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Identification of *Brucella* spp. in feral swine (*Sus scrofa*) at abattoirs in Texas, USA

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Summary

Various tissues, nasal swabs, urine and blood samples were collected from 376 feral swine at two federally inspected abattoirs in Texas during six separate sampling periods in 2015. Samples were tested for *Brucella* spp. by culture and serology. *Brucella* spp. were cultured from 13.0% of feral swine, and antibodies were detected in 9.8%. Only 32.7% of culture-positive feral swine were also antibody positive, and 43.2% of antibody-positive feral swine were culture positive. Approximately, the same number of males (14.0%) and females (12.1%) were culture positive, and slightly more males (10.5%) than females (8.7%) were antibody positive. Our results indicate that serology likely underestimates the prevalence of feral swine infected, and that those who come in contact with feral swine should be aware of the symptoms of infection with *Brucella* spp. to ensure prompt treatment.

KEYWORDS

abattoir, *Brucella* spp., brucellosis, feral swine, slaughter, *Sus scrofa*

1 | INTRODUCTION

Feral swine (*Sus scrofa*) are becoming an increasing problem across the United States as they continue to expand their populations through a combination of natural range expansion, accidental escape and intentional release (Bevins, Pedersen, Lutman, Gidlewski, & Deliberto, 2014). Populations of feral swine now exist in more than 35 states, but the largest populations occur in Texas where they have been well established for the past several decades throughout most of the state (Mapston, 2007) and are estimated to exceed two million (Rollins, Higginbotham, Cearly, & Wilkins, 2007). Several years ago, Texas landowner damage due to feral swine according to a survey was reported to be $7,515 over the lifetime ownership of the land by the respondent (Adams et al., 2005), and likely has increased since the survey was conducted.

Feral swine cause extensive damage to agricultural crops and forest plantations, destroy pastures and native plants, imperil threatened and endangered species and cause irreversible damage to sensitive environments (Campbell & Long, 2009). A conservative estimate from approximately 10 years ago of the economic costs of feral swine damage and control in the United States placed it at $1.5 billion per year (Pimentel, 2007). More recent analyses estimate the economic impact of feral swine damage to be much higher (Anderson, Slootmaker, Harper, Holderieath, & Shwiff, 2016). Efforts to remove feral swine populations once established are often met with limited success especially in states with populations as large and extensive as those found in Texas (Dickson, Mayer, & Dickson, 2001). In some states, hunting and bounty programmes have been implemented in an attempt to reduce feral swine populations. However, both tend to create an incentive for intentional release of feral swine rather than serving to decrease populations as complete elimination would ultimately result in destroying

the source of income (Bevins et al., 2014). Abattoirs that process feral swine are another approach, which have been used to address feral swine issues in some states. At least seven states slaughter and process feral swine (Dr. Robert Boyle, USDA-Food Safety Inspection Service, personal communication). In Texas, the feral swine population is so extensive and agricultural crop damage issues are already so widespread (Mapston, 2007) that such facilities are unlikely to create a new market for feral swine. However, in states where eradication efforts might still be an option, abattoirs may result in anthropogenic movement of feral swine to previously free areas, thus creating additional problems.

As feral swine are known to carry numerous zoonotic pathogens and parasites (Meng, Lindsay, & Sriranganathan, 2009; Witmer, Sanders, & Taft, 2003), we were interested in assessing the occupational hazard posed by feral swine to abattoir employees. Specifically, we were interested in the prevalence of *Brucella* spp. as unconfirmed reports of the pathogen had been reported in an employee and a federal inspector at two different abattoirs. These reports, along with increasing interest in feral swine zoonoses, prompted our interest in quantifying the risk to abattoir employees. Consequently, we collected multiple tissues, nasal swabs, urine and blood from feral swine at two federally inspected slaughter facilities in Texas during six different months in 2015 for assessment of *Brucella* spp. by culture and serology.

Impacts

- Of 376 feral swine sampled at two abattoirs, 13% of feral swine were culture positive for *Brucella* spp.
- Approximately 9.8% of feral swine were antibody positive, suggesting that serology underestimates the prevalence of *Brucella* spp. in feral swine.
- Abattoir employees who slaughter feral swine should wear personal protective equipment, and be aware of the signs and symptoms of *Brucella* spp. infection to ensure prompt treatment should they become infected.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Feral swine were sampled at two federally inspected slaughter facilities in Texas. Due to confidentiality concerns, the facilities are referred to simply as Facility A and Facility B. Sample size was determined based on detecting *Brucella* spp. at a prevalence of 2.5% (estimate for Texas, USDA-Wildlife Services, unpublished data) with 95% confidence, a test sensitivity of 80% and specificity of 90%. As culture is considered the gold standard, these are conservative estimates of sensitivity and specificity. A "population" of 86,000 (approximate number of feral swine slaughtered at the two facilities each year) was used to calculate the target sample size of 325. Approximately 62 samples were collected (31 at each) in January, March, May, August, October and December of 2015 resulting in tissue and serum samples from 376 feral swine.

2.2 | Blood

Two to three 15-ml Vacutainers of blood were collected from each feral swine immediately after euthanasia. The Vacutainers were placed on their side at room temperature for approximately 1 hr, and then transferred to a cooler. Once sample collection was complete, the blood was centrifuged for 15 min at 125 *g*. Serum was transferred to 2-ml cryogenic vials and stored refrigerated until shipping. In addition, an aliquot of blood (approximately 1 ml) was transferred to a blue-top buffered citrate tube immediately after collection to prevent separation.

2.3 | Tissues

One nasal swab was collected from each animal using the BBL™ CultureSwab transport media collection system (Becton Dickinson and Company, Sparks, MD, USA). The applicator was inserted into the nasal cavity of both nostrils using a circular motion to cover as much of the mucosal surfaces as possible. A urine sample was collected from each animal by extracting urine directly from the bladder with a 3-ml syringe, and then transferring it to a cryogenic vial. A disposable scalpel and disinfected forceps were used to collect the submandibular, parotid, medial retropharyngeal (head), tracheobronchial, gastrohepatic (body), axillary or inguinal lymph nodes (peripheral), spleen and the reproductive tract. Tissues were placed in a Whirl-Pak® bag by region of the body and labelled with a barcode unique to the individual feral swine. All samples were transferred to a cooler shortly after collection and shipped to the laboratory on the same day or the following day.

2.4 | Serology testing

All sera were tested at the National Veterinary Services Laboratories (NVSL) in Ames, Iowa. Serum from each feral swine was tested for antibodies to *Brucella* spp. with the buffered antigen plate agglutination test (BAPA), competitive enzyme-linked immunosorbent assay (cELISA), complement fixation, fluorescence polarization assay (FPA), the rivanol test, plate agglutination, tube agglutination and card tests. Each test was conducted according to standard procedures (Nielsen, 2002; Nielsen et al., 1999). Animals were considered antibody positive if two or more of the serological assays tested positive. Suspects were considered positives for the purposes of calculating prevalence.

2.5 | Tissue culture

Culture for all tissues except the spleen was performed at the Agricultural Research Service in Ames, Iowa. Nasal swabs and 500 µl of urine were inoculated directly onto Kuzdas Morse agar plates (KM) upon arrival at the laboratory and incubated at 37°C in 5% CO_2 for 7 days. All other tissues submitted for bacteriologic culture were frozen at −20°C until testing. With the exception of reproductive tissues,

lymphatic tissues were isolated by blunt dissection according to region of the body. For individual lymph nodes within a sampling region of the body, and bulbourethral/seminal vesicles or uterine tissues (reproductive), approximately one-gram aliquots of tissue were individually ground in 2 ml of phosphate-buffered saline (pH = 7.2) using sterile glass grinders. One to five samples each were processed from the head, body and peripheral regions. Aliquots (100 μl) of each tissue suspension were plated onto KM and incubated at 37°C in 5% CO_2 for 7 days.

Whole blood and spleen were tested at Texas A&M University's College of Veterinary Medicine in College Station, Texas. Culture of the spleen samples was performed as previously described (Farrell, 1974). Briefly, multiple spleen sections were weighed and one gram of tissue was homogenized in 1 ml of peptone saline using an OMNI® TH homogenizer and OMNI® Rotor-Stator Generator Probes. A volume of 100 μl of homogenized spleen was plated onto Farrell's agar media in duplicate. Whole blood (100 μl) was plated directly onto Farrell's agar media in duplicate. All plates were incubated at 37°C for up to 4 weeks, and bacterial growth was monitored for up to 4 weeks.

2.6 | Culture identification

Brucella isolates were identified on the basis of colony morphology, growth characteristics and a real-time PCR assay using *Brucella*-specific primers and probe to the omp2A region of the *Brucella* (Alton, Jones, Angus, & Verger, 1988; Lee, Olsen, & Bolin, 2001). An individual was considered culture positive if *Brucella* spp. was identified in any of the tissues, nasal swabs, whole blood or urine.

2.7 | Molecular detection of *Brucella* spp. in spleen and whole blood

Confirmation of *Brucella* colonies was performed by conventional polymerase chain reaction (PCR). To identify *Brucella* genus strains, primer sets consisted of vjbRf/vjbRr (5′-ACTACTTTGCCATTGACCCG-3′/5′-AGTGAAAACCGTACAACCCG-3′), amiCf/amiCr (5′-GGTCGAGCAGGATATCGGT-3′/5′-GAGGGGGCTTGTTTCGCAC-3′) and virB12f/virB12r (5′-CGCTGTCTCTCTGGCCGCTT-3′/5′-CTTATGGTGATCCTGCTGGC-3′) were used to amplify 212-, 700- and 413-bp fragments of vjbR (BMEII1116), amiC (BMEI1056) and virB12 (BMEII0036) genes, respectively. A multiplex PCR approach using species-specific primer sets were used to confirm that all isolates corresponded to *B. suis*. Primers sets consisted of GI1f/GI1r (5′- CATTCGTCCATCGTGATGTATT-3′/5′-AGAAAATGAAGCGCCTGAAG-3′), G15f/G15r (5′-GCGGGAGAATATGCTTGAAA-3′/5′-AAAATACCGGGCTGGTTCAC-3′) and InCPf/InCPr (5′-GGGATGGTTTTGGTCAGGTA-3′/5′-TCTCAATGGACACGCGAATA-3′) were used to amplify 109-, 364/553- and 203-bp fragments of GI1 (BMEI0899), GI5 (BMEII0221) and InCP (BRA0366). Specifically, 553- and 203-bp fragments of GI5 (BMEII0221) and InCP (BRA0366), respectively, amplify in *B. suis* and *B. canis*, but 109-bp fragment of GI1 (BMEI0899) will only amplify *B. canis* and not *B. suis*, allowing distinction between these species. PCR was conducted with an initial denaturing at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 57°C for 2 min and 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were analysed using 1.5% agarose gel electrophoresis. Ethidium bromide-stained gel bands were imaged using an auto-image analyser.

2.8 | Statistical analysis

Descriptive statistics and exact binomial 95% confidence intervals were generated using Microsoft Excel. Sensitivity and specificity estimates were calculated using culture results as the gold standard (Godfroid, Nielsen, & Saegerman, 2010).

3 | RESULTS

3.1 | Culture

Of 376 feral swine that were sampled, 49 (13.0%) were culture positive for *Brucella* spp. Of these, 16 (32.7%) were antibody positive. Head, body and peripheral lymph nodes were more likely to be positive than urine, nasal swabs, whole blood, spleen or reproductive tract (Table 1). Approximately, the same number of culture-positive feral swine was collected at Facility A (*n* = 25) and Facility B (*n* = 24). The majority of culture positives were collected from adults (81.6%; 40 of 49) and the remainder from subadults (*n* = 9). Approximately, the same number of males (*n* = 28) and females (*n* = 21) was culture positive (Table 2).

3.2 | Serology

Brucella antibodies were detected in 9.8% (*n* = 37) of all feral swine tested. Of these, 43.2% were culture positive. *Brucella* antibody prevalence ranged from 4.0 to 11.2% with the eight different serological tests (Table 3). The sensitivity and specificity across all serological tests based on the culture results were

TABLE 1 Apparent prevalence with 95% confidence intervals (CI) of *Brucella* spp. detected in various feral swine tissues by culture (*n* = 376)

Tissue (*n*)	No. of positive	% positive (95% CI)
Urine (341)	7	2.1 (1.0–4.2)
Nasal swab (373)	1	0.3 (0.05–1.5)
Head LN (372)	20	5.4 (3.5–8.2)
Body LN (371)	17	4.6 (2.9–7.2)
Peripheral LN (374)	18	4.8 (3.1–7.5)
Reproductive LN (372)	6	1.6 (0.7–3.5)
Any lymph nodes (376)	49	13.0 (10.0–16.8)
Spleen[a] (251)	3	1.2 (0.4–3.5)
Whole blood[a] (251)	0	0 (0–1.5)

[a]Tested by both culture and polymerase chain reaction.

TABLE 2 Comparison of *Brucella* spp. serology and culture results for feral swine sampled at two different slaughter facilities (n = 376) in Texas by different variables with 95% confidence intervals (CI)

Variable (n Facility A, B)	Serology			Culture		
	Facility A	Facility B	Combined	Facility A	Facility B	Combined
Sex						
Female (73, 100)	17.8 (10.7–28.1)	24.0 (16.7–33.2)	21.4 (15.9–28.1)	9.6 (4.7–18.5)	14.0 (8.5–22.1)	12.1 (8.1–17.8)
Male (113, 87)	15.9 (10.3–23.8)	19.5 (12.6–29.1)	17.5 (12.9–23.4)	15.9 (10.3–23.8)	11.5 (6.4–19.9)	14.0 (9.9–19.5)
Unknown (1, 1)	0 (0–79.4)	100 (20.7–100)	50.0 (9.5–90.6)	0 (0–79.4)	0 (0–79.4)	0 (0–65.8)
Age class						
Adult (153, 144)	19.0 (13.5–25.9)	25.0 (18.6–32.7)	21.9 (17.6–26.9)	13.7 (9.2–20.1)	13.2 (8.6–19.7)	13.5 (10.0–17.8)
Subadult (30, 43)	6.7 (1.8–21.3)	14.0 (6.6–27.3)	11.0 (5.7–20.2)	13.3 (5.3–29.7)	11.6 (5.1–24.5)	12.3 (6.6–21.8)
Juvenile (4, 1)	0 (0–49.0)	0 (0–79.4)	0 (0–43.5)	0 (0–49.0)	0 (0–79.4)	0 (0–43.5)
Unknown (1, 0)	0 (0–79.4)	N/A	0 (0–79.4)	0 (0–79.4)	N/A	0 (0–79.4)
Month						
January (32, 34)	3.1 (0.6–15.7)	11.8 (4.7–26.6)	7.6 (3.3–16.5)	9.4 (3.2–24.2)	23.5 (12.4–40.0)	16.7 (9.6–27.4)
March (25, 30)	36.0 (20.2–55.5)	43.3 (27.4–60.8)	40.0 (28.1–53.2)	12.0 (4.2–30.0)	6.7 (1.8–21.3)	9.1 (3.9–19.6)
May (35, 30)	34.3 (20.8–50.9)	36.7 (22.0–54.5)	35.4 (24.9–47.5)	25.7 (14.2–42.1)	16.7 (7.3–33.6)	21.5 (13.3–33.0)
August (31, 32)	0 (0–11.0)	0 (0–10.7)	0 (0–5.8)	3.2 (0.6–16.2)	3.1 (0.6–15.7)	3.2 (0.9–10.9)
October (33, 30)	12.1 (4.8–27.3)	33.3 (19.2–51.2)	22.2 (13.7–33.9)	18.2 (8.6–34.4)	20.0 (9.5–37.3)	19.1 (11.2–30.4)
December (32, 32)	15.6 (6.9–31.8)	12.5 (5.0–28.1)	14.1 (7.6–24.6)	9.4 (3.2–24.2)	6.3 (1.7–20.2)	7.8 (3.4–17.0)
Overall (188, 188)	16.5 (11.9–22.5)	22.3 (17.0–28.8)	19.4 (15.7–23.7)	13.3 (9.2–18.9)	12.8 (8.7–18.3)	13.0 (10.0–16.8)

N/A, Not applicable.

TABLE 3 Prevalence of *Brucella* spp. with 95% confidence intervals (CI) of 376 feral swine sera tested for antibodies with the buffered antigen plate agglutination test (BAPA), competitive enzyme-linked immunosorbent assay (cELISA), complement fixation test (CFT), fluorescence polarization assay (FPA), the rivanol, plate agglutination (PAT), standard tube test (STT) and card test. Samples with incomplete agglutination at any dilution were identified as suspect and were counted as positive in this table. Sensitivity and specificity estimates were calculated based on using the culture results from this study as the gold standard

Serological test	No. of positive	% Positive (95% CI)	Calculated sensitivity (Previously published sensitivity range[a])	Calculated specificity (Previously published specificity range)
BAPA	19	5.1 (3.3–7.8)	24.5 (76.2–95.6)	97.9 (96.4–99.3)
cELISA	24	6.4 (4.3–9.3)	28.6 (97.5–100)	96.9 (99.7–99.8)
CFT	30	8.0 (5.7–11.2)	32.7 (23.0–97.1)	95.7 (30.6–100)
FPA	22	5.9 (3.9–8.7)	28.6 (99.0–99.3)	97.6 (96.9–100)
Rivanol	20[b]	5.3 (3.5–8.1)	22.4 (50.5–100)	97.2 (21.9–100)
PAT	42[c]	11.2 (8.4–14.8)	26.5 (50.9–80.4)	91.1 (97.5–99.6)
STT	39[d]	10.4 (7.7–13.9)	34.7 (29.1–100)	93.3 (99.2–100)
Card	15[e]	4.0 (2.4–6.5)	22.4 (74.3–99.0)	98.8 (7.4–100)

[a]Published sensitivity and specificity values based on data from Fosgate et al. (2002) and Nielsen (2002).
[b]Includes 11 suspect samples.
[c]Includes 31 suspect samples.
[d]Includes 18 suspect samples.
[e]Includes one suspect sample.

40.8% and 83.8%, respectively. The highest antibody prevalence was detected using the plate agglutination test (11.2%), standard tube test (10.4%) and complement fixation (8.0%). At least one positive was detected with each of the serological tests although the results were positive on all eight serological tests for only 12 samples (Table 4).

3.3 | Prevalence trends

The antibody prevalence was similar at Facility A (9.0%; 95% CI: 5.7–14.0) and Facility B (10.6%; 95% CI: 7.0–15.9). The culture prevalence was also similar at Facility A (13.3%; 95% CI: 9.2–18.9) and Facility B (12.8%; 95% CI: 8.7–18.3). There were no significant differences

TABLE 4 Sera reactions of 376 feral swine to *Brucella* spp. using the buffered antigen plate agglutination test (BAPA), competitive enzyme-linked immunosorbent assay (cELISA), complement fixation (CF), fluorescence polarization assay (FPA), the rivanol, plate agglutination, tube agglutination and card test. Samples with incomplete agglutination at any dilution were identified as suspect and were counted as positive in this table

BAPA	cELISA	CF	FPA	Rivanol[a]	Plate[b]	Tube[c]	Card test[d]	No. of samples
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	303
Negative	Negative	Negative	Negative	Negative	**Positive**	Negative	Negative	14
Positive	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	12
Negative	Negative	Negative	Negative	Negative	Negative	**Positive**	Negative	11
Negative	Negative	Negative	Negative	Negative	**Positive**	**Positive**	Negative	8
Negative	Negative	Negative	Negative	**Positive**	Negative	Negative	Negative	5
Negative	Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	3
Negative	**Positive**	**Positive**	**Positive**	Negative	Negative	Negative	Negative	3
Negative	**Positive**	Negative	Negative	Negative	Negative	Negative	Negative	2
Negative	Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	2
Positive	Negative	Negative	Negative	Negative	**Positive**	Negative	**Positive**	1
Negative	**Positive**	**Positive**	Negative	Negative	Negative	Negative	Negative	1
Negative	**Positive**	**Positive**	**Positive**	Negative	Negative	**Positive**	Negative	1
Positive	Negative	Negative	Negative	Negative	**Positive**	**Positive**	Negative	1
Negative	Negative	**Positive**	Negative	Negative	**Positive**	**Positive**	Negative	1
Positive	**Positive**	**Positive**	**Positive**	Negative	**Positive**	Negative	**Positive**	1
Positive	Negative	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	Negative	1
Positive	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	Negative	1
Negative	**Positive**	**Positive**	**Positive**	**Positive**	Negative	**Positive**	Negative	1
Positive	Negative	**Positive**	Negative	Negative	**Positive**	**Positive**	Negative	1
Positive	**Positive**	**Positive**	Negative	Negative	**Positive**	Negative	Negative	1
Negative	**Positive**	**Positive**	Negative	Negative	Negative	**Positive**	Negative	1
Negative	Negative	Negative	Negative	Negative	Negative	Negative	**Positive**	1

[a]11 suspect samples.
[b]31 suspect samples.
[c]18 suspect samples.
[d]One suspect sample.

in antibody or culture prevalence between gender, age or collection month at either facility (Table 2).

4 | DISCUSSION

Although we collected various sample types to culture for *Brucella* spp., there was no one-sample type or even a particular region of the body that was consistently culture positive. Although whole blood and nasal swabs do not appear to be appropriate samples for culturing *Brucella* spp., our results imply that a sampling regime that does not include a variety of sample types will result in an underestimation of the actual prevalence. It also suggests that infection with *Brucella* spp. in feral swine is not necessarily localized to one particular region of the body, and anyone handling feral swine should be aware of the risk of exposure.

Despite sampling at two different facilities, there was no significant difference between the two in serologic or culture prevalence. In fact, the only observed difference was that August was the only collection month with significantly lower antibody (no positives) and cultural prevalence (<4%). Neither of the two facilities was climate-controlled and during the week in August that samples were collected, daytime temperatures exceeded 100°F. The possibility that extreme heat caused an overgrowth of contaminating bacteria that obscured the recovery of *Brucella* spp. cannot be excluded (Boraker, Stinebring, & Kunkel, 1981), even though the samples were placed in a cooler shortly after collection.

Although antibody (9.8%) and culture prevalence (13.0%) of *Brucella* spp. was similar overall in the feral swine we tested, only 32.7% of the culture-positive feral swine were antibody positive on any of the serological tests, and the sensitivity and specificity were much lower when comparing each of the serological tests independently (Table 3). Although it is common to report suspects as positives, it did impact the antibody prevalence and apparent effectivity of the serological test. There are several known limitations of existing serological diagnostics. This includes (i) detection of both false negatives and false positives,

(ii) cross-reactivity with other pathogens, (iii) the tests were originally validated in cattle, (iv) the tests have traditionally been utilized as herd tests in domestic swine, (v) the tests were developed using *B. abortus* not *B. suis* antigen and (vi) the loss of antibody titres over time (Godfroid et al., 2010; Weiner, Iwaniak, & Szulowski, 2012). Given these limitations, it is not surprising that serology underestimates infection.

In a study conducted in several U.S. states, feral swine lymph nodes were cultured for *Brucella* spp. resulting in 11.5% (n = 183) culture positive, and 25.3% of corresponding serum was antibody positive (Pedersen et al., 2014). Although the culture prevalence was similar to the prevalence we identified (13.0%), the antibody prevalence was much higher which may have been due to the targeted collection of feral swine samples from counties previously identified as antibody positive (Pedersen et al., 2014). Other studies in the United States have estimated *Brucella* spp. antibody prevalence in feral swine between 3.5 and 4.3% with localized areas of higher prevalence (Bevins et al., 2014; Pedersen et al., 2012). In Texas, the antibody prevalence in feral swine seems to vary widely from 1 to 24% depending on region of the state (Campbell et al., 2008; Musser, Schwartz, Srinath, & Waldrup, 2013; Wyckoff, Henke, Campbell, Hewitt, & VerCauteren, 2009). It is possible that some of this variation was also due to the different serological tests utilized between studies. In our study, we compared the results of eight different serological tests to each other and used the culture results to determine the infection status of the animal, which is often not possible due to the time, effort and cost required to collect and test numerous samples. The wide variation in congruence between culture and serology suggests the need for developing better diagnostics for detection in feral swine to ensure awareness of human risk.

There are very few studies that have analysed the humoral response of *Brucella* spp. in swine, and those that do exist consist of a few studies conducted in domestic swine inoculated with either *B. suis* or *B. abortus* S19 vaccine strain (Hutchings, Delez, & Donham, 1946; Kernkamp & Roepke, 1948). In the studies involving *B. suis* infection, sows were inoculated intravaginally or orally with a dose of $1–5 \times 10^{10}$ colony-forming units, and the humoral response persisted from 101 to 139 days post-inoculation (Hutchings et al., 1946). This is different from what is typically observed in cattle infected with *B. abortus* strain 2,308 where antibodies can be detected for more than a year, whereas animals vaccinated with *B. abortus* S19 strain typically have a short duration of antibody response. In cattle, antibodies to S19 typically can be detected from 6 to 12 weeks (Aguirre et al., 2002; Draghi et al., 2010; Nielsen et al., 2007; Sutherland, 1984), but in swine, antibodies can be detected for <4 weeks (Kernkamp & Roepke, 1948). Persistence of *Brucella* titres has been reported in cattle and *Brucella* can generally be cultured from about 50% of antibody-positive cattle (Roffe et al., 1999). However, this does not appear to be the case in swine, as either not all culture-positive swine retain a titre or the current *Brucella* serologic tests do not identify all serologically positive swine. Serological methods that currently exist may not be applicable for domestic and feral swine as they were developed for cattle, and as such, additional research to develop serological tests specifically for swine is recommended.

Despite detection of *B. suis* and *B. abortus* in feral swine in proximity to cattle (Musser et al., 2013), a spatial and temporal association, but not transmission, is all that has been established. However, *B. suis* infection in cattle complicates surveillance for *B. abortus* as antibody responses cannot be differentiated between the two strains (Olsen & Hennager, 2010). Although *B. suis* infection does not cause abortions in cattle typically observed with *B. abortus*, it is associated with a high incidence of retained placentas and shedding in the milk (Olsen & Hennager, 2010). Eliminating the infection in cattle is also desirable to prevent human infection caused by consumption of unpasteurized milk.

Prior to efforts to eradicate brucellosis in the United States, *B. suis* infections were common among abattoir workers; now human infections are less common (Giurgiutiu et al., 2009), but brucellosis is still one of the most commonly reported zoonosis worldwide (Seleem, Boyle, & Sriranganathan, 2010). In the United States, the North Carolina Department of Environment, Health and Natural Resources reported a total of 22 cases of brucellosis in employees with exposure to the pork processing plant kill floor in 1991 and 1992 (Trout et al., 1995). A subsequent investigation conducted by the Centers for Disease Control's National Institute for Occupational Safety and Health determined that 19% of kill floor workers (n = 154) had evidence of recent or persistent brucellosis, and 53% of the cases (n = 30) were identified in employees who had not previously been diagnosed, leading to a recommendation that the plant process only brucellosis-free swine (Trout et al., 1995). Although commercial swine in the United States maintain brucellosis-free status, antibody prevalence of brucellosis in feral swine can be up to 14.4% (Pedersen et al., 2012), meaning that employees at abattoirs who slaughter feral swine are at increased risk of exposure to *Brucella* spp. At a minimum, personal protective equipment and health monitoring programmes should be provided to employees working in these facilities.

Feral swine hunters and especially those with hunting dogs are also at an increased risk of exposure. Hunters are perhaps at the highest risk of exposure while field dressing animals. However, *B. suis* infection has been reported in hunting dogs exposed to feral swine (Ramamoorthy et al., 2011), and it is suspected that infected hunting dogs can subsequently transmit the infection to humans (Barr, Eilts, Roy, & Miller, 1986). Evidence of transmission to a human from a dog that was exposed to infected domestic swine has been reported (Nicoletti, Quinn, & Minor, 1967), thus further supporting the possibility. In addition to the risk to owners of infected dogs, veterinary staff should be aware of the possibility of *B. suis* infection when treating dogs with reproductive tract signs, back pain or lameness, and should take appropriate precautions to prevent transmission (Mor et al., 2016).

Our results suggest that there is a large gap in congruency between *Brucella* spp. serology and culture in feral swine and that development of serological tests designed to specifically detect *B. suis* in feral swine with high sensitivity and specificity is warranted. Additional outreach to abattoir workers, veterinarians, hunters and wildlife biologists is recommended to ensure that appropriate precautions are taken to prevent infection or if symptoms develop that proper post-exposure care and prompt treatment are pursued.

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