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Monitoring infected dogs after a canine brucellosis outbreak

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ABSTRACT

Many episodes of canine brucellosis in dog kennels have been reported but recently an outbreak that involved pets and their owners has been described. The purpose of this study was to confirm that the outbreak had a common source and evaluate the evolution of 4 dogs involved in this outbreak after the measures implemented that included a survey of 41 animals from the same area. The variable number of tandem repeat (VNTR) analysis indicated that the *B. canis* isolated from the human clustered together with the isolates collected from the canine pups. Two dogs continued with bacteremia after the first antibiotic therapy and from one of them *B. canis* was also isolated from urine showing the importance of the later in the infection dissemination. In an effort to protect the public, stray dogs should be controlled and educational programs about the risk of this zoonotic disease should be implemented.

1. Introduction

Asymptomatic infected dogs can harbour *Brucella canis* for long periods of time [1]. The male prostate and epididymides that contain these bacteria may disseminate the disease in semen at the time of a breeding as well as vaginal discharges of infected females [2]. Both dog sexes excrete *B. canis* in urine and it has been demonstrated experimentally that this could serve as a route of infection [3,4]. It has been stressed that infected dogs be removed from kennels and euthanized or neutered and treated with antibiotics [5,6]. Although *B. canis* is sensitive *in vitro* to a variety of antibiotics, antibiotic therapy of infected dogs has resulted in failures and relapses. A single antibiotic regimen was reported as unsuccessful and was not recommended [7,8]. The combination of doxycycline with gentamicin has been used, however 2 or 3 courses of treatment separated by 1–2 months may be required [5]. Better success was achieved from a combinations of gentamicin–ciprofloxacin–doxycyclyne–rifampin [9] and doxycycline–enrofloxacin–rifampin–streptomycin for control ocular inflammation [10]. More recently, enrofloxacin has been suggested as an alternative drug for the treatment of dogs [11].

Many episodes of canine brucellosis have been reported in dog kennels, but recently an outbreak in Buenos Aires, Argentina that involved pets and their owners was also described [12].

The purpose of this study was to evaluate the evolution of dogs involved in this outbreak following control measures that included confinement, castration, antibiotic therapy and a brucellosis survey of 41 dogs from the same area. In order to confirm that the outbreak had a common source, variable number of tandem repeat (VNTR) analysis was performed [13].

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The results of this study were partially presented at the 64th Brucellosis Research Conference, Buenos Aires, Argentina, September 21st to 23rd, 2011.

2. Materials and methods

2.1. Dogs implicated in the outbreak

The outbreak involved a 4-year-old bitch with a history of abortion. In 2006 the bitch gave birth to weak puppies that died 3 days after birth and in the last pregnancy in 2008 had given birth to 5 puppies, two of which were born dead. Two of three (2 males and 1 female) apparently normal puppies were adopted by different families.

After the outbreak the bitch, 3 puppies and one cohabitant male were removed from their homes to the Anthropozoonosis Center, Veterinary and Preventive Medicine Division, TRES DE FEBRERO, Buenos Aires (ACVPM) [12]. Serological tests were positive for the bitch and the 3 remaining puppies and *B. canis* was isolated from the blood cultures of all four dogs while the cohabitant male tested negative [12].

2.2. Treatment

One month after confinement the dogs including the cohabitant were neutered and 2 months later were treated with iv enrofloxacin (7 mg/kg) daily for 30 days after which the bitch died. A second treatment was prescribed 16 months after admission but the dose was increased to 10–12 mg/kg daily for 30 days.

2.3. Serological tests

All dogs were tested with serological assays. For detection of R-*Brucella* antibodies rapid slide agglutination test (RSAT) and indirect ELISA (iELISA) were performed following previously established procedures [14], using antigens prepared at National Laboratories and Institutes of Health Administration (ANLIS). Each test included positive, weak positive and negative sera as controls. For detection of S-*Brucella* antibodies, the buffered plate agglutination test (BPAT) was run following established protocols [14].

2.4. Bacteriological studies

During castration, blood culture, urine, and testicle biopsy from all animals, including the cohabitant, were taken for bacteriology studies. *Brucella* organisms were isolated from fluids and tissues following procedures described previously [15]. The bacterial strains isolated were typed as recommended by the International Committee on Bacterial Nomenclature (ICBN) Subcommittee on Taxonomy of the Genus *Brucella* [16] at ANLIS and by a combinatorial PCR, according to a protocol described previously [17].

2.5. VNTR typing

Relationships between isolates F5/10–29 from human and F1/10–31, F1/10–32, F1/10–33, F1/11–1 and F1/11–2 from dogs were examined by cluster analysis using the categorical coefficient and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) implemented in Bionumerics Version 5.1 (Applied Maths, Belgium). Profiles of type strain *B. canis* RM6/66 were included for comparative purposes. Seventeen VNTR loci were targeted for amplification and subsequent allele size determination by running amplicons on a ABI 3130xl genetic analyzer (Applied Biosystems, Life Technologies, CA) following the methods outlined by Whatmore et al. [13]. Fragment analyses and final allele designation was based on amplicon size and determined in GeneMapper® Software (Applied Biosystems, Life Technologies, CA). For BioNumerics, the analyses were conducted with the *B. canis* reference strain and the isolates collected during this study. In both cases a weighted analyses was undertaken as described by Al Dahouk et al. [18].

2.6. Survey of dogs in the area

Two months after a free neuter program for animals of both sexes was run in the neighbourhood where the outbreak had occurred. During 35 days forty-one dogs were castrated and serum samples taken for serological testing. All were clinically examined and data on reproductive history and contact with canine roamers were obtained from the dog owners.

3. Results

Twenty days before the castration of dogs the first pup died and *B. canis* was isolated from the spleen, auxiliary lymph nodes, thymus, liver and pleurae after authopsy [12]. The cause of death was probably due to generalized location of bacteremia. *B. canis* was isolated from the blood culture, urine and testicle of pup No. 2, and from the urine and testicle of pup No. 3 (Table 1) but cohabitant dog tested negative. Eight months after *B. canis* was isolated from blood culture of puppies 2 and 3, urine cultures of both puppies tested negative.

Twelve months after admission, new samples were taken and *B. canis* was isolated from blood culture and urine of puppy No. 3. Clinical examination of the dogs showed generalized weight loss, probably due to captivity where homemade food was changed by dry pellets.

Thirteen months after admission the bitch died but since the cadaver was not kept frozen, the cause of death was not determined and not attempt to recover *Brucella canis* was made due to generalized contamination.

A new treatment scheme was prescribed 16 months after admission which consisted of increasing the doses of enrofloxacin to 10–12 mg/kg daily for 30 days. Studies of pups No. 2 and No. 3 include data from 1, 3, 14 and 20 months after the second antibiotic treatment.

The male living in the same home with the pups and their mother tested serologically and bacteriologically negative in all tests throughout this period. Because the outbreak was located in the Libertador (LIB) neighbourhood (Fig. 1), a survey (21 dogs) was implemented in this area and adjoining vicinities Loma Hermosa (LH) 16 dogs, Martin Coronado (MC) 1 dog, P. Podestá (PP) 1 dog, E. de

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Table 1
Serology and bacteriological findings of dogs.

Case	Age	Date*	Serology			Bacteriology								
			BPAT	RSAT	IELISA (%P)	Blood culture	U	T	S	ALN	TH	L	P	Isolations	Strains
Bitch	4y	0	(*)	Pos	53	Pos	ND							*B. canis*	F5/10–32
		8	Neg	Pos	39	Neg	Neg								
		12	(*)	Pos	56	Neg	Neg								
Died		13							Con	Con		Con	Con		
Puppy 1	2m	0	Neg	Pos	62	Pos	ND							*B. canis*	F5/10–33
Died		1							Pos	Pos	Pos	Pos	Pos	*B. canis*	
Puppy 2	2m	0	Neg	Pos	48	Pos	ND							*B. canis*	F5/10–31
		1	Neg	Pos	51	Pos	Pos	Pos						*B. canis*	
		8	Neg	Pos	39	Pos	Neg							*B. canis*	
		12	Neg	Pos	46	Neg	Neg								
		17	Neg	Pos±	33	Neg	Neg								
		19	Neg	Pos±	30	Neg	Neg								
		30	Neg	Neg	27	Neg	ND								
		36	Neg	Neg	27	Neg	ND								
Puppy 3	2m	0	Neg	Pos	39	Pos	ND							*B. canis*	
		1					Pos	Pos						*B. canis*	
		8	Neg	Pos	47	Pos	Neg							*B. canis*	F1/11–1
		12	Neg	Pos	70	Pos	Pos							*B. canis*	F1/11–2
		17	Neg	Pos	56	Neg	Neg								
		19	Neg	Pos	59	Neg	Neg								
		30	Neg	Pos	78	Neg	Neg								
		36	Neg	Pos	86	Neg	Neg								

*: months after beginning of the case consultation; BPAT: buffered plate antigen test; ND: not done; m: months; y: years; RSAT: rapid slide agglutination test; IELISA cut off (%*P*) > 29; Pos: positive; Neg: negative; NT: not done; U: urine; T: testicle; S: spleen; ALN: auxiliary lymph nodes; TH: thymus; L: liver; P: pleurae; (*): non specific agglutination; Con: contaminated.

Table 2
Serological findings of 9 positive dogs.

Dog	Neighbourhood	Habits	Sex	Age	Breed	BPAT	RSAT	IELISA	Clinical findings
1	LIB	Home	F	2y	MB	Neg	Pos	75	None
2	LIB	Home	F	2y	MB	Neg	Pos	59	None
3	LIB	RD	M	5y	MB	Neg	Pos	47	None
4	LIB	RD	F	5y	MB	(*)	Pos	44	2 birth, pups died latter
5	LIB	RD	F	ND	MB	(*)	Pos	32	None
6	LH	RD	F	4y	MB	(*)	Pos	52	1 birth, pups died
7	LH	RD	F	4y	MB	Neg	Pos	35	None
8	LH	Home	F	7y	MB	Neg	Pos	85	Distemper
9	MC	Home	F	8y	MB	Neg	Pos	36	None

PP: Pablo Podesta; LIB: Libertador; LH: Loma Hermosa; Home: dogs with owners; RD: dogs allowed to roam freely; MB: mix breeds; ND: no data; M: male; F: female; Pos: positive; Neg: negative; IELISA positive %*P* > 29; (*): non specific agglutination; y: years.

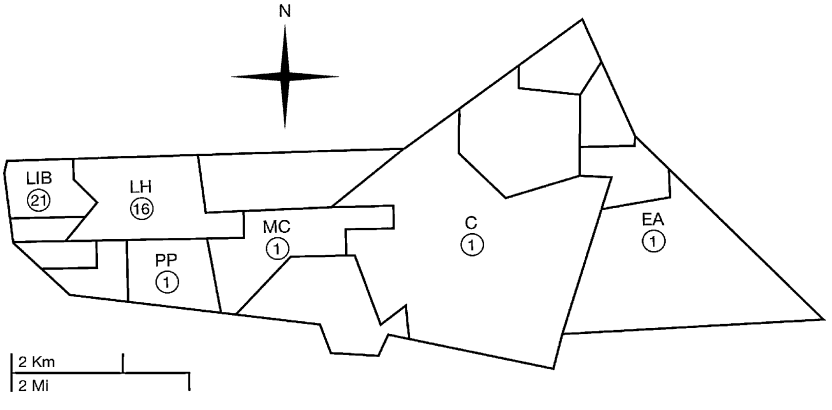

Fig. 1. Source of 41 dogs castrated in 6 neighbourhoods.

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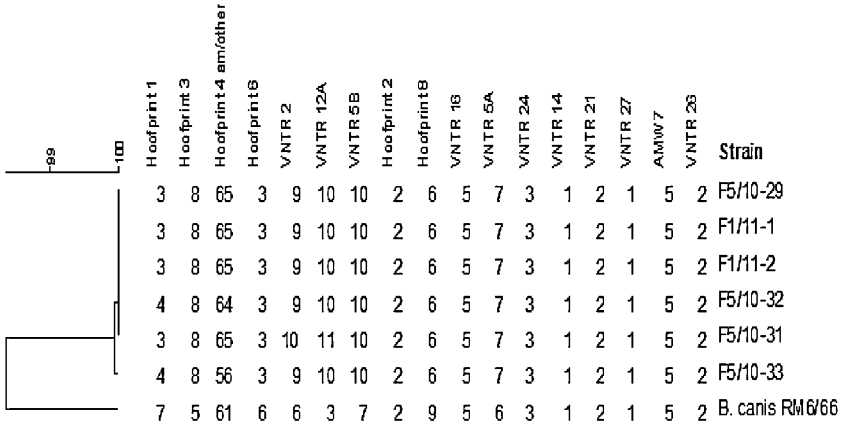

Fig. 2. Relationship between isolates from the outbreak as determined by VNTR analysis (Whatmore et al. [13]). Profile obtained from the reference strain RM6/66 is included for comparison.

los Andes (EA) 1 dog and Caseros (C) 1 dog. These 41 dogs were neutered and studied by serological tests; 9 dogs aged between 2 and 8 years (8 female and 1 male) were positive, of which 2 had a history of whelped weak pups with subsequent mortality and 1 had had distemper. All these positive dogs were mixed breeds; 4 of them lived with their owners and 5 were allowed contact with canine roamers. Of the 9 positive animals, 5 were from LIB, 3 from LH and 1 from MC (Table 2).

Conventional biochemical tests as well as combinatorial PCR were consistent with *B. canis* and VNTR results indicated that the *B. canis* isolate from the human (F5/10–29) clustered together with four other isolates collected from the pups (F5/10–31, 32, 33, F1/11–1 and 2). Here, the isolates from the pupps were identical to the *B. canis* isolate from the human at 13 of the 17 VNTR loci considered (Fig. 2). Differences in alleles were apparent at 4 of the VNTR loci namely, VNTR12a, Hoofprint 1, VNTR2 and Hoofprint 4 (Fig. 2). In all cases the differences in allele calls at these loci were based on a one step change. The *B. canis* reference isolate and the isolates collected during this study indicated that the later were the same as the reference isolate at 8 VNTR loci while they were different at 9 VNTR loci.

4. Discussion

The problem of stray dogs infected with canine brucellosis and its implications for public health has been amply reported [1,5]. In Buenos Aires a serological study of 219 dogs from lower class neighbourhoods and slums found 7.3% anti-*B. canis* antibodies and in 3 cases *B. canis* were isolated, indicating a health hazard for the human population [19]. More recently, 224 dogs tested for canine brucellosis in the context of a free neuter program in another area of Buenos Aires found 10.7% serologically positive dogs and *B. canis* was isolated in 2 cases [20]. Since infected dogs have been shown to remain bacteremic for long periods of time, these results also suggest a risk of human infection in this area. From 1996 to 2010, 292 strains of *B. canis* have been isolated in our laboratory from dogs, mainly from Buenos Aires.

Free neuter programs are in progress in different neighbourhoods of the city every year. Our survey in the neighbourhood that included castration found 9 (21.9%) serologically positive dogs and 5 (23%) in LIB, but bacteriological studies were not conducted. Of the 41 dogs castrated 33 were females of which 8(25%) were serologically positive.

Usually the most important source of contagion is contact with aborted foetal and vaginal discharges from females and semen and/or urine from infected males [3,4]. In this report pup No. 2 continued with bacteremia after the first antibiotic therapy whereas after the second treatment bacteriological studies in both blood and urine samples tested negative and the serological test titre decreased. However, *B. canis* were isolated from the blood culture of pup No. 3 after the first antibiotic therapy and also from blood and urine 12 months after admission, showing the potential importance of the latter in *B. canis* dissemination in spite of castration.

After the second treatment this pet had negative cultures but the serological titre remained high, probably indicating *B. canis* localization. Studies performed 30 months after admission showed that pup No. 2 was serologically negative, but pup No. 3 had increased titres.

It is important to highlight that these puppies exhibited a good overall condition, good temperament and no sign of disease during confinement on the ACVPM premises and have been requested for adoption. It has been stressed that dogs with brucellosis may recover spontaneously as soon as a year after infection but it is more common for recovery to take place after 2–3 years, whereas some dogs may remain chronically infected for at least 5 years [21].

The VNTR based typing schemes have been described as a tool to identify the possible origin of infections and/or recognize outbreaks that reflect a common source [18]. On the basis of more variable loci, this assay could differentiate isolates from restricted sources. From the VNTR analysis it is apparent that the *B. canis* isolate from the human and the pupps are related as they only differed at four VNTR loci.

Several options of dog population management have been proposed [22]. In an effort to protect public health, stray dogs should be controlled and educational programs

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on the potential risk of this zoonotic disease should be implemented. *Brucella canis* infection should be included in differential diagnosis for dogs, regardless of previous history or neuter status.

Disclosure statement

No competing financial interests exist.

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