



ORIGINAL ARTICLE

Detection of *Brucella* spp. during a serosurvey of pig-hunting and regional pet dogs in eastern Australia

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Brucellosis is a zoonotic disease with worldwide distribution. *Brucella suis* serotype 1 is thought to be maintained in the Australian feral pig population, with disease prevalence higher in Queensland (Qld) than New South Wales (NSW). Pig hunting is a popular recreational activity in rural Qld and NSW, with feral pigs in these states thought to carry *B. suis*. Brucellosis associated with *B. suis* has been diagnosed in dogs engaged in pig hunting in some of these areas. A total of 431 dogs from northern Qld and north-west NSW were recruited. Two distinct cohorts of clinically healthy dogs were tested – (1) 96 dogs from central, north and far north Queensland actively engaged in pig-hunting and (2) 335 dogs from rural and remote north-west NSW that were primarily companion (non-pig hunting) animals. Serum samples were tested for antibodies to *Brucella* spp. using the Rose Bengal test (RBT) test followed by complement fixation testing (CFT) for RBT-positive samples. A subset of samples was retested using RBT and CFT. Seven dogs were considered seropositive for *B. suis* from Qld and remote NSW, including 4/96 (4.2%; 95% CI 3.5% to 4.3%) from the pig-hunting cohort and 3/335 (0.9%) from the regional pet dog cohort. The use of RBT and CFT in dogs to detect anti-*Brucella* antibodies requires validation. Veterinarians treating pig-hunting dogs and physicians treating pig hunters in central, north and far north Qld need to be aware of the zoonotic risk posed by *B. suis* to these groups.

Keywords *Brucella suis*; dogs; pig hunting; Queensland; Rose Bengal; veterinary science

Abbreviations CDC, Centers for Disease Control and Prevention; CFT, complement fixation test(ing); EMAI, Elizabeth Macarthur Agricultural Institute; IQR, interquartile range; NSW, New South Wales; PCR, polymerase chain reaction; Qld, Queensland; RBT, Rose Bengal test(ing); RSPCA NSW, Royal Society for the Prevention of Cruelty to Animals New South

Wales; SAT, serum agglutination test; USA, United States of America; X, crossbreed

Aust Vet J 2022;100:360–366

doi: 10.1111/avj.13172

Some members of the *Brucella* genus can cause brucellosis, one of the most important global zoonotic diseases.¹ *Brucella* spp. is Gram-negative aerobes that act as facultative intracellular pathogens targeting the mononuclear phagocytic system and reproductive tracts of mammals and amphibians,² with sporadic incidental infections found in humans exposed to infected animals or their products.³ Although widespread, brucellosis is considered a neglected disease by the World Health Organization, with 500,000 cases of human brucellosis reported annually.⁴ Like many febrile bacterial diseases, human brucellosis is likely under-reported, with some estimates suggesting that 5,000,000–12,500,000 cases occur annually.⁵

Globally, most cases of human brucellosis are caused by *Brucella melitensis*, *Brucella abortus*, *Brucella suis* and *Brucella canis*. *B. melitensis* is maintained by sheep, goats and camels, with *B. abortus* carried by cattle, water buffalo, elk and bison. Most human infections arise from these two organisms, often due to exposure to infected animals around the time of parturition, particularly in areas like the Middle East where food production is mainly from small-holdings where pasteurisation of milk and cheese is suboptimal.^{6–9} *B. canis* is maintained by domestic dogs, with transmission primarily venereal.¹⁰ *B. melitensis*, *B. abortus* and *B. canis* are all considered exotic to Australia, however *B. suis* is endemic.¹¹

The last reported case of *B. suis* in a domestic pig in Australia was in Queensland (Qld) in 1991, although it persists in the feral pig population.¹² There are an estimated 25 million feral pigs in Australia, with most of these found in Qld and New South Wales (NSW).¹³ Feral pigs in both Qld (seroprevalence 1.85%–10.5%) and NSW (seroprevalence 0%–3%) have been found to be seropositive to *B. suis*,^{14–17} and most human infections in Australia are reported in Qld and from individuals who had direct or indirect contact with feral pigs.^{11, 18, 19}

B. suis has also been detected in dogs used for pig hunting, a popular recreational activity in rural Qld and NSW.^{20, 21} In dogs, *B. suis* infection can cause disease in the reproductive system, axial or appendicular skeleton, potentially resulting in lameness, abortion, testicular and epididymal enlargement, prostatic abscessation, back pain and/or discospondylitis.²² Transmission of *B. suis* from dog to human has been recorded in Australia,²³ with the zoonotic nature of

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the organism presenting a risk to pig hunters, veterinarians, veterinary nurses, and laboratory staff exposed to heavy inoculums at surgery, necropsy, during culture in the veterinary laboratory, or after abortion of pregnant bitches.^{20, 24}

As feral pigs are known to carry *B. suis* in Qld and NSW^{14–17} and pig-hunting dogs are at an increased risk for contracting *B. suis* in south-east Qld and north-west NSW,^{20, 21} this study sought to expand our understanding of the exposure risks of *B. suis* in two distinct populations of dogs: pig-hunting dogs in central, north and far north Qld and non-pig hunting (companion) dogs in north-west NSW. Based on findings from previous serosurveys, it was hypothesised that greater than 5% of pig-hunting dogs in central, north and far north Qld would be seropositive to *B. suis* but none of the companion dogs in north-west NSW would be. We theorised that the risk of contracting *B. suis* is associated with engaging in the activity of pig-hunting, rather than incidental environmental exposure alone.

Materials and methods

Sample population: Pig-hunting dogs

Ten veterinary clinics from central, north and far north Qld were invited to participate in this study firstly by email, then in person. These clinics were chosen based on their location in rural and regional areas, as it was believed they would more likely have pig hunter clientele. Eight clinics from Sarina, Clermont, Proserpine, Charters Towers, Tully, Innisfail, Malanda and the Atherton Tableland participated and collected samples between August and November 2018 (Figure 1). All dogs enrolled had written owner consent, were 6-months-of-age or older and were actively used for pig hunting at the time of sampling. Medical histories for each patient

were obtained from the participating clinics, with the following data captured: age, sex and reproductive state (entire or neutered), breed and vaccination history ('C3' – contains canine distemper virus, canine adenovirus 1 and canine parvovirus antigen; 'C5' – C3 plus canine parainfluenza virus and *Bordetella bronchiseptica* antigen; *Leptospira interrogans* serovar Copenhageni; *Leptospira interrogans* serovar Australis). Ethics approval for all animal handling and sampling was obtained from The University of Sydney Animal Ethics Committee (approval number 2018/1341).

A registered veterinarian performed a physical examination and deemed each dog to be healthy prior to sampling, with no evidence of back or joint pain or testicular swelling (in entire male dogs). Whole blood (3–5 mL) was collected via cephalic venepuncture, stored on ice and allowed to clot; serum was harvested, aliquoted and stored at -20°C until testing was performed.

Sample population: Regional pet dogs

Dogs were enrolled from eight remote communities in western NSW participating in the Royal Society for the Prevention of Cruelty to Animals NSW (RSPCA NSW) Indigenous Community Companion Animal Health Programs (ICCAHPs; Figure 1). All dogs were pets and their owners provided written consent. Serum samples were obtained between September 2016 and May 2018, with the breed, sex and age of dogs recorded. Clients of other RSPCA NSW ICCAHPs participated in another research project exploring relationships with dogs which included questions such as the number of dogs owned, diet, sleeping place, use of pets for hunting and whether pets had bred in the previous 5 years.²⁵ All samples were obtained under general anaesthesia while sterilisation surgery was taking place. Ethics approval was obtained from The University of Sydney Animal Ethics Committee (approval numbers 2016/1044 and 2017/1140).

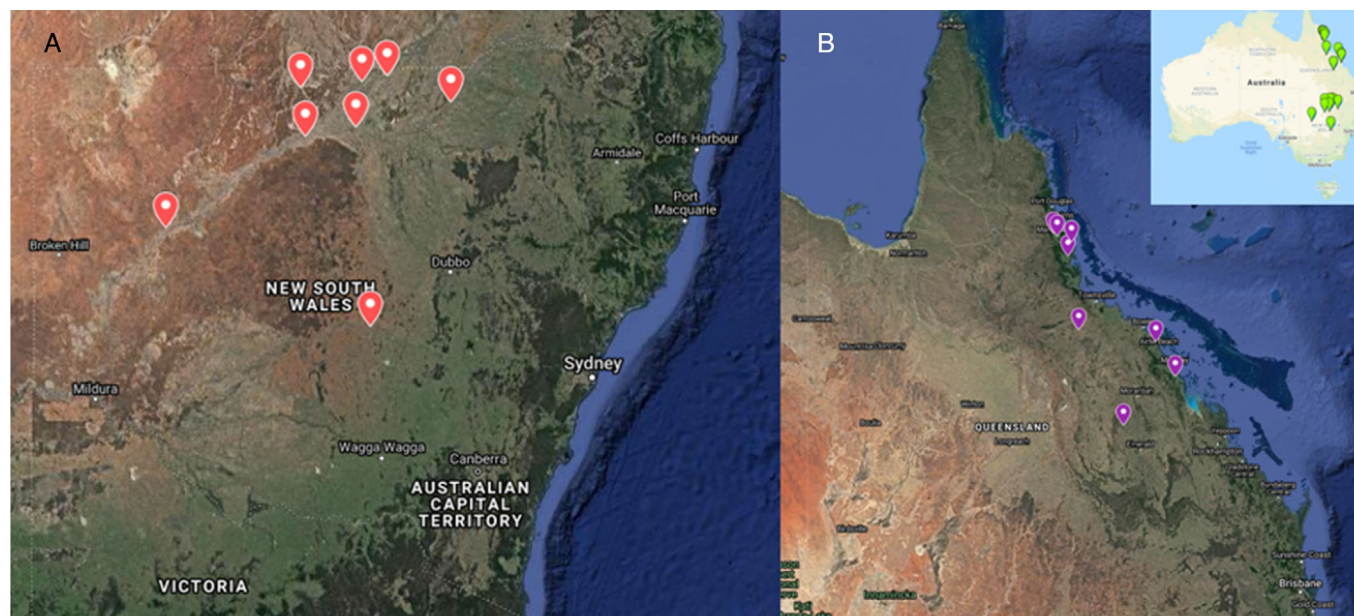


Figure 1. Map of Australia indicating the regions where dogs were sampled for anti-*Brucella* spp. antibodies in serosurveys between 2016 and 2018 (A—regional dogs in NSW, B—pig-hunting dogs in Qld).

A total of 3.5 mL of whole blood was obtained from each dog by jugular or cephalic venepuncture and placed in an evacuated serum separator tube. After clot formation at room temperature, serum tubes were centrifuged at 2000 g for 10 min. Serum was then transferred in 0.5 mL aliquots to sterile cryogenic tubes and stored at -20°C until tested.

Laboratory testing

The pig-hunting dog samples underwent Rose Bengal testing (RBT) in 2019 using the bioMérieux Brucelloslide-Test at SydPath, St Vincent’s Hospital, Darlinghurst, NSW, 2010. Samples that tested RBT-positive were shipped for complement fixation testing (CFT) at Elizabeth Macarthur Agricultural Institute (EMAI), Woodbridge Road, Menangle, NSW, 2568. In 2021, all positive pig-hunting dog samples with sufficient stored serum underwent repeat RBT and CFT at EMAI. A random selection of negative pig-hunting dog RBT samples originally tested in 2019 was also retested with both RBT and CFT at EMAI in 2021. The regional pet dog samples underwent RBT at EMAI in 2018, using the IDEXX Pourquier® Rose Bengal Ag test kit, and if RBT-positive, underwent CFT at EMAI. In 2021, all positive, regional pet dog samples with sufficient stored serum and several randomly selected negative samples underwent repeat RBT and CFT at EMAI.

The RBT is a rapid slide or card agglutination test that uses killed *B. abortus* organisms, stained red with Rose Bengal, as the antigen to detect anti-*Brucella* spp. antibodies. Although *B. abortus* is exotic to Australia, it is a so-called smooth *Brucella* species and can therefore be used to indirectly identify *B. suis* antibodies, as there is no direct serological test for *B. suis*. As RBT is more sensitive at detecting IgG, whereas CFT is more dependent on IgM, both tests can be useful in a serosurvey examining exposure to *B. suis*.²⁶⁻²⁸

RBT was used to screen all samples, although different test kits were used in the two laboratories. Any positive or inconclusive results were subsequently subjected to CFT at EMAI as a secondary test, according to EMAI guidelines for *Brucella* testing. Although there are data on the sensitivity and specificity of RBT and CFT in cattle (RBT - Se 89.7%, Sp 96.9%; CFT - Se 80.9%, Sp 99.9%²⁶; and RBT in humans (RBT - Se 87.4%, Sp 100%²⁷; for detecting anti-*Brucella* spp. antibodies, no published data exist on the sensitivity and specificity of these tests for anti-*B. suis* antibodies in canines. SydPath classified RBT results as either positive or negative using the bioMérieux kit, and EMAI currently

classifies a positive RBT as either 1+, 2+ or 3+ using the IDEXX kit. For CFT, EMAI pre-treat serum to inactivate complement at a 1:3 dilution with Kolmar for 30 min at 53°C. EMAI uses a ≥1/8 dilution as the cut-off titre for a positive diagnosis of anti-*Brucella* spp. antibodies with CFT, although this is empiric, and the technique has not been systematically validated.

The Commonwealth Department of Health Public Health Laboratory Network’s case definition for human brucellosis list definitive laboratory evidence as:

- 1 Isolation (i.e., positive culture) of *Brucella* species from a sterile site, or
- 2 Seroconversion or significant increase in *Brucella* antibody titres in acute and convalescent sera

Table 1. Location of dogs tested for *Brucella* antibodies between 2016 and 2018 as part of a serosurvey

Town	State	No. dogs tested (number positive for <i>Brucella</i> antibodies)	% Total tested
Atherton	Qld	5	1.2
Charters Towers	Qld	6	1.4
Clermont	Qld	29 (2 positive)	6.7
Innisfail	Qld	5	1.2
Malanda	Qld	30 (1 positive)	7.0
Proserpine	Qld	17 (1 positive)	4.0
Sarina	Qld	1	0.2
Tully	Qld	3	0.7
Bourke	NSW	78 (1 positive)	18.1
Brewarrina	NSW	101 (2 positive)	23.4
Collarenebri	NSW	34	7.9
Condobolin	NSW	32	7.4
Enngonia	NSW	25	5.8
Goodooga	NSW	19	4.4
Weilmoringle	NSW	26	6.0
Wilcannia	NSW	20	4.6
Total		431 (7 positive)	100

Towns, where a *Brucella* seropositive dog was identified, are shaded.

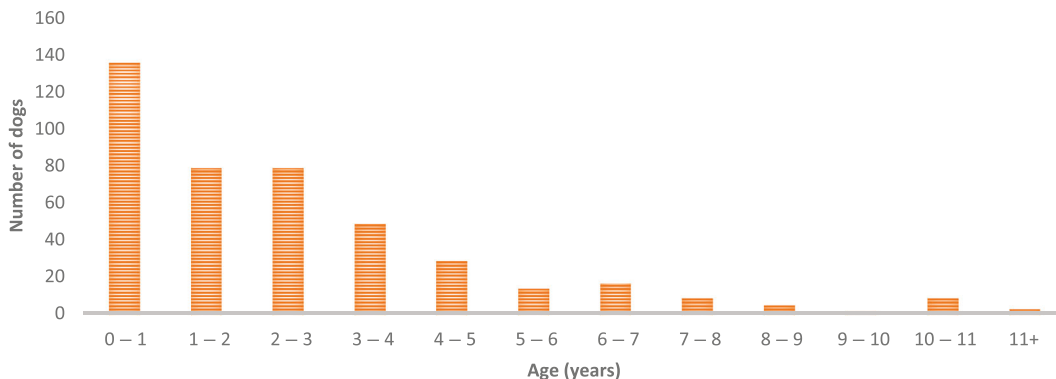


Figure 2. Age of dogs sampled in a serosurvey from 2016 to 2018 from Queensland and New South Wales.

Table 2. Positive and inconclusive *Brucella* results of dogs tested with CFT and RBT as part of a serosurvey in central, north and far north Qld and north-west NSW between 2016 and 2018

Location (state)	Breed	Age (years)	Sex	Neutered	RBT (original)	RBT (repeat)	CFT (original)	CFT (repeat)	<i>Brucella</i> spp. status
Malanda (Qld)	Cattle Dog X	2	Female	Entire	Positive	2+	1/64	1/32	Positive
Clermont (Qld)	Ridgeback X	7	Male	Entire	Positive	1+	1/8	Anticomplementary	Positive
Clermont (Qld)	Bull Arab X	7	Female	Entire	Positive	2+	Anticomplementary	Anticomplementary	Positive
Proserpine (Qld)	Bull Arab X	3	Female	Entire	Positive	N/A	N/A	N/A	Positive ^a
Bourke (NSW)	Chihuahua X	0.5	Female	Entire	1+	1+	Anticomplementary	<1/4	Positive
Brewarrina (NSW)	Terrier X	2	Female	Entire	1+	1+	1/4	1/4	Positive
Brewarrina (NSW)	Terrier X	1	Male	Entire	1+	N/A	<1/4	N/A	Positive ^a
Proserpine (Qld)	Wolfhound X	2	Female	Entire	Positive	Negative	<1/4	<1/4	Inconclusive
Bourke (NSW)	Mastiff X	4	Male	Entire	Negative	1+	N/A	1/4	Inconclusive
Bourke (NSW)	Bull Arab	5	Male	Entire	1+	Negative	Anticomplementary	Anticomplementary	Inconclusive
Bourke (NSW)	Wolfhound X	2	Male	Entire	1+	Negative	Anticomplementary	<1/4	Inconclusive
Wilcannia (NSW)	Mastiff X	4	Female	Entire	1+	Negative	Anticomplementary	Anticomplementary	Inconclusive
Wilcannia (NSW)	Labrador	2	Female	Entire	1+	Negative	1/4	Anticomplementary	Inconclusive
Wilcannia (NSW)	Maltese X	2	Female	Entire	1+	Negative	Anticomplementary	<1/4	Inconclusive
Condobolin (NSW)	Maltese X	2.5	Female	Entire	1+	Negative	<1/4	<1/4	Inconclusive
Condobolin (NSW)	Maltese X	4	Female	Entire	1+	Negative	Anticomplementary	Anticomplementary	Inconclusive
Condobolin (NSW)	Kelpie X	0.8	Female	Entire	1+	Negative	<1/4	<1/4	Inconclusive
Condobolin (NSW)	Maltese X	0.5	Female	Entire	1+	Negative	<1/4	<1/4	Inconclusive
Condobolin (NSW)	Maltese X	1	Female	Entire	1+	Negative	<1/4	<1/4	Inconclusive
Condobolin (NSW)	Cattle Dog	0.8	Male	Entire	1+	Negative	1/4	<1/4	Inconclusive
Brewarrina (NSW)	Pointer X	2	Female	Entire	1+	Negative	<1/4	<1/4	Inconclusive
Goodooga (NSW)	Chihuahua	1	Female	Entire	1+	Negative	Anticomplementary	Anticomplementary	Inconclusive
Collarenebri (NSW)	Kelpie X	0.3	Male	Entire	1+	Negative	Anticomplementary	Anticomplementary	Inconclusive
Collarenebri (NSW)	Cattle Dog X	0.3	Male	Entire	1+	Negative	Anticomplementary	Anticomplementary	Inconclusive

^a Due to insufficient serum this sample was unable to be retested after initial RBT.

EMAI subjectively classified a positive RBT as either 1+, 2+ or 3+, based on the strength of the reaction and colour change observed. X, crossbred; N/A, not applicable as not tested.

Suggestive criteria include:

- 1 A single high titre of a specific *Brucella* antibody²⁹

Due to the variation in serologic diagnostic criteria used by laboratories for brucellosis in dogs, we utilised the following criteria for the dogs in our serosurvey:

- 1 **Negative result:** RBT negative on both original and repeat testing
- 2 **Inconclusive result:** RBT positive on either original or repeat testing, but not both
- 3 **Positive result:** RBT positive on both original and repeat testing, or RBT positive on original testing only (for samples that only underwent one round of testing)

While CFT results were available for each dog, they were not used in our diagnostic criteria due to concerns about the suitability of CFT as a screening tool for *Brucella* exposure in dogs.

Data analysis

Data was mapped using Google MyMaps (<https://google.com/maps>). The neuter status of positive and negative dogs in Qld and the

number of male and female dogs in both cohorts were compared using two-tailed Fisher's exact tests. A significant level of $P < 0.05$ was used.

Results

Sample population: Total

A total of 431 dogs were sampled between 2016 and 2018 from across Qld and north-west NSW (Table 1). The majority (335/431; 78%) of dogs tested were from NSW. The age of the study cohort was skewed, with most dogs sampled less than 4 years of age (343/431; median age 2 years; IQR 0.5–3 years; Figure 2). Overall, there were more females sampled than males (239/431; $P = 0.027$; Fishers Exact test, two-tailed). Seven dogs across the study cohort were seropositive (7/431; 1.6%), and 17 dogs returned inconclusive results (17/431; 3.9%).

Sample population: Pig-hunting dogs

Four dogs were seropositive from the 96 pig-hunting dogs sampled (4.2%; 4/96; 95% CI 3.5% to 4.3%; Table 2), with one dog returning

an inconclusive result. The four *Brucella* spp. seropositive pig-hunting dogs were from the far north Qld region of Malanda and central Qld regions of Clermont and Proserpine. More than 80% (80/97) of the dogs sampled from Qld, including all seropositive cases, were sexually intact. There was no relationship between the likelihood of a pig-hunting dog being entire and *Brucella* spp. seropositive (Fisher's exact test; two-tailed; $P = 1$).

Sample population: Regional pet dogs

Of the 335 dogs from NSW sampled, three were seropositive (3/335; 0.9%) with a further 16 returning inconclusive results (Table 2). The three positive regional pet dogs were from the remote NSW regions of Bourke and Brewarrina. All dogs from NSW were sexually entire at the time of sampling due to their enrolment in a sterilisation program.

Discussion

This is the first published report of *Brucella* detection in a serosurvey of dogs in central, north and far north Queensland. Four of the pig-hunting dogs sampled were seropositive, with one of them recording repeated positive RBT results at two different laboratories and a high CFT titre of 1/64. These four dogs were in geographically distinct areas separated by hundreds of kilometres, with one in Malanda on the Atherton Tablelands in far north Qld, two in Clermont, central Qld and one in Proserpine, coastal central Qld (Table 1). All dogs were clinically normal at the time of sampling, and thus may have recently recovered from an infection or have subclinical brucellosis, possibly with *B. suis* organisms constrained to a very limited focus in a lymph node in the patient. Brucellosis is a notifiable animal disease in Queensland, meaning veterinarians who suspect a dog may be infected with *B. suis* are required by law to notify Biosecurity Queensland.³⁰ However, diagnosing dogs based on serological results alone is problematic. Serology may result in false positives, due to cross-reactions such as the known *Yersinia enterocolitica* O:9 lipopolysaccharide O-chain cross-reaction,²⁹ or false negatives, for example, if a *Brucella* infection is localised in the body or if the serological tests being used aren't validated for the test subject. Additionally, practical limitations and expenses relating to shipping samples often mean suspected canine cases are treated symptomatically by veterinarians in Queensland rather than subjected to confirmatory testing (J. Lee, Pers Comm., 23/08/21).

We hypothesised that greater than 5% of the pig-hunting dog cohort would be seropositive for *B. suis*, based on previous serosurveys of pig-hunting dogs in south-east Qld and NSW.³¹ Our results were close to the expected seroprevalence, with 4.2% of pig-hunting dogs from central, north and far north Qld seropositive. Although our findings reinforced our hypothesis, it is likely there is regional variation in the presence and prevalence of *B. suis* infections in feral pigs across Australia,^{14–16} impacting the *B. suis* seroprevalence in pig-hunting dogs. Feral pigs are not a homogenous population across Australia³² and with *B. suis* mainly spread between pigs via bodily fluids and sexual reproduction, the transmission of *B. suis* normally requires close contact between infected groups of pigs, although the bacteria can survive in cool, moist soil for a period of time.^{33, 34} Additionally, the historic rate of *B. suis* in the feral pig population in

Qld may have been overestimated. Previous studies have found between 1.8% and 10.5% of feral pigs in Qld seropositive for *B. suis*,^{15, 16} although this does not mean they were all actively shedding organisms in the environment. Indeed, of the 3% of feral pigs found to be seropositive in an NSW serosurvey, none was positive on serum culture or polymerase chain reaction (PCR).¹⁴

The high number of dogs initially positive on RBT (Table 2), particularly regional pet dogs unlikely to have had meaningful contact with feral pigs, prompted us to retest all RBT-positive samples plus several negative samples using both RBT and CFT, although the appropriateness of using both RBT and CFT is unsubstantiated. Unfortunately, two RBT-positive samples were unable to be retested due to insufficient serum. On the second round of testing and based on our serological diagnostic criteria for a positive, inconclusive, and negative result, we found seven seropositive dogs and 17 inconclusive results, with all the inconclusive results testing RBT-positive on the initial round of testing. While the pig-hunting dog results of four seropositive and one inconclusive was expected, the high number of inconclusive results in NSW regional pet dogs was unexpected. While these dogs lived in rural and remote regions in an area of the state that has previously recorded *B. suis* infected dogs,^{20, 31} they were primarily pet or companion animals confined to a yard and only a small number was used infrequently for hunting, although some were allowed to free roam.²⁵ According to the previously published survey of dog owners, about half of the dogs sampled had been bred in the past 5 years and almost three-quarters of respondents fed their dogs with raw meat on various occasions.²⁵ The three seropositive NSW dogs were small breeds generally unsuitable for pig hunting, with one less than 12 months of age. This raises questions about their serostatus, however, infection at parturition or via ingestion of infected raw meat is possible and has been previously recorded.²⁴

The variability in results between the first and second rounds of RBT and CFT highlights the difficulty in interpreting *Brucella* spp. serological results. Both tests rely on technician interpretation, with the 'weak positive' of the RBT (classified as +1 by the EMAI technical manual) representing an equivocal result. EMAI uses a cut-off dilution of 1/8 for the CFT, as a CFT result of 1/4 is particularly difficult to read. However, an analysis of the literature shows considerable variation in the use of rapid diagnostic kits for *Brucella* spp. serological testing, including varying definitions of a positive or negative case.^{28, 29, 35} Additionally, there is no published sensitivity or specificity for either RBT or CFT to detect antibodies against *Brucella* spp. in dogs.

At the time of testing, and out of an abundance of caution, EMAI used a cascading approach to a positive diagnosis – that is, only samples positive on RBT were tested with the 'confirmatory' CFT. We opted to not refer to CFT in our diagnostic criteria and developed a more nuanced approach to RBT for establishing laboratory evidence of *Brucella* seropositivity in canines. While the World Organisation for Animal Health (OIE) recommends both RBT and CFT as useful tests for general surveillance studies,³⁴ they note that these tests may not be useful in all species and in all situations, and each test must be validated for fitness in the tested species. Importantly, they note that CFT is less sensitive than RBT and is only recommended as a

complementary test for diagnosing *B. suis* infection.³⁴ Since our samples were tested, EMAI has changed its diagnostic methodology to now test all canine samples with both RBT and CFT, regardless of the initial RBT result. Further deliberation on the usefulness of RBT and CFT as a screening tool for *B. suis* in canines is required.

With human health laboratories in Australia moving away from rapid slide agglutination tests for *Brucella* spp. and moving towards the serum or tube agglutination test (SAT; S. Repousis, Pers Comm., 18/10/21), it would be worthwhile for veterinary diagnostic laboratories to review their current serological tests and assess if they are still fit for purpose, particularly for diagnosing disease in animals like dogs. As it stands, we advise against using CFT as a 'confirmatory' test after screening with RBT in canine serosurveys. The Commonwealth Department of Health considers SAT the 'gold standard' serological test,²⁹ as it is simple to perform and is widely utilised by public health agencies including the Centers for Disease Control and Prevention (CDC) in the USA.³⁶ Unfortunately, due to limited sample volumes after repeated RBT and CFT, we were unable to perform the SAT on our samples. Further research to establish the sensitivity and specificity of RBT, CFT and SAT as serological tests for *Brucella* spp. in dogs is required. With brucellosis a notifiable disease in dogs in Qld and NSW, and veterinary clinicians potentially making long-term treatment decisions, including euthanasia, based on serological results, it is vital for veterinary diagnostic laboratories to review their serological testing for *B. suis* in dogs and for veterinarians to reinforce serological results with clinical presentation, bacterial culture or repeat serology.

While our study cohorts were not representative samples of the dog population in eastern Australia, our research showed that pig-hunting dogs in Queensland were at risk for *B. suis* exposure, echoing earlier research that showed pig-hunting dogs from south-west Qld and northern NSW have an increased risk of contracting *B. suis*.^{20, 31} Our regional pet dog population, however, also demonstrated low rates of *Brucella* spp. exposure, in contrast to earlier studies on pet dogs.^{37, 38} Feeding feral pig meat to dogs is a risk factor for *B. suis* infection,²⁰ therefore it is possible that exposure for both pig-hunting dogs and regional pet dogs in remote regions came from contaminated pig meat.

There are several limitations associated with fixed time-point 'snapshot' serosurveys such as the one used in the current study. We were only able to determine past exposure from our samples and were unable to establish the timing of the exposure or whether this exposure initially caused clinical or subclinical disease. Taking subsequent blood samples from the seropositive dogs would have allowed us to monitor their serostatus over time and determine if they developed clinical signs of brucellosis. Additionally, pig-hunting dogs are known to be transported long distances to facilitate hunting expeditions,³⁹ meaning the location where *B. suis* seropositivity was diagnosed may not have been the site of exposure. Although feeding raw feral pig meat is a known risk factor for brucellosis in dogs,²⁰ we were unable to determine if this risk was present in our study cohorts.

Veterinarians who treat pig-hunting dogs in Qld and NSW should be aware of the zoonotic risks presented by these dogs, as they have also been previously found to have high rates of *Coxiella burnetii* and *Leptospira* spp. exposure.^{40, 41} Pig hunters should be aware of the risks posed by pig-hunting to their dogs and take appropriate

preventative measures to protect both themselves and their dogs. Finally, further research is required to identify more reliable serological tests for *B. suis* in dogs.

Acknowledgements

The authors would like to thank Debbie Marriott from SydPath, St Vincent's Hospital for facilitating the RBT testing at St Vincent's, and the staff at EMAI Serology, particularly Monica Suann, for running and re-running our samples. Open access publishing facilitated by The University of Sydney, as part of the Wiley - The University of Sydney agreement via the Council of Australian University Librarians.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest or sources of funding for the work presented here.

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(Accepted for publication 1 May 2022)