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Brucella suis infection in domestic pigs in Sardinia (Italy)

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SUMMARY

During a 4-year (2007–2010) survey, the presence of *Brucella suis* infection in domestic pigs in Sardinia was investigated. Serum samples were collected from breeding pigs located on 108 commercial farms with documented reproductive problems and analysed using the Rose Bengal (RBT) and complement fixation (CFT) tests for screening and confirmation of *Brucella*, respectively. Of the 1251 serum samples analysed by RBT, 406 sera, originating from 36 farms, were positive for *B. suis*. CFT was positive in 292/748 sera analysed, confirming positivity in all 36 pig herds. Pigs with international complement fixation test units per ml (ICFTU/ml) values ⩾160 were slaughtered, and their organs collected for bacteriological examination and testing by polymerase chain reaction (PCR). *Brucella* spp. strains were isolated in culture from 13/502 organs analysed, and subsequently identified as *B. suis* biovar 2. PCR detected positivity to *Brucella* spp. in 19/285 organs analysed. These results confirm the presence and emergence of *B. suis* infection in domestic pigs in Sardinia.

Key words: *Brucella suis*, domestic pig, epidemiology, Italy, Sardinia.

INTRODUCTION

Brucella suis, currently recognized as having five biovars, is the aetiological agent of porcine brucellosis, a disease that affects domestic and feral pigs. This condition is clinically silent in most cases, but, when apparent, it is characterized by abortion at any stage of gestation as a result of chronic inflammatory lesions of the reproductive organs. *B. suis* infection is of widespread occurrence in wild boars, albeit with a generally low prevalence, while in domestic pigs it is considered a re-emerging disease in some countries as a consequence of spillover from wild boars to outdoor-reared pigs [1]. In addition, once introduced into a pig herd, *B. suis* infection spreads easily and is difficult to eliminate [2]. Of the three biovars causing infection in pigs, *B. suis* biovar 2 is the most common in Europe, where it plays an economically important role as the causative agent of reproductive losses. *B. suis* biovar 2 can also be isolated in wild boars with mild or no clinical signs and is known to cause disease in hares (*Lepus europaeus*) [3]. However, *B. suis* biovar 2 is considered to have a low zoonotic potential since it seems unable to infect healthy humans [4–6], although some human cases have been reported [3, 5]. By contrast, *B. suis* biovars 1 and 3 are spread throughout the world, and are highly pathogenic for humans causing severe disease [2, 7, 8]. Of the other known biovars, biovar 4 can

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be transmitted to cattle, horses, Canidae, and occasionally to humans, while biovar 5, occasionally isolated from rodents in Eastern Europe, can be transmitted to humans also causing disease [9]. In the European Communities, porcine brucellosis is listed as one of the compulsorily notifiable diseases [10]. In Italy, data about porcine brucellosis are quite scarce and seropositivity to *Brucella* spp. in wild boar has been exclusively reported in previous studies [11, 12], while *B. suis* biovars 1 and 2 have been isolated in tissue culture [13]. To our knowledge, no previous studies describing the presence of *Brucella* spp. in domestic pigs in Italy are available in the literature.

In Sardinia, pig farming officially includes 4852 farms and 169 278 pigs (ISTAT data, 2010). In fact, three different production systems are involved: (i) regular commercial farms, (ii) regular family-consumption farms, and (iii) irregular free-range pigs. The first mentioned production system consists of farms rearing pigs for breeding and/or slaughter for profit. The second system consists of farms having a maximum permitted number of four pigs kept for fattening and in-home slaughter and consumption. Under this system, breeding and slaughter for profit are forbidden. The latter production system consists of pigs kept in semi-wild conditions, which are not subjected to mandatory registrations, and which are occasionally captured for illegal slaughter. The exact number of irregular free-range pigs is unknown but it is believed to be limited.

The aim of this survey was to investigate and confirm, over a 4-year period (2007–2010), the presence of *B. suis* in pig farms reporting reproductive problems in Sardinia (Italy).

METHODS

Study population

Our survey was conducted from 2007 to 2010 on pig farms with reproductive problems such as infertility, frequent/infrequent abortion, small litter size, stillbirths, fetal mummification, decreased farrowing rates, and poor conception rates, in order to detect *B. suis* infection. One hundred and eight regular commercial pig farms agreed to participate in this survey. All monitored pig farms were included only once in this survey. Enrolled pig farms had a number of breeding animals per farm (ranged 6–229, mean 12·3 ± 24·2), of which most were small size herds, with only three having more than 50 breeder animals.

Survey design, sample collection and processing

All animals involved in the breeding that occurred on the enrolled farms were tested for seropositivity to *Brucella* spp. using the Rose Bengal test (RBT) as the screening test. All serum samples from farms with at least one RBT-positive sample were further analysed using the complement fixation test (CFT) to confirm the presence of *Brucella* spp. All pigs with CFT-confirmed seropositivity and an international complement fixation test units per ml (ICFTU/ml) value ⩾160, were slaughtered, and the organs collected for both bacteriological examination and, beginning in 2008, testing by polymerase chain reaction (PCR). Spleen, uterus (or testicles if male), and retropharyngeal lymph nodes were the target organs sampled. Other organs such as tonsils, fetus (brain), and the mesenteric, mediastinal, and parotid lymph nodes were also collected, when possible. The collected organs were mantained at 4 ± 1 °C until bacteriological examination, and if PCR was not performed immediately, tissues were frozen and stored at -20 °C.

Serological tests

The RBT and CFT were performed according to the World Organization for Animal Health (OIE) standard procedures [14, 15]. They are described as follows.

RBT

Briefly, 30 μl of serum sample and 30 μl of antigen of *B. abortus* strain 99 Weybridge (Istituto 'G. Caporale', Teramo, Italy) were dispensed in the appropriate wells of plastic plates, and carefully mixed. Subsequently, the plates were subjected to agitation using a circular motion oscillator set at about 30 beats/min for 4 min, and read for agglutination immediately after. Serum samples were considered positive when agglutination was evident.

CFT

Briefly, using a standard 96-well microtitre plate, 25 μl of diluted inactivated (58 ± 2 °C for 30 min) sera were placed in three rows of the plate. Volumes of 25 μl CFT buffer were added to the wells of the first and

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third rows. Next, five serial doubling dilutions were performed by transferring 25 μl volumes of serum from the third row onwards. Volumes of 25 μl of antigen were added to each well, except in the anti-complementary control row. Volumes of 25 μl of complement were added to each well. The plates were incubated at 37 ± 1 °C for 30 min and 25 μl of sensitized sheep red blood cells were added to each well. The plates were re-incubated at 37 ± 1 °C for 30 min. The results were read after the plates were centrifuged at 1000 ***g*** for 10 min at 4 °C to allow unlysed cells to settle. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis. The absence of anti-complementary activity was checked for each serum in the first row. Serum samples were considered positive for ICFTU/ml values ⩾20.

Bacteriological examination

Bacteriological examinations were performed in accordance with the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* [14, 15]. Briefly, tissue portions (30–50 g) were homogenized in 0·9% saline solution and 1 ml was inoculated into Farrell's medium (obtained by addition of 5% horse serum and Brucella selective supplement (Oxoid, UK) to Brucella medium base (Oxoid), and incubated at 37 ± 2 °C in a 5–10% CO_2 atmosphere for up to 6 weeks. Once a week, 0·5 ml inoculated Farrell's medium was subcultured on serum-dextrose agar (SDA) and incubated at 37 ± 2 °C in a 5–10% CO_2 atmosphere. SDA was prepared by the addition of 5% horse serum (Oxoid) to Brucella medium base (Oxoid). Plates were checked every 48 h up to a maximum of 8 days. Suspected colonies were subjected to microscopic control (morphology, Gram-staining, motility), catalase and oxidase tests, and agglutination assay with anti-*Brucella* serum (Sclavo Diagnostics, Italy). Recovered *B. suis* isolates were sent to the National Reference Centre for Brucellosis for identification of biovars.

PCR assay

Processing of samples

A Wizard Genomic DNA Purification commercial kit (Promega, USA) was used for the extraction of total DNA from organs. Briefly, 300 μl of sample homogenates were added to the appropriate tube containing a Nuclei Lysis Solution (600 μl) and incubated at 65 ± 2 °C for 30 min. A volume of 200 μl of protein precipitation solution was added to each tube. After centrifugation for 4 min at 14 000 ***g*** (Fresco 17 Centrifuge, Thermo Scientific, USA), 600 μl of supernatant were transferred into new tubes for DNA extraction, mixed in 600 μl of isopropanol, incubated at −20 °C for 60 min, then centrifuged at 12 000 ***g*** for 20 min. After discarding supernatants, all tubes were incubated with 600 μl of 70% ethanol and centrifuged for 1 min at 14 000 ***g***, then ethanol aspirated. Finally, the DNA pellets were resuspended in 50 μl of DNA rehydration solution. The rehydrated DNA was incubated at 65 °C for 60 min, and denaturated at 95 °C. The extracted DNA from each homogenate was aliquoted and stored at –20 °C until further use.

Primers

The specific oligonucleotide primers OG157 (5′-GGC ATGAACCGCTGTCC-3′) and OG158 (5′-CTTCCG GGGCGAGTTG-3′) (Sigma-Aldrich, USA) that allowed amplification of a fragment of ∼141 bp, were used in this survey. *B. melitensis* strain NCTC 10 123 was used as a positive control.

PCR conditions

The assay was performed in a final volume of 25 μl of a mixture containing Platinum PCR Mix (Invitrogen, USA) with ∼50 ng of template DNA and 1 μl of PCR primers (200 nM). The amplifications were performed in a thermal cycler (MyCycler Thermal Cycler, Bio-Rad, USA), with the following steps: initial denaturation at 94 °C for 2 min, cycling at 94 °C for 30 s, 55·5 °C for 30 s, 72 °C for 2 min for 30 cycles with a final extension at 72 °C for 4 min. The products (5 μl or 7 μl) were analysed by electrophoresis through a 2% agarose gel 1 × TBE [100 mM Tris-HCl (pH 8·0), 90 mM boric acid, 1 mM disodium EDTA]. The gel was stained with SYBR Safe DNA Gel Stain (Molecular Probes, Invitrogen) (1×) and visualized under UV light. The Smart Ladder (Invitrogen) was used as a molecular size standard.

Statistical analysis

For each laboratory test employed, the results were summarized as number and percentages with 95% confidence intervals (CIs) of positive and negative samples. The statistical analysis was performed with Minitab release 12·1 (Minitab Ltd, UK).

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Ethical statement

The collection of biological samples was performed in accordance with the national guidelines for animal welfare and only after the farmers' informed consent.

RESULTS

RBT findings

During the 4-year survey, 108 pig farms with documented reproductive problems were enrolled. Animals from 36/108 farms (33·3%) were seropositive. A total of 1251 sera were tested with the RBT, and positivity was detected in 406 samples (32·5%, 95% CI 29·9–35·0). The percentages of seropositivity at the herd level ranged from 5·6% to 100·0% (mean 38·2 ± 28·9) (data not shown). The number of farms and serum samples tested with RBT are detailed by year in Table 1.

CFT findings

A total of 748 sera coming from the 36 farms with positivity to *Brucella* spp. detected by the RBT, were tested with the CFT for confirmation. The CFT detected positivity to *Brucella* spp. in 292 sera (39·0%, 95% CI 35·5–42·5). The percentages of seropositivity at herd level ranged from 7·1% to 84·6% (mean 41·3 ± 29·9) (data not show). Overall, the positivity to *Brucella* spp. detected by RBT was confirmed by CFT for 275 sera (36·8%, 95% CI 33·3–40·2), while 131 sera positive by RBT showed a ICFTU/ml value <20 by CFT. Of the 342 sera negative by RBT, 17 showed a ICFTU/ml value ⩾20 and 325 were confirmed negative. Overall, seropositivity to *Brucella* was confirmed in each of the 36 pig farms. The results of the CFT on sera testing positive for *Brucella* spp. by RBT, detailed by year, are given in Table 2.

Bacteriological findings

Bacteriological examinations were performed on 502 organs, sampled from 139 pigs with a ICFTU/ml value ⩾160. *Brucella* spp. strains were isolated in culture from 13 organs (five uteri, three mesenteric, two mediastinal and two retropharyngeal lymph nodes, and one tonsil) collected from six pigs. All 13 strains isolated were subsequently identified as *B. suis* biovar 2. The results of the examined organs are given in Table 3.

PCR assay findings

Beginning in 2008, PCR was performed on the organs collected. Two hundred and eighty-five organs were investigated by PCR using primer pairs specific for *Brucella* spp. Nineteen organs (seven uteri, four tonsils, one spleen, three mesenteric, two mediastinal, and two retropharyngeal lymph nodes) were positive. PCR-positive organs were found in 12 pigs. By culture comparison, all 13 culture-positive organs were also positive by PCR; six additional organs were found to be PCR positive but culture negative; these organs were two uteri, three tonsils and one spleen. The results of the organs examined by PCR are given in Table 4.

DISCUSSION

To our knowledge, this is the first survey on *B. suis* infection in domestic pigs in Italy. The results of this survey, obtained from domestic pigs reared in regular commercial farms, are in agreement with recent data concerning wild boars [16], and show that porcine brucellosis caused by *B. suis* is present in Sardinia. Our results indicated that about 33% of the herds with reproductive problems enrolled in this survey, showed seropositivity to *Brucella*.

Culture results indicate that *B. suis* was the only *Brucella* species detected in pigs in Sardinia. Indeed, after starting from an endemic condition, Sardinia has continued to maintain its officially declared status as free from brucellosis caused by *B. melitensis* in sheep and goats, and by *B. abortus* in cows and buffalo, which was first recognized in 1998 [17]. This goal has been achieved by means of national eradication programmes conducted on these species. By contrast, no eradication programmes have been carried out in pigs for porcine brucellosis.

Serological positivity confirmed by CFT was relatively high at a serum (pig) level. In addition, results showed seroprevalences at the farm level ranged from 7·1% to 84·6%. This wide variation in the percentages between farms, and high prevalences at farm level are not surprising. They can be explained by the fact that most of the enrolled farms had a small number of breeding animals, which usually have a quite long reproductive career, and, thus, a wide possibility of spreading infection in the herd. These aspects are common in pig farming in Sardinia.

However, it should be pointed out that recent data on *B. suis* infection in domestic pigs in European countries are very scarce, whereas the majority of data comes from surveys carried out on wild boars.

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Table 1. *Number of pig farms and blood sera with positive and negative Rose Bengal test (RBT), data from years 2007–2010*

	Pig farms monitored by RBT		Blood sera tested with RBT				
				Positive		Negative	
Year	Total monitored (*N*)	No. with positivity	Total *N*	*n*	% (95% CI)	*n*	% (95% CI)
2007	33	12	410	180	43·9 (39·1–48·7)	230	56·1 (51·3–60·9)
2008	30	11	384	43	11·2 (8·0–14·4)	341	88·8 (85·6–92·0)
2009	22	6	261	141	54·0 (48·0–60·1)	120	46·0 (39·9–52·0)
2010	23	7	196	42	21·4 (15·7–27·2)	154	78·6 (72·8–84·3)
Total	108	36	1251	406	32·5 (29·9–35·0)	845	67·5 (65·0–70·1)

CI, Confidence interval.

Table 2. *Number and percentage of blood sera with positive and negative Rose Bengal test (RBT) and complement fixation test (CFT) per year, from pig farms with at least one seropositivity detected by the RBT, data from years 2007–2010*

				Blood sera tested with CFT			
	Blood sera tested with RBT			Positive		Negative	
Year	Result	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
2007	Positive	180	57·3 (51·9–62·8)	115	63·9 (56·9–70·9)	65	36·1 (29·1–43·1)
	Negative	134	42·7 (37·2–48·1)	7	5·2 (1·5–9·0)	127	94·8 (91·0–98·5)
	Total	314	100·0	122	38·9 (33·5–44·2)	192	61·1 (55·8–66·5)
2008	Positive	43	30·1 (22·6–37·6)	8	18·6 (7·0–30·2)	35	81·4 (69·8–93·0)
	Negative	100	69·9 (62·4–77·4)	1	1·0 (0·0–3·0)	99	99·0 (97·0–100·0)
	Total	143	100·0	9	6·3 (2·3–10·3)	134	93·7 (89·7–97·7)
2009	Positive	141	77·5 (71·4–83·5)	118	83·7 (77·6–89·3)	23	16·3 (10·2–22·4)
	Negative	41	22·5 (16·5–28·6)	8	19·5 (7·4–31·6)	33	80·5 (68·4–92·6)
	Total	182	100·0	126	69·2 (62·5–75·9)	56	30·8 (24·1–37·5)
2010	Positive	42	38·5 (29·4–47·7)	34	81·0 (69·1–92·8)	8	19·0 (7·2–30·9)
	Negative	67	61·5 (49·8–73·1)	1	1·5 (0·0–4·4)	66	98·5 (95·6–100·0)
	Total	109	100·0	35	32·1 (23·3–40·9)	74	67·9 (59·1–76·7)
Total	Positive	406	54·3 (50·7–57·8)	275	67·7 (63·2–72·3)	131	32·3 (27·7–36·8)
	Negative	342	45·7 (42·2–49·3)	17	5·0 (2·7–7·3)	325	95·0 (92·7–97·3)
	Total	748	100·0	292	39·0 (35·5–42·5)	456	61·0 (57·5–64·5)

CI, Confidence interval.

In fact, *B. suis* had been investigated and isolated in domestic pigs only in Croatia by Cvetnic *et al.* [18]. These authors detected seropositivity in 0·8% of domestic pigs tested, and isolated *Brucella* spp. strains from 88 (58·3%) serologically positive examined pigs. These were mostly identified as biovar 2, but two strains, isolated from aborted piglets, resulted as biovar 3. In Italy, data about porcine brucellosis concerns only wild boars. In the Piedmont Region (northwest Italy), Gennero *et al.* [11] recovered 63 isolates of *B. suis* from 940 tissue specimens, of which only one isolate was identified as biovar 2, whereas the others were identified as biovar 1. Bergagna *et al.* [12], also in the Piedmont Region, isolated *B. suis* biovar 2 from 198/1841 culture tested animals. In the Abruzzo Region (central Italy),

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Table 3. *Number of pigs and organs with positive and negative results by bacteriological examination for the detection of* B. suis *sampled from years 2007–2010*

	Total examined (*N*)	Positive		Negative	
		n	% (95% CI)	*n*	% (95% CI)
Pigs	139	6	4·3 (0·9–7·7)	133	95·7 (92·2–99·1)
Organs					
Tonsils	33	1	3·0 (0·0–8·9)	32	97·0 (91·0–100·0)
Spleen	139	0	0·0	139	100·0
Uterus	123	5	4·1 (0·6–7·6)	118	95·9 (92·4–99·5)
Retropharyngeal lymph nodes	120	2	1·7 (0·0–4·0)	118	98·3 (96·0–100·0)
Parotid lymph nodes	7	0	0·0	7	100·0
Mesenteric lymph nodes	28	3	10·7 (0·0–22–2)	25	89·3 (77·2–100·0)
Mediastinal lymph nodes	41	2	4·9 (0·0–11·5)	39	95·1 (88·4–100·0)
Fetus	8	0	0·0	8	100·0
Testicles	3	0	0·0	3	100·0
Total	502	13	2·6 (1·2–4·0)	489	97·4 (96·0–98·8)

CI, Confidence interval.

Table 4. *Number of pigs and organs with positive and negative results by PCR for the detection of* Brucella *spp. sampled from years 2008–2010*

	Total examined (*N*)	Positive		Negative	
		n	% (95% CI)	*n*	% (95% CI)
Pigs	54	12	22·2 (11·1–33·3)	42	77·8 (65·2–90·4)
Organs					
Tonsils	44	4	9·1 (0·6–17·6)	40	90·9 (82·0–99·8)
Spleen	54	1	1·9 (0·0–5·4)	53	98·1 (94·5–100·0)
Uterus	50	7	14·0 (4·4–23·6)	43	86·0 (75·6–96·4)
Retropharyngeal lymph nodes	41	2	4·9 (0·0–11·5)	39	95·1 (88·4–100·0)
Parotid lymph nodes	2	0	0·0	2	100·0
Mesenteric lymph nodes	42	3	7·1 (0·0–14·9)	39	92·9 (84·8–100·0)
Mediastinal lymph nodes	43	2	4·7 (0·0–10·9)	41	95·3 (88·9–100·0)
Fetus	6	0	0·0	6	100·0
Testicles	3	0	0·0	3	100·0
Total	285	19	6·7 (3·8–9·6)	266	93·3 (90·4–96·2)

CI, Confidence interval.

De Massis *et al*. [13] isolated a *B. suis* biovar 2 strain in culture from a female wild boar. In these studies, the seroprevalences ranged from 0% to 20%. In Sardinia, in particular, Addis *et al.* [16] found a seroprevalence of 6·11%. By contrast, Ebani *et al.* [19] found no seropositivity in sera of wild boars from the Tuscany Region (central Italy), and ascribed this to established eradication programmes.

Current opinion suggests that in domestic pigs *B. suis* has become uncommon in developed countries as in Europe, showing a low prevalence and only sporadic outbreaks in domestic animals. By contrast, porcine brucellosis is considered a re-emerging disease in some countries, such as in South-East Asia and in South America, as consequence of spillover from wild boars to outdoor-reared pigs [1, 2, 6]. Our results suggest that *B. suis* is also a potential emerging infection in domestic pigs in developed countries, in particular where *B. suis* infection is present in wild boar populations.

In interpreting the data presented in this paper, two additional aspects should be taken into account. First, the possible occurrence of false seropositive reactions, resulting from infections by some cross-reacting bacteria, such as *Yersinia enterocolitica* O:9 [20]. However, no *Y. enterocolitica* O:9 strain was isolated from the organs examined. Second, the analysed

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samples came from pig herds with reproductive problems. Therefore, the results obtained do not properly reflect the real epidemiological condition of *B. suis* infection in domestic pigs in Sardinia, nor should it be considered that the reproductive problem is due to this microbial agent in all *B. suis* seropositive pigs. In addition, it should be considered that small size herds are common in Sardinia. In such herds, where boar lending for natural mating is a common practice, reproductive failure can go unnoticed, or not be presented for diagnosis, and therefore pass undetected.

Therefore, the real prevalence of *B. suis* infection in domestic pigs in Sardinia is far from being known, but, based on our results, it is worthy of consideration, and is in need of further investigation to better depict it. The data arising from this survey confirms the presence of *B. suis* infection in pig farms in Sardinia, suggesting that this microbial agent must be taken into account as a potential reproductive pathogen in domestic pigs. All the isolates recovered from the organs in our survey were identified as biovar 2, which is mostly responsible for reproductive losses but with low zoonotic potential [3, 21]. *B. suis* biovar 1, previously isolated in Sardinia from the uterus of a female wild boar (E. Bandino, 2007, personal communication), was not isolated in this study. Therefore, further studies will be necessary to confirm or exclude the presence of *B. suis* biovar 1, highly pathogenic for humans, in pigs in Sardinia.

B. suis infection in domestic pigs is mainly caused by venereal transmission during mating, and its higher prevalence in the reproductive organs than in other organs suggests that genital excretion plays a role in its transmission [1, 5, 8]. Our results support this evidence since both bacteriological culture and PCR mainly detected *B. suis* in the uterus, rather than in other organs. Moreover, in recent outbreaks of porcine brucellosis in Europe, wild boars, together with hares, were identified as the source of transmission of *B. suis* to pigs reared commercially, and wild animals have been recognized as acting as a reservoir of infection in some areas [2, 4]. The transmission of *B. suis* from wild boars to domestic pigs has never been conclusively proven, but since direct contact and mating have been documented to occur between them, this is a possible means for the spread of bacteria from wild to domestic pigs [18, 22]. Furthermore, pig enclosure locations, unsuitable conditions for enclosure fencing, and pig-breeding characteristics have proven to be important risk factors for contact between wild boars and domestic pigs [22].

In Sardinia, the importance of these risk factors needs to be investigated, along with the presence of irregular free-range pigs, since, not only are they exposed to a greater likelihood of contact with wild boars, on account of their unauthorized movements, they might act as a 'bridge' between wild boars and domestic pigs. In fact, in Sardinia irregular free-range pigs have been shown to play an important epidemiological role in the maintenance of several infectious diseases affecting swine, such as African swine fever and trichinellosis [23, 24]. Furthermore, in Italy, and in particular in Sardinia, a rise in the number and density of the wild boar population in the last few years has been recorded, leading to a greater likelihood of contact between domestic pigs and wild boars.

CONCLUSIONS

The results obtained in this survey in domestic pigs, are in agreement with recent data from wild boars [16], and show that porcine brucellosis caused by *B. suis* is circulating widely in the domestic pig population in Sardinia. This, along with the relatively high percentage of positive cases, and that *B. suis* was isolated from pigs on farms showing reproductive problems, strongly suggests that *B. suis* is an emerging infectious disease in domestic pigs in Sardinia. The lack of data on *B. suis* infection in domestic pigs suggests that this disease is taken into lesser account in some quarters. Thus, the current epidemiological situation of porcine brucellosis suggests the need for control and eradication programmes for this infection.

Control and eradication programmes must consider the risk factors of this disease and existing local conditions, and must be based on: (i) an increase in the awareness of the infection risk for more exposed subjects, such as farm and slaughterhouse workers, and wild boar hunters; (ii) the identification and elimination of the domestic pig herds that are positive to *B. suis* infection; (iii) the improvement of management systems eliminating the irregular free-range pig herds; (iv) the improvement of breeding conditions for domestic pigs; (v) the prevention of contact between wild boars and domestic pigs; (vi) the heightened surveillance of *B. suis* in wildlife.

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DECLARATION OF INTEREST

None.

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