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Brucella abortus in the bitch: subclinical infection associated with urinary excretion

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SUMMARY

Brucella abortus infection in the bitch, associated with an aborted bovine fetus, is described. Clinical signs were absent, but the organism was isolated from many sites in the body, including the urine, after death. The response of the dog to *B. abortus* infection and its possible role as a vector of the disease is discussed.

INTRODUCTION

Brucella abortus infection in the dog has been recorded relatively infrequently. Cases of abortion have been described in the USA (Morse *et al.* 1953), France (Philippon, Roumy & Renoux 1969), Germany (Schwarz, 1954; Ehrlein, Schimmelpfennig & Bisping, 1963) and Great Britain (Taylor, Renton & McGregor, 1975; Bicknell, Bell & Richards, 1976).

Polyarthritis was recorded by Clegg & Rorrison (1968) and undulating fever and arthritis by McErlean (1966), although in the latter case diagnosis was based on serological evidence only.

An open case, characterized by fever, muscle stiffness and arthritis, and by isolation of the organism from blood and urine, was described by Hall (1974).

Symptomless infection in male farm dogs with isolation of the organism at death from the spleen was recorded in Canada by Prior (1976).

We wish to describe a case of symptomless infection in a pregnant bitch associated with urinary excretion of *B. abortus*.

CASE HISTORY

During an investigation into a breakdown in a Brucellosis Accredited herd in which a 7-month bovine fetus had been aborted it was suggested by the farmer's wife that the farm dog might be responsible for transmission of infection from a neighbouring farm. The dog was included in the routine examination and somewhat unexpectedly *Brucella abortus* was isolated from a vaginal swab submitted to the Veterinary Investigation Centre, Wolverhampton.

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The animal was a 5-year-old Collie bitch, which appeared to be perfectly healthy and had whelped seven normal puppies 14 days previously. Further enquiries into its habits indicated that it was not a wanderer, stayed near the farm buildings all day and slept in a kennel at night. It was fed on a proprietary dog-meat and biscuit but was known to drink waste milk and to lick fetal fluids and placentae of calving cows. It was also known to have had access for several hours to the aborted, brucella-infected fetus, 5 weeks before sampling and 3 weeks before whelping. After consideration of these factors and the herd history, it was considered most likely that the dog had become infected from the fetus and that infection had entered the herd some time previously in another way.

Because of the danger of infection to young children on the farm, the bitch and puppies were destroyed and the carcasses made available for post-mortem examination. In view of the rarity of reports of the condition, it was decided to carry out a detailed bacteriological examination, to determine the distribution of infection throughout the body, and to assess the risk of transmission to other animals and man.

MATERIALS AND METHODS

Post-mortem examination of the bitch revealed no gross pathological changes. The whole uterus and portions of brain, lung, liver, spleen, kidneys and mammary gland were removed for bacteriological examination. Cultures were also made *in situ* from bone marrow, clotted blood, the urinary bladder and the vagina.

The palatine tonsils, and the mandibular, pharyngeal, mediastinal, gastric, mesenteric, supramammary and internal iliac lymph nodes were removed; these were dipped in methylated spirit and ignited to sterilize the surface and then homogenized in Griffiths tubes for bacteriological examination and guinea-pig inoculation. The tonsils and mandibular and pharyngeal nodes were mixed to form one batch; gastric and mesenteric nodes were bulked similarly; the other nodes were homogenized separately.

After culture direct from the bladder, urine was removed for guinea-pig inoculation and further examination. Rectal contents were mixed with antibiotic solution containing 100 international units penicillin and 100 μg streptomycin per ml, and allowed to stand overnight in the refrigerator before inoculation. A portion of mammary tissue was removed and homogenized for guinea-pig inoculation.

Post-mortem examination of the seven puppies showed no obvious pathological changes. Portions af liver, spleen and lung tissue were removed from each carcass and homogenized together for bacteriological examination and guinea-pig inoculation.

The bacteriological media used included 5% sheep blood agar (Oxoid Base), serum dextrose agar with antibiotic (Jones & Morgan, 1958) and the medium of Farrell (1974). Plates were incubated at 37 °C in 12% CO_2 and examined daily.

Young guinea-pigs were injected intramuscularly with 1 ml of homogenized tissue or other material and killed after 6 weeks. Blood samples were taken at death for serological examination and the spleens removed for bacteriological

Table 1. *Summary of cultural and biological examination*

Tissue	Blood agar	SDA	Farrells	Guinea-pig
Uterus	+	+	+	N.T.
Liver	−	−	−	N.T.
Spleen	−	−	−	N.T.
Lung	−	−	−	N.T.
Kidney	−	−	−	N.T.
Brain	−	+	+	N.T.
Bone marrow	−	−	−	N.T.
Blood	−	−	−	N.T.
Vagina	+	+	+	N.T.
Urine	−	−	+	+
Faeces	−	−	−	−
Mammary gland	−	−	−	+
Tonsil: mandibular pharyngeal L.N.'s	−	+	+	+
Internal iliac	−	−	+	+
Supramammary	+	−	+	+
Mediastinal	−	−	+	+
Gastric and mesenteric	+	+	+	+
Puppy viscera	−	−	−	−

+, *B. abortus* isolated; −, *B. abortus* not isolated; N.T. No test.

examination. A blood sample taken at death from the bitch was also examined serologically.

After isolation of *B. abortus* from the urinary bladder, an attempt was made to determine the concentration of organisms in the urine, a portion of which had been retained at 4 °C in a refrigerator.

Quantities ranging from 0·2–0·5 ml were pipetted onto plates of Farrell's medium and colony counts made after 3 days incubation.

RESULTS

Organisms with the morphological and cultural characteristics of *B. abortus* were isolated on culture from the uterus, urinary bladder, vagina and brain tissue and from all the groups of lymph nodes examined. No isolations were made from puppy material. All cultures were sent to the Diseases of Breeding department, Central Veterinary Laboratory, Weybridge, and were identified as *B. abortus*, biotype 2. This was the same biotype as that isolated from the bovine fetus.

Guinea-pigs inoculated with material from all the lymph-node samples, from the mammary tissue and from urine were positive for *B. abortus* on bacteriologial examination and serology. Table 1 summarizes the bacteriological and biological examinations.

The blood sample from the bitch gave the following results: Rose Bengal Plate Test, +; serum Agglutination Test, + 1/640; complement fixation test, + + + + 1/400; Coombes Test, + + 1/1280; using *B. abortus* (strain 99) antigens. A plate test for *B. canis* proved negative.

Only one series of colony counts was carried out on the urine because of the

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concurrent growth of other organisms, particularly *Proteus vulgaris*. The average figure obtained was $2{\cdot}45 \times 10^2$ organisms/ml.

DISCUSSION

The available evidence suggests that the bitch became infected in late pregnancy and approximately 5 weeks before euthanasia, following access to highly infective material. No signs of illness were ever observed and the pregnancy was not affected, yet culture results indicated the presence of the organism in a wide variety of sites, presumably following an initial bacteraemia. Such extensive isolations have not been recorded in natural infections in the dog, but experimental work has shown the very variable response of this animal to *B. abortus* infection, as indicated by clinical signs, serological response and isolation of the organism.

Van der Hoeden (1933) infected dogs by the oral, conjunctival and intracutaneous route. In a group of 23 dogs, clinical signs were virtually absent; a good serological response was obtained in almost every case; isolations of Brucella were made from a variety of sites at autopsy, and, in one case only, from the urine during life.

Feldman, Bollman & Olson (1935) infected five dogs with *Brucella abortus*: two by the intravenous route and three by mouth. There were no clinical signs and the serological response as measured by the serum agglutination test was variable with a more rapid response and much higher titres following intravenous inoculation. Attempts to isolate Brucella were made in four dogs at autopsy, by inoculation of guinea-pigs with spleen or mesenteric lymph node material, but all proved negative.

Morse, Kowalczyk & Beach (1951) carried out two series of experiments in dogs to simulate natural infection. In the first experiment they fed aborted fetuses and placentae from infected cows to a group of 15 dogs. Bacteriological examinations were made of blood, urine and faeces at weekly intervals and after death. Bone-marrow, and homogenates of viscera and lymph nodes were examined bacteriologically and in some cases inoculated into guinea-pigs. Clinical signs were apparently rare. Two pregnant bitches aborted but *Brucella* was not recovered from the discharges; two bitches produced normal healthy litters. *Brucella* was recovered from the urine of two bitches, at 2 h and 8 h after infection and from the blood in only one animal 48 h after infection. *B. abortus* was isolated from lymph nodes only, in a total of nine dogs. Guinea-pig inoculations produced positive results in two cases only, one of which was negative on culture. Serological response as measured by agglutination test was low and only present in a small proportion of the group. In the second experiment they fed milk, naturally infected with *B. abortus*, to a group of 14, nine-week-old puppies, on an *ad libitum* basis, killing the animals between 43 and 112 days. During the course of the experiment serum agglutinins were not detected and isolation attempts on blood, urine, faeces and a wide range of tissues were completely negative.

None of the experimental work quoted above includes any estimate of the size of the infecting doses, but one must assume that they were high, particularly

where the feeding of aborted fetuses and placentae was involved. If this is so, then the lack of clinical signs produced, the variable agglutinin responses, and the irregular pattern of isolation of the organism indicate a marked resistance of the dog to infection. Feldman *et al.* (1935) observed that *B. abortus* 'fails to prosper within the tissues of the dog' and this would seem to sum up the information derived from both natural and experimental infections.

The most significant finding in the case recorded here was the isolation of *B. abortus* from the urine. Although the estimated concentration of organisms was quite low ($2{\cdot}45 \times 10^2$/ml) it was theoretically high enough to infect fully susceptible non-vaccinated cattle if sufficient quantity were voided onto food; McEwen, Priestley & Paterson (1939) in experiments to determine an infective dose for heifers found that $1{\cdot}4 \times 10^3$ organisms were sufficient to infect two out of nine non-vaccinated, pregnant animals.

A risk to human health also existed. Direct contact with infective material is well recognized as a source of infection (Henderson & Hill, 1972) and children in particular could be in danger when handling an infected dog, particularly one that appeared healthy. In the case described by Hall (1974) the dog was incontinent and the author comments on the risk to adults in cleaning fouled floors. However, surveys of dairy-farming communities (Henderson & Hill, 1972) have shown a very low prevalence of clinical as opposed to subclinical infection, suggesting a reasonable degree of natural resistance in the human being. In this case, human contact with the dog was minimal, and the family remained in good health.

The eradication of bovine brucellosis in Gt Britain is well advanced, but until it is completed there will continue to be risk of spread of infection to other animals. In the case of the dog its dual role as farm working animal and pet could enable it to act occasionally as a vector of the disease, to the detriment of control and eradication schemes and to human health.

REFERENCES

Bicknell, S. R., Bell, R. A. & Richards, P. A. (1976). *Brucella abortus* in the bitch. *Veterinary Record* **99**, 85.

Clegg, F. G. & Rorrison, J. M. (1968). *Brucella abortus* infection in the dog: a case of polyarthritis. *Research in Veterinary Science* **9**, 183.

Ehrlein, H. J., Schimmelpfennig, I. R. & Bisping, W. (1963). Ein Beitrag zur Brucellose des Hundes. *Deutsche Tierärztliche Wochenschrift* **70**, 353.

Farrell, I. D. (1974). The development of a new selective medium for the isolation of *Brucella abortus* from contaminated sources. *Research in Veterinary Science* **16**, 280.

Feldman, W. H., Bollman, J. L. & Olson, C. (1935). Experimental brucellosis in dogs. *Journal of Infectious Diseases* **56**, 321.

Hall, R. F. (1974). A case of open brucellosis in the dog. *Veterinary Record* **94**, 454.

Henderson, R. J. & Hill, D. M. (1972). Subclinical brucella infection in man. *British Medical Journal* iii, 154.

Hoeden, J. van der (1933). Pathogenesis of brucellosis Bang. *Journal of Comparative Pathology* **46**, 232.

Jones, L. M. & Morgan, W. J. B. (1958). A preliminary report on a selective medium for culture of Brucella, including fastidious types. *Bulletin of the World Health Organization* **19**, 200.

McErlean, B. A. (1966). Undulating fever, posterior paresis and arthritis in a dog apparently due to brucellosis. *Veterinary Record* **79**, 567.

McEwen, A. D., Priestley, F. W. & Patterson, J. D. (1939). An estimate of a suitable infective dose of *Brucella abortus* for immunisation tests on cattle. *Journal of Comparative Pathology* **52**, 116.

Morse, E. V., Kowalczyk, T. & Beach, B. A. (1951). The bacteriologic aspects of experimental brucellosis in dogs following oral exposure. 1. Effects of feeding aborted fetuses and placentas to adult dogs. *American Journal of Veterinary Research* **12**, 219.

Morse, E. V., Ristic, M., Witt, L. E. & Wipf, L. (1953). Canine abortion apparently due to *Brucella abortus*. *Journal of the American Veterinary Medical Association* **122**, 18.

Philippon, A., Roumy, B. & Renoux, G. (1969). Un cas de brucellose canine à *Brucella abortus*. *Bulletin de l'Académie Vétérinaire de France* **42**, 923.

Prior, M. G. (1976). Isolation of *Brucella abortus* from two dogs in contact with bovine brucellosis. *Canadian Journal of Comparative Medicine* **40**, 117.

Schwarz, H. (1954). Verwerfen beim Hund infolge Abortus Bang. *Monatshefte für Veterinärmedizin* **7**, 152.

Taylor, D. J., Renton, J. P. & McGregor, A. B. (1975). *Brucella abortus* biotype 1 as a cause of abortion in a bitch. *Veterinary Record* **96**, 428.