Isolation, Histopathological and molecular detection of Yesinia Pseudotuberculosis infection in Sheep in Turkey.

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Isolation, Histopathological and molecular detection of Yersinia Pseudotuberculosis infection in Sheep in Turkey.

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Abstract

The aim of the present study was to detect causes of abortion in sheep and early neonatal death of lambs and treatment trial. Diagnosis of Yersinia pseudotuberculosis was made on the basis of bacteriological from the stomach contents, lung, liver, and peritoneal fluid and pathological findings. Additionally, in order to confirm the presence of Yersinia pseudotuberculosis nested PCR was performed. In treatment trials Amoxycillin were given 1ml/10kg BW. This is the first case report of Yersinia pseudotuberculosis causing abortion in sheep in Turkey. As a result of this study it is suggested that Yersinia pseudotuberculosis should be considered in the differential diagnosis of causes of ovine abortion and early neonatal death of lambs in this country.

Key words: Yersinia pseudotuberculosis, lambs, PCR, treatment, Diyarbakir.
Introduction

Yersinia pseudotuberculosis is a Gram-negative organism that causes enteritis and septicaemia (Taffs and Glynis, 1983; Brown and Davis 1989). Yersiniosis has been reported from wild animals and birds, rodents, ruminants, carnivores, non-human primates, man and occasionally farm animals (Brown et al., 2007; Busato et al., 1999; Juste et al., 2009). The clinical symptoms and pathologic changes vary among the different host species (Zhang et al., 2008). The common symptoms of the disease in ruminants, and other mammals are necrotizing, ulcerative enteritis and mesenteric lymphadenitis with fever, anorexia, vomiting, and diarrhea (Callinan et al., 1988; Gill, 1966; Hum et al., 1997; Foster et al., 2008). Septicemia due to yersiniosis can lead to the involvement of visceral organs, especially liver and spleen. The infection can lead to abortion, stillbirth or birth of weak or healthy newborn (Corbel et al., 1992) in sheep, goats, pigs and cows (Novoslavskij et al., 2010; Otter, 1996; Riet-Correa et al., 1990; Seimiya et al., 2005; Slee and Button, 1990). Experimental infection produced a purulent placentitis in most ewes and focal hepatic and myocardial necrosis in newborn lambs. Rarely, the organism would cause other pathological changes, such as pneumonia in cattle (Juste et al., 2009), epididymo-orchitis of rams (Slee and Button, 1990) or ocuglandular syndrome in (goats Wessels et al., 2009). The organism can be shed by asymptomatic animals (Brown et al., 2007).

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Material and Method

Material

The materials of the study were consisted of three fetus and four lambs which were died approximately two days after birth from a two hundred sheep flock in Diyarbakir between August and November 2010. Fetuses and lambs were examined according to standard necropsy procedures. Tissues were collected for microbiological examination under precautions to prevent extraneous or cross-contamination of individual tissues.

Microbiology

Liver, lung and gastric contents of aborted fetus and died lambs were cultured on blood agar and MacConkey agar. Culture plates were incubated at 37°C in both aerobic and anaerobic atmospheres for 24-48 h. Suspect *Yersinia* sp. colonies were identified by the following: Gram reaction, motility at 22 and 37°C, fermentation of glucose, lactose. VITEK 2 GN (colorimetric) card (Biomerieux SA France) identification kit was used for identification.

Pathology

Three fetus and four lambs were necropsied. Samples of liver, mesenteric lymph node and small and large intestines were fixed in 10% neutral buffered formalin.

PCR assay for detection of *Yersinia pseudotuberculosis*

DNA for Polimeraze Chain Reaction (PCR) was isolated by a Purification Kit (Vivantis, GF-BA). PCRs performed in 50μl volumes containing 5μl of template DNA, 0,1mM each of the four deoxynucleoside triphoshates, 5μl of 10 X PCR buffer, 3mM MgCl2, 0,1μl of each primer, and 0.5U of Tag DNA polymerase. The PCR amplifications carried out at 94°C for 5 min as an initial denaturation step and then subjected to 30 cycles consisting of 1 min at 94°C,1 min at 55°C for detection of inv (inv-1-5'-TAA GGG TAC TAT CTC GGC GGA GGA-3', inv-2-5'-CGT GAA ATT AAC CGT CAC ACT-3') at 55 °C for detection of Ypf-20210-wzz-5'-GGT GAT GAG CAA GTT CAA G-3', Ypr-20538-wzz-5'-GCT AAA TCC ACT GCT CGC TG-3' (Bogdanovich et al., 2007).
2003) 1 min at 72 ºC, followed by a final 5 min extension step at 72 ºC. 10μl volume of each PCR products was loaded onto a 1.5% agarose gel containing 0.05μl/ml of ethidium bromide solution. The gel was visualized on an UV board. A negative control with sterile water instead of DNA template was also prepared. 100bp DNA Ladder (Fermentas SM 0241) was used as the molecular size marker.

Results

Microbiology
Yersinia pseudotuberculosis was isolated as pure growth from the stomach contents, lung, liver, and peritoneal fluid. Any other bacteria or Chlamydia weren’t isolated on tissue culture of lung, liver, kidney, brain, and stomach contents. The caused agent was identified as Yersinia pseudotuberculosis strains by the VITEK 2 Compact device (Fig.1). The testing of Vitek 2 demonstrated that Yersinia pseudotuberculosis was susceptible to Amoxycillin, Ampicillin, Tetracyclin, Lincocin-spectinomycin, Sulphamethoxazole-trimetophrim but resistant to Eritromycin, Penicillin G.

PCR assay
Yersinia pseudotuberculosis was detected with nested PCR method. Amplified products were shown figure 2.

Figure 1. Yersinia pseudotuberculosis.

Pathology
Three fetus and four lambs which were necropsied, had gross lesions characterized by peritoneal and sometimes pericardial serous or sanguineous fibrinous fluid, enlarged mesenteric lymph nodes, marked edema of the mesentery, edema and thickness of the wall of the bowel and abomasum, and a fibrinous or hemorrhagic enteritis of the small and large intestine, which was severe in the ileum. Histopathological examinations of liver, kidney and lung indicated to non significant pathology. In the small and large intestines there were multi-focal, heavy inflammatory cell infiltrates, consisting of neutrophils, macrophages, lymphocytes and plasma cells within the lamina propria.

Figure 2. M:100 bp DNA Ladder; Lane 1, Negative control (sterile water); Lane 2, 295 bp amplified product (inv); Lane 3, Negative control (sterile water); Lane 4, 418 bp amplified product (Ypf-20210-wzz,Ypr-20538-wzz).

Treatment
In treatment trials bacterium was sensitive at Amoxycillin 1ml/10kg BW (Amoxycillin, Clamoxyl LA Pfizer).

Discussion
In ruminants, clinical diseases by Y. pseudotuberculosis associated with abortion (Hannam, 1993; Otter, 1996), (mastitis Juste et al., 2009) and enterocolitis (Seimiya et al., 2005; Slee
and Button, 1990). There were few reports of Y. pseudotuberculosis abortion in sheep UK (Baird et al., 1997), Australia (Callinan et al., 1988; Slee and Skillbeck, 1992), New Zealand (Hodges et al., 1984) and USA (Brown and Davis, 1989).

This study considered to be the first case of abortion and early neonatal death of lambs due to intrauterine Yersinia Pseudotuberculosis infection in Turkey. The course of the infection and its histopathology suggested that the organisms produced a relatively slowly evolving infection which reached the stage of producing placental failure and fetal death. These results indicated that strains of Y. Pseudotuberculosis with natural pathogenicity could produce placental infection and abortion in pregnant sheep.

This is consistent with observations by Karbe and Erikson, (1984), (Corbel et al., 1992), Jerrett and (Slee, 1989). The history of pathological signs, results of bacterial culture and PCR of Y pseudotuberculosis as the etiologic agent in this research provide that there were similarities between our results and previously reports by Gill (1966), (Martins et al., 1998), (Iwata et al., 2008), (Thoerner et al., 2003), Welsh and Stair, (1993).

The epidemiology of Yersiniosis is complex and the factors leading to clinical disease are unknown. The organism may be shed in the faeces by clinically normal animals in a herd and by other species such as rodents and birds in the environment (Witte et al., 1985; Riet-Correa et al., 1990). Yersinia pseudotuberculosis and Yersinia enterocolitica have been isolated from ovine abortion cases (Karbe and Erikson, 1984; Otter, 1996). Infection of ewes by Y. pseudotuberculosis can lead to abortion, stillbirth or birth of weak or healthy lambs. Infection by Y. enterocolitica resulted in placentitis and abortion, in subsequent normal pregnancies, Y. pseudotuberculosis causes septicaemia, tissue micro-abscesses and enteritis in lambs and young pigs and sporadic abortions in sheep, and cattle (Givens and Marley, 2008). At necropsy, typical gross lesions included peritoneal serous or sanguineous, fibrinous exudate, enlarged mesenteric lymph nodes, marked mesenteric oedema and fibrinous or haemorrhagic enteritis. Histologically, there was severe fibrinonecrotic enteritis with mixed inflammatory cell infiltration of the lamina propria. Y pseudotuberculosis was isolated from the mesenteric lymph nodes. The syndromes occurred in winter and stress appeared to be a contributing factor. The gross and histopathological findings were similar to those previously reported (Karbe and Erikson, 1984; Otter and Callaghan, 2008) and may assist in diagnosis.

The prevalence of Yersiniosis in domestic animals may be higher than reported as a consequence of the nonspecific signs of infections as well as the serologic cross reactions that lead to confusion with other bacterial infections (Hodges et al., 1984; Witte et al., 1985; Corbel et al., 1992; Juste et al., 2009) Early diagnosis is important for successful treatment and reducing stress factors may prevent clinical Yersiniosis. Tetracyclines, neomycin, and lincomycin-spectinomycin are reported to be effective against most isolates of Y pseudotuberculosis and can be used until results of antibiotic sensitivity tests are available (Taffs and Glynis, 1983; Bin-Kun et al., 1994). Since yersiniosis is a potential zoonosis known to cause gastrointestinal infections in humans, care should be taken when handling infected animals (Martins et al., 1998). In this study treatment trials showed recovery in animals were by giving antibiotics to which the bacterium was sensitive and so there were no more abort after treatment. This is consistent with observations from field researches (Sanford 1995; Juste et al., 2009).

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This investigation indicates that Yersiniosis could be important in sheep and lambs, especially under stressful conditions.
conditions in Turkey. Further studies should be involved more animals in Turkey to determine the importance and extent of *Y. pseudotuberculosis* infection in lambs and sheep as well as its prevalence and economic significance.

References


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