

Original Research Article

Histological changes in canine placenta during acute brucellosis

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A B S T R A C T

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Brucellosis is a disease which is caused by *Brucella* sp. and generally affects animals and is transferrable to humans. This paper is a study about the changes seen in canine placenta when affected by acute brucellosis. Three *Brucella* suspected placental tissues from a single bitch were first confirmed for the presence of brucellosis and then grossed, processed by automated tissue processor, sectioned and stained by haematoxylin and eosin stain. The slides were then examined under a light microscope. Important changes were seen throughout the tissue particularly in blood vessels and all the three samples showed similar changes. Only a few studies have been made on histology of canine placenta in brucellosis thus these findings will help better understanding of brucella pathology.

Introduction

Brucellosis is a disease that generally occurs in animals such as cows, pigs, dogs, cattles and goats. *Brucella* are gram negative, non-motile, non-spore forming coccobacilli bacteria (Dr Delia Skan, August 2004). The genus *Brucella* comprises at least 8 species named according to the preferred mammal hosts (Maria-Jesus Grillo, 2012). *B.melitensis*, *B.abortus*, *B.suis* and *B.canis* are the most important species and they preferentially infect goats and sheeps, bovines and swine and dogs respectively (Moreno E and Moriyon, 2006). Cross-infection within species adds to the complexity of the disease (Ettinger *et al.*, 1995). Around the world more than 500,000 human infections

are reported annually (Pappas *et al.*, 2006). It is one of the major zoonotic diseases in the world so it is transferrable to humans by drinking of unsterilized milk or meat of the infected animals or coming in very close contact with their secretions. Human to human transfer can be due to sexual contact or from mother to child, is rare but possible (Dr Delia Skan, August 2004). Unpasteurized milk is a common source of infection, as is inhalation from carcasses among abattoir workers (Godfroid, 2005). Brucellosis is an important human disease in many parts of the world and yet it is often unrecognized and frequently goes unreported (Dean *et al.*, 2012).

The disease is always caused by infection with a *Brucella* sp. and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens (Vallbracht *et al.*, 2004).

Brucellosis is a major disease with predilection to infect placenta and foetal membrane (Rahman Md. Siddiquir and Baek Byeong Kirl, April 2008). In dogs it is spread by breeding and coming in contact with aborted fetuses. Dogs can be infected by four species of *Brucella* (*B.melitensis*, *B.suis*, *B.canis*, and *B.abortus*) (Bruce Hollett, 2006). This disease can be treated with antibiotics, as in humans, but it is difficult to cure (Thakur, 2002). The three placental sample used by us were from the same bitch. The most common clinical feature of brucellosis in animals are placentitis and abortion (Silva *et al.*, 2000). She was suspected of brucellosis when she gave birth to still-born and aborted fetuses as these are few of the symptoms of brucellosis in canines. This suspicion was confirmed when biochemical tests and molecular tests were positive. They were then taken for histopathological processes and development of slide.

Materials and Methods

Determination of brucellosis in the three placental samples

Permission from Institutional Ethical Committee has been taken for this study. The three placental tissues were inoculated in *Brucella* agar/broth for 4 days at 37°C in 5% CO₂ (ESCO celculture CO₂ incubator) for the production of colonies. The samples were then tested for positive Brucellosis using routine culture and identification tests (gram staining, oxidase test, catalase test, urease test, triple sugar

iron (TSI) test reactions), and molecular tests by a *Brucella* specific Real Time PCR protocol in Light Cycler 2.0, These tests confirmed that all the three placental samples had brucellosis and were suitable for further study on their histological changes.

Histological processing of the collected placenta

The three placenta samples from the bitch were fixed in 10% neutral-buffered formal-saline for more than 24 hours. The tissues were then grossed and put for its processing (Automated tissue processor) which included dehydration in alcohols, cleaning in xylene and infiltration and embedded in paraffin wax.

The tissues embedded in the paraffin wax were sectioned using a microtome (Leica RM 2235) taking series section of 4 µm and those were then mounted on glass slides. These slides were then stained in haematoxylin and eosin (H&E) and were examined under a light microscope for microscopic histological changes (Meador *et al.*, 1988).

Result and Discussion

The placental tissues showed many changes which were noted as follows. In two of the placental tissues, were areas of necrotising placentitis with scattering of sloughed epithelial cells. This feature is seen in figure 1. Calcification was also seen but in few areas of all the three samples as seen in figure 2. In one placenta (the third placenta) all the changes were significantly more than the changes in the other two placenta.

In the blood vessels, vasodilation was present in very low amounts in all three but showed polymorphic infiltration in the

Table.1 Generalized placental histological changes due to brucellosis.

| Changes | Sample 1 | Sample 2 | Sample 3 |
|---|-----------------|-----------------|-----------------|
| Placentitis | 1+ | 1+ | 2+ |
| Necrotising placentitis | 1+ | 1+ | 2+ |
| Necro-purulent placentitis | 0 | 0 | 0 |
| Necro-hemorrhagic placentitis | 0 | 0 | 0 |
| Necrosis of trophoblastic epithilium lining | 0 | 0 | 0 |
| Necrosis of trophoblastic chorionic villi | 0 | 0 | 0 |
| Necrotic cellular debris | 0 | 0 | 0 |
| Sloughed epithilial cells | 1+ | 1+ | 1+ |
| Calcification | 1+ | 1+ | 2+ |

Table.2 Blood vessel changes during brucellosis

| Changes | Sample 1 | Sample 2 | Sample 3 |
|---------------------------------|-----------------|-----------------|-----------------|
| Vasodilation | 1+ | 1+ | 1+ |
| Polymorph infiltration in wall | 2+ | 2+ | 2+ |
| Polymorph infiltration in lumen | 2+ | 2+ | 2+ |
| Focal necrotizing panarteritis | 2+ | 2+ | 2+ |

Table.3 Other general changes during brucellosis.

| Changes | Sample 1 | Sample 2 | Sample 3 |
|-----------------------------------|-----------------|-----------------|-----------------|
| Focal infiltration of neutrophils | 1+ | 1+ | 2+ |
| Bacterial colonies | 0 | 0 | 0 |
| Micro-abscess | 0 | 0 | 0 |

Figure.1 Necrotizing Placentitis seen on the upper left hand side

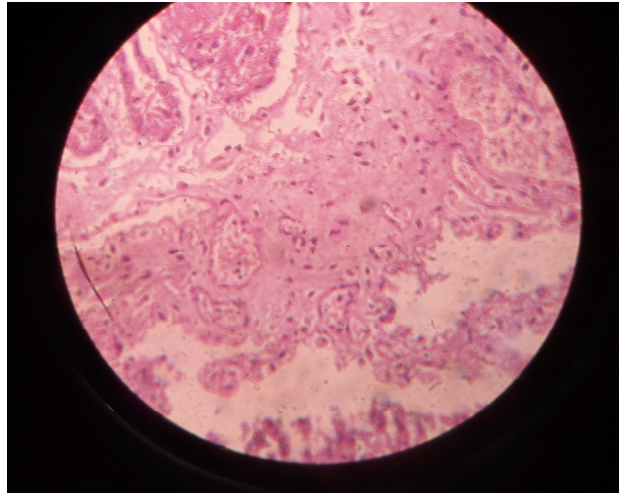


Figure.2 The numerous violet clustered dots are sites of calcification which prove that the samples were affected by brucellosis for a long time.

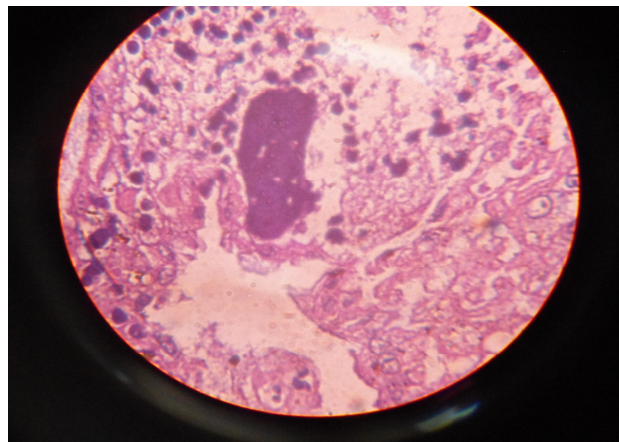
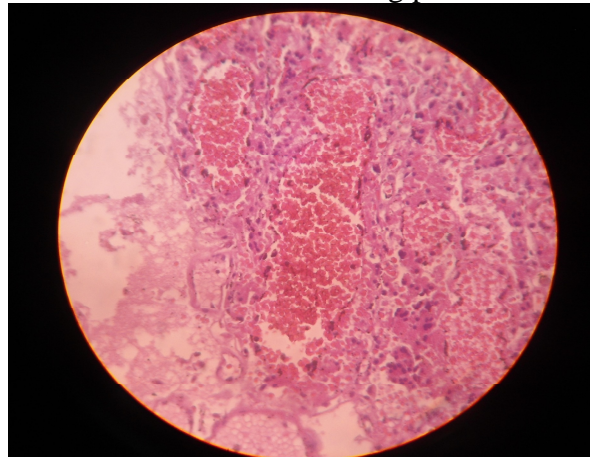


Figure.3 The polymorph infiltration in the lumen and wall is clearly seen along with vasodilation and necrotising panarteritis.



wall and lumen in a moderate degree. The affected placentae also had focally necrosed panarteritis. All these are clearly seen in figure.3. There was low focal infiltration of neutrophils in two of the samples but in one placenta it was present comparatively more with bacterial colonies. Micro abscess were absent in all the three placentae. The detailed findings are shown in Table 1, Table 2 and Table 3 (1+ corresponds to mild, 2+ corresponds to moderate, 3+ corresponds to high changes) and in Figs 1,2 and 3 .

The histological changes showed similar pattern of changes which are commonly found in placenta during Canine Brucellosis. There are focal and generalized inflammatory changes, and prominent vascular changes. The calcification seen, refers that the bitch was infected with brucellosis for a long time. Canine brucellosis is an important health problem and in this study some aspects of histological changes in placenta in brucellosis have been explored. There has not been many detailed study on the histology of placenta in canines affected by brucellosis.

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