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# A serological and bacteriological survey of dogs to detect *Brucella* infection in Lomas de Zamora, Buenos Aires province

G. LÓPEZ[1], S. M. AYALA[2], A. M. EFRON[2], C. F. GÓMEZ[1], N. E. LUCERO[2]*

[1]*Zoonosis Center, Municipalidad de Lomas de Zamora, 12 de octubre 1060, (1828) Banfield, Provincia de Buenos Aires, Argentina;* [2]*Brucellosis Service, Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) "Dr. C. G. Malbrán", Avda. Vélez Sarsfield 563, (1281) Ciudad Autónoma de Buenos Aires, Argentina.*
**Correspondence: E-mail: nidia@elsitio.net*

## ABSTRACT

Canine brucellosis caused by *Brucella canis* is a disease of the reproductive tract that may cause miscarriage in females, infection of the sexual organs in males and infertility in both sexes. The prevalence of brucellosis in dogs is unknown and little has been done to control the disease, except in certain breeds and some commercial dog kennels. In the course of a free neuter program in Lomas de Zamora, Buenos Aires province, prevalence of antibodies to *Brucella* sp., bacteriological isolation and clinical observations were performed. Of 224 dogs studied, 33 (14.7%) were found positive for the rapid slide agglutination test (RSAT), 24 (10.7%) of which were confirmed by IELISA. Of the 33 RSAT positive, 17 (51.5%) blood cultures were done, and *B. canis* were isolated from 2 cases. Since infected dogs have been shown to remain bacteremic for prolonged periods, our results also suggest a risk of human infections in this area.

**Key words:** *Brucella canis*, brucellosis, canine brucellosis

## RESUMEN

**Estudio serológico y bacteriológico de brucelosis en perros de Lomas de Zamora, provincia de Buenos Aires.** La brucelosis canina causada por *Brucella canis* es una infección que afecta el tracto reproductivo de los perros y que puede provocar abortos en las hembras, infección de los órganos sexuales en los machos e infertilidad en ambos sexos. Se desconoce la prevalencia de esta enfermedad y las medidas de control se aplican sólo en algunos criaderos comerciales. Durante un programa de castración gratuita de perros llevado a cabo en el Partido de Lomas de Zamora (Gran Buenos Aires) se realizaron estudios clínicos, serológicos y bacteriológicos en 224 animales. En la población estudiada, la prueba de microaglutinación rápida en portaobjetos (RSAT) resultó positiva en suero de 33 (14,7%) perros y fueron confirmadas por IELISA en 24 (10,7%) de ellos. A 17 de los 33 perros positivos mediante RSAT (51,5%) se les practicaron hemocultivos y en 2 casos se aisló *B. canis*. Debido a que los perros infectados permanecen bacteriémicos durante períodos prolongados, constituyen un riesgo para la salud pública.

**Palabras clave:** *Brucella canis*, brucelosis, brucelosis canina

Although there have been reports of brucellosis cases in dogs by contact exposure to livestock infected with *Brucella abortus, Brucella melitensis* and *Brucella suis*, canine brucellosis is caused mainly by *Brucella canis* (4, 14). This disease of the reproductive tract may cause miscarriage in females, infection of the sexual organs in males, as well as infertility, dyscospondilitis and uveitis in both sexes. *B. canis* is spread by contact with the semen or vaginal discharge of an infected animal or by contact with mammary secretions or miscarried puppies. Infected, non-pregnant females are usually asymptomatic, except for enlarged lymph nodes. There is no fever associated with the infection, though intermittent bacteremia can persist for two years or longer (5).

The global prevalence of brucellosis in dogs is unknown (8), and little has been done to control the disease, except in certain breeds and some commercial dog kennels.

The purpose of the present survey of urban dogs in an area of Buenos Aires province was to ascertain the prevalence of antibodies to *Brucella,* as well as to isolate the bacteria and gather clinical data. Since the infection can be transmitted to humans, another objective was to determine the potential risk to residents of the area.

A free neuter program (NP), that included dogs and cats of both sexes, aged between six months through 10 years, was implemented by the Municipality of Lomas de Zamora (LZ), province of Buenos Aires in 1992 and has continued to date offering owners the free neutering/ castration of their pets, mainly if they are in contact with roamers.

LZ with 13 neighborhoods (Figure 1), has an estimated population of 642,000 people and 34,839 dogs [1 dog

per 3.5 inhabitants] (1). A survey of 224 dogs (220 females and 4 males) was run in 11 of the 13 neighborhoods in the course of a NP.

All dogs were clinically examined at the time of neutering/castration and sera were collected, but blood cultures could only be obtained from 166 animals, since some owners did not cooperate.

Information on dogs included name, size, age, breed, reproductive history, contact with other animals and owner data. Because of the threat of human transmissions, if the dogs tested positive for brucellosis, the persons in contact with them were phoned and offered a brucellosis serological diagnosis.

For detection of antibodies against smooth *Brucella* spp., the buffered plate agglutination test (BPAT), the Rose Bengal test (RBT) and the standard tube agglutination test (SAT), were run as previously described (9), with antigens prepared at ANLIS "Dr. C. G. Malbrán", by using the *B. abortus* 1119-3 strain.

For detection of antibodies against rough *Brucella* spp., the rapid slide agglutination test (RSAT) was performed (3), with serial sera dilutions to determine the end point titre. Briefly, 10 µl of serum dilution were mixed with 10 µl of antigen on a 25 x 75 mm glass slide for 1-2 min and results were read with a 10X microscope objective including a control standard serum with a known titre. This antigen was prepared at ANLIS "Dr. C. G. Malbrán" from the (M-) variant strain of *B. canis.*

As a confirmatory technique, the IELISA was run with the antigen obtained from the (M-) variant of *B. canis* by the procedure reported previously (10). The lyophilised protein A/G, horseradish peroxidase conjugated, was obtained from ImmunoPure, Pierce Lb. and was used at 1:20,000 after testing various working dilution ranges with strong positive, weak positive and negative sera. The antigen diluted in 0.06 M sodium carbonate buffer (pH 9.6) was passively coated at 50 µl/well, incubated for 18 h at room temperature (RT) and then washed five times in 0.01 M phosphate buffered saline containing 0.05% tween 20, pH 7.2 (PBS/T). Control and test sera were added at 1:100 in PBS/T, 50 µl/well, for 1 h at RT. After five washes in PBS/T, the appropriated diluted protein A/G, horseradish peroxidase conjugated, was added, 50 µl/well, and incubated for 1 h at RT. Finally, 100 µl of chromogenic substrate [4.0 mM hydrogen peroxide and 1.0 mM 2,2´-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt in 0.05 M citrate buffer, pH 4.5] per well was added and the $OD_{414}$ measured in a photometer (Labsystems Multiskan EX microplate reader) by use of 100 µl of chromogenic substrate in a plate as a control for the microplate reader. The test is positive when color develops, the cut-off value being > $OD_{414}$ 0.281.

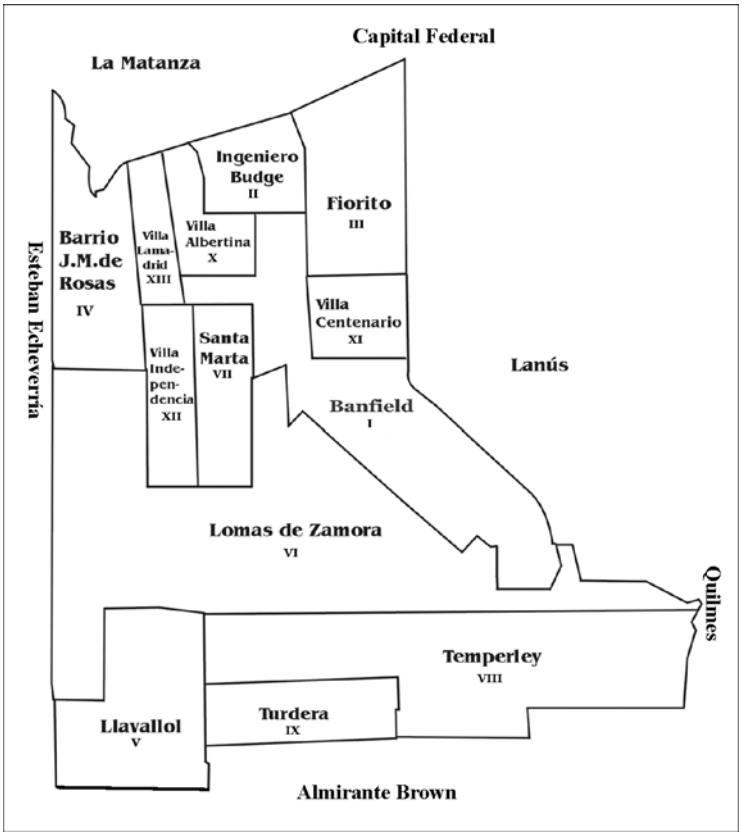


**Figure 1.** Neighborhoods of Lomas de Zamora, province of Buenos Aires

*Brucella* organisms were isolated from blood cultures and the strains typed as recommended by the International Committee on Bacterial Nomenclature (ICBN) Subcommittee on Taxonomy of the Genus *Brucella* (7) at ANLIS "Dr C.G. Malbrán".

It was suggested that *B. canis* brucellosis posed no serious problem for the health of the general population, but pets and strays dogs could be infected with this microorganism. Seroprevalence rates of approximately 6% have been reported in the USA where stray dogs are allowed to roam freely (6). In 1980, Myers and Varela Díaz (15) found that 30.5% of sera from stray dogs in a county of Buenos Aires province presented agglutination antibodies to *B. canis,* while 6.0% of animals had positive *B. canis* cultures. In 2008, Boeri *et al*. (2), studied 219 dogs in lower class neighbourhoods and slums of the city of Buenos Aires and detected anti-*B. canis* antibodies in 7.3% of dogs and *B. canis* was isolated from 3 cases.

**Table 1.** Clinical, serological and bacteriological findings from 33 RSAT positive dogs.

| Dog | Sex | Age | Breed | BPAT | RSAT | IELISA | Blood Culture | Clinical Findings |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 3y | H. Doberman | Neg | Pos± | 0,190 | Neg | Neg |
| 2 | F | 1y | H. Shepherd | Neg | Pos± | 0,124 | Neg | Neg |
| 3 | F | 2y | H | Pos± | 32 | 0,808 | ND | Neg |
| 4 | F | 1y | H | Neg | Pos± | 0,308 | Neg | Neg |
| 5 | F | 7y | H | Neg | 2 | 0,713 | Neg | Neg |
| 6 | F | 2y | H | Neg | 16 | 0,714 | ND | Neg |
| 7 | F | 3y | H | Neg | Pos± | 0,263 | Neg | Neg |
| 8 | F | NI | H | Neg | 16 | 0,706 | Neg | Neg |
| 9 | F | 5y | H | Neg | Pos± | 0,284 | Neg | Neg |
| 10 | F | 9m | H | Pos± | 128 | 0,955 | Neg | Neg |
| 11 | F | 3y | H | Neg | Pos± | 0,706 | Neg | Tumor |
| 12 | F | 6y | H | Neg | Pos | 0,786 | ND | Neg |
| 13 | F | 6y | H | Neg | Pos± | 0,652 | ND | Neg |
| 14 | F | 10m | H | Neg | Pos± | 0,517 | ND | Neg |
| 15 | F | 5y | H | Neg | Pos± | 0,234 | ND | Neg |
| 16 | F | 6y | H | Neg | Pos± | 0,277 | ND | Neg |
| 17 | F | 2y | H | Neg | Pos± | 0,133 | ND | Neg |
| 18 | F | NI | H | Pos± | Pos± | 0,844 | ND | Neg |
| 19 | M | NI | H | Neg | Pos± | 0,294 | ND | Renal nephrosis |
| 20 | F | 9m | H | Neg | Pos± | 0,296 | ND | Neg |
| 21 | F | 1y | H | Neg | Pos± | 0,215 | ND | Neg |
| 22 | F | 2y | H | Neg | Pos | 1,500 | Neg | Neg |
| 23 | F | 2y | H | Pos± | 32 | 0,979 | *B. canis* | Neg |
| 24 | F | 8m | H | Neg | Pos± | 0,303 | Neg | Neg |
| 25 | M | 4y | G.Shepherd | Pos | Pos | 0,369 | ND | Neg |
| 26 | F | 11m | H | Neg | Pos± | 0,293 | Neg | Neg |
| 27 | F | 3y | H | Neg | Pos± | 0,269 | Neg | Neg |
| 28 | F | 2y | H | Neg | Pos | 0,899 | ND | Neg |
| 29 | F | 1y | H | Neg | Pos± | 0,241 | Neg | Neg |
| 30 | F | 2y | H | Neg | 32 | 0,541 | ND | Neg |
| 31 | F | 9m | H | Neg | 2 | 0,454 | ND | Neg |
| 32 | F | NI | H | Neg | 2 | 0,671 | *B. canis* | Miscarriages |
| 33 | F | 2y | H | Neg | 32 | 1,010 | Neg | Neg |

M: male
F: female
Pos±: weakly positive
ND: not done
IELISA cutoff: $OD_{414}$ >0,281
H: hybrid
NI: no information
m: months-old
y: years-old

Human infection by *B. canis* often goes undiagnosed. However, the isolation of *B. canis* from a boy hospitalised for unexplained fever has recently been reported (11).

In the present study we surveyed 224 dogs (7 mo-10 yrs) from an urban area, in the course of a NP, determining the prevalence of antibodies to smooth and rough *Brucella* spp., as well as bacteriological isolation and clinical data. The NP offers owners free neutering of their pets in order to prevent uncontrollable reproduction.

In our study, all 224 dogs (220 females and 4 males) were tested by BPAT; 4 were weakly positive and 1 was positive. These 5 sera were tested by RBT and sera N° 3, 10, 18 and 25 were negative; serum N° 23 was weakly positive but tested negative to SAT. These results were probably due to cross-reactivity with the rough-*Brucella* species, because all these sera were positive to RSAT and IELISA. Some overlapping in the detection of antibodies against rough and smooth *Brucella* strains has been reported (13).

The sera of 33 (14.7%) (31 females and 2 males) of the 224 dogs reacted positively to RSAT, a practical screening test, while 24 (10.7%) of these dogs [22 females and 2 males] showed positive IELISA titres (Table 1). IELISA is useful as a confirmatory test and has demonstrated a specificity and sensitivity of 100% when the cut-off value of $OD_{414}$ 0.281 was selected (10). Because the 9 sera negative by IELISA were probably from dogs at an early stage of the infection or showing false positive results, they were retested using 2-mercaptoethanol (2ME). Equal volume of serum was mixed with 25 µl of 0.2M 2ME and 50 µl of RSAT antigen was added (5). Seven sera were negative, two weakly positive but new samples could not be obtained.

Blood culture was done only for 166 (74.1%) dogs and for 17 from 33 (51.5%) RSAT positive dogs; *B. canis* was isolated from 2 females that tested positive to RSAT and IELISA, (11.7%) (Table 1). Bacteriological studies have been considered specific but, intermittent periods of abacteriemia may occur.

None of the dogs had previously been tested for brucellosis and the owners were asked about their dogs' clinical history.

**Table 2**. Clinical findings in dogs that tested serologically[(1)] and bacteriologically negative for brucellosis

| n | Sex | Breed | Blood culture | Clinical findings |
|---|---|---|---|---|
| 172 | F | H | 132 | 12 |
| 3 | F | Pekinese | 3 | 1 |
| 12 | F | G. Shepherd | 11 | Neg |
| 1 | F | Rottweiler | ND | Neg |
| 1 | F | S. Husky | 1 | Neg |
| 2 | M | H | 2 | Neg |
| 191 | | | 149 | 13 |

F: female M: male
H: hybrid ND: not done
Neg: negative
(1) BPAT, RSAT and IELISA negative results

**Table 3.** Dogs neutered and tested in the 13 neighborhoods of Lomas de Zamora District

| Neighborhood | n | Number of positive samples BPAT[(1)] | RSAT[(1)] | IELISA[(1)] | *B. canis culture*[(1)] |
|---|---|---|---|---|---|
| I | 70 | 2 | 12 | 8 (11.4%) | |
| II | 22 | | 1 | 1 (4.5%) | |
| III | 7 | | 1 | | |
| IV | 0 | | | | |
| V | 4 | | | | |
| VI | 74 | 3 | 9 | 9 (12.1%) | 2 |
| VII | 3 | | | | |
| VIII | 19 | | 2 | 1 (5.2%) | |
| IX | 0 | | | | |
| X | 14 | | 6 | 4 (28.5%) | |
| XI | 7 | | 1 | 1 (14.2%) | |
| XII | 3 | | 1 | | |
| XIII | 1 | | | | |
| Total | 224 | 5 | 33 | 24 | 2 |

(1)positive results

Only three of the positive dogs presented pathological clinical findings, such as tumor and miscarriage (two females), while one male had renal nephrosis, with polyurea and increased creatinine level.

Of 191 dogs (189 females and 2 males) that tested negative (Table 2), 13 females (1 Pekinese and 12 hybrids) presented clinical pathological findings: 6 had miscarried in the last pregnancy, 3 whelped weak pups with subsequent mortality, 1 had purulent vaginal discharge after parturition, 1 presented hepatosplenomegaly, 1 had polycystic ovary (syndrome) and 1 had canine distemper. We agree with previous reports that suggest that clinical signs are not adequate to diagnose canine brucellosis; however, the animal should be tested for this disease whenever there is a history of abortion or poor reproductive performance, dyscospondilitis and uveitis in either sex (6).

Cases of this infection have been reported in many breeds, sometimes in the pet- shop trade (6). Of the 24 dogs positive to RSAT and IELISA in our study, one male was a German Shepherd and the others were mixed breeds in daily contact with roamers.

Table 3 shows the 13 neighborhoods of Lomas de Zamora District and the distribution of neutered and tested dogs. Neighborhood X, with many roamers, presented 28.5% of IELISA positive sera while neighborhoods I, VI and XI ranged from 11.4 to 14.2%. The infection was probably located in neighborhood X, from which it spread to areas including I, VI and XI. The other two neighborhoods (II and VIII), located to the north and south presented 4.5% and 5.2% positive cases, respectively.

No cases were detected in some neighborhoods (V, VII, and XIII) with middle class residents considered to be more responsible toward their pets, whereas neighborhoods III, and XII had not IELISA positive dogs.

The owners of 46 dogs (33 RSAT positive and 13 with clinical findings) were canvassed. Only five answered and were tested serologically, but refused the clinical examination and follow- up. Two owners of dog 2 and the owner of dog 3 (Table 1) tested negative for brucellosis. The owner of a female with canine distemper that tested serologically and bacteriologically negative for brucellosis, also tested negative, but the owner of dog 22 (Table 1) was positive to both RSAT and IELISA (12) (data not shown).

In the present study, we used the convenience sampling approach focused on dogs whose owners had consented to the neutering. The limitation of this approach was the lack of representation because the owners had refused to castrate the males.

However, since infected dogs have been shown to remain bacteriemic for prolonged periods (6), the findings of this study also suggest a risk of human infection in this area.

The disease may persist in field and urban dogs if no eradication programs are implemented. If infected dogs increasingly contaminate the environment, they could become a threat to public health.

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