescence. The diagnosis was confirmed on 6 June by cell infection and molecular biology techniques. Typing of the virus, through partial nucleoprotein gene and polymerase gene sequencing, showed that it was a lyssavirus of the European bat lyssavirus type 1 (EBLV-1) species, subtype b, very similar to the EBLV-1b viruses previously isolated in the Centre region. This virus has 99.2% homology with a viral strain previously isolated in Bourges (Cher *département*) in 2009.

The French network for epidemiological surveillance of animal rabies was set up following the discovery of the first case of rabies in a fox on 28 March 1968.

Objectives

The primary objective of this network for outbreak surveillance is to enable early detection of the presence of a rabies infection by carrying out a diagnosis of any animals that are suspect (clinical signs suggestive of rabies, human contamination by a bite, scratch or licking on mucous membranes or damaged skin) or found dead without reason, so as to rule out rabies.

Players in the surveillance programme

The partners in the surveillance network call on specialists from the fields of health (coordinated by the Directorate General for Health), agriculture (coordinated by the General Directorate for Food), and the environment (coordinated by the Ministry of Ecology, Sustainable Development and Energy). The Chiroptera Group of the French Society for the Study and Protection of Mammals (SFEPM) plays a vital role in the collection of bat specimens (Picard-Meyer *et al.*, 2013b).

The population monitored

As France is rabies-free, but nonetheless exposed due to the regular introduction of cases of imported rabies and the presence of rabies in bats, the primary objective of the epidemiological surveillance network is to monitor for rabies in pets (particularly biting dogs and cats) and wild animals (especially bats).

Surveillance procedures

Pets: This surveillance depends primarily on the presentation to the veterinary practitioner of animals suspected of rabies or animals that bite or scratch. A biting or scratching animal is defined as an "animal susceptible to rabies that, irrespective of where the incident occurred, has bitten or scratched someone" (Article R223-25-5° of the CRPM) and must be placed under the supervision of a mandated veterinarian (Ministerial Order of 21 April 1997). Even if it has been properly vaccinated against rabies, a biting or scratching animal must be placed under veterinary surveillance, because while the protection conferred by anti-rabies vaccination is extremely high, it is not absolute. The surveillance period is statutorily set at fifteen days for biting or scratching pets and thirty days for wild animals that have been tamed or kept in captivity, taking into account the longer pre-symptomatic carrying period sometimes observed in certain species (Ministerial Order of 21 April 1997). During the surveillance period, the animal must be presented three times to the same mandated veterinarian. During the surveillance period, the animal may not be euthanised (except with the agreement of the veterinary services or in cases of force majeure), nor may it be vaccinated against rabies. In the event of the death or euthanasia of a biting or scratching animal during this period, a diagnosis of rabies must be carried out by the NRC.

Wild carnivores: It is recommended that anyone finding a wild animal dead, injured or sick should not handle it and should contact the veterinary services of the *département* concerned. The system for monitoring rabies in bats is based on an epidemiological surveillance network coordinated by the Nancy Laboratory for Rabies and Wildlife (ANSES) in partnership with the Chiroptera Group of the SFEPM, consisting of volunteers and veterinary practitioners. This network, which was strengthened in 2000, is an adaptation of the existing organisation for the epidemiological surveillance of animal rabies. The surveillance of rabies in bats is based on the diagnosis of rabies in the corpses of bats found, most often, in an environment close to humans. Approximately 70% of the bats are sent by the network of chiropterologists, directly or *via* members of the public who contact the volunteers by calling their bat-rescue service ("SOS chauves-souris"), or the SFEPM's Chiroptera Group (http://www.sfepm.org/groupeChiropteres.htm). Bats are a protected species in metropolitan France, so they may neither be killed, nor handled, nor transported, even after death, without official authorisation granted by the Ministry of Ecology.

Diagnosis

The French surveillance network sends samples to two laboratories. The NRC of the IPP is mobilised when human contamination is suspected, i.e. if at least one of the four following conditions is met:

- a bite resulting in broken skin,
- scratching,
- licking of damaged skin (broken or scratched skin),
- projection of saliva on mucous membranes.

If this is not the case, the samples are sent to the Nancy Laboratory for Rabies and Wildlife (ANSES), the NRL for rabies.

These two laboratories use the reference techniques recommended by the OIE (OIE, 2012, Rabies chapter) and the WHO (Meslin *et al.*, 1996) and undertake phylogenetic identification of the virus strain in the event of positive diagnosis, providing information about the species and the type of virus (canine or from bats) and its geographical origin, which is of use for epidemiological investigations and for the implementation of management measures, especially in cases where rabies has been imported.

Health control measures

Rabies management is based on the management of animals that have been in contact with a rabid animal or one suspected to have rabies. The conditions and characteristics of contact are defined by the provisions of the CRPM, which specifically describes the identification of infected and potentially infected animals.

The classification of carnivorous animals as infected or potentially infected depends on the probability of contact between the carnivore and an animal known to be rabid, and this probability of contact is assessed by the DDecPP.

The management of infected animals is based on the Ministerial Order of 9 August 2011, which stipulates that infected animals not properly vaccinated at the time of infection must be euthanised.

The management of possibly infected animals is based on Article R. 223- 34 of the CRPM. Appropriate measures determined by the Director of the DDecPP are taken with consideration for the species of lyssavirus infecting the animal recognised as rabid, and the vaccination status of the potentially infected animals.

Regulatory References

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Case of indigenous rabies in a bat in the Loir-et-Cher *département*

The NRL diagnosed a second serotine bat as infected with a lyssavirus on 24 June 2014. This diagnosis using immunofluorescence was confirmed on 25 June using molecular biology techniques and on 26 June using cell infection. Typing of the virus, through partial nucleoprotein gene and polymerase gene sequencing, showed it was a genotype 5 (EBLV-1), subtype b lyssavirus, with 98.8% homology with a strain isolated in a bat from Spain and 98.2% homology with two EBLV-1b strains previously isolated in the Doubs and Meuse *départements*.

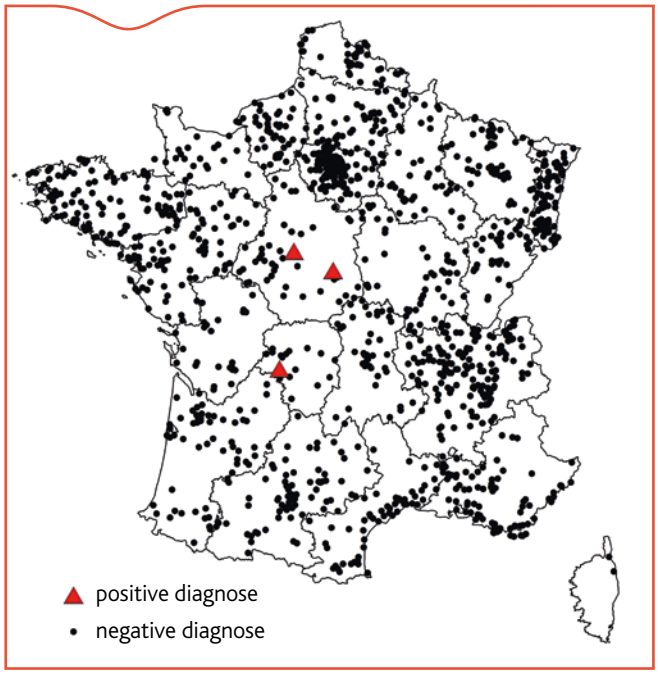

Figure 1. Geographical distribution of positive and negative diagnoses of rabies in metropolitan France for 2014

Case of indigenous rabies in a bat in the Haute-Vienne *département*

On 12 September 2014, a serotine bat received at the NRL was diagnosed as positive for rabies using immunofluorescence. The diagnosis was confirmed in the days that followed using cell infection and molecular biology techniques. Typing of the virus, through partial nucleoprotein gene and polymerase gene sequencing, showed it was a genotype 5 (EBLV-1), subtype b lyssavirus, with 98.7% homology with the EBLV-1b strain isolated three months prior from a serotine bat in the Loir-et-Cher *département*.

Discussion

Currently, most recorded cases of animal rabies in metropolitan France involve bats (57 cases since 2001). Nonetheless, cases of illegally imported infected pets (ten cases since 2001) are reported on a regular basis despite the implementation of strict regulations. Rabies thus remains a significant ongoing threat to animals in France and more broadly in Europe (Cliquet *et al.*, 2014): since 2001, 22 alerts have been recorded in Europe, including twelve from Morocco. In this context, a one-day event entitled "Rabies, a highly topical disease" was jointly organised on 9 October 2014 by the Ministry of Agriculture, Food and Forestry and ANSES as part of World Rabies Day. This day of awareness-raising on the health risks of the disease and the risk of its introduction into France brought together over a hundred scientists, stakeholders involved in the surveillance and management of carnivorous animal movements, health professionals, veterinarians, and air and maritime transport operators.

Table 1. Regional distribution of animal species examined for diagnosis of rabies in France in 2014

Metropolitan Regions	Animal species									
	Cats	Dogs	Bats	Foxes	Equidae	Cattle	Goats	Monkeys	Other domestic species	Other wild species
Alsace	33	22	84	5						
Aquitaine	30	35	8							
Auvergne	17	22	12	6		1				1
Basse-Normandie	9	15		3						
Bourgogne	14	27	3	4		1				1
Bretagne	36	61	45	1						1
Centre	10	28	9	2						3
Champagne-Ardenne	15	23	18	1		1				
Corse	3	2	1							
Franche-Comté	11	11	21	1	1	1				1
Haute-Normandie	24	14	20							
Île-de-France	135	54	4		1					3
Languedoc-Roussillon	36	27	30	2						3
Limousin	1	13	1	1		1				
Lorraine	29	23	56	2					1	2
Midi-Pyrénées	29	58	9	2						2
Nord Pas de Calais	21	15	3							3
Pays de la Loire	31	38	29	3		1		1	1	1
Picardie	26	25	11	4						1
Poitou-Charentes	14	12	2							
Provence-Alpes-Côte d'Azur	53	32	11	1					1	1
Rhône-Alpes	84	54	45	4					2	6
Overseas territories										
Guadeloupe		1								
La Réunion	1									
Guyane	3	10	37			2	1			
Martinique	1	4								
General total	666	626	459	42	2	8	1	1	5	29

The number of suspected cases and therefore analyses undertaken for the detection of rabies is high, and similar from one year to another for each animal category (Servat *et al.*, 2014), which reflects a good level of vigilance among the parties involved. Moreover, the geographical distribution of these suspected cases remains fairly homogeneous, suggesting satisfactory coverage of the entire national territory.

The cases of rabies recorded each year in bats demonstrate the need to maintain a high level of information, prevention and vigilance on the part of the population and of mandated veterinarians regarding the risk related to these specific epidemiological cycles. Since 1989, 67 bats have been found to be infected with lyssaviruses in France. The serotine bat, the principal species infected with EBLV-1 in Europe, accounts for 64 of these 67 cases of rabies recorded in France. The recent discovery in Europe of the new BBLV (Bokeloh bat lyssavirus) (Picard-Meyer *et al.*, 2013b; Dacheux *et al.*, not published) and LLBV (Lleida bat lyssavirus) lyssaviruses, combined with the annual detection of infected bats, underlines the need to maintain and strengthen epidemiological surveillance in all regions for effective management and to raise the awareness of at-risk individuals. It is therefore well worth intensifying the collection of bats for diagnosis and in particular of target species such as serotine bats (carriers of EBLV-1), Natterer's bats (supposed carriers of BBLV), bent-wing bats (carriers of LLBV) and Daubenton's bats (carriers of EBLV-2).

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Table summarizing regulated diseases in France

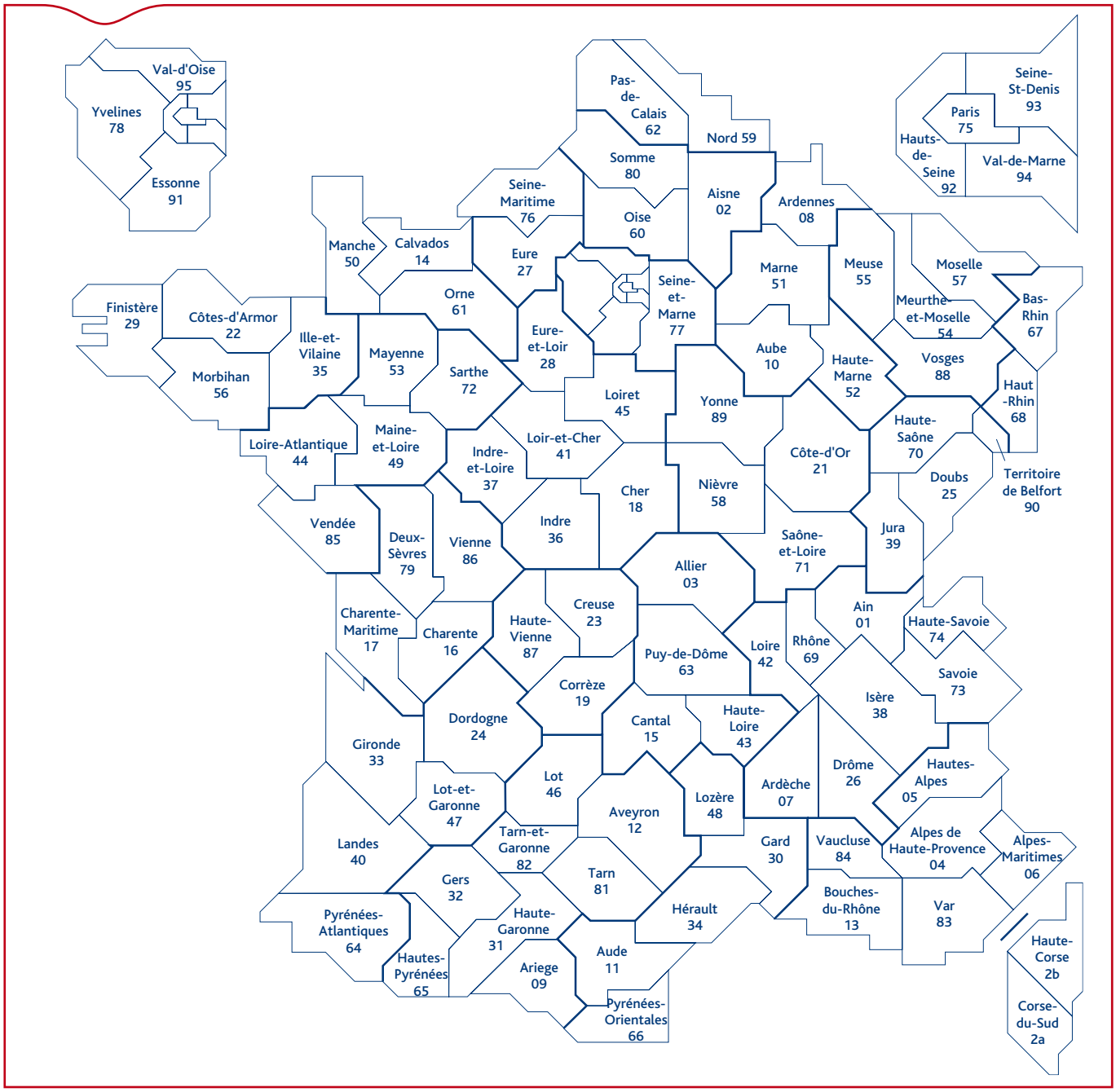
	Disease	Farms (animals) investigated	Farms (animals) with non-negative results	Clinical/ pathological suspicions	Incident outbreaks 2014* (%)	Change in incidence 2014-2013	Prevalence 2014* (%)
Cattle	Bovine tuberculosis	13,714 (690,754)	1,279 (3,932)	532	**105 (0.050%)**	-7	190 (0.09%)
	Bovine brucellosis	173,326	701 (790)	36,777 (65,743)	**0**	0	0
	Enzootic bovine leukosis	36,141	48	2	**2 (<0,001%)**	-2	2 (<0.001%)
	Infectious bovine rhinotracheitis	175,924	17,227	0	**3,331 (1,9 %)**	+0.2%	17,227 (9.8%)
	Hypodermosis (Warble flies)	9,873	39	0	**0**	-1	0
	Classical form of bovine spongiform encephalopathy	(1,043,572)	0 (0)	0	**0**	0	0
	Atypical forms of bovine spongiform encephalopathy	(1,043,572)	3 (3)	0	**2 L-BSE (<0.0002%) 1 H-BSE (<0.0002%)**	+2 L-BSE -1 H-BS	<0.0002%
	Anthrax	SO	SO	14	**0**	-1	0
	Foot-and-mouth disease	SO	SO	4	**0**	0	0
	Bluetongue	216 (1,149)**	46 (82)**	33 (187)**	**31*** **	-114***	31***
Ruminants	Brucellosis in small ruminants	36,226 (1,361,339)	12 (24)	2,541 (4,891)	**0**	0	0
	Classical scrapie in sheep	(10,103 animals at slaughterhouse + 29,851 animals at rendering)	0	0	**0**	-1	0% at slaughterhouse 0% at rendering
	Classical scrapie in goats	(8,681 animals at slaughterhouse + 51,876 animals at rendering)	0	0	**0**	-2	0% at slaughterhouse 0% at rendering
	Atypical scrapie in sheep	(10,103 animals at slaughterhouse + 29,851 animals at rendering)	1 sheep at slaughterhouse 4 sheep at rendering	0	**5**	-5	0.010 % at slaughterhouse 0.013 % at rendering
	Atypical scrapie in goats	(8,681 animals at slaughterhouse + 51,876 animals at rendering)	5 goats at rendering	0	**5**	2	0% at slaughterhouse 0.01 % at rendering
Pigs	Classical swine fever	1,783 (16,060)	10 (15)	3	**0**	0	0
	Aujeszky's disease	2,213	15	3	**0**	0	0
	Porcine brucellosis	NA	NA	5	**7**	0	Not determined

	Disease	Farms (animals) investigated	Farms (animals) with non-negative results	Clinical/ pathological suspicions	Incident outbreaks 2014* (%)	Change in incidence 2014-2013	Prevalence 2014* (%)
Poultry	Avian *influenza* in poultry and captive birds	721	17	15	**0**	0	0
	Newcastle disease in poultry and captive birds	NA	NA	7	**0****	0	0
	Pullorum disease-fowl typhoid	NA	NA	0	**0**	0	0
	Salmonella in reproduction flocks for broilers and layers of *Gallus gallus*(in growing and laying periods)	3,751	58	NA	**15**	+7 (0.17%)	0.4%
	Salmonella in flocks of layers of *Gallus gallus* (in growing and laying periods)	7,315	120	NA	**67**	+30 (0.43%)	0.92%
	Salmonella in reproduction flocks of *Meleagris gallopavo* (in growing and laying periods)	1,279	6	NA	**4**	+1 (0.07%)	0.31%
	Salmonella in broiler flocks of *Gallus gallus* and *Meleagris gallopavo*	71,414	489	NA	**489**	+50 (0.08%)	0.68%
Horses	Equine infectious anaemia	NA	2 Equines	0	**2**	+1	Not determined
	Contagious equine metritis	NA	0	0	**0**	0	0
	Equine viral arteritis	NA	1 Equine	0	**1**	+1	Not determined
Fish	IHN	NA	NA	0	**2**	plus 2	Not determined
	VHS	NA	NA	3	**3**	-10	Not determined
	KHV	NA	NA	2	**2**	+1	Not determined
Bees	American foulbrood	NA	NA	241	**79**	-130	Not determined
	Nosemosis	NA	NA	20	**0**	-5	Not determined
Other animals	Rabies in carnivores	NA	NA	1,292 (dogs and cats)	**0**	-1	0
	Rabies in bats	NA	NA	459 (number of bats analysed)	**3**	+2	Not determined

* Herds for which a Prefectural declaration of infection (APDI) was issued in 2014
** Excluding Corsica
*** All outbreaks of bluetongue in 2014 concerned Corsica and were serotype 1
*** No outbreak of Newcastle disease in poultry but there were 3 cases of paramyxovirus in captive pigeons (one may be a vaccine strain).
NA: Not applicable

Reference map

The following map identifies the *départements* referred to, either by name or by number, in the different articles.



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