ARTICLE

Brucella canis Isolates from Canadian Dogs

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Abstract

Eleven *Brucella canis* isolates from Canadian dogs were characterized by dye and antibiotic sensitivity, phage susceptibility, urease and H_2S production, CO_2 requirement, and reaction with monospecific A,M, and R anti-*Brucella* antiserum. The isolates could be separated into two distinct groups. One group had a sensitivity pattern similar to that seen with the American type strain RM666, while the other group had a pattern identical to that of a Mexican strain, Mex 51. Epidemiological studies supported contraction of infections in the United States and Mexico respectively. The characteristics of all isolates were stable after repeated subculture indicating that strain differences could serve as useful epidemiological markers and supporting division of the species into two biovars.

Résumé

Souches de *Brucella canis*, isolées chez des chiens du Canada

Les auteurs ont déterminé les caractéristiques de 11 souches de *Brucella canis*, isolées chez des chiens du Canada. Ils utilisèrent à cette fin la sensibilité aux colorants et aux antibiotiques, la susceptibilité aux bactériophages, la production d'uréase et de H_2S, les besoins en CO_2 et la réaction avec les antisérums monospécifiques A, M et R contre *Brucella*. Ils réussirent à séparer les isolats en deux groupes distincts. Le premier afficha un profil de sensibilité semblable à celui de la souche RM666 du type américain; quant au second, son profil de sensibilité s'apparentait à celui de la souche mexicaine Mex 51. Une étude épizootiologique permit de conclure que la contamination se fit respectivement aux États-Unis et au Mexique. Les caractéristiques de tous les isolats demeurèrent stables après plusieurs cultures successives, indice que les différences entre les souches pourraient servir de marqueurs épizootiologiques utiles et qu'il convient de diviser l'espèce en deux biotypes.

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Introduction

Brucella canis infection in the dog is often asymptomatic. Clinical signs, when they occur, may be limited to a generalized peripheral lymphadenopathy with or without infertility. In the female, abortion usually occurs late in gestation and is characterized by fetal autolysis and a dark colored vaginal discharge lasting from one to six weeks. Some bitches may whelp stillborn or weak pups. Surviving pups have a generalized lymphadenopathy and can carry infection to maturity. The infected bitch continues to have normal estrous cycles. Mature male dogs may develop orchitis and epididymitis resulting in testicular enlargement. In chronic infections, unilateral or bilateral testicular atrophy is a more common sequel. Semen abnormalities are evident by five weeks postinfection. Scrotal enlargement due to the accumulation of fluid in the tissues and scrotal dermatitis from licking and secondary infection may occur. Less frequently observed clinical aspects in the dog include discospondylitis, multifocal pyogranulomatous dermatitis, and ocular conditions such as corneal edema and endophthalmitis. A comprehensive review of the clinical and epidemiological aspects of canine brucellosis is available (1).

A variety of slide agglutination, tube agglutination, and agar gel immunodiffusion tests have been described for the diagnosis of *B. canis* in dogs (2,3,4,5,6). Almost all have low specificity with false positive rates ranging from about 10% to almost 60%. Recently developed agar gel immunodiffusion procedures using cytoplasmic protein antigens show improved sensitivity and specificity, and may lead to more reliable routine tests. However, at present, the commonly used serological tests for *B. canis* should be interpreted with caution.

The definitive diagnostic procedure for canine brucellosis is the isolation of *B. canis*. Tissues for culture include blood, lymph nodes, spleen, liver, kidney, bone marrow, testicular tissue, semen, urine, vaginal discharges, milk, placenta, uterus, and aborted fetuses. Hemoculture, though not always successful, can be useful as the bacteremic phase in untreated dogs may persist for up to five years (2).

The first reported isolates of *B. canis* were made from Beagles in the northeastern United States in 1966 (7). The disease is now generally accepted as having worldwide distribution (1). In Canada, clinical and serological evidence indicates that *B. canis* occurs sporadically or exists at very low levels (8,9,10). This paper is, to our knowledge, the first published report

TABLE 1
Comparison of the Dye and Antibiotic Sensitivities of *Brucella canis* Field Strains from Canada with Strain RM666 and Mex 51

	Thionin			Basic Fuchsin			Thionin Blue		Safranin-O		Penicillin (Units/mL)					
Isolate	A	B	C	A	B	C	D	E	F	G	2.5	5	10	15	20	25
Poodles:																
BA 28A,B,C																
BA 49B,C,E	−	(+)	+	−	(+)	+	−	(+)	−	(+)	(+)	−	−	−	−	−
F,G																
Retriever:																
C11-T2																
C12-T5	(+)	+	+	+	+	+	(+)	+	+	+	+	+	+	(+)	−	−
C13-T4																
MEX51	(+)	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(+)	−
RM666	(+)	+	+	−	+	+	−	(+)	−	−	(+)	−	−	−	−	−

\+ = growth
(+) = poor growth
− = no growth

A = 1:25,000
B = 1:50,000
C = 1:100,000
D = 1:500,000
E = 1:1,000,000
F = 1:5,000
G = 1:10,000

of *B. canis* in Canadian dogs based on actual recovery of the organism. We describe the characteristics of eleven isolates of *B. canis* (from western Canada in 1982 and 1983) compared with the type strain RM666 and with Mex 51, a strain originating in Mexico.

Materials and Methods

Isolates — Suspected field isolates of *B. canis* were submitted for confirmation to the Animal Pathology Laboratory, Agriculture Canada, in Saskatoon by the Reference Laboratories Branch, Animal Health Division, Alberta Agriculture, in Edmonton. Isolates BA28A, B and C, and isolates BA49B,C,E,F and G originated from the same kennel and were received in March of 1982. They came from five Poodles showing clinical or serological evidence of *B. canis* infection. The organism was recovered at necropsy from uterus, vagina, and pooled samples of spleen. Isolates C11-T2, C12-T5 and C13-T4 were received in April and May of 1983. They were from a seven-year-old Labrador retriever with weight loss, lethargy, and unilateral testicular swelling. The organism was initially recovered from urine and at necropsy from kidney and pooled liver and spleen. All eleven isolates in this study were obtained using routine bacteriological procedures. Tissues were seared and sterile scissors used to open the seared surface. Inoculum was removed using either a sterile glass rod or a sterile swab. Tissue inoculum and urine-soaked swabs were streaked directly onto blood agar (5% sheep's blood in a trypticase soy agar base) and MacConkey agar, both incubated aerobically at 35°C, and blood agar and chocolate agar, both incubated in 5% CO_2 at 35°C. Colonies were evident at two to three days under CO_2 and three to four days aerobically on both blood and chocolate agar. The type strain RM666 was obtained from Dr. G. Brown, Reagents Section, National Veterinary Services Laboratories, Ames, Iowa. Strain Mex 51 was obtained from Dr. C. Rigby, Animal Diseases Research Institute, Nepean, Ontario.

Tests Performed — All eleven isolates were identified as *Brucella* sp. by Gram's stain, lack of hemolysis, nonfermentation of lactose, production of catalase, and agglutination with antirough *Brucella* antiserum. The isolates were then examined using accepted procedures (11) as well as an extended range of dye and antibiotic concentrations, additional phage types and safranin-O sensitivity tests. Tests performed were as follows: CO_2 requirement; H_2S production; urease production; growth in the presence of thionin or basic fuchsin (1:25,000, 1:50,000, 1:100,000, 1:125,000, 1:150,000, 1:200,000), thionin blue (1:500,000 and 1:1,000,000), safranin-O (1:5,000 and 1:10,000), penicillin (1, 2.5, 5, 10, 15, 20 and 25 U/mL); meso-erythritol (1 and 2 mg/mL); lysis by Tbilisi (Tb), Firenze (Fi), S708, Weybridge (Wb), Berkeley$_2$ (BK2), rough (R), rough ovis (R/O), and rough canis (R/C) phages, and agglutination by monospecific anti-*Brucella* A, M and R antisera.

Controls — Quality control consisted of monitoring media, antisera, phages and incubator conditions using the following cultures: *B. abortus* biovar 1, strain 544; *B. abortus* biovar 2, strain 86/8/59; *B. abortus* biovar 4, strain 292; *B. abortus* strain 19; *B. suis* biovar 1, strain 1330; *B. suis* biovar 4, strain 40; *B. ovis* strain 63/290; *B. canis* strain RM/666.

Results

The submitted isolates did not require CO_2, produced trace amounts of H_2S, hydrolysed urea in 15 minutes, grew in the presence of meso-erythritol and reacted with anti-*Brucella* R antiserum but not with anti-*Brucella* A or M antisera on the tube agglutination test. The results of the dye and antibiotic tests are summarized in Table 1. All eight isolates from the Poodles

had the same growth characteristics on dye and antibiotic media. They did not grow on either thionin or basic fuchsin at a concentration of 1:25,000, and grew poorly at 1:50,000. On thionin blue, there was reduced growth at 1:1,000,000 and total inhibition at 1:500,000. Similarly, on safranin-O, reduced growth was observed at 1:10,000 and total inhibition at 1:5,000. There was reduced growth on penicillin plates at 2.5 U/mL, and total inhibition at 5 U/mL. The type strain, RM666, gave almost identical results. The control strain Mex 51 and the other three isolates (Labrador retriever) grew in the presence of all dye concentrations tested, as well as on penicillin plates at concentrations of 10 U/mL. Reduced growth on higher concentrations of penicillin was also observed with these strains. On the basis of dye and antibiotic sensitivity tests, Mex 51 and these three isolates appeared identical and formed a group distinctly different from that seen with RM666 and the other eight isolates.

Phages Tb [Routine Test Dilution (RTD) and 10^4RTD], Fi(RTD and 10^4RTD), S708(RTD), Wb(RTD), and BK2(RTD) did not lyse any of the isolates. Rough canis (RTD and 10^4RTD) lysed all isolates. Phages R and R/O occasionally had a weak lytic effect at 10^4 RTD but not at RTD. This phenomenon was observed with all isolates except Mex 51.

Discussion

The most frequently described strains of *B. canis* resemble RM666, the type strain for the species. Characteristics of this strain include resistance to thionin (1:25,000) but sensitivity to basic fuchsin (1:50,000), safranin-O (1:5,000), thionine blue (1:500,000), and penicillin (5 U/mL) (11,12). Recently, several strains of *B. canis* have been described that grow in the presence of basic fuchsin (1:25,000) (13,14). A number of these strains, including Mex 51, originated in Mexico. Our examination of Mex 51 in parallel with RM666 showed that Mex 51 consistently grew on concentrations of basic fuchsin, safranin-O, thionin blue, and penicillin that were inhibitory to RM666. Without exception, dye and antibiotic results for the Canadian field isolates matched those of either RM666 or Mex 51. The eight isolates from the kennel exhibited the same characteristics as RM666 while the isolates from the Labrador retriever were indistinguishable from Mex 51.

The properties of RM666, Mex 51, and all field strains remained unchanged after seven to ten subcultures under laboratory conditions and storage at −70°C in litmus milk. This stability indicates that strain differences might be useful epidemiological markers. Histories available with these 11 isolates support this concept. Poodles from the kennel had extensive contact with dogs from the United States, where typical (RM666) strains are predominant. The Labrador retriever spent approximately 4.5 months in Mexico and the southwestern United States prior to his return to Canada and the development of canine brucellosis.

Brucella canis is usually classified as H_2S negative (12,15). It will, however, slowly produce H_2S after several days of growth (16). In our system RM666, Mex 51 and all field strains produced trace amounts of H_2S after five days.

The smooth phages Tb, Fi, S708, Wb, and BK2 did not lyse any of the *B. canis* isolates, while R/C (RTD and 10^4RTD) lysed all of them. These results are expected for this group of phages. The frequency of occurrence of the weak lytic effect produced by R/O(10^4RTD) was twice that of R(10^4RTD). Variations in the specificity of the rough phages, particularly the R/O group, are known to occur and may be reflected in these observations (13).

All field and control strains of *B. canis* described here grew on serum-dextrose-tryptose agar with added meso-erythritol at 1 and 2 mg/mL. Brucellae that are inhibited by erythritol can be detected on this medium but actual erythritol utilization is difficult to assess. The growth of RM666 in trypticase soy broth was not enhanced by the addition of erythritol and it was concluded that RM666 does not utilize this substrate (12). Recent oxidative-metabolic studies have determined that Mexican isolates can utilize erythritol and confirmed that RM666 cannot, leading to the conclusion that differences in erythritol utilization and basic fuchsin sensitivity are sufficient to warrant division of the species into two biovars (14). The results obtained here support this suggestion.

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