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

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By

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A Dissertation Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine and Animal Science Universiti Putra Malaysia

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................. ii

LIST OF TABLES ........................................................................... vii

LIST OF PLATES ........................................................................... ix

LIST OF FIGURES ........................................................................ xi

ABSTRACT .................................................................................... xii

ABSTRAK ..................................................................................... xv

CHAPTER

1 INTRODUCTION ........................................................................ 1

2 LITERATURE REVIEW ............................................................. 5
   Pasteurella haemolytica .......................................................... 5
   Pneumonic Pasteurellosis ....................................................... 6
   Pneumonic Pasteurellosis Status in Malaysia ......................... 9
   Background of Pasteurella Vaccination in Malaysia ............... 10
   Mucosal Immunity in Respiratory Tract ................................. 13

3 ATTEMPTS TO STIMULATE THE BRONCHUS-ASSOCIATED LYMPHOID TISSUE IN THE LUNGS OF GOATS VIA ORAL ADMINISTRATION OF PASTEURELLA HAEMOLYTICA A2
   Introduction ........................................................................... 24
   Materials and Methods ......................................................... 25
      Animals ............................................................................... 25
      Inoculum ............................................................................ 26
      Experimental Design .......................................................... 26
      Sample Collection and Processing ...................................... 27
      Immunoperoxidase Procedure ............................................. 28
   Results ................................................................................... 29
      Responses by the BALT ....................................................... 29
      Responses by the IgA-Producing Cells in the BALT ........... 30
      Responses by the Mesenteric Lymph Nodes ....................... 31
4 INTRANASAL STIMULATION OF THE BRONCHUS-ASSOCIATED LYMPHOID TISSUE OF GOATS AND ITS EFFECT ON IN VITRO COLONISATION OF PASTEURELLA HAEMOLYTICA A2

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>36</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>37</td>
</tr>
<tr>
<td>Animals</td>
<td>37</td>
</tr>
<tr>
<td>Inoculum</td>
<td>38</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>38</td>
</tr>
<tr>
<td>The Study of BALT Reactions</td>
<td>39</td>
</tr>
<tr>
<td><em>In Vitro</em> Colonisation Technique</td>
<td>40</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>41</td>
</tr>
<tr>
<td>Results</td>
<td>42</td>
</tr>
<tr>
<td>Responses in the Number and Size of BALT</td>
<td>42</td>
</tr>
<tr>
<td><em>In Vitro</em> Colonisation by <em>Pasteurella haemolytica</em> A2</td>
<td>46</td>
</tr>
<tr>
<td>Discussion</td>
<td>47</td>
</tr>
<tr>
<td>Summary</td>
<td>49</td>
</tr>
</tbody>
</table>

5 CELLULAR AND HUMORAL RESPONSES IN THE RESPIRATORY TRACT OF GOATS FOLLOWING INTRANASAL STIMULATION USING FORMALIN-KILLED PASTEURELLA HAEMOLYTICA A2

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>51</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>52</td>
</tr>
<tr>
<td>Animals</td>
<td>52</td>
</tr>
<tr>
<td>Inoculum</td>
<td>53</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>53</td>
</tr>
<tr>
<td>Serology</td>
<td>54</td>
</tr>
<tr>
<td>Studies on BALT Response</td>
<td>55</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>56</td>
</tr>
<tr>
<td>Results</td>
<td>56</td>
</tr>
<tr>
<td>Antibody Responses in Lung Lavage Fluid</td>
<td>56</td>
</tr>
<tr>
<td>Serum Antibody Responses</td>
<td>57</td>
</tr>
<tr>
<td>Responses by the BALT</td>
<td>59</td>
</tr>
<tr>
<td>Discussion</td>
<td>63</td>
</tr>
<tr>
<td>Summary</td>
<td>65</td>
</tr>
</tbody>
</table>
VACCINATION TRIAL AGAINST PNEUMONIC PASTEURELLOSIS IN SHEEP AND GOATS USING A NEWLY DEVELOPED PASTEURELLA SPRAY VACCINE

Introduction ................................................................. 99
Materials and Methods .................................................... 100
Sheep ........................................................................... 100
Goats ............................................................................ 100
Vaccine Preparation ......................................................... 101
Experimental Design ......................................................... 102
Statistical Analysis ............................................................ 103
Results ............................................................................ 104
Serological Response by Sheep ............................................. 104
Serological Response by Goats ............................................. 105
Incidences of Pneumonic Pasteurellosis in
Sheep Farm ...................................................................... 107
Incidences of Pneumonic Pasteurellosis in
Goat Farm .......................................................................... 108
Bacteriology ...................................................................... 108
Discussion ......................................................................... 108
Summary ........................................................................... 112

GENERAL DISCUSSION .......................................................... 114

BIBLIOGRAPHY .................................................................. 123

APPENDICES ..................................................................... 134

VITA .................................................................................. 141

PUBLICATIONS .................................................................. 142
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>The number of bronchus associated lymphoid tissue (BALT) and their average size in the lungs of goats following different treatments</td>
<td>30</td>
</tr>
<tr>
<td>3.2</td>
<td>The effects of oral administration of formalin-killed <em>Pasteurella haemolytica</em> A2 on the total number of cells and the percentage of IgA-producing cells in BALT of goats</td>
<td>31</td>
</tr>
<tr>
<td>3.3</td>
<td>The effects of oral administrations of formalin-killed <em>Pasteurella haemolytica</em> A2 on the size and cortex:medullar ratio of mesenteric lymph nodes of goats</td>
<td>32</td>
</tr>
<tr>
<td>4.1</td>
<td>Average size and number of bronchus-associated lymphoid tissue (BALT) in the lungs of goats following either a single intranasal exposure to live (group 1) or dead <em>Pasteurella haemolytica</em> A2 (group 2) or a double intranasal exposures to live (group 3) or dead <em>Pasteurella haemolytica</em> A2 (group 4)</td>
<td>42</td>
</tr>
<tr>
<td>5.1</td>
<td>The average number of bronchus associated lymphoid tissue (BALT) in lungs of goats exposed twice to formalin-killed <em>Pasteurella haemolytica</em> A2</td>
<td>60</td>
</tr>
<tr>
<td>6.1</td>
<td>The average number and size of bronchus-associated lymphoid tissue (BALT) in the lungs of goats following intranasal exposure to formalin-killed <em>Pasteurella haemolytica</em></td>
<td>74</td>
</tr>
<tr>
<td>6.2</td>
<td>The effects of dexamethasone on the average number of cells and the average percentage of IgA-producing cells in BALT of goats exposed to formalin-killed <em>Pasteurella haemolytica</em> A2</td>
<td>75</td>
</tr>
<tr>
<td>7.1</td>
<td>Average clinical score and extent of lung lesions after challenge with <em>Pasteurella haemolytica</em> A2</td>
<td>88</td>
</tr>
</tbody>
</table>
The bronchus associated lymphoid tissue (BALT) of goats exposed twice to the dead *Pasteurella haemolytica* A2 showing two large-sized nodes containing numerous cells. Lymphoid nodule within the lamina propria of a bronchus. HE x400.............. 44

The bronchus associated lymphoid tissue (BALT) of a goat exposed twice to the live *Pasteurella haemolytica* A2 showing a large node containing numerous cells. Lymphoid nodule located external to the muscularis mucosa. HE x400.............. 44

The bronchus associated lymphoid tissue (BALT) of a control, unexposed goat showing small nodule containing several cells. Lymphoid aggregates associated with a bronchus. HE x400.... 45

The bronchus associated lymphoid tissue (BALT) of a goat exposed once with live *Pasteurella haemolytica* A2 showing small nodule similar to those of control, unexposed goats. Lymphoid aggregate located external to the muscularis mucosa. HE x400 ................................................................. 45

The bronchus associated lymphoid tissue (BALT) of goat at two weeks after the first intranasal exposure to formalin-killed *Pasteurella haemolytica* A2 showing small nodular-type BALT. HE x400 ................................................................. 61

The bronchus associated lymphoid tissue (BALT) of goat at two weeks after the second intranasal exposure to formalin-killed *Pasteurella haemolytica* A2. The BALT are many and larger, containing more cells than previously. HE x400 ...................... 61

The bronchus associated lymphoid tissue (BALT) of goat at three weeks after the second intranasal exposure to formalin-killed *Pasteurella haemolytica* A2. The BALT is markedly large, containing numerous cells. HE x400........................... 62
5.4 The bronchus associated lymphoid tissue (BALT) of goat at four weeks after the second intranasal exposure to formalin-killed *Pasteurella haemolytica* A2 showing large nodular-type BALT. HE x400 .................................................. 62

6.1 The bronchus associated lymphoid tissue (BALT) of a goat killed at 1 day post-dexamethasone treatment showing a small aggregate with few cells. HE x400 .................................................. 76

6.2 The bronchus associated lymphoid tissue (BALT) of a goat killed at 21 days post-dexamethasone treatment showing a markedly small aggregate with few cells. HE x400 .................................................. 76

6.3 The bronchus associated lymphoid tissue (BALT) of a goat exposed to formalin-killed *Pasteurella haemolytica* A2 without the dexamethasone treatment showing a large nodule with numerous cells. HE x400 .................................................. 77

6.4 The bronchus associated lymphoid tissue (BALT) of a control untreated goat showing a small nodule. HE x400 .................................................. 77

7.1 Normal lungs of goat without any lesions or vaccination history .................................................. 90

7.1 Lungs of goat vaccinated with the intranasal spray vaccine and challenged with live *Pasteurella haemolytica* A2 showing relatively no pneumonic lesions .................................................. 90

7.2 Lungs of goat vaccinated with the oil adjuvant vaccine and challenged with live *Pasteurella haemolytica* A2 showing small area of pneumonia of approximately 10% .................................................. 91

7.3 Lungs of a control unvaccinated goat challenged with live *Pasteurella haemolytica* A2 showing extensive area of pneumonia. Approximately 40% of the lung area were affected .................................................. 91
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>The colonisation of Pasteurella haemolytica A2 in the lung tissues of goat with different treatments. Group 1 and 2 were intranasally exposed once to live or dead P. haemolytica A2 respectively while groups 3 and 4 were intranasally exposed twice to live or dead P. haemolytica A2 respectively.</td>
<td>47</td>
</tr>
<tr>
<td>5.1</td>
<td>The serum antibody (IgM, IgA, IgG) responses in the lung lavage fluid of goats following intranasal exposures to formalin-killed Pasteurella haemolytica A2.</td>
<td>57</td>
</tr>
<tr>
<td>5.2</td>
<td>The serum antibody (IgM, IgA, IgG) responses following intranasal exposures of goats to formalin-killed Pasteurella haemolytica A2.</td>
<td>58</td>
</tr>
<tr>
<td>5.3</td>
<td>The size of bronchus associated lymphoid tissue (BALT) and the number of lymphocytes in the BALT responses by goats exposed twice to formalin-killed Pasteurella haemolytica A2.</td>
<td>59</td>
</tr>
<tr>
<td>7.1</td>
<td>Serum antibody levels of goats following intranasal vaccination (group 1), intramuscular vaccination (group 2) and mixed vaccination (group 3). Group 4 was the unvaccinated control.</td>
<td>93</td>
</tr>
<tr>
<td>8.1</td>
<td>IgG antibody response in serum of sheep following intranasal spray vaccination of killed Pasteurella haemolytica A2. Note the drop in IgG level below the cut-off point on week 4 after second vaccination due to Haemonchus contortus. The incidence of pneumonic pasteurellosis also increase during that period of time.</td>
<td>105</td>
</tr>
<tr>
<td>8.2</td>
<td>IgG antibody responses in serum goats following intranasal spray of killed Pasteurella haemolytica A2 and alum precipitate vaccination subcutaneously. Note the incidence of pasteurellosis in the farm before vaccination (week –2 and 0) and the increase in IgG level during outbreak of pasteurellosis in the farm at week 6.</td>
<td>106</td>
</tr>
</tbody>
</table>
Abstract of the dissertation presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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June 1998

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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Jun 1998

Pengerusi: Prof Madya Dr Mohd Zamri Saad
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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 ketidakpopuleran vaksin di kalangan penternak adalah antara sebab-sebab yang dikenalpasti menyebabkan penyakit ini berterusan. Memandangkan selama ini pemvaksinan sistemik gagal untuk membuka hasil yang memuaskan, sudah sampai masanya kajian ke atas peranan keimunan mukosral pada trakus pernafasan dalam mengawal radang peparu pasteurellosis dipertimbangkan semula.

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 dikeloloni 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 semburaan intranasal menghasilkan perlindungan yang baik terhadap penyakit pasteurellosis pneumonia. Gerakbalas antibodi sistemik yang tinggi dan ketara di dapat 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 viscosity 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 (Kaltreider, 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 (Kaltreider, 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 immunosuppressive 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.
Pasteurella haemolytica

*Pasteurella haemolytica* is a Gram-negative, small, non-motile, coccobacillus 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.