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Human *Brucella canis* outbreak linked to infection in dogs

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SUMMARY

The zoonotic risk of *Brucella canis* has been considered fairly high for persons who handle breeding dogs in kennels or are exposed to infected animals. Transmission to humans in other circumstances has been thought to be rare. We describe an uncommon outbreak of brucellosis caused by *B. canis* which, to the best of our knowledge, is the first reported in the literature. This outbreak involved six persons (three children and three adults), a bitch and three puppies which had close daily contact with the family. The clinical symptoms of the index case led to an erroneous diagnosis and the infection would have gone undiagnosed if culture had not been positive. This report aims to increase awareness of medical personnel of the need to order screening tests for children, immunodeficient persons or pregnant women presenting with fever of unknown origin, unexplained spleen or liver enlargement or other systemic signs. The emerging zoonotic potential of this disease in urban areas and the need to coordinate canine brucellosis surveillance systems should be evaluated.

Key words: *B. canis* infection, brucellosis outbreak.

INTRODUCTION

Brucella canis is a Gram-negative, aerobic, intracellular coccobacillus first recognized in 1966 [1], isolated from tissue and vaginal discharges of infected dogs. Cultures are always in the rough or mucoid phase on primary isolation. The *Brucella* Subcommittee meeting in Munich in 1978 [2] recommended that it be given full species status.

Outbreaks of canine abortions caused by *B. canis* have been widely reported [3–5] describing clinical signs ranging from asymptomatic to abortion and testicular atrophy [6].

The zoonotic risk is relatively high in persons who handle breeding dogs in kennels and are exposed to reproductive tissues and fluids of infected dogs [6]. Transmission to humans in other circumstances has been considered rare [7] with only 30 cases reported worldwide since the first isolation in the late 1960s [6]. Other authors consider *B. melitensis* and

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B. suis more virulent for humans than *B. abortus* and *B. canis* [8].

In this paper we present results from an uncommon outbreak of human brucellosis, which to the best of our knowledge is the first reported in the literature to be caused by *B. canis*.

MATERIALS AND METHODS

Index case

A 17-month-old boy was admitted to the Paediatrics Department of the Eva Perón Hospital of San Martín, Buenos Aires with a 72 h history of watery diarrhoea, watery vomiting, fever (39 °C) and dehydration 5%. Gastroenteritis was suspected because of vomiting without diarrhoea 1 week earlier which had resolved spontaneously. Co-proculture was negative and CSF by lumbar puncture was normal, no organisms growing in culture of this sample. Laboratory tests showed leukocytes $7{\cdot}3 \times 10^9$/l, lymphocytes 29·4%, monocytes 9·6% and granulocytes 61%. Red cell count was 5×10^9/l, haemoglobin 12·3, haematocrit 36%, platelets $2{\cdot}77 \times 10^5$, creatinine 0·30, urea 0·12 and glycaemia 0·9. Iontophoresis: potassium 3·4, sodium 130 and chloride 101.

The patient received oral glucose electrolyte solution therapy and 100 mg/kg ceftriaxone twice a day. After 3 days of antibiotic treatment he showed remarkable clinical improvement, antibiotic therapy was suspended and the patient released from the hospital. Three days afterwards the two blood cultures (Bact Alert, bioMérieux, France) were positive and a small non-mobile Gram-negative coccobacillus was isolated. The strain grew without producing acid on triple sugar iron (TSI) agar and was positive to urease, nitrate reduction and oxidase tests. It was tentatively identified as *B. canis* and sent to our laboratory for confirmation. Conventional brucellosis serological tests had not been performed on the patient's serum.

Epidemiological investigation

Because of suspicion of canine brucellosis the family of the index case was traced. They had two dogs, a male and a female; the latter had recently had five puppies, two stillborn. The dogs and three puppies were clinically examined and tested for brucellosis. The family had kept one female puppy and given the two males to two other families. The index case family (10 members), six members of the family in contact with one male puppy and seven of the family with the second puppy were tested for brucellosis.

Serological tests

For detection of smooth *Brucella* antibodies: we ran the buffered plate agglutination test (BPAT), Rose Bengal test (RBT), standard tube agglutination test (STAT) and complement fixation test (CFT) using antigens prepared at ANLIS by Dr C. G. Malbrán with *B. abortus* 1119-3 strain. Competitive ELISA (CELISA) were performed as previously reported [9] with antigen (S-LPS from *B. abortus* 1119-3) and the mAb standardized and supplied by the Brucellosis Centre of Expertise and OIE Reference Laboratory, Animal Diseases Research Institute (ADRI), Canada. The test is positive when $\%I > 28$.

For detection of rough *Brucella* antibodies we used rapid screening agglutination test (RSAT) to detect anti-*B. canis* antibodies, including a control standard serum with each test. This antigen was prepared at ANLIS by Dr C. G. Malbrán from the (M-) variant strain of *B. canis*.

Indirect ELISA (IELISA) with *B. canis* antigen was used as a confirmatory test for the detection of human [10] and dog [11] anti-*B. canis* antibodies including positive, weak positive and negative sera as controls in each plate. In order to detect human anti-*B. canis* antibodies we used a previously established cut-off value of $\%P > 27$; for dogs the value was $\%P > 29$. A recombinant protein combining immunoglobulin-binding sites of proteins A and G conjugated with horseradish peroxidase was used for assessment of antibodies to rough lipopolysaccharide in dogs and humans [10].

Clinical isolates

For dog blood cultures we used monophasic commercial liquid medium Hemo *Brucella* (Britania SA, Argentina).

Bacteriological studies

The strains isolated were identified and typed by CO_2 requirement and its agglutination pattern with monospecific anti-A, anti-M and anti-R sera. *Brucella* cultures are smooth or rough, agglutinated by their respective antisera. Smooth-form cultures may be examined for their predominant agglutinogen A

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Table 1. *Serology, bacteriology and clinical findings in human cases and dogs*

				B. abortus antigen				*B. canis* antigen					
								CELISA		IELISA			
Family	Case	Age	Date*	BPAT	RBT	STAT	CFT	%I	RSAT	%P	Blood culture	Strain isolated	Clinical findings
1	1	17 mo.	0								Pos	*B. canis*	Fever, diarrhoea, Vomiting
			1	Neg	Neg	Neg	Neg	19	Pos	74			
			4	Neg	Neg	Neg	Neg	10	Pos	56			
	2	5 yr	0	Neg	Neg	Neg	Neg	21	Pos	75			
			3	Neg	Neg	Neg	Neg	13	Pos	61			
	3	42 yr	0	Neg	Neg	Neg	Neg	28	Pos	55			
			3	Neg	Neg	Neg	Neg	15	Pos	30			
2	4	40 yr	0	Neg	Neg	Neg	Neg	28	Pos	100			
	5	5 yr	0	Neg	Neg	Neg	Neg	17	Pos	64			
			1	Neg	Neg	Neg	Neg	18	Pos	60			
			2	Neg	Neg	Neg	Neg	15	Pos	30			
3	6		0	Neg	Neg	Neg	Neg	16	Pos	45			
	Dog 1		0	Neg					Pos	53	Pos	*B. canis*	
	Puppy 1		0	Neg					Pos	62	Pos	*B. canis*	
	Puppy 2		0	Neg					Pos	48	Pos	*B. canis*	
	Puppy 3		0	Neg					Pos	51	Pos	*B. canis*	

CELISA, Competitive ELISA; IELISA, indirect ELISA; BPAT, Buffered plate agglutination test; CFT, complement fixation test; Neg, negative; Pos, positive; RBT, Rose Bengal test; RSAT, rapid slide agglutination test; STAT, standard tube agglutination test.
CELISA cut-off (I%) >28; Dogs IELISA cut-off (%P)>29; Human IELISA cut-off (%P) >27.
* Date = months after first consultation.

(*B. abortus*, *B. suis*) or M (*B. melitensis*) but rough-form cultures are agglutinated by unabsorbed antisera prepared with *B. canis* or *B. ovis* cultures. Urease test, production of H_2S, growth on dyes, erythritol and penicillin sensitivity and lysis by Tb, Wb and R/C phages were performed following procedures previously described and included typed *Brucella* strains of each species in all tests [12, 13]. Colony morphology was studied initially by direct observation, acriflavine test and staining of colonies with Crystal Violet.

PCR of strains isolated

A previously described [14] combinatorial PCR was performed. DNA of strains was amplified using puReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Science Corp., USA). We were able to identify *B. canis* when the primer BCSP31 and omp31 were amplified and amplicons of omp2b and omp2a were detected by *B. canis*-specific primers but not by *B. abortus*-specific primers.

RESULTS

Serological tests using *B. abortus* 1119-3 antigen were negative for the patients, but when *B. canis* antigen was used, three members of the index case family, two of the second family and one of the third family tested positive with titres declining over time (Table 1). The bitch and three puppies also tested positive (Table 1).

Five strains were isolated: one from the index case and four from dogs in contact with him. Conventional biochemical tests performed on strains were consistent with *B. canis* (Table 2); subsequently, combinatorial PCR confirmed these results.

After confirmation of strain as *B. canis* the index case was cited and placed on 5 mg/kg trimethoprim–sulfamethoxazole/20 mg/kg rifampicin daily for 6 weeks. Seventy-two hours later he presented mild fever and nasal congestion probably due to an associated viral infection. At the end of antibiotic therapy he was much better. His 5-year-old brother with positive RSAT and IELISA was examined clinically showing palpable spleen and 19200 wbC. He suffered from an episode of vomiting lasting for 3 days one

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Table 2. *Differential characteristics of strains isolated and species of genus* Brucella

				Growth on media with					Agglutination in sera[f]			Lysis by phages[g]		
Strain	Colonial morphology	CO_2 requirement	H_2S production	Thionin[a]	Basic fuchsin[a]	Safranin O[b]	i-erythritol[c]	Penicillin[d]	Urease[e]	A	M	R	Tb	Wb	R/C
Human 1	Rough	−	−	+/−	+/−	−	+	−	++	−	−	+	−	−	+
Dog	Rough	−	−	+/−	+	−	+	−	++	−	−	+	−	−	+
Puppy 1	Rough	−	−	+/−	+	−	+	−	++	−	−	+	−	−	+
Puppy 2	Rough	−	−	+/−	+	−	+	−	++	−	−	+	−	−	+
Puppy 3	Rough	−	−	+/−	+	−	+	−	++	−	−	+	−	−	+
B. melitensis 16M*	Smooth	−	−	+	+	+	+	+	+	−	+	−	−	−	−
B. suis 1330*	Smooth	−	+	+	−	−	+	−	++	+	−	−	−	+	−
B. abortus 544*	Smooth	+	+/−	−	+	+	+	+	−	+	−	−	+	+	−
B. canis RM6/66*	Rough	−	−	+	−	−	+	+	++	−	−	+	−	−	+

[a] 20 μg/ml; [b] 100 μg/ml; [c] 1 mg/ml; [d] 5 IU/ml in base medium; [e] Bauer's method; [f] A, A monospecific antiserum; M, M monospecific antiserum; R, rough *Brucella* antiserum; [g] at routine test dilution (RTD).

* Reference strains.

month previously and at the time of the examination presented asthenia and loss of appetite. He was placed on 5 mg/K trimethoprim–sulfamethoxazole/20 mg/kg rifampicin daily for 40 days. Two months later he had recovered. The mother with positive RSAT and IELISA reported asthenia as her only symptom and after clinical examination no treatment was indicated. Neither signs nor symptoms of relapse were detected during the follow-up period (6 months) in the outpatient service where index case, his brother and mother were evaluated.

The mother of the second family which had been in contact with puppies reported headache, asthenia, myalgias and nausea over the last 2 months but refused clinical examination. Her 5-year-old daughter had suffered from fever of unknown origin, vomiting and diarrhoea 2 months previously. The girl had a high (>64) c-reactive protein and was placed on 5 mg/kg trimethoprim–sulfamethoxazole/20 mg/kg rifampicin daily for 40 days but therapy was abandoned after 3 weeks. One member of the third family that received a puppy was positive to RSAT and IELISA, asymptomatic and after clinical examination no therapy was indicated.

DISCUSSION

No clinical signs are pathognomonic of canine brucellosis, although reproductive failure and infertility should be suspected. Transmission is mainly through contact with vaginal discharges, abortion materials and fluids of bitches and semen and/or urine of males [3]. Since clinical examinations are inadequate for diagnosis, isolation of the organism and serological tests are the only reliable way to confirm a presumptive diagnosis. In this case the bitch had a history of abortion 3 years previously, gave birth to weak puppies which died after 3 days one year later, but was never diagnosed with brucellosis. Of the last pregnancy in 2008, two puppies were born dead and three (two males, one female) were apparently normal.

This situation, associated with a recent study of 219 dogs in lower-class neighbourhoods and slums of Buenos Aires with a high rate of unmet basic needs, which found anti-*B. canis* antibodies in 7·3% of dogs and *B. canis* isolations in three cases, indicates a health hazard for the population exposed [15]. More recently 224 dogs tested for canine brucellosis in the context of a free neuter programme in another area of Buenos Aires found 10·7% serologically positive dogs while *B. canis* was isolated in two cases [16]. Since

infected dogs have been shown to remain bacteraemic for long periods of time, these results also suggest a risk of human infection in this area.

Considering the few reports of human global cases in the past 20 years, *B. canis* is probably either not tested for or not reported [5]. Since routine human brucellosis diagnosis does not include *B. canis* investigation, infection with this species may be more widespread than is currently suspected [10].

This outbreak involved six persons (three children, three adults) and four dogs living in close contact with the family. The clinical symptoms of the index case led to an erroneous diagnosis and the infection would have remained undiagnosed if culture had not been positive. Human brucellosis is usually described as a disease with protean manifestations and should be suspected, especially in endemic areas. Awareness of canine brucellosis in humans is low, including knowledge of its transmission potential and its medical consequences. Identification of a human case should prompt investigation in order to enable early detection and treatment of all patients. This report aims to increase the awareness of medical personnel of the need to order screening tests for children, immunodeficient persons or pregnant women presenting fever of unknown origin, unexplained spleen or liver enlargement or other systemic signs.

Because this study was based on clinical observations of this case alone, we were unable to evaluate the contribution *B. canis* to other possible cases in the same neighbourhood. All ages were affected, probably because of close contact with the bitch and puppies; however, children, particularly those aged <6 years might be more exposed because they play with dogs more often and are less protected. The family may have become infected by the latest whelping because they were in contact with the puppies whereas the two previous episodes were miscarriages.

After the outbreak, all dogs were removed from the house to the Anthropozoonosis Centre where one puppy died. *B. canis* was isolated from spleen, axillary lymph nodes, thymus, pleurae and liver after autopsy. No strain was isolated from mediastinal lymph nodes. The bitch and two surviving puppies were neutered, placed on antibiotic therapy and checked periodically by serological and bacteriological tests.

Control measures including examination of dogs in the neighbourhood and a campaign for information and education of the community were developed by the Anthropozoonosis Centre. The emergence of this urban outbreak also demonstrates the importance of coordinating canine brucellosis surveillance systems.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Carmichael LE.** Canine brucellosis: Isolation, diagnosis, transmission. In: *Proceedings of the 71st Annual Meeting of the US Livestock Sanitary Association*. Richmond, VA: US Livestock Sanitary Association, 1968, pp. 517–527.
2. **Osterman B, Moriyon I.** International committee on systematics of prokaryotes, subcommittee on the taxonomy of Brucella. Minutes of the meeting, 17 September 2003, Pamplona, Spain. *International Journal of Systematic and Evolutionary Microbiology* 2006; **56**: 1173–1175.
3. **Carmichael LE.** *Brucella canis*. In: Nielsen K, Duncan JR, eds. *Animal Brucellosis*. Boca Raton, FL: CRC Press, 1990, pp. 335–350.
4. **Carmichael LE, Green EG.** Canine brucellosis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*. Philadelphia: Saunders, 1990, pp. 573–584.
5. **Carmichael LE, Shin SJ.** Canine brucellosis: a diagnostician's dilemma. *Seminars in Veterinary Medicine and Surgery* (*Small Animal*) 1996; **11**: 161–165.
6. **Hollett RB.** Canine brucellosis: outbreaks and compliance. *Theriogenology* 2006; **66**: 575–577.
7. **Wanke MM.** Canine brucellosis. *Animal Reproduction Science* 2004; **82–83**: 195–207.
8. **Young EJ.** An overview of human brucellosis. *Clinical Infectious Disease* 1995; **21**: 283–290.
9. **Lucero NE, *et al.*** Competitive enzyme immunoassay for diagnosis of human brucellosis. *Journal of Clinical Microbiology* 1999; **37**: 3245–3248.
10. **Lucero NE, *et al.*** Diagnosis of human brucellosis caused by *Brucella canis*. *Journal of Medical Microbiology* 2005; **54**: 457–461.
11. **Lucero NE, *et al.*** Sensitivity and specificity of an indirect enzyme-linked immunoassay for the diagnosis of *Brucella canis* infection in dogs. *Journal of Medical Microbiology* 2002; **51**: 656–660.
12. **Corbel MJ, Banai M.** Genus *Brucella*, Meyer and Shaw 1920, 173AL. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, eds. *Bergey's Manual of Systematic Bacteriology*. New York: Springer, 2005, vol. 2, pp. 370–386.

13. **Alton GG, *et al.*** Bacteriological methods. In: *Techniques for the Brucellosis Laboratory*. Paris, France: Institut National de la Recherche Agronomique, 1988, pp. 13–61.
14. **Imaoka K, *et al.*** Simultaneous detection of the genus *Brucella* by combinatorial PCR. *Japanese Journal of Infectious Disease* 2007; **60**: 137–139.
15. **Boeri E, *et al.*** Canine brucellosis in dogs in the city of Buenos Aires. *Medicina* (*Buenos Aires*) 2008; **68**: 291–297.
16. **López G, *et al.*** A serological and bacteriological survey of dogs to detect *Brucella* infection in Lomas de Zamora, Buenos Aires province. *Revista Argentina de Microbiología* 2009; **41**: 97–101.

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