J. Vet. Malaysia (2005) 17 (2) 29 - 32

EXPERIMENTAL INFECTION OF GOATS BY *PASTEURELLA MULTOCIDA* B:2

M. Zamri-Saad, N.M.D. Zaitun, A. Ernie and M.S. Shafarin

Facultyof Veterinary Medicine, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

SUMMARY

An experiment was carried out to determine whether goats can be infected by *Pasteurella multocida* B:2, the causative agent of haemorrhagic septicaemia of cattle and buffaloes. Thirty-six healthy local goats were divided into four groups consisting of nine goats per group. Goats of groups 1 and 3 were inoculated intranasally with 1ml inoculum containing 10^9 CFU of live *P. multocida* B:2. Goats of groups 2 and 4 were not infected, but were exposed to the infected animals by keeping goats of group 2 together with group 1 while group 4 together with group 3. Immediately post-infection, goats in groups 3 and 4 were treated with dexamethasone at the rate of 1mg/kg for three consecutive days. Three goats from each of the four groups were killed on days 7, 14 and 21 post-infection. One goat from group 3 died percutely on day 2 post-infection, showing lesions typical of haemorrhagic septicaemia. Other two goats from the same group were weak. None of the goats in groups 1, 2 and 4 succumbed to the disease but most exhibited signs of mild respiratory tract infection. Post-mortem examinations revealed that 20 (53%) goats had mild, acute pneumonia affecting less than 7% of the lung, suggesting that *P. multocida* B:2 is not a primary pathogen of the respiratory tract. Four (11%) goats of group 3 had pulmonary oedema and congestion, indicating that dexamethasone enhanced chances of goats being infected by *P. multocida* B:2. *P. multocida* was successfully re-isolated from lungs and nasal swabs of goats from all groups, and heart blood of goats from group 3 but not from the lymph nodes and tonsils. The re-isolation of *P. multocida* B:2 was successfully made for up to 14 days p.i. in groups without dexamethasone but for up to 21 days following dexamethasone treatment. Transmission of *P. multocida* B:2 to the in-contact goats occurred at a rate of 40%.

Keywards: *Pasteurella multocida* B:2, infection, transmission, goats

INTRODUCTION

Pasteurella multocida serotype B:2 (Carter:Heddleston) is the bacterium responsible for haemorrhagic septicaemia (HS) in Asia, while serotype E:2 causes the disease in Africa. It is an acute, fatal septicaemic disease affecting buffaloes and cattle, characterised by a short clinical course lasting a few hours with signs of severe depression, dypsnoea, submandibular oedema and recumbency, followed by death (Horadagoda *et al.*, 2001). The high-morbidity, high-mortality nature of this disease renders it to be the most important disease economically in Southeast Asia, where it is epidemic (Joseph, 1979). Annual losses of USD400-6000 in Indonesia, USD 1.4 million in Laos and approximately USD1.0 million in Malaysia have been reported recently (Verma nd Jaiswal, 1998). The disease has been known to occur in many parts of the world, but is more frequently reported in Middle and North East, central and South Africa, and some European countries, besides Southeast Asia.

Although haemorrhagic septicaemia is commonly observed in buffaloes and cattle, it has also been reported in other species of animals. The range of hosts includes American bison, sheep and swine, deer, elephants, yaks, horses and camels. Goats have been reported to be naturally infected with haemorrhagic septicaemia (Interior, 1993). This study describes experimental infection of *P. multocida* serotype B:2 in goats.

MATERIALS AND METHODS

Animals

Thirty-six clinically healthy local Katjang goats of about 8 months of age were selected. Prior to the start of the experiment, nasal swabs were collected to ensure that the goats were free from *P. multocida* B:2, treated with anthelmintic (Levamisol, Boehringer) and were housed together. They were fed daily with cut grass and commercial goat pellets while drinking water was available *ad libitum*.

Inoculum

Stock culture of *P. multocida* B:2, isolated from a previous outbreak of haemorrhagic septicaemia in cattle, was used. The bacteria was sub-cultured onto blood agar at 37°C for 24 hours before five uniform-sized colonies were selected and incubated in the brain heart infusion broth for 18 hours at 37°C. The viable count of the organism was $1x10^9$ CFU/ml.

Experimental procedures

The goats were divided into four groups consisting of nine goats per group. Goats of groups 1 and 3 were inoculated intranasally with 1 ml of the inoculum containing $1.0x10^9$ CFU of *P. multocida* B:2. Goats of groups 2 and 4 were not inoculated but exposed to the infected animals by keeping goats of group 2 with group 1 and goats of group 4 with group 3. Immediately after inoculation, goats of groups 3 and 4 were injected intramuscularly with dexamethasone at the rate of 1mg/kg for three consecutive days.

Following the infection, clinical signs and rectal temperature were monitored daily and recorded. Post-mortem examination was carried out on all dead goats. Death within 48 hours post-infection was classified as peracute, between 3 to 7 days as acute, between 8 to 14 days as subacute and between 15 to 21 days as chronic infection. On days 7, 14 and 21 post-infection, three goats from each group were killed for post-mortem examination. The experimental procedure was in accordance with the guidelines of the Committee of Institutional Animals, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Sample processing

Samples of lung, lymph node, tonsil, nasal swab and heart blood were taken aseptically for bacteriological examination. They were cultured for the presence of *P. multocida* on blood agar plates at 37° C for 24 hours.

RESULTS

Clinical observations

Only one goat of group 3 succumbed to the disease on day 2 post-infection. The affected animal was found weak and having fever. Two other goats in group 3 were weak with fever. No sign of clinical haemorrhagic septicaemia was exhibited by other goats. However, all goats, either infected or exposed, showed signs of mild respiratory tract infection. They were coughing and sneezing with nasal discharge; the highest body temperature recorded was 38.5° C.

Post-mortem examinations

The goat of group 3 that died 48 hours post-infection showed congestion of most internal organ, including lung, spleen and liver. There was evidence of pulmonary oedema and hydrothorax. Two other goats of the same group showed severe pulmonary congestion and oedema with slight hydrothorax. Examinations of other animals revealed no sign of generalised congestion, haemorrhage or oedema that indicated haemorrhagic septicaemia.

Table1: Percentage of pneumonic lung lesions in goats following different treatment by *P. multocida* B:2

Animal group	Treatment	Average Lung Lesion (%)
1	Intranasal	5.4
2	Exposed	3.9
3	Intranasal with Dexamethasone	5.7
4	Exposed with Dexamethasone	4.6

Note: The differences in the average of lung lesions between the different groups were insignificant ($p>0.05$)

However, the lungs of 20 (53%) goats of various groups showed varying degrees of acute pneumonia ranging from 1-15% (Table 1). The pneumonic lesion was confined to the right apical lobe of the lungs with only two goats showing the lesions in the left apical lobe. The lung lesions were observed until 14 days post-infection.

Bacteriological examination

P. multocida B:2 was re-isolated in pure cultures from lungs and nasal swabs of goats of groups 1 and 2 at days 7 and 14 post-infection, but for up to day 21 post-infection from goats of groups 3 and 4. Successful re-isolations were obtained from 5 (56%) goats of group 1, 4 (45%) goats of group 2, 5 (56%) goats of group 3 and 3 (33%) goats of group 4 (Table 2). Transmission of *P. multocida* to exposed goats occurred at an average rate of 40%. Re-isolation of *P. multocida* B:2 from heart blood was successful in two goats of group 3.

DISCUSSION

The results of this study indicate that goats are susceptible but relatively resistant to clinical disease of haemorrhagic septicaemia following intranasal exposure. The evidenced that only one goat was infected and treated with dexamethasone died peracutely of the disease supports this observation. This has been observed earlier by Wijewardana *et al.* (1986). Two other goats with the same treatments were weak with pulmonary congestion and oedema, indicating a milder form of haemorrhagic septicaemia. However, the clinical signs of respiratory tract infection, gross lesions in the lungs and re-isolation of *P. multocida* B:2 proved that goats can be infected by and transmit the organism. This is in agreement with earlier observations (Loganathan and Chandrasekaran, 1992).

The pneumonic lesions were acute but mild, involving approximately 7% of the lung and confined to the right apical lobe. This is similar to the mild acute lung lesions reported in experimental haemorrhagic septicaemia of cattle (Zamri-Saad and Saharee, 1990; Erler and Schimmel, 1993; Graydon *et al.*, 1993), indi

Table 2: Re-isolation of *P.multocida* 6:B from organ samples of infected and in-contact goats

Animal group	Days p.i	Animal ID	Re-isolation rate
1	7	2344	+
		2577	+
		1401	-
	14	2576	+
		2341	+
		2581	+
	21	2579	-
		1461	-
		2348	-
2	7	1405	-
		2582	+
		0269	+
	14	2578	+
		2345	-
		0268	+
	21	1464	-
		1403	-
		0244	-
3	7	0242	+
		0267	+
		0246	+
	14	0261	-
		0250	-
		0247	+
	21	0249	-
		0262	-
		0265	+
4	7	0245	-
		0201	-
		0248	+
	14	0241	+
		0263	-
		0266	-
	21	0243	-
		0270	-
		0264	+

cating that *P. multocida* B:2 is not a primary lung pathogen. It may use the lung as a route to cause septicaemia since earlier reports showed that *P. multocida* B:2 reside in the pneumocytes (Horagoda and Belak, 1990), survive intracellular environment and undergo exocytosis (Galdiero *et* al., 2001) while dexamethasone treatment failed to enhance the severity of lung lesions.

Most successful re-isolations of *P. multocida* were made from the nasal swab and lungs. None of the goats had the organism in the tonsils or lymph nodes, contrasting the previous studies in cattle and buffaloes (Wijewardana *et al.*, 1986; De Alwis *et al.*, 1990; Saharee *et al.*, 1993). Re-isolation was successful from 56% of the infected goats and from 40% of the exposed goats but dexamethasone treatment prolonged the presence of *P. multocida* in goats to 21 days post-infection. Similarly, dexamethasone increased the number of goats carrying *P. multocida* in the exposed group. A stressful condition has been shown to enhance bacterial infectivity leading to disease (Zamri-Saad *et al.*, 1991; Saharee *et al.*, 1993). The complete absence of the organism between days 14 and 21 post-infection may be explained by the clearance mechanism of the respiratory system, which support further the earlier claims that goats are relatively resistant to *P. multocida* B:2 (Wijewardana *et al.*, 1986) and *P. multocida* B:2 is not a primary pathogen of the respiratory tract (Zamri-Saad and Saharee, 1990). It appears that *P. multocida* B:2 is able to enter and multiply in the goat tissues but is unable to resist the host defense mechanism leading to failure in causing severe damage to the host. This is known as subclinical infection (Baskerville, 1981). Dexamethasone treatment prolonged infection and survival of the organism leading to clinical disease (Loganathan and Chandrasekaran, 1992).

REFERENCES

Baskerville, A. (1981). Mechanisms of infection in the respiratory tract. *New Zealand Vet. J.* **29**: 235-238

De Alwis, M.C.L., Wijewardana, T.G, Gomis, A.I.U. and Vipulasiri, A.A. (1990). Persistence of the carrier status in haemorrhagic septicaemia (*Pasteurella multocida* serotype 6:B infection) in buffaloes. *Trop. Anim. Hlth. Prod.* **22**: 185-194

Erler, W. and Schimmel, D. (1992). The effect of the *Pasteurella multocida* toxin in calves. Pasteurellosis in Production Animals. ACIAR Proceedings No. 43. pp. 103-104

Galdiero, M., De Martino, L., Pagnini, U., Pisciotta, M. and Galdiero, E. (2001). Interaction between bovine endothelial cells and *Pasteurella multocida*: association and invasion. *Res. Microbiol.* **152**: 57-65

Graydon, R.J., Patten, B.E. and Hamid, H. (1992). The pathology of experimental haemorrhagic septicaemia in cattle and buffalo. Pasteurellosis in Production Animals. ACIAR Proceedings No. 43. pp105-107

Horadagoda, N.U. and Belak, K. (1990). Demonstration of *Pasteurella multocida* Type 6:B (B:2) in formalin-fixed paraffin-embedded tissues of buffaloes by the peroxidase anti-peroxidase (PAP) technique. *Acta Veterinaria Scandanavica* **31**: 493-495

Horadagoda, N.U., Hodgson, J.C., Moon, G.M., Wijewardana, T.G. and Eckersall, P.D. (2001). Role of endotoxin in the pathogenesis of haemorrhagic septicaemia in the buffalo. *Microbiol. Pathogenesis* **30**: 171-178

Interior, M.M. (1993). Country Report: Philippines. Pasteurellosis in Production Animals. ACIAR Proceedings No.43, pp. 254-256

Joseph, P.G. (1979). Haemorrhagic septicaemia in Peninsular Malaysia. *Kajian Veterinar* **11**:65-79

Loganathan, P. and Chandrasekaran, S. (1992). Clinicopathological changes in goats experimentally infected with *Pasteurella multocida* and *Pasteurella haemolytica*. Proceedings of the National IRPA Seminar, pp. 20-21

Saharee, A.A., Salim, N.B., Rasedee, A. and Jainudeen, M.R. (1993). Haemorrhagic septicaemia carriers among cattle and buffalo in Malaysia. Pasteurellosis in Production Animals. ACIAR Proceedings No. 43. pp. 89-91

Verma, R. and Jaiswal, T.N. (1998). Haemorrhagic septicaemia vaccines. *Vaccine* **11/12**: 1184-1192

Wijewardana, T.G., De Alwis, M.C.L., and Vipulasiri, A.A. (1986). An investigation into the possible role of the goat as a host in haemorrhagic septicaemia. *Sri Lanka Vet. J.* **34**: 24-32

Zamri-Saad, M. and Saharee, A.A. (1990). The pathology of experimental haemorrhagic septicaemia in cattle. *Trop. Vet.* **8**: 1-7

Zamri-Saad, M., Jasni, S., Nurida, A.B and Sheikh-Omar, A.R., 1991. Experimental infection of dexamethasone-treated goats with *Pasteurella haemolytica* A2. *Bri. Vet. J.* **147**: 565-568