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

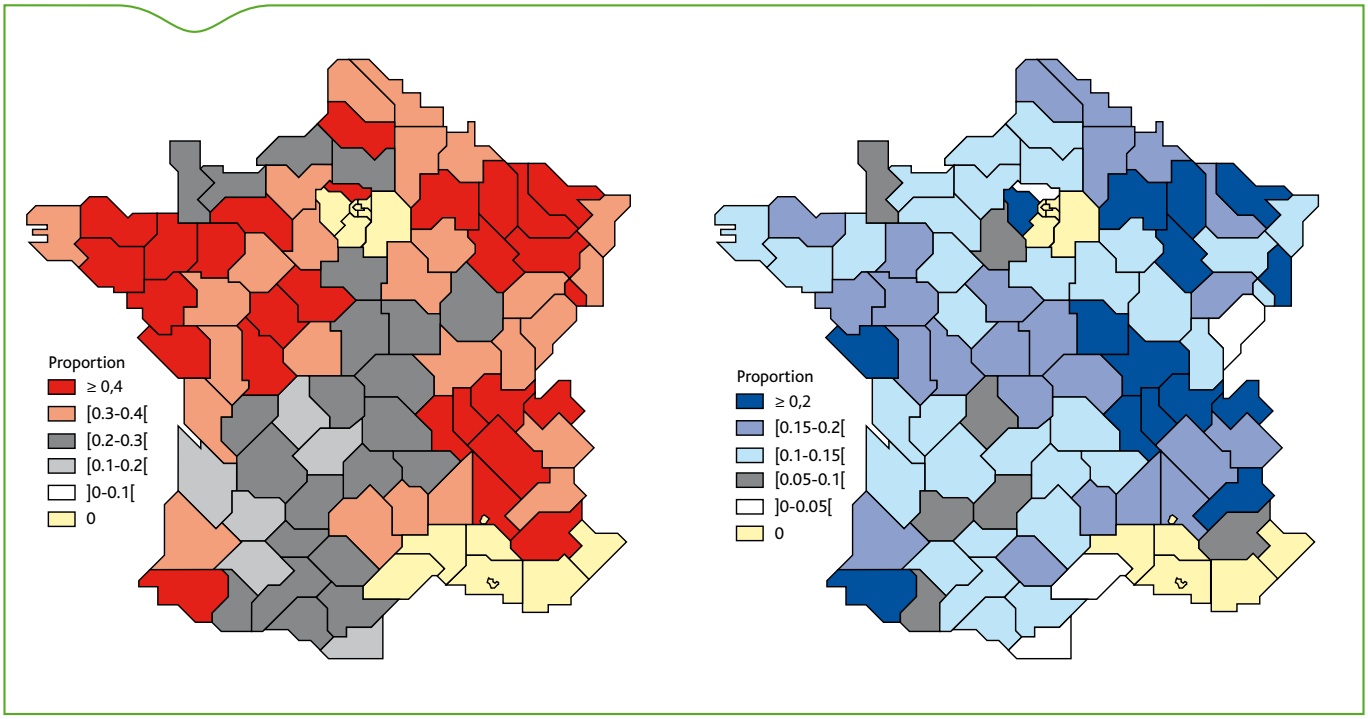

Figure 1. Departmental distribution of the proportion of breeders making declarations in dairy (left, in red) and beef farms (right, in blue)

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.

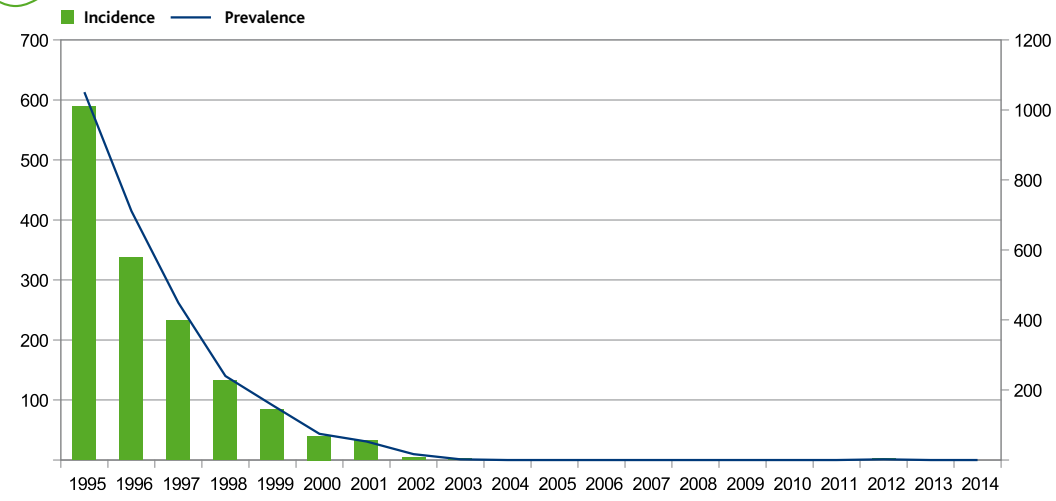

Figure 2. Annual figures for incidence and prevalence of herds infected with bovine brucellosis in France from 1995 to 2014

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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