ELSEVIER

Animal Reproduction Science 82–83 (2004) 195–207

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Canine brucellosis

M.M. Wanke*

Facultad de Ciencias Veterinarias Area de Teriogenología, Chorroarín 290 (1427), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina

Abstract

This review discusses the prevalence, etiology, pathogenesis, clinical findings, diagnostic methods, therapy, management and public health considerations of *Brucella canis* infection in dogs. Canine brucellosis is a contagious infection produced by a gram-negative coccobacilus called *Brucella canis*. The main sources of infection are vaginal fluids of infected females and urine in males. Routes of entry are venereal, oronasal, conjunctivae mucosa and placenta. The most significant symptoms are late abortions in bitches, epididymitis in males and infertility in both sexes, as well as generalized lymphadenitis, discospondylitis and uveitis. Diagnosis is complex because serology can give false positive results and chronic cases can give negative results, needing to be complemented with bacteriological studies. No antibiotic treatment is 100% effective and the infection often recurs in animals apparently treated successfully. Infected animals must be removed from the kennels and no longer used for breeding. Preferably, males should be castrated and females spayed. Human contagion is not frequent, although it has been reported, and is easily treated.

Keywords: Canine brucelosis; *Brucella canis*; Clinical findings; Diagnostic; Treatment

1. Introduction

Since Leland Carmichael's first isolation of *Brucella canis* in 1966 (Carmichael, 1966), canine brucellosis has been recognized as the cause of great economic loss in kennels. Even today, it is difficult to establish a true diagnosis of this disease and to convince breeders that their animal's normal reproductive career has ended.

It is especially common in the southern states of the United States (Wooley et al., 1977), Central and South America; surveys of its prevalence have been carried out in Mexico (Flores Castro and Segura, 1976), Brazil (Azevedo et al., 2003), Argentina (Baruta et al., 2001) and Chile (Zamora et al., 1980). In Europe, it has been reported in Germany (Weber and

* Tel.: +54 11 4258 8607; fax: +54 11 4524 8480.
E-mail address: wanke@fvet.uba.ar (M.M. Wanke).

0378-4320/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2004.05.005

Schliesser, 1977), Spain (Rodriguez Ferri et al., 1982; Mateu de Antonio and Martin Castillo, 1993), Italy (Ciuchini et al., 1982), Czechoslovakia (Sebek et al., 1976), Poland (Pilaszek and Pilaszek, 2000) and from a kennel in France in 1966 (Fontbonne and Garrin-Bastuji, 1966). In Asia, the disease has been reported in India (Srinivasan et al., 1992), Philippines (Baluyut and Duguies, 1997), Korea (Park and Oh, 2001), Japan (Katami et al., 1991), China (Jiang, 1989), Turkey (Diker et al., 1987), Malaysia (Joseph et al., 1983) and Taiwan (Tsai et al., 1983) and in Africa in Nigeria (Adesiyun et al., 1986). A survey of its prevalence was also made in Ontario, Canada (Bosu and Prescott, 1980).

This is a contagious disease with venereal and oral modes of transmission that produces late abortions in females, epididymitis and prostatitis in males. It leads to infertility in both sexes as well as lymphadentitis and diskospondylitis as extragenital symptoms (Carmichael and Kenney, 1968; Carmichael, 1976; Barton, 1977; Johnson and Walker, 1992; Berthelot and Garin Bastuji, 1993; Carmichael, 1999). Diagnosis is difficult because of unstable serum antibody titers that vary from individual to individual as well as between different methods used for their detection. Furthermore, many dogs remain asymptomatic despite being infected, which makes owners unwilling to accept that their dog is ill and should not be used for breeding. Finally, antibiotic treatment has been shown to be efficient in some cases; however, no treatment is 100% effective. In many cases, relapses have occurred after treatment, with the animal appearing "cured" (in terms of bacterial elimination). Consequently, making it is ethically unacceptable to keep a treated animal for breeding purposes. Cases of human infection, mainly of laboratory technicians who have had direct contact with the bacteria and owners of infected animals, have been reported (Swenson et al., 1972; Munford et al., 1975; Godoy et al., 1979; Ramacciotti, 1980; Carmichael and Green, 1990).

2. Etiology

Despite the description of cases of brucellosis in dogs caused by four of the six species of the genus *Brucella*, three of these (*Brucella melitensis*, *Brucella suis*, *Brucella abortus*), produce only occasional infections in individual animals, while *B. canis* is of epidemiological importance. *B. canis*, isolated for the first time by Leland Carmichael (Carmichael, 1966), is a gram-negative coccobacilus that is differentiated from the other species of the genus *brucella* (except *Brucella ovis*) in that it forms rugose colonies (Carmichael and Bruner, 1968; Carmichael and Green, 1990; Berthelot and Garin Bastuji, 1993). It grows in common culture media including triptose agar and does not require CO_2 for culture. It affects all breeds of dogs and can occasionally affect human beings.

3. Transmission

Contagion is mainly through vaginal secretions, both during estrus and parturition, abortion, and post-partum in the fetus, placenta and lochia, where bacteria can be found in concentrations up to 10^{10} per ml (Carmichael and Green, 1990). Males excrete bacteria in their semen. Although both sexes excrete bacteria in urine, the concentrations

in male urine are higher, reaching 10^3–10^6 bacteria/ml of urine (Serikawa et al., 1981; Carmichael and Joubert, 1988). For this reason, urine from a male is more dangerous as a source of infection. Excretion of bacteria through urine starts at 4–8 weeks after infection.

Bacterial concentration in milk is high. However, its importance in disseminating the disease is controversial. Some authors consider that milk is unimportant as a vehicle of infection since the pups become infected in the uterus (Carmichael and Green, 1990), while others consider milk to be dangerous because of its potential for environmental dispersion. Low concentrations of bacteria have also been isolated from saliva, nasal and ocular secretions, and from feces, albeit of low importance as a source of infection (Weber and Christoph, 1982). In addition, cages, equipment and people in contact with infected dogs have been reported as sources of infection (Johnson and Walker, 1992).

4. Pathogenesis

The routes of entry for the pathogen are genital, oronasal or conjunctivae mucosa. It is also noted that cohabitation with infected males can result in infection (Serikawa et al., 1981; Carmichael and Joubert, 1988). After *Brucella* gain entry into the animal, they are phagocytized by macrophages and other phagocytic cells and taken to lymphatic organs (lymph nodes and spleen) and genital organs where they reproduce. Bacteriemia develops 1–4 weeks after infection and persists for at least 6 months and then, intermittently, up to 64 months (Carmichael, 1979; Carmichael et al., 1983; Carmichael and Joubert, 1987; Carmichael and Green, 1990). Bacteria reach target organs through the blood and produce the pathological changes typical of the disease (Fig. 1).

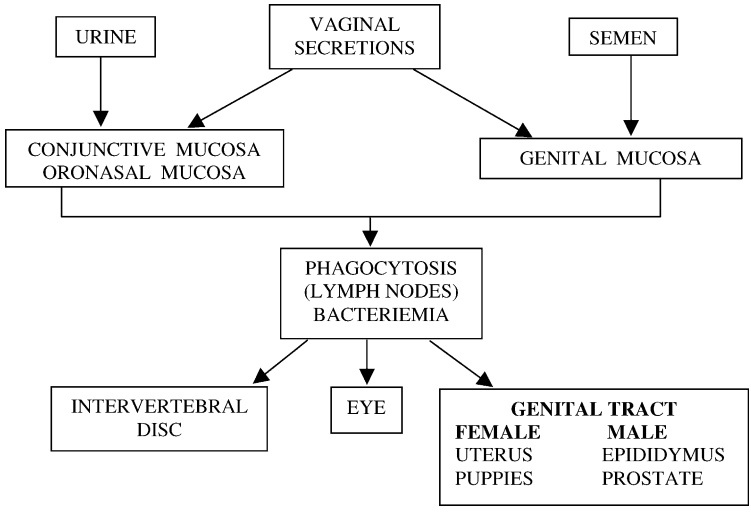

Fig. 1. Pathogenesis of canine brucellosis.

5. Clinical symptoms

General symptoms of brucellosis are not very evident. Pyrexia is rarely present. Loss of shiny coat, general deterioration of condition or intolerance to exercise can be noted in some animals, but these changes are not manifested in most cases. Moreover, symptoms vary according to the organ affected.

5.1. Genital tract

The classical symptom of canine brucellosis is late abortion, between 30 and 57 days of gestation, with a higher frequency noted between days 45 and 55 (Carmichael and Kenney, 1968). Aborted puppies usually appear partially autolysed and show the characteristic lesions of a generalized bacterial infection: subcutaneous edema, congestion and hemorrhage of the subcutaneous abdominal region, sero-sanguinous peritoneal fluid loss, with focal infiltration of lymphoid cells and degenerative lesions in liver, spleen, kidneys and intestines. The bitch continues to excrete a brownish or green-gray discharge over a long period (Carmichael and Kenney, 1968). An infected bitch can experience several consecutive abortions and, in some cases, can also give birth to weak pups which die either a few hours or up to a month after parturition. Sometimes, apparently normal puppies are born which develop the disease at a later date (Lewis et al., 1973; Nicoletti, 1989). The main clinical manifestation of the disease before the dog reaches puberty is a generalized lymphadenitis (Carmichael and Green, 1990). Such animals should not be kept in the kennel. Embryo resorptions can occur and are interpreted as conception failure.

The most frequent manifestations of infection in the male are severe epididymitis and prostatitis. During the acute stage, the epididymis increases in size and is accompanied by evidence of pain, and the presence of sero-sanguinous fluid in the tunica. Frequent licking of the scrotum produces edema and dermatitis that often becomes contaminated with non-hemolytic staphylococci. The epididymis decreases in size in the chronic phase and becomes hard and the testes often display atrophy. Orchitis is noted infrequently and cases of testicular necrosis with ulcerative scrotal dermatitis, from which *B. canis* was isolated, have also been reported (Schoeb and Morton, 1978).

Testicular damage initiates an autoimmune response that produces antisperm antibodies that can be found in blood serum and seminal plasma (Serikawa et al., 1984b) from 11 to 14 weeks post-infection, with the agglutinating activity and titer varying with each dog (Serikawa et al., 1981). Auto-agglutination of sperm is often observed from 18 weeks post-infection (Serikawa et al., 1984a). These findings suggest that sterility due to canine brucellosis is associated with an autoimmune phenomenon. Generally, the males become sterile. Whether they become sterile or not, they can continue to excrete bacteria in the seminal fluid (Nicoletti, 1989).

Epididymitis generally develops around 5 weeks post-infection and the presence of neutrophils and macrophages in semen and evidence of teratospermia with spermatozoa showing deformed acrosomes, swollen mid-pieces and retained protoplasmic droplets, are also detected at about this stage. Other sperm anomalies, including coiled tails and detatched heads, are observed at 16 weeks post-inoculation and head to head agglutination is detected at 18–27 weeks (Serikawa et al., 1984a). Accumulations of inflammatory cells, comprising

macrophages with phagocytized sperm, surrounded by masses of neutrophils are seen later. In dogs with bilateral testicular atrophy, azoospermia is a common occurrence (Serikawa et al., 1984a).

Lymphadenitis, especially involving the retropharyngeal and inguinal nodes, appears in both sexes, although generalized lymphadenitis and follicular hyperplasia of the spleen, are also commonly detected. Examination of aspirates or biopsies of lymphatic ganglions generally reveals lymphatic hyperplasia with a large number of plasmatic cells. Other symptoms detected relatively frequently include discospondylitis accompanied by acute pain in the vertebral column, lameness and, if there is compression of the medulla, paresis and ataxia (Kerwin et al., 1992). These symptoms rapidly yield to antibiotic treatment (Kerwin et al., 1992). *B. canis* can also produce anterior uveitis (Saegusa et al., 1977) and, occasionally, isolated cases of polygranulomatous dermatitis, meningoencephalomyelitis (Purvis, 1981) and endocarditis (Ying et al., 1999).

6. Diagnosis

The only method that provides a definitive diagnosis of brucellosis in a dog is the bacteriological isolation of the microorganism. Nevertheless, this is not always possible and an unequivocal diagnosis is not reached in some cases. The different types of serological diagnosis vary in sensitivity and specificity, leading to false positives and negatives, depending upon the stage of the disease and the antigen or the method used for testing. Clinical data and anamnesis must be used in conjunction with serology and bacteriology to reach a definite diagnosis.

6.1. Serology

Antibodies against *Brucella* can be detected at 2 weeks post-infection (Weber and Schliesser, 1975; Weber and Krauss, 1977; Weber, 1980; Weber and Christoph, 1982). These are developed against the wall and cytoplasmic proteins of the bacteria. The methods used for detection vary in sensitivity, specificity and complexity. There are five serological diagnostic methods used in practice today.

Rapid slide macro and microagglutination test with and without the addition of 2-mercaptoethanol (2-ME).

Tube agglutination test with and without the addition of 2-mercaptoethanol (2-ME).
Agar gel immunodiffusion.
ELISA tests using cell wall antigen or cytoplasmic protein antigen.
Indirect immunofluorescence.

Other methods used generally in research have been complement fixation, and counter-immuno-electrophoresis.

Complement fixation has been described by Alton et al., 1975 and Weber and Krauss (1977). There is a good correlation between this test and the TAT Weber and Krauss (1977). It is not routinely used because canine sera often has anti-complement activity (Alton et al., 1975; Weber and Krauss, 1977).

Antigens used in these reactions can be lipopolysaccharides (LPS) of the bacterial cell wall of *B. ovis*, the pathogenic strain of *B. canis* (RM 6/66) or the less mucoid strain (M−) of *B. canis* described by Carmichael and Kenney (1968). In general, published work in this area shows that tests using antigens made from *B. ovis* and *B. canis* (RM 6/66) lead to 62% false positive reactions, while those made using the M− strain, give fewer false positive reactions. The false positives result from the IgM that cross-reacts with other bacteria including *Streptococci*, *Stafilococci* and *Pseudomonas* (Zoha and Carmichael, 1982a,b; Carmichael et al., 1984; Carmichael and Joubert, 1987). If the reaction is positive, it should be repeated adding 2-mercaptoethanol (2-ME) that destroys the IgM. If this reaction is negative, it should be repeated at least 15 days later to make sure that the false positive was due to a cross reaction and not to an early infection, where the switch from IgM to IgG had not yet occurred. Since the M− strain antigen causes fewer false positive reactions than the other two strains mentioned above, it is better to work with this bacterial antigen (Carmichael and Joubert, 1987).

Agar gel immunodiffusion and ELISA can also be carried out with antigens that only have the cytoplasmic proteins common to *B. abortus* and *B. canis*. In general, antibodies against the LPS appear earlier (Moon et al., 1994) and disappear shortly after bacteriemia has ceased, whereas antibodies raised against the cytoplasmic antigens tend to persist longer (from 6 months to a year) and allow the detection of chronic cases (Zoha and Carmichael, 1982a,b; Carmichael et al., 1984). This is an important factor since, in some serologically negative males, the presence of bacteria has been detected in the epididymis and prostate (Moore and Kakuk, 1969; Flores Castro et al., 1977). Although antibodies can be detected from two weeks post-infection, no serological method is completely precise before 12 weeks post-infection (Zoha and Carmichael, 1982b).

6.1.1. Rapid slide agglutination test

This is the simplest microscopic method to use in practice. This test is sensitive and can be performed at the early stage of infection. Since false negatives are rare, it can be used as a filter to discard negative animals and carry out more specific tests on those that are positive. There is a commercial test available (D-Tec CB; Symbiotics Corp., Kansas City, Missouri, USA) that uses a suspension of *B. ovis* stained with Rose Bengal that cross reacts with *B. canis* antigen and gives a high percentage of false positives, even with the addition of 2-ME. The antigen for microagglutination prepared with *B. canis* M−, is more specific and false positives are rare (Carmichael and Joubert, 1987; Damp et al., 1973).

6.1.2. Tube agglutination test

This test has the advantage of being semi-quantitative; however, it is not very specific. A titer of 2:200 or more is considered to provide evidence of infection (Nicoletti and Chase, 1987a; Moon et al., 1994). A positive case can be detected 2–4 weeks post-exposure (Carmichael and Kenney, 1968). When titers are low, the animals are considered to be suspect and the diagnosis should be confirmed 2 weeks later. The serum should not be derived from hemolized blood as it can give cross reactions (Carmichael and Kenney, 1970; Carmichael and Green, 1990). The same test, with the addition of 2-ME, can be applied later, but the result is more specific (Moore and Kakuk, 1969). No commercial tests are

available for this method. The technique has been described by Alton et al. (1975), and Carmichael and Kenney (1968).

6.1.3. Agar gel immunodiffusion

This technique has been described by Zoha and Carmichael (1982b). When cell wall antigens are used, positive cross-reactions are common. However, there are specific *B. canis* antigen precipitation bands which can be distinguished from those produced by other bacterial antigens by analyzing the way in which the precipitation lines are joined in this test if serum from a positive control is also included (Zoha and Carmichael, 1982b). The test can be also carried out using cytoplasmic antigens; this test is more specific but turns positive later.

6.1.4. ELISA tests

These tests have been developed both with the cell wall antigens from *B. canis* (M− and RM 6/66) and with the cytoplasmic antigens of *B. abortus* that are common to all strains of the genus *Brucella* (Baldi et al., 1997). No commercial tests are available. The latter has the advantage that these antigens do not show cross-reactivity with bacteria belonging to any genus other than *Brucella*, but gives positive results with any bacteria of the *Brucella* genus. Antigens with protein fractions of this antigen have recently been developed (Baldi et al., 1994). In our experience, this test is more sensitive. Positive cases can be identified early in infected animals and the results continue to be positive in animals which have been treated with antibiotics to eliminate bacteriemia (Baldi et al., 1994; Baldi et al., 1997; Wanke et al., 2000; Wanke et al., 2002). ELISA tests carried out with cell wall antigens of the M− *Brucella* strain are highly specific but a little less sensitive (Serikawa et al., 1989; Mateau de Antonio et al., 1993), while those performed with the wild strain RM 6/66 display a high percentage of false positives (Mateau de Antonio et al., 1993).

6.1.5. Indirect immunofluorescence

This test has proved to be more sensitive and specific than agglutination tests (Weber and Hussein, 1976). However, results from Cornell University's Diagnostic Laboratory indicate a high rate of false positive reactions with the IFA test. A commercial kit available produced by Eurokit SRL, Gorizia, Italy is available.

Among the laboratories that diagnose *Brucella canis* in the USA and appear in the official list of the AAVLD (American Association of Veterinary Laboratory Diagnosticians), six use indirect immunofluorescence, three AGID, two 2-ME TAT and one RSAT. None use complement fixation. These serological procedures are summarized and compared in Table 1.

6.2. Bacterial cultures

Culturing the bacteria from samples collected from a suspected case is the only way to confirm that the animal has been infected with *B. canis*. It is the best method for the diagnosis of early infection in dogs that have not received antibiotic treatment. Unfortunately, a negative result does not confirm the absence of infection or of a cure, since the bacteria can be temporarily absent from the tissue cultured.

Table 1
Serologic tests for *Brucella canis* (Modified from Johnson, 1995)

Serologic test	Antigen	Time frame for positive results	Comments
2-ME-RSAT	Cell wall	8–12 weeks after infection to 3 months after the animal is abacteriemic	Very sensitive, false positive results are common. Few (1%) false negative results reported; easy and fast
2-ME-TAT	Cell wall	10–12 weeks infection to 3 months after the animal is abacteriemic	Semi-quantitative: false positive results are possible
AGID test	Cell wall	12 weeks after infection to 4 months after the animal is abacteriemic	Test procedure is complex: more specific than 2-ME-RSAT
AGID test	Cytoplasmic	12 weeks after infection to 36 months after the animal is abacteriemic	Most specific serologic test but not sensitive; detects chronic cases when other tests give negative results
ELISA	Cell wall (*B. canis* M−)	Unknown (expect time to be similar to that observed with the TAT)	Very specific; less sensitive than TAT; limited availability
ELISA	Cytoplasmic	Unknown (expect time to be similar to that observed with AGID (cytoplasmic))	Very sensitive and specific; detects chronic infections. Limited availability

The easiest material to culture is blood since, in addition to being easy to obtain, it is "sterile", which allows uncontaminated cultures to be obtained. Aerobic media are used for blood culture and they are incubated for 9 days at 37 °C. Sub-cultures in triptose–agar, generally adding polimixin B and bacitracin to the media to inhibit growth of contaminants, are made on days 3, 6 and 9 of incubation (Nicoletti, 1989). Bacteriemia appears between 2 and 4 weeks post-infection and persists for about 6 months, becoming intermittent over at least a year (generally 2–5 years) in untreated dogs (Carmichael, 1979; Carmichael et al., 1983; Carmichael and Joubert, 1987). Frequently, the semen is contaminated, as are vaginal secretions during estrus and after abortions. A high concentration of microorganisms can be found in the placentae, even though the aborted fetuses may not show any bacteria. Urine samples have cultured positive for the organism even in animals in which no bacteria were found in the blood. This test is more reliable and easier to carry out in males because female urine is often contaminated with other microorganisms (Serikawa et al., 1978). The best organs for isolation, for biopsies or at post-mortem examination, are lymph nodes, prostate, spleen, medulla and, sometimes, liver and testes (Weber and Schliesser, 1975).

7. Treatment

B. canis are intracellular bacteria, which means that antibiotics cannot reach it adequately. In addition, it is sensitive to relatively few antibiotics (Nicoletti and Chase, 1987b). Many different antibiotics have been tried, alone or in combination, and none have been 100% effective in eradicating the disease. Bacteriemia has been eliminated in some cases and

negative titers of antibodies, especially of those raised against the bacterial cell wall, have been obtained; however, bacteria remain alive in the tissues. We have seen a case in which a recently infected dog gave negative titers immediately after treatment, but at that same time we were able to isolate the bacteria from his semen (Wanke et al., 2000). Frequently, titers reappear much later after infection and at different times after the termination of the treatment. In females, this often happens at estrus and after stressful situations if the animal has not been spayed. Even untreated females may give birth, but the puppies are born infected, thus keeping the disease in the kennels. Males normally remain sterile because of testicular lesions but, even so, can continue to transmit the disease. Generally, treatments with only one drug have not been successful.

7.1. Treatments that show relative success

The following treatments have been relatively successful.

1. Oral tetracycline (30 mg/kg) twice daily for 28 days and intravenous streptomycin (20 mg/kg) once daily, for 14 consecutive days at the beginning of the treatment. Out of 105 dogs subjected to this treatment, 81 proved to be negative (Nicoletti, 1991).
2. Oral tetracycline (30 mg/kg) three times a day for 30 days and IM streptomycin 20 mg/kg on days 1–7 and 24–30 of the treatment. Of the 19 dogs receiving this treatment, 14 became negative with one cycle of this treatment and two more with two cycles, and the last three were put down (Nicoletti and Chase, 1987b).
3. Minocycline (10 mg/kg) twice daily, combined with IM streptomycin (4.5 mg/kg) for 7 days (Flores Castro and Carmichael, 1981).
4. Long-acting oxitetracycline (20 mg/kg IM) once a week, for 4 weeks, acompanied by daily injections of streptomycin for the first 7 days. Of the 24 dogs treated 21 became negative (Zoha and Walsh, 1982). No success was obtained with ampicillins (Lewis et al. 1973). Finally, we have found that a 4-week treatment regimen with enrofloxacine gives good results (Wanke, unpublished observations).

8. Infection control in kennels

When abortions, infertility or epididymitis are detected in dogs during a routine inspection of dog kennels, it is necessary to carry out an immediate serological test for *Brucella* on the affected animals. If positive, the following steps are recommended:

1. Quarantine the establishment during the period of eradication of the disease. If dogs previously from the same kennel are brought back to the kennel, they can re-infect the kennel.
2. Carry out serological tests and blood cultures on all the animals of the kennel.
3. Identify the source of the infection (matings, new animals, etc.).
4. Remove all animals tested positive from the kennel. Physical separation of healthy dogs from the infected ones, even maintaining strict measures of hygene, is not enough to avoid the propagation of the disease (Carmichael and Joubert, 1988). For this reason, it is recommended that all infected animals are taken away from the kennel. All animals tested

positive must be neutered, treated with antibiotics using any of the above mentioned schemes, and taken away from the kennels.
5. Treat all dogs tested negative for 1 month with tetracycline and streptomycin and carry out monthly control by eliminating any new positives from the group until no more positives are obtained for 3 consecutive months. New cases can be expected during the first 5 months.
6. Clean the kennels of the infected dogs well with quaternary ammonia and iodides. The bacteria, which do not live outside the dog very long, can be easily eliminated with common disinfectants (quaternary ammonia and iodides). The situation changes if it is in organic material where they survive longer. For this reason, rigorous cleaning of the kennel is of utmost importance (Johnson and Walker, 1992).
7. Continue testing every 3 months for a year and establish a good preventative plan to avoid the appearance of new outbreaks. Although cases of partially successful treatments have been reported, no treatment has been 100% effective and the puppies born from bitches with chronic brucellosis, if they survive, often are infected. These develop the disease after puberty and disseminate the organisms. Because of this, it is essential that dogs tested positive are not kept as breeders, even though they are of high genetic value.

9. Prevention

All females must be routinely tested serologically before mating. All males should be subjected to similar testing before mating. Males and females should be subjected to at least one test per semester to minimize the risk of infection. All new animals (male or female) that enter the kennel should be examined and should be quarantined for 8–12 weeks. No animals exposed to the infectious agent, or those displaying symptoms similar to those of brucellosis, should be bought.

10. Public health significance

The incidence of the illness in human beings is not exactly known. Although *B. canis* affects man, there are few cases reported and the illness, generally from close contact with material from abortions or secretions during estrus, is often mild (Shin and Carmichael, 1999). There have been cases of infection in laboratories directly involved in work with the bacteria (Godoy et al, 1979). The illness starts with prolonged fever, enlarged lymph glands (Shin and Carmichael, 1999), pharyngitis (Swenson et al., 1972), joint pain (Godoy et al., 1979) shivering and weight loss (Munford et al., 1975). Infected people respond rapidly to antibiotic treatments including tetracyclines alone, or in combination with streptomycin or ampicillin (Ramacciotti, 1980; Shin and Carmichael, 1999).

References

Adesiyun, A.A., Abdullahi, S.U., Adeyanju, J.B., 1986. Prevalence of *Brucella abortus* and *Brucella canis* antibodies in dogs in Nigeria. J. Small Anim. Pract. 27, 31–37.

Alton, G.G., Jones, L.M., Pietz, D.E., 1975. Laboratory techniques in brucellosis, second ed. World Health Organization, Geneva, Switzerland.

Azevedo, S.S., Vasconcellos, S.A., Alves, C.J., Keid, L.B., Grasso, L.M.P.S., Mascolli, R., Pinheiro, S.R., 2003. Inquerito sorologico e fatores de risco para a brucelose por *Brucella canis* em caes do municipio de Santana de Parnaiba, Estado de Sao Paulo. Pesq. Vet. Bras. 23, 156–160.

Baldi, P.C., Wanke, M.M., Loza, M.E., Fossati, C.A., 1994. *Brucella abortus* cytoplasmic proteins used as antigens in an ELISA potentially useful for the diagnosis of canine brucelosis. Vet. Microbiol. 41, 127–134.

Baldi, P.C., Wanke, M.M., Loza, M.E., Monachesi, N., Fossati, C.A., 1997. Diagnosis of canine brucellosis by detection of serum antibodies against an 18 kDa cytoplasmic protein of *Brucella* spp. Vet. Microbiol. 57, 273–281.

Baluyut, C.S., Dugui es, M.V., 1997. A serological survey for *Brucella canis* agglutinins in dogs using the macroscopic tube agglutination test. Philip. J. Vet. Med. 16, 93–101.

Baruta, D.A., Ardoino, S.M., Riesco, S.R., Marengo, M.L., Brandan, J.L., Oriani, D.S., 2001. Determinacion de anticuerpos prevalentes contra brucellas especificas e inespecificas en caninos en la ciudad de Gral. Pico (La Pampa). Selec. Vet. 9, 415–418.

Barton, C.L., 1977. Canine brucellosis. Vet. Clin. N. Am. 7, 705–710.

Berthelot, X., Garin Bastuji, B., 1993. Brucelloses canines. Point Vet. 25, 125–129.

Bosu, W.T.K., Prescott, J.F., 1980. A serological survey of dogs for *Brucella canis* in Southwestern Ontario. Can. Vet. J. 21, 198–200.

Carmichael, L.E., 1966. Abortion in 200 Beagles. J. Am. Vet. Med. Assoc. 149, 1126.

Carmichael, L.E., Bruner, D.W., 1968. Characteristics of a newly-recognized species of *Brucella* responsible for infectious canine abortions. Cornell Vet. 48, 579–592.

Carmichael, L.E., Kenney, R.M., 1968. Canine abortion caused by *Brucella canis*. J. Am. Vet. Assoc. 152, 605–616.

Carmichael, L.E., Kenney, R.M., 1970. Canine brucellosis: the clinical disease, pathogenesis, and immune response. J. Am. Vet. Med. Assoc. 156, 1726–1734.

Carmichael, L.E., 1976. Canine brucellosis: an annotated review with selected cautionary comments. Theriogenology 6, 105–116.

Carmichael, L.E., 1979. Brucellosis caused by *Brucella canis*. In: Steele, J.H. (Ed.), CRC Hand-Book Series of Zoonoses. CRC Press Inc., Boca Raton, FL, vol.1, pp. 185–194.

Carmichael, L.E., Zoha, S.J., Flores Castro, R., 1983. Biological properties and dog response to a variant (M−) strain of *Brucella canis*. Dev. Biol. Stand. 56, 649–656.

Carmichael, L.E., Zoha, J., Flores-Castro, R., 1984. Problems in the serodiagnosis of canine brucellosis: dog responses to cell wall and internal antigens of *Brucella canis*. Dev. Biol. Stand. 56, 371–383.

Carmichael, L.E., Joubert, J.C., 1987. A rapid slide agglutination test for the serodiagnosis of *Brucella canis* infection that employs a variant (M−) organism as antigen. Cornell Vet. 77, 3–12.

Carmichael, L.E., Joubert, J.C., 1988. Transmission of *Brucella canis* by contact exposure. Cornell Vet. 78, 63–73.

Carmichael, L.E., Green, E.G., 1990. Canine brucellosis. In: Greene, C.E. (Ed.), Infectious Diseases of the Dog and Cat. W.B. Saunders Co, Philadelphia, PA, pp. 573.

Carmichael, L.E., 1999. Brucelosis canina causada por B canis: enfermedad clínica problemas en inmunodiagnostico, Rev. Med. Vet. (Buenos Aires) 80, 102–106.

Ciuchini, F., Sala, V., Pistoia, C., Piccininno, G., Pievaroli, A., Fantini, C., 1982. Brucellosi da *Brucella canis*: rilievi anticorpali su cani della citta e del suburbio di Roma. Clin. Vet. 105, 138–144.

Damp Jr., S.C., Crumrine, M.H., Lewis, G.E., 1973. Microtiter plate agglutination test for *Brucella canis* antibodies. Appl. Microbiol. 25, 489–490.

Diker, K.S., Aydin, N., Erdeger, J., Ozyurt, M., 1987. A serologic survey of dogs for *Brucella canis* and *Brucella abortus* and evaluation of mercaptoethanol microagglutination test. Ankara Univ. Vet. Fak. Derg. 34, 268–276.

Flores Castro, R., Segura, R., 1976. A serological and bacteriological survey of canine brucellosis in Mexico. Cornell Vet. 66, 347–352.

Flores Castro, R., Suarez, F., Pfeiffer, C., Carmichael, L.E., 1977. Ramirez. J. Clin. Microbiol. 6, 591–597.

Flores Castro, R., Carmichael, L.E., 1981. *Brucella canis* infection in dogs: treatment trials. Rev. Latinoam. Microbiol. 23, 75–79.

Fontbonne, A., Garrin-Bastuji, B., 1966. An outbreak of *Brucella canis* infection in a French Kennel. In: Abstract book of the Third International Symposium on Reproduction of Dogs, Cats and Exotic Carnivores, pp. 79.

Godoy, A.M., Neves, J., Peres, J.N., Barg, L., 1979. Sobre um caso de infeccao humana por *Brucella canis* em laboratorio. Arq. Esc. Vet. UFMG 31, 141–145.
Jiang, F.X., 1989. A survey on canine brucellosis in Wusu county. Chin. J. Vet. Sci. Technol. 1, 18–19.
Johnson, C.A., Walker, R.D., 1992. Clinical signs and diagnosis of *Brucella canis* infection. Compend. Cont. Educ. Pract. Vet. 14 (763/767), 770–772.
Johnson, C., 1995. Management of *Brucella canis* outbreaks in breeding kennels. In: Kirk, R.W. (Ed.), Current Veterinary Therapy XII. W.B. Saunders Co., Philadelphia, pp. 1094–1097.
Joseph, P.G., Mahmud, Z.B.H., Sirimanne, E.S., 1983. Canine brucellosis in Malaysia: a first report. Kajian Vet. 15, 17–22.
Katami, M., Sato, H., Yoshimura, Y., Suzuki, T., Suzuki, Y., Nakano, K., Saito, H., 1991. An epidemiological survey of *Brucella canis* infection of dogs in the Towada area of Aomori prefecture (Japan). J. Vet. Med. Sci. 53, 1113–1115.
Kerwin, S.C., Lewis, D.D., Hribernik, T.N., Partington, B., Hosgood, G., Eilts, B.E., 1992. Diskospondylitis associated with *Brucella canis* infection in dogs: 14 cases (1980–1991). J. Am. Vet. Med. Assoc. 201, 1253–1257.
Lewis Jr., G.E., Crumrine, M.H., Jennings, P.B., Fariss, B.L., 1973. Therapeutic value of tetracycline and ampicillin in dogs infected with *Brucella canis*. J. Am. Vet. Med. Assoc. 163, 239–241.
Mateau de Antonio, E.M., Martin Castillo, M., 1993. Encuesta seroepidemiologica frente a *Brucella canis* y brucelas de tipo liso en perros. Med. Vet. 10, 241–246.
Mateau de Antonio, E.M., Martin, M., Soler, M., 1993. Use of indirect enzyme-linked immunosorbent assay with hot saline solution extracts of a variant (M−) strain of *Brucella canis* for diagnosis of brucellosis in dogs. Am. J. Vet. Res. 54, 1043–1046.
Moon, J.S., Park, Y.H., Jung, S.C., Ku, B.G., Jang, G.C., Shin, S., Lee, S.I., Lee, J.M., Shin, S.J., 1994. Serological tests in canine brucellosis. RDA J. Agric. Sci. Vet. 36, 614–621.
Moore, J.A., Kakuk, T.J., 1969. Male dogs naturally infected with *Brucella canis*. J. Am. Vet. Med. Assoc. 155, 1352.
Munford, R.S., Weaver, R.E., Patton, C., Feeley, J.C., Feldman, R.A., 1975. Human disease caused by *Brucella canis*. A clinical and epidemiologic study of two cases. J. Am. Med. Assoc. 231, 1267–1269.
Nicoletti, P., Chase, A., 1987a. An evaluation of methods to diagnose *Brucella canis* infection in dogs. Compend. Cont. Educ. Pract. Vet. 9, 1071–1073.
Nicoletti, P., Chase, A., 1987b. The use of antibiotics to control canine brucellosis. Compend. Cont. Educ. Pract. Vet. 9, 1063–1066.
Nicoletti, P., 1989. Diagnosis and treatment of canine brucelosis. In: Kirk, R.W. (Ed.), Current Veterinary Therapy X. W.B. Saunders Co., Philadelphia, pp. 1317–1320.
Nicoletti, P., 1991. Further studies on the use of antibiotics in canine brucellosis. Compend. Cont. Educ. Pract. Vet. 13, 944.
Park, C.K., Oh, J.Y., 2001. Bacteriological and serological investigation of *Brucella canis* infection of dogs in Taegu city, Korea. Kor. J. Vet. Res. 41, 67–71.
Pilaszek, J., Pilaszek, M., 2000. Wystepowanie i zwalczanie brucelozy psow. Magazyn-Weterynaryjny 9, 28–29.
Purvis, D.S., 1981. Isolation of *Brucella canis* from canine with pyogranulomatous dermatitis. In: Abstracts of the 81st Annual Meeting of the American Society for Microbiology, pp. 309.
Ramacciotti, F., 1980. Primer aislamiento de "*Brucella canis*" en humano por hemocultivo efectuado en la Republica Argentina (Buenos Aires). Rev. Med. Vet. 61, 49–54.
Rodriguez Ferri, E.F., Suarez Fernandez, G., Dominguez Rodriguez, L., Pozo Vegas, M., 1982. Estudio serológico de perros procedentes de áreas urbanas y rurales en relación con la brucelosis. An. Inst. Nac. Invest. Agr. Gan. 17, 123–139.
Saegusa, J., Ueda, K., Goto, Y., Fujiwara, K., 1977. Ocular lesions in experimental canine brucellosis. Jpn. J. Vet. Sci. 39, 181–185.
Schoeb, T.R., Morton, R., 1978. Scrotal and testicular changes in canine brucellosis: a case report. J. Am. Vet. Med. Assoc. 172, 598–600.
Sebek, Z., Zykora, I., Holda, J., Komarek, J., 1976. Serologicky prukaz *Brucella canis* v chovu laboratornich psu plemene Beagle v Ceskoslovenska. Cesk. Epidemiol. Mikrobiol. Imunol. 25, 129–136.
Serikawa, T., Muraguchi, T., Nakao, N., Irie, Y., 1978. Significance of urine-culture for detecting infection with *Brucella canis* in dogs. Jpn. J. Vet. Sci. 40, 353–355.

Serikawa, T., Muraguchi, T., Yamada, J., Takada, H., 1981. Long-term observation of canine brucellosis: excretion of *Brucella canis* into urine of infected male dogs. Exp. Anim. 30, 7–14.

Serikawa, T., Kondo, Y., Takada, H., Yamada, J., 1984a. Head-to-head type auto-spermagglutination with IgA antibody to acrosome induced by *Brucella canis* infection. Jpn. J. Vet. Sci. 46, 40–48.

Serikawa, T., Takada, H., Kondo, Y., Muragachi, T., Yamada, J., 1984b. Multiplication of *Brucella canis* in male reproductive organs and detection of autoantibody to spermatozoa in canine brucellosis. Dev. Biol. Stand. 56, 295–305.

Serikawa, T., Iwaki, S., Mori, M., Muraguchi, T., Yamada, J., 1989. Purification of a *Brucella canis* cell wall antigen by using immunosorbent columns and use of the antigen in enzyme-linked immunosorbent assay for specific diagnosis of canine brucellosis. J. Clin. Microbiol. 27, 837–842.

Shin S.J., Carmichael, L.E., 1999. Canine brucelosis caused by *Brucella canis*. In: Carmichael, L. (Ed.), Recent Advances in Canine Infectious Diseases. International Veterinary Information Service, Ithaca, NY (http://www.ivis.org).

Srinivasan, V.K., Nedunchelliyan, S., Venkataraman, K.S., 1992. Prevalence of canine brucellosis in urban and rural areas of Tamilnadu. Indian J. Vet. Med. 12, 39.

Swenson, R.M., Carmichael, L.E., Cundy, K.R., 1972. Human infection with *Brucella canis*. Ann. Intern. Med. 76, 435–438.

Tsai, I.S., Lu, Y.S., Isayama, Y., Sasahara, J., 1983. Serological survey for *Brucella canis* infection in dogs in Taiwan and the isolation and identification of *B. canis*. Taiwan J. Vet. Med. Anim. Husb. 42, 91–98.

Wanke, M.M., Monachesi, N.E., Loza, M.E., Rutter, B., Baldi, P.C., Fossati, C.A., 2000. Determinacion de anticuerpos contra proteinas citoplasmáticas de *Brucella* por ELISA para la detección de casos crónicos de brucelosis canina. Selec. Vet. 8, 308–311.

Wanke, M.M., Delpino, M.V., Baldi, P.C., 2002. Comparative performance of ELISA assay using cytosolic or outer membrane antigens of *Brucella* for the serodiagnosis of canine brucellosis. Vet. Microbiol. 88, 367–375.

Weber, A., Schliesser, T., 1975. Serologischer und kultureller Nachweis von *Brucella canis* bei Beagle-Hunden einer Versuchstierhaltung. Zentralbl. Veterinarmed. 22B, 403–410.

Weber, A., Hussein, N.A., 1976. Der indirekte Immunfluoreszenztest zum Nachweis von *Brucella canis*-Infektionen bei Beagle-Hunden. Zentralbl. Veterinarmed. 23, 151–157.

Weber, A., Krauss, H., 1977. Zum serologischen Nachweis von *Brucella canis*-Infektionen bei Hunden mittels Komplementbindungsreaktion. Zentralbl. Veterinarmed. 24B, 746–752.

Weber, A., Schliesser, T., 1977. The occurrence of antibodies to *Brucella canis* in domestic dogs in the Federal Republic of Germany. J. Clin. Microbiol. 6, 591–597.

Weber, A., 1980. Serologischer Nachweis von *Brucella canis*-Infektionen mittels vergleichender Durchfuhrung der Langsamagglutination bei 37 deg C und 50 deg C. Zentralbl. Veterinarmed. 27B, 412–418.

Weber, A., Christoph, H., 1982. Untersuchungen zur naturlichen Ubertragung von *Brucella canis* bei Hunden. Fortschr. Veterinarmed. 35, 351–355.

Wooley Jr., R.E., Brown, J., Shotts, E.B., Blue, J.L., Dreesen, D.W., 1977. Serosurvey of *Brucella canis* antibodies in urban and rural stray dogs in Georgia. Vet. Med. Small Anim. Clin. 72, 1581–1584.

Ying, W., Nguyen, M.Q., Jahre, J.A., 1999. *Brucella canis* endocarditis: case report. Clin. Infect. Dis. 29, 1593–1594.

Zamora, J., Alonso, O., Martin, R., 1980. Brucelosis canina en Valdivia Chile. Estudio serológico y bacteriológico en perros de ciudad. Zentralbl. Veterinarmed. 27, 149–153.

Zoha, S.J., Carmichael, L.E., 1982a. Properties of cell wall antigens of virulent *Brucella canis* and a less mucoid variant of reduced pathogenicity. Am. J. Vet. Res. 43, 171–174.

Zoha, S.J., Carmichael, L.E., 1982b. Serological responses of dogs to cell wall and internal antigens of *Brucella canis* (*B. canis*). Vet. Microbiol. 7, 35–50.

Zoha, S.J., Walsh, R., 1982. Effect of a two-stage antibiotic treatment regimen on dogs naturally infected with *Brucella canis*. J. Am. Vet. Med. Assoc. 180, 1474–1475.