UNIVERSITI PUTRA MALAYSIA

THE ROLE OF THE RESPIRATORY MUCOSAL IMMUNITY IN PROTECTION AGAINST *PASTEURELLA HAEMOLYTICA* A2 INFECTION IN GOATS

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By

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Chairman: Assoc Prof Dr Mohd Zamri Saad

Faculty: Veterinary Medicine and Animal Science

Pneumonic pasteurellosis is one of the most important and devastating diseases in sheep and goats, causing great economic losses to small ruminant industry worldwide. The disease is caused either by *Pasteurella haemolytica* or *Pasteurella multocida* and *Pasteurella haemolytica* A2 is the most common isolate from sheep and goats in Malaysia, comprising approximately 38 per cent of the isolates from pneumonic lung lesions.

The disease is best controlled by vaccination, and systemic vaccination has been used for years with limited success. Stress, improper vaccination program and unpopularity of the vaccine among the farmers are some of the

reasons that have been associated with the persistence of the disease. Since the systemic vaccination failed to give promising results, studies on the role of mucosal immunity of the respiratory tract in controlling pneumonic pasteurellosis should timely be reviewed.

In this study, the bronchus-associated lymphoid tissue (BALT) in the lungs has been successfully stimulated by double intranasal exposures to either live or formalin-killed *Pasteurella haemolytica* A2 at two weeks interval. The size of BALT and number of lymphocytes in the BALT were significantly increased as early as week 2 post-exposure and remained high until week 4 post-exposure. At the same time, the level of IgA against *Pasteurella haemolytica* A2 increased significantly as early as week 1 post-first exposure and reached a peak level at week 6 post-exposure. The IgM appeared to be present for a short while, at week 3 post-exposure before the levels started to decline in the following week. Initially, the IgG increased gradually and insignificantly before it reached significantly high level at week 4 post-exposure, and remained high at weeks 5 and 6 at the time when the numbers of BALT continued to increase.

This study also revealed that intranasal stimulation of BALT was able to protect the lungs from colonization by *Pasteurella haemolytica* A2 during an *in vitro* study, thus prevent the lung surface from being adhered and invaded by

the organism. However, dexamethasone treatment which is similar to the effect of steroid released under stressful conditions, significantly reduced the number and size of the BALT, thus significantly reduced the percentage of IgA-producing cells.

Vaccination trial on goat farm using the pasteurella spray vaccine intranasally showed good protection towards pneumonic pasteurellosis. Significant high levels of systemic antibody responses were also noted during the period of vaccination trial. The incidence of pneumonic pasteurellosis in the farm was markedly reduced.

Abstrak disertasi yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Doktor Falsafah

PERANAN KEIMUNAN MUKOSA PERNAFASAN DALAM MELINDUNGI KAMBING TERHADAP JANGKITAN *PASTEURELLA HAEMOLYTICA* A2

Oleh

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Pasteurellosis pneumonia adalah salah satu penyakit terpenting pada kambing dan bebiri, mengakibatkan kerugian ekonomi yang nyata pada industri ruminan kecil serata dunia. Penyakit ini disebabkan oleh sama ada *Pasteurella haemolytica* atau *Pasteurella multocida*, dan *Pasteurella haemolytica* A2 merupakan isolat yang paling kerap diasingkan daripada kambing dan bebiri di Malaysia, merangkumi kira-kira 38 peratus isolat yang diperolehi daripada lesi pneumonia.

Penyakit ini boleh dikawal dengan baik melalui pemvaksinan, dan pemvaksinan sistemik telah digunakan selama bertahun-tahun dengan kejayaan yang terhad. Ketegasan, program pemvaksinan yang tidak sesuai dan ketidakpopularan vaksin di kalangan penternak adalah antara sebab-sebab yang dikenalpasti menyebabkan penyakit ini berterusan. Memandangkan selama ini pemvaksinan sistemik gagal untuk membuahkan hasil yang memuaskan, sudah sampai masanya kajian ke atas peranan keimunan mukosal pada trakus pernafasan dalam mengawal radang peparu pasteurellosis dipertimbangkan semula .

Dalam kajian ini, tisu limfoid berkait bronkus (BALT) dalam peparu telah berjaya dirangsangkan melalui dedahan berganda kepada *Pasteurella haemolytica* A2 yang hidup atau dimatikan dengan formalin melalui intranasal. Dedahan berganda dibuat dalam jangkamasa selang 2 minggu. Saiz BALT dan bilangan sel limfosit dalam BALT bertambah dengan ketara seawal 2 minggu selepas dedahan, dan mengekalkan ketinggiannya sehingga minggu ke 4 selepas dedahan. Pada masa yang sama, tahap IgA terhadap *Pasteurella haemolytica* A2 meningkat dengan ketara seawal minggu pertama selepas dedahan, dan mencapai kemuncaknya pada minggu ke 6 selepas dedahan. IgM hadir cuma sebentar, iaitu pada minggu ke 3 selepas dedahan, sebelum menurun pada minggu berikutnya. Pada peringkat awal, IgG meningkat beransur-ansur sebelum mencapai peningkatan bermakna pada minggu ke 4,

dan mengekalkan ketinggiannya pada minggu ke 5 dan 6, iaitu masa di mana bilangan BALT turut meningkat.

Kajian ini juga menunjukkan bahawa rangsangan melalui intranasal ke atas BALT mampu melindungi peparu daripada dikoloni oleh *Pasteurella haemolytica* A2, seterusnya menghalang permukaan peparu daripada dilekati dan dicerobohi organisma berkenaan.

Walau bagaimanapun, rawatan oleh dexamethasone yang mempunyai kesan yang sama seperti steroid sewaktu tegasan, mengurangkan bilangan dan saiz BALT dan turut mengurangkan peratusan sel yang menghasilkan IgA. Pemvaksinan yang dilakukan di ladang ternakan kambing menggunakan vaksin semburan intranasal menghasilkan perlindungan yang baik terhadap penyakit pasteurellosis pneumonia. Gerakbalas antibodi sistemik yang tinggi dan ketara di dapati berlaku sewaktu kajian dijalankan Pengurangan kadar penyakit pasteurellosis pneumonia di ladang berkenaan juga dapat dilihat dengan jelas.

CHAPTER 1

INTRODUCTION

Pneumonic pasteurellosis is one of the most important and devastating diseases affecting small ruminants worldwide. The disease is caused by either *Pasteurella haemolytica* or *Pasteurella multocida* (Donachie, 1993), although the former is frequently isolated from such cases in goats (Gilmour, 1992).

There are two biotypes of *Pasteurella haemolytica*, namely biotype A and T. Clinically, biotype A is associated with pneumonic pasteurellosis and comprises serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14 and 16. Biotype T, that comprises serotypes 3, 4, 10 and 15, is associated with systemic disease in young lambs. About 10 percent of the *Pasteurella haemolytica* serotypes remain untypable (Donachie, 1993). The development of the disease has been known to be associated with various stress factors particularly the environmental factors such as climate, transportation, nutrition and housing (Jubb *et al.,* 1985; Zamri-Saad *et al.,* 1989; Jasni *et al*., 1991). Concurrent infections with parainfluenza type III virus, herpesvirus and *Haemonchus contortus* have also been shown to predispose animals to pneumonic pasteurellosis (Gilmour *et al*., 1993; Zamri-Saad *et al*., 1993a).

Various studies have been conducted regarding the use of vaccines to control pneumonic pasteurellosis in Malaysia (Zamri-Saad *et al.*, 1989; Chandrasekaran *et al.*, 1991; Zamri-Saad *et al.,* 1993b) however the results were inconclusive and the disease remains a major threat to the goat and sheep industry. Imported vaccines, such as HEPTAVAC-P (Hoechst, UK), Ovipast (Hoechst (M) Sdn. Bhd.) and Carovax (Wellcome, UK) have been used to control the disease with limited success rate (Wan Mohamed *et al.,* 1988). A locally produced formalin-killed oil adjuvant vaccine, incorporating *Pasteurella haemolytica* and *Pasteurella multocida* of unknown serotypes was also used unsuccessful in controlling the disease (Zamri-Saad *et al.*, 1989). However, an improved oil adjuvant vaccine that incorporating the locally isolated *Pasteurella haemolytica* A7 and *Pasteurella multocida* types A and D showed ability to protect the lambs when challenged with either *Pasteurella haemolytica* A2 or A7 (Zamri-Saad *et al.*, 1993b). Although the locally produced oil adjuvant vaccine gave better protection, its thick viscousity lead to difficulties in administration and caused swelling at the site of administration and lameness with uneventful recovery in approximately ten percent of vaccinated animals (Jamaludin, 1993).

The locally produced oil adjuvant vaccine has been shown to be able to stimulate good antibody response (Zamri-Saad *et al.*, 1993c). However, most sheep in various farms in Malaysia showed low antibody titre against pasteurellosis (Zamri-Saad *et al.* 1993d) and does not protect the animal from being contacted with pneumonic pasteurellosis, thus the disease

incidence remained high in many farms. This is probably due to the unpopularity of the oil adjuvant vaccine among farmers (Jamaludin, 1993) and the improper vaccination program provided by the vaccine manufacturer, which has been recognised as one of the contributing factors in vaccination failure (Zamri-Saad, 1996).

The respiratory tract, which is the route of infection for *Pasteurella haemolytica* (Gilmour *et al.,* 1991), is one of the mucosal tissues in the body which armed with mucosal immunity (Kaltrieder, 1976). Anderson *et al.* (1986) stated the importance of stimulating the pulmonary lymphoid tissue with effective methods that will provide substantial local cellular and antibody mediated immune responses (Kaltrieder, 1976).

Other factors that contribute to the success of vaccination include the method of vaccine administration (Mosier, 1993). Thus, with the uncertainties and difficulties encountered in systemic vaccination of animals against pneumonic pasteurellosis, other methods for the control of pneumonic pasteurellosis should perhaps be explored.

The objectives of this study are:

1. to stimulate the mucosal immunity in the respiratory tract of goats following either with intranasal or oral exposures to *Pasteurella haemolytica* A2.

2. to determine the mucosal immune responses following intranasal exposures to formalin-killed *Pasteurella haemolytica* A2.
3. to verify the immunosuppresive effect of dexamethasone on mucosal immunity of the respiratory tract following intranasal exposure to formalin-killed *Pasteurella haemolytica* A2.
4. to determine the protective role of mucosal immunity against intratracheal challenge with live *Pasteurella haemolytica* A2.

CHAPTER 2

LITERATURE REVIEW

Pasteurella haemolytica

Pasteurella haemolytica is a Gram-negative, small, non-motile, cocco-bacillus bacteria with slight pleomorphism and occasional bipolar staining (Adlam, 1989). The organism can easily be recognised upon cultivation on blood agar and show various sizes of beta zone haemolysis. Bovine blood appears to be superior for the demonstration of the organism when compared with ovine or horse blood (Soltys, 1979).

The organism can be separated into two biotypes, A and T (Donachie, 1993). Biotypes A and T from cases of ovine pneumonia can be distinguished based on different cultural, biochemical and pathological characteristics (Soltys, 1979). De Alwis (1993) recorded that the differences between biotypes A and T depend on their ability to ferment arabinose and not trehalose and vice versa. Both serotypes are heterologus in nature and sixteen serotypes can be differentiated by an indirect haemagglutination (IHA) test which distinguishes

sixteen different capsular antigens in the two biotypes (Donachie, 1993). Biotype A comprises serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14 and 16, while the biotype T contains serotypes 3, 4, 10 and 15. Ten percent remain untypable (Donachie, 1993).

All serotypes are pathogenic and serotype A2 is the most prevalent, comprises around 38% of all *Pasteurella haemolytica* isolates (Donachie, 1993). The most common serotypes isolated from pneumonic pasteurellosis of sheep and goats in Malaysia are serotypes A2, followed by A7 and A9 (Bahaman *et al.*, 1991). *Pasteurella haemolytica* serotypes A11 and A12 were also isolated from cases in Malaysia (Bahaman *et al.,* 1991).

Pneumonic Pasteurellosis

Pasteurella haemolytica can be found in nasopharynx of healthy animals as normal flora (Gilmour, *et al.,* 1991; De Alwis, 1993). Although the organism are present in the nasopharynx area, the animal are not clinically affected with pneumonic pasteurellosis (Mosier, 1993). Colostral immunity in lambs can last for approximately 4 to 5 weeks (Gilmour and Gilmour, 1989), but the duration of immunity following natural infection has not been fully investigated adequately (Mosier, 1993). The prevalence of respiratory infections and associated economic losses continues to be very high although various attempts have been

made to control the disease. It is difficult to control respiratory disease since respiratory infections often occur as a result of synergistic interactions between various pathogens (Busse, 1991). Many carriers of *Pasteurella haemolytica* will not always develop pasteurellosis and remained clinically healthy (Gilmour and Gilmour, 1989). This is because the exposure of respiratory pathogenic organism per se is not sufficient enough for the development of disease. Furthermore, the respiratory tract provides a variety of integrated defence mechanisms including mechanical non-immunological and immunological, which function in concert to prevent development of respiratory tract infections (Stratton, 1986). However, failure of any one or more of the lung defence mechanisms disrupts this stage of homeostasis resulting in pulmonary infection (Babiuk and Campos, 1993).

Various stress factors such as transportation, climate and housing stress (Jubb, *et al.*, 1985; Zamri-Saad, *et al.*, 1989; Jasni, *et al.*, 1991) could alter the normal homeostasis of the host. This favour the organism in such a way that it replicates much higher levels and migrates to the lower lung without being cleared by the normal defence mechanisms. The prevalence and numbers of *Pasteurella haemolytica* in the nasopharynx increase during stressful conditions, predisposing the host to pneumonic pasteurellosis (Gilmour, 1993). Outbreaks of the disease usually are sporadic and unpredictable (De Alwis, 1993; Gilmour, 1989) and is unlikely to develop a long lasting flock resistance in the farm.