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 Gasconbreed 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 \in 22,025 for surveillance and control of porcine brucellosis. Laboratory costs amounted to \in 16,592 for health

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 control measures and veterinary costs were \in 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

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 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

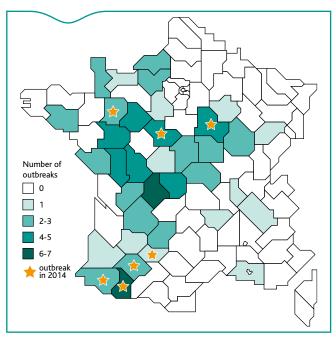


Figure 1. Geographic distribution of confirmed brucellosis outbreaks in pig holdings in France from 1993 to 2014 and location of confirmed outbreaks in 2014

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.

References

Bronner, A., Marcé, C., Fradin, N., Darrouy-Pau, C., Garin-Bastuji, B., 2011. Bilan de la surveillance de la brucellose porcine en France en 2010 : détection de foyers chez des porcs de race locale. Bull. Epid. Santé Anim. Alim. 46 : 39–40.

Marcé, C., Garin-Bastuji, B., 2012. Brucellose porcine en France en 2011 : sept foyers dont deux en race locale. Bull. Epid. Santé Anim. Alim., 54 : 41-43.

Marcé, C., Garin-Bastuji, B., 2013. Brucellose porcine en France en 2012 : trois foyers dont un en race locale. Bull. Epid. Santé Anim. Alim., 59 : 44-46.

Marcé, C., Garin-Bastuji, B., Jaÿ, M., Pozzi, N., 2014. Brucellose porcine en France en 2013 : trois foyers en race locale. Bull. Epid. Santé Anim. Alim., 64 : 42-44.