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Response of Goats to the Different Routes of Infection by *Pasteurella multocida* B: 2

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Abstract: Haemorrhagic Septicaemia (HS) is an important tropical disease of cattle and buffaloes caused by *Pasteurella multocida* B:2. It usually leads to outbreaks causing acute deaths. This study was conducted to determine the response shown by goats following different routes of infection by *P. multocida* B: 2. Twenty healthy local goats were selected and divided into four equal groups before they were injected with dexamethasone at the rate of 1 mg kg^{-1} for four consecutive days. On day 4, goats of groups 1, 2 and 3 were inoculatedintratracheal, subcutaneous and intranasal, respectively with 1 ml of inoculum containing 10^9 cfu mL of live *P. multocida* B: 2. Goats of group 4 were the negative control receiving no treatment. During the 4-week experimental period, two (40%) goats of group 1 were killed for humane reason; one within 24 h and the other at day 4 post-infection. Three (60%) goats of group 2 were killed, each on days 2, 5 and 14post-infection while two (40%) goats of group 3 were killed on days 20 and 32 post-infection. Post-mortem examination revealed that goats that were killed within the first 2 days post-infection showed lesions typical of HS. Goats that were killed between days 3 and 7 showed evidence of acute pneumonia while those that were killed between days 8 and 14 p.i. showed subacute pneumonia affecting 25% of the lungs. *P. multocida* B: 2 was successfully isolated from the lungs, lymph nodes, spleens, tonsils, heart blood, livers and subcutaneous fluid of goats of groups 1 and 2 that were killed peracutely. Isolation was successful from the heart blood and subcutaneous fluid of goat of group 2 that were killed acutely on day 5. However, isolation was unsuccessful from goats that were killed after day 14 of infection.

Key words: *Pasteurella multocida* B: 2, infection, goats, routes, inoculum, pneumonia

INTRODUCTION

Haemorrhagic Septicaemia (HS) is an acute disease ofcattle and buffalo in the tropic. It is caused by *Pasteurella multocida* B: 2, characterised by a short clinical course (Biswas *et al*., 2004). The clinical signs include severe depression, pyrexia, submandibular oedema and dyspnoea followed by recumbency and death (Horadagoda *et al*., 2001). HS has also been reported in other animal species including sheep, swine,deer, elephant, yaks, camels and horses. Goats have been reported to be naturally infected by this disease (Joseph, 1979).

The disease is generally associated with stressful conditionssuch as wet and humid weather condition in the tropics, poor husbandry practices orrearing under stressful free-range system. The natural route of infection is through inhalation while successful experimental infection had been made via intranasal and subcutaneous inoculations (Alwis *et al*., 1990). Earlier studies revealed that goats are suitable as animal model to study infections by *P. multocida* A and D (Zamri *et al*., 1996).

The present investigation was carried out to determine the response shown by goats following different routes of infection by *P. multocida* B: 2.

MATERIALS AND METHODS

Animals: Twentyclinically healthy local goats were selected. Priorto the start of the experimental, nasal swabs were collected to ensure that the goats were free of *P. multocida* B: 2, treated with anthelmintic (Levamisol, Boehringer) and were housed together. They were fed daily with cut grass and commercial pellet while drinking water was available *Ad libitum*.

Inoculum: Stock culture of *P. multocida* B: 2, isolated from previous outbreak of HS in cattle was used. The bacteria was sub-cultured onto blood agar at 37°C for 24 h before four uniform-sized colonies were selected and incubated into brain heart infusion broth for 16 h at 37°C. The viable count of the organism was determined as 1×10^9 cfu mL.

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Experimental procedures: The goats were divided intofour groups consisting of 5 goats per group before all goats were injected intramuscularly with dexamethasone at the rate of 1 mg kg^{-1} for four consecutive days. At day 4 of dexamethasone treatment, goats of group 1 was inoculated intratracheal, group 2 subcutaneous and group 3 intranasal with 1 mL of the inoculum containing 1×10^{10} cfu mL of live *P. multocida* B: 2. Goats of group 4 remained as control uninfected.

Following the infection, clinical signs were monitored daily and recorded. Those that were weak and recumbent were killed. Post-mortem examination was carried out on all dead goats. Death within 48 h post-infection was classified as peracute, between 3 and 7 days as acute, between 8 and 14 days as subacute and after 15 days as chronic infection. After one month, all goats were killed for post-mortem examination.

Sample processing: Samples of lung, lymph node, tonsil, nasal swab, spleen, heart blood, liver and subcutaneous fluid were taken aseptically for bacteriological isolation. They were cultured for the presence of *P. multocida*on blood agar and incubated at 37°C for 24 h. Gram stain and biochemical tests were used to identify *P. multocida*.

Cultures suspected of *P. multocida* B: 2 was confirmed by PCR assay. Multiplex PCR was conducted by using two primer sets designated from the sequence of the clones KMTI (KMT1T7- 5'-ATCCGCTATTT ACCCAGTGG-3' and KMT1SP6- 5'-GCTGTAAACGAA CTCGCCAC-3')and 6b (KTT2- 5'-AGGCTCGTTTGG ATTATGAAG-3' and KTSP61- 5'-ATCCGCT AACACACTCTC-3') for the specific detection of *P. multocida*and HS-causing serogroup B isolates (Townsend *et al*., 1998). Briefly, 25 μL reaction mixture containing 1x PCR buffer, 2.0 mM MgCl2, 200 μM concentration of each dNTP, 20 pmol of each primers, 1 U *taq*DNA polymerase was prepared and one colony was picked from the plate as a template and re-suspended in the PCR mixture. The reaction mixture was subjected to amplification in a Thermal Cycler (Eppendorf) according to the following programme: Initial denaturation at 95°C for 4 min, denaturation at 95°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 45 s, which was repeated 30 cycles and a final extension of 72°C for 6 min. Amplified products were separated by agarose gel electrophoresis (1.0% agarose in 1×TBE) at 70 V for 1 h and 30 min and stained with ethidium bromide. The DNA band was observed under UV transilluminator and photographed (Alpha imager).

RESULTS

Clinical observations: Following experimental infection by *P. multocida* B: 2, 4 (20%) goats were inactive with fever (40°C). One goat of group 1 was recumbent 18 h post-infection and was killed within 24 h. Another goat of the same group was killed on day 4 after showing fever and inactive since day 1. Two goats of group 2 were killed on days 2 and 5 showing signs of fever and inactivity. One goat of group 2 and two goats of group 3 showed neither signs of respiratory tract infection nor septicaemia but showed progressive weight lost. They were killed on days 14 and 21, respectively.

Gross pathology: All dead goats showed various degree of congestion of most internal organs. The goat of group 1 that was killed within 24 h showed acute pneumonia affecting less than 5% of the lung, congested spleen, trachea and lung. The goat that that was killed on day 4 showed lesions of acute pneumonia, affecting the right apical and intermediate lobes of the lung. There was also severe pulmonary oedema, severely congested liver, moderately congested lung and trachea.

The goat of group 2 that was killed on day 2 showed typical lesions of HS with petechial haemorrhage at the injection side, severe pulmonary congestion and oedema and hydrothorax. There was evidence of acute pneumonia on the right and left apical lobe and congested trachea. The goat that was killed on day 5 revealed congestion of internal organs with subacute pneumonia affecting 25% of the right lung. The injection site showed moderately engorged subcutaneous blood vessel with moderate hydrothorax and pulmonary congestion and oedema. Other lesions included congested tonsil, spleen,liver and submandibular lymph node. The goat that was killed on day 14 showed reduction in body weight with moderate hydrothorax and hydropericardium. There was chronic pneumonia affecting 40% of the lung with slightly congested trachea.

The two goats of group 3 that were killed on day 21 showed chronic fibrinous pneumonia affecting 20% of the right apical lobe. The remaining goats showed no significant lesions. None of the control untreated goat had lesions.

Bacterial isolation: Following PCR assay, *P. multocida* B: 2 was successfully isolated from lung, spleen, liver, tonsil, heart blood, subcutaneous fluid and lymph node of

Table 1: Detection of *P. multocida* B:2 from organs of dead goats

Animal ID	Animal group	Days p.i.	Organs with *P.multocida* B:2
0231	1	Within 24 h	Lung
			Spleen
			Lymph node
2342	1	4	Lung
			Spleen
			Liver
			Lymph node
			Tonsil
			Heart blood
2360	2	2	Lung
			Spleen
			Liver
			Lymph node
			Tonsil
			Subcutanelous fluid
			Heart blood
2378	2	5	Subcutanelous fluid
			Heart blood

goat of group 2 that was killed on day 2 but not from goat that was killed on day 14. Similarly, *P. mutocida* B: 2 was isolatedfrom the heart blood and subcutaneous fluid of goat that was killed on day 5. The goat of group 1 that was killed within 24 h revealed the presence of *P. multocida* B: 2 from lung, spleen and lymph node while the goat that was killed on day 4 also showed the presence of *P. multocida* B: 2 in liver, tonsil and heart blood. Other goats did not have *P. multocida* B: 2 (Table 1).

DISCUSSION

This study indicates that goats areable to succumb to experimental HS following subcutaneous and intratracheal routes of infection by *P .multocida*B:2. Two types of infection were recorded; peracute and acute infections. Similar observations have been reported in cattle, buffaloes and goats following experimental infection by *P. multocida* B:2 (Loganathan and Chandrase Karan, 1992; Graydon *et al*., 1993; Horadagoda *et al*., 2002). In general, the subcutaneous route of infection was considered the best method of experimental recreation of the disease in goats, as those of cattle since 3 (60%) goats needed to be killed peracutely (Zamri and Saharee, 1990). Intratracheal route took longer time resulting in slightly less death rate. Intranasal route, however, failed to successfully recreate the disease when the goats died after 21 days and without *P. multocida* B:2. Similar observations were made in cattle (Alwis *et al*., 1990). A study in cattle and buffaloes revealed that subcutaneous inoculation resulted in death within 24-31 and 60 h for buffaloes and cattle, respectively compared to goats that took 48 h (Graydon *et al*., 1993).

Traditional method of bacterial identification revealed that *P. multocida* was successfully isolated from organs of goats for up to 14 days. However, based on PCR, *P. multocida* B: 2 was able to localise in goat for only one week. Most successful isolation of *P. multocida* B: 2 were made from the heart blood, subcutaneous fluid, lymph nodes, tonsils, livers, spleens and lungs of goats that died acutely. The tonsil and lymph node have been identified as the organs where *P. multocida* B: 2 can be readily isolated in cattle and buffaloes (Wijeard ana *et al*., 1986; Alwis, 1999; Townsend *et al*., 1998). However, this study failed to isolate *P. multocida* B: 2 from any of the nasopharynx sample, contrastingthe results of previous study in cattle and buffaloes (Townsend *et al*., 1998).

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