CASE REPORT
JIVA 20 (1) April 2022
https://doi.org/10.55296/JIVA/20.1.2022.82-86

Received: 19/02/2022
Accepted: 16/03/2022

BRUCELLA ABORTUS INFECTION IN A NEAPOLITAN MASTIFF DOG: A CASE REPORT

Athira K.*[1], Shyma V.H.[2], Justin Davis K.[2], Vijayakumar K.[3] and Jayakumar C.[4]

PhD Scholar[1],Assistant Professors[2,4], Professor and Head[3],
Department of Veterinary Epidemiology and Preventive Medicine [1,2,3]
Department of Animal Reproduction Gynaecology and Obstetrics[4]
College of Veterinary and Animal Sciences, Mannuthy- 680 651, Thrissur, Kerala
Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, India.
**Corresponding author: athira27shiva@gmail.com*

ABSTRACT

A five-year-old Neapolitan mastiff was presented with a history of abortion at 55th day of gestation with greenish grey vaginal discharge. The haemato-biochemical parameters revealed anaemia, leucocytosis, hypoalbuminemia and hyperglobulinemia. Microscopic examination of Stamp stained smear revealed red coccobacillary organisms suggestive of *Brucella* spp. Paired sera samples were collected on the day of presentation and three weeks after abortion. These sera samples on RBPT showed agglutination within four minutes. DNA extracted from the aborted tissues yielded amplicons specific for *B. abortus* on PCR. The animal was successfully treated with Enrofloxacin at the dose rate of 5 mg/kg at 12 hour interval orally for 30 days.

Keywords: *Brucella abortus*, Leucocytosis, Neapolitan mastiff, Stamp staining

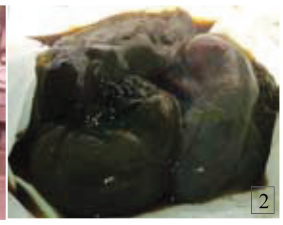
INTRODUCTION

Canine brucellosis is a chronic infectious zoonotic disease of dogs caused by *Brucella* spp. and one of the leading cause of infertility in domestic canids, more specifically in breeding kennels worldwide (Wanke, 2004; Hollett, 2006). Brucella (*B. abortus, B. suis and B. melitensis)* can get transmitted to dogs through ingestion of infected placenta from other domesticated animals (Talukder *et al*., 2011). Even though brucellosis does not bring about any change in timing, duration of oestrous and breeding in dogs, it is considered as the major cause of infertility and abortion in dogs. In 75 per cent of cases, the classic signs of brucellosis are late term abortion, more common from 45-55 days of gestation. This paper reports a case of abortion due to *B. abortus* infection in a Neapolitan mastiff and its molecular detection.

CASE HISTORY AND OBSERVATIONS

A five-year-old female Neapolitan Mastiff dog (Fig. 1) was referred to the University Veterinary Hospital (UVH), Kokkala (Thrissur district of Kerala), with a history of abortion on 55th day of gestation with greenish grey vaginal discharge. The aborted pups (Fig. 2) were partially autolysed with serosanguineous peritoneal fluid. All the vital parameters of the bitch were within the normal range. On a detailed signalment, the owner reported that the bitch was fed with raw milk and had a history of consecutive abortions. The haematological and biochemical parameters revealed anaemia (red blood cells - 3.49x10^{6}/ul haemoglobin - 8.5 g/dL), leucocytosis (22.9x10^{3}/ul, hypoalbuminemia (1.39 g/dL) and hyperglobulinemia (6.92 g/dL). Microscopic examination of Stamp stained aborted foetal stomach content's smear revealed red coccobacillary organisms against the blue background suggestive of *Brucella* spp. (Alton *et al.,* 1975) (Fig. 3). The paired sera samples were collected on day of presentation and 21st day post abortion. Rose Bengal plate test (RBPT) showed agglutination within four minutes in both the sera samples collected. DNA was extracted from the uterine discharges and foetal stomach contents and subjected to Brucella genus specific PCR and identified as *Brucella* spp. This product was again subjected to identify the species of the organism and revealed it as *B. abortus* which yielded an amplicon size of 136 base pair (Fig. 4). Thus the case was diagnosed as abortion due to *B. abortus* and confirmed by sequencing.


1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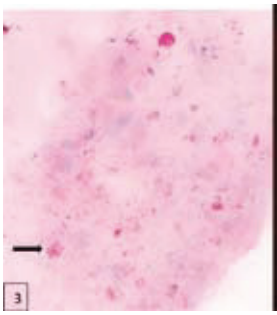
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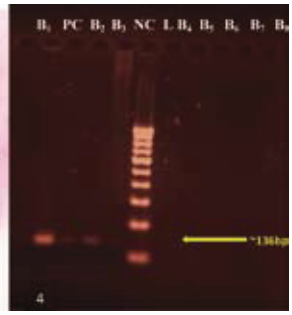
4

TREATMENT AND DISCUSSION

Johnston *et al.* (2001) opined that pet animals tested positive for Brucella should be neutered and housed singly. The treatment regimens included minocycline (25 mg/kg once daily orally (PO) for 14 days), dihydrostreptomycin (5 mg/kg intramuscularly (IM) twice daily for 7 days), tetracycline (30 mg/kg PO twice daily for 21 days) and streptomycin (20 mg/kg IM once daily for 14 days). Treatment with antibiotics should be continued to decrease bacteraemia and subsequent shedding of the organism.

Wanke *et al.* (2006), conducted a clinical trial with Enrofloxacin at the dose rate of 5 mg/kg at 12-hour interval, orally for 30 days in all dogs of a kennel with history of abortion. They opined that treatment did not completely eliminate *B. canis*, but it was successful in avoiding further abortions, transmission of the disease to newly whelped puppies and maintained fertility in bitches. In this case, the animal was treated successfully with Enrofloxacin at the dose rate of 5 mg/kg at 12-hour interval given orally for 30 days. In the subsequent whelping, the bitch whelped three live pups, which were found negative for brucellosis using RBPT and no further abortions was reported.

Beagle fever or canine brucellosis is an important contagious disease in pet dogs, with primary effects on reproduction, and considered to be the major causes of infertility and abortion in stray as well as pet dogs (Faigel, 1969; Carmichael, 2018). In India, the first report of Brucella infection in dogs was that of Pillai *et al.* (1991) from Chennai.

From the contaminated mucosal sites, brucellae are phagocytized by tissue macrophages and transported to the site of multiplication (*i.e.* lymphatic and genital tract tissues) (Carmichael, 2018). In females the principal sign is abortion generally after 45 to 55 days of gestation, characterized by grey-green vaginal discharge and partially autolyzed aborted pups (Kustriz, 2003). Haematological and biochemical disturbances in canine brucellosis included leucocytosis due to a neutrophilia, degenerative left shifts and hyperglobulinemia with concomitant hypoalbuminemia (Cosford, 2018).

Canine brucellosis should be considered in the differential diagnosis whenever there was a history of abortion in female dogs or poor reproductive performance in either sex (Carmichael, 2018). Mol *et al*. (2020) clearly demonstrated that the diagnosis of canine brucellosis remained a challenge even when multiple tests were employed. For better diagnosis of Brucella infection, Saxena *et al.* (2015) suggested a combination of RBPT and enzyme linked immunosorbent assay (ELISA) to be used. But in this case, a combination of RBPT and PCR helped in proper diagnosis of the case.

Inability of many antimicrobials to attain adequate concentrations at intracellular level had made treatment of brucellosis a difficult task. Wanke (2004) recommended that all male and female dogs should be routinely tested serologically before mating and in Brucella tested positive cases; breeding should be avoided, even in dogs with high genetic value.

CONCLUSION

A Neapolitan mastiff bitch with a history of abortion at 55th day of gestation with greenish grey vaginal discharge was diagnosed with *Brucella abortus* infection using Stamp staining, RBPT and confirmed by PCR. The animal was treated with Enrofloxacin at the dose rate of 5 mg/kg at 12-hour interval given orally for 30 days. The above treatment was successful in avoiding further abortions, transmission of the disease to newly whelped puppies and maintaining fertility in bitches.

ACKNOWLEDGEMENT

The authors are thankful to the Head, University Veterinary Hospital, Kokkala and authorities of Kerala Veterinary and Animal Sciences University for providing all facilities for the completion of this work.

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