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Short Communication

HAEMORRHAGIC SEPTICAEMIA LIKE DISEASE IN A BUFFALO

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SUMMARY

Bacteriological studies were conducted on a buffalo brought to Teaching Veterinary Clinical Complex suspected to be a case of hemorrhagic septicemia like disease. Sample from upper respiratory tract was collected with the nasal swab under sterile condition for bacteriological studies. Greyish white coloured round, smooth, glistening colony appeared. Microscopic examination after Gram staining revealed gram positive cocci seen in pairs which were confirmed to be as *Streptococcus pneumonia*. The bacteria did not show agglutination with an anti-*Pasteurella multocida* B:2 specific agglutinating monoclonal antibody.

Hemorrhagic septicemia (HS) is often acute and fatal disease primarily occurring in water buffaloes and cattle but occasionally in other domestic and wild mammals (De Alwis 1999). Late reporting of disease, indiscriminate antibiotic therapy prior to sampling result into submission of improper sampling that lead to failure to demonstrate the bacteria and even molecular test like PCR fails to demonstrate the bacterial DNA. Therefore such casual outbreaks reported as HS cases/outbreaks despite no experimental evidence of bacteria. However, several other bacterial and viral infections as well as *P.multocida* type A may cause pneumonia and lead to death. It is therefore important to look for other causes of pneumonia and death in cases/outbreak reported as HS like diseases. A bacteriological study was conducted on a buffalo suffering from fever and pneumonia and clinically declared as HS like disease.

A buffalo brought to Teaching Veterinary Clinical Complex with the history of respiratory distress, brisket edema since one week, inappetance for last three weeks, reduced milk yield from sixteen liters to nil, less water intake and red colour urine. Clinical examination revealed congested mucous membrane, swelling of prescapular lymph node, catarrhal nasal discharge, high fever of 106°F and glucosuria. X-ray examination showed nodular lesions in lungs (Fig.1) indicating a chronic type of infection. Nasal swab from upper respiratory tract collected aseptically. Samples were collected aseptically from each nostril. The swabs were incubated overnight at 36-37°C in brain heart infusion broth. The broths were streaked on casein sucrose yeast (CSY) agar supplemented with 5% sheep blood and incubated again overnight at 36-37°C.The blood agar plates examined for hemolysis and characteristics of bacterial colony. Bacterial smears were prepared from each different bacterial colony and stained with gram staining and preliminary identification done.

Streptococcus genus specific PCR was performed using 1 µl (17ng/µl) of genomic DNA as template and Phusion® High-Fidelity PCR Master Mix along with 0.5 µ M of each primer i.e. Str1 [5-GTACAGTTGCTTCAGGACGTATC-3] and Str2 [5-ACGTTCGATTTCATCATCACGTTG-3 (Picard *et al.,* 2004). The thermal cycling parameters for PCR amplification was consisted of initial denaturation at 98°C for 30 seconds followed by 35 cycles of 98°C for 10 seconds, 55°C for 15 seconds, 72°C for 30 seconds and of final extension of 72°C for 10 minutes.

Purification, isolation and identification of the bacteria were done by streaking on CSY blood agar. A large diffuse colony and small grayish bacterial colony appeared. Alpha type of hemolysis was seen on CSY blood agar plate. The gram staining of bacterial smear prepared with small bacterial colony showed gram positive cocci in pairs (Fig.2) described in preliminary identification as *Streptococcus pneumoniae*. The bacterial smear of large diffused colony showed large gram positive and suggestive of bacillus considered as contaminant.

The bacterium of small colony was purified by picking a single small colony carefully and then streaking on casein sucrose yeast blood agar. Pure, single, small grayish bacterial colonies appeared on CSY agar after overnight incubation at 36-37°C. Further studies for demonstration of bacterial capsule and PCR confirmation were done with purified small bacterial colony. Virulence of bacteria demonstrated by the negative staining of bacterial smear with nigrosine showed the presence of capsule (Fig.3). PCR confirmation was done and *Streptococcus* specific product of 197 bp was amplified (Fig 4).

Pneumonia is the most frequently occurring respiratory affection in domestic animals, since the etiologic agents are bacteria, viruses or viruses complicated with bacteria (Allen *et al.*, 1991). Among the various respiratory affections, pasteurellosis is the most common cattle respiratory disease caused by *P. multocida* and commonly isolated from bovine respiratory diseases (Dabo *et al.*, 2007). Other pneumonic pathogens but less frequently could be recovered from pneumonic lungs are *Staphylococcus aureus*, *Streptococcus pneumoniae* (Beiter *et al.*, 2006) and *E.coli* spp. (Wessely *et al.*, 2005). Streptococcal pneumonia is primarily pathogen of human but also reported in rabbit. Perusal of scientific literature did not show any report of demonstration of *Streptococcus*

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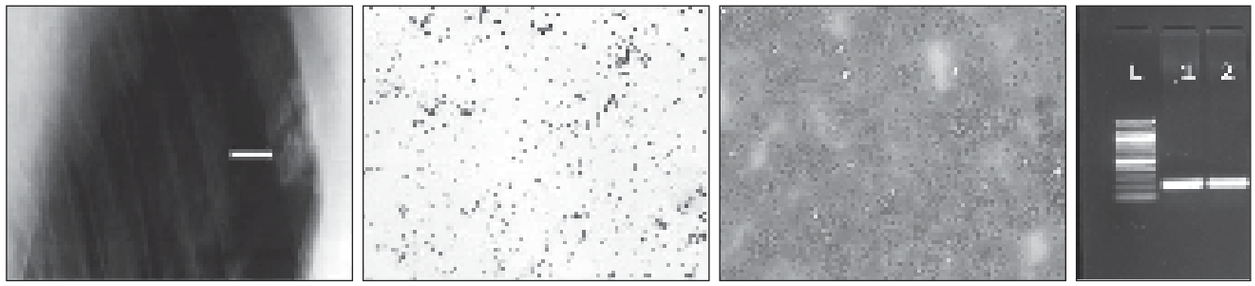

Fig.1-4. Radiological examination showed nodular lesions in lungs. 2. Small bacterial colony showed gram positive cocci in pairs. 3.The negative staining of bacterial smear from small colony with nigrosine showed the presence of capsule. 4. Agarose gel showing amplification of Streptococcus specific gene of 197 bp. (L-100bp Ladder, Lane 1- positive control, Lane 2- Field sample)

pneumonia in buffaloes suffering from pneumonia. Demonstration of capsulated *Streptococcus pneumoniae* established it to be the cause of pneumonia in the buffalo.

Pneumonia is a leading cause of loss to ruminants throughout the world, where several causative agents and contributory factors appear to be involved (Novert, 2002; Yener *et al.*, 2005). It is concluded that when *P.multocida* B:2 is not demonstrated, in such HS like disease, other bacterial or viral etiological agents may also be investigated as cause of pneumonia. Environmental stress leading to immune compromised state of animal is an important factor in epidemiology of HS and such conditions of environmental stress may also be conducive to infections due to otherwise noninfectious opportunistic pathogens. Such opportunistic pathogens may cause severe pneumonia and septicemia leading to death of animal.

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