UNIVERSITI PUTRA MALAYSIA

DETERMINATION SEVERITY OF PNEUMONIA AND RESPONSES OF HEAT SHOCK PROTEIN-70 CONCENTRATION IN VACCINATED AND NON-VACCINATED PNEUMONIC GOATS

DHARSHINI A/P MASLAMANY

Ip
FPV 2018 24

DETERMINATION SEVERITY OF PNEUMONIA AND RESPONSES OF HEAT SHOCK PROTEIN-70 CONCENTRATION IN VACCINATED AND NON-VACCINATED PNEUMONIC GOATS

DHARSHINI A/P MASLAMANY

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

in partial fulfilment of the requirement for the

Degree of Doctor of Veterinary Medicine,

Universiti Putra Malaysia,

Serdang, Selangor Darul Ehsan

MARCH 2018

It is hereby we have read this project paper entitled "Determination Severity of Pneumonia and Responses of Heat shock protein-70 Concentration in Vaccinated and Non-Vaccinated Pneumonic Goats", by Dharshini A/P Maslamany and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

ASSOC. PROF DR. FAEZ FIRDAUS JESSE BIN ABDULLAH

DVM (UPM), PhD (UPM)

Senior Lecturer

Department of Veterinary Clinical Studies

Faculty of Veterinary Medicine

(Supervisor)

____

PROFESSOR DR. ABD WAHID HARON

DVM (UPM), PhD (IRELAND)

Senior Lecturer

Department of Veterinary Clinical Studies

Faculty of Veterinary Medicine

(Co-Supervisor)

ACKNOWLEDGEMENTS

First and foremost, I would like to express my utmost gratitude to my supervisor, Assoc. Prof Dr. Faez Firdaus Jesse Bin Abdullah for his guidance, help and undivided attention while doing this project.

I would like to acknowledge my co-supervisor, Prof Dr Abd Wahid Haron for their contributions toward the better understanding with my project and sharing knowledge throughout the project.

Sincere thanks to my groupmates (Kalai Vaani, Hafizin), staffs of Large Animal Ward, UVH and Mr. Mohd Jefri NorSidin of Clinical Studies Laboratory of Faculty of Veterinary Medicine, Universiti Putra Malaysia for their assistance for sample collection and owner of each farms who allows me to collect sample from their animal.

Special mention to my parents, family and my friend Sanchita for supporting me to complete this project successfully.

CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

HSP	Heat Shock Protein
ELISA	Enzyme-Linked Immunosorbent Assay
LPS	Lipopolysaccharide
RVM	Recombinant Vaccine for Mannheimiosis
G	Gauge
^{0}C	Degree Celsius
mL	Milliliter
rpm	Revolutions per minute
HPR	horseradish peroxide
OD	Optical density
SPSS	Statistical Product and Service Solutions
P	Probability

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kursus VPD 4999 - Projek Tahun Akhir.

PENENTUAN KETERUKAN PNEUMONIA DAN TINDAK BALAS "HEAT SHOCK PROTEIN-70" DALAM KAMBING YANG DIJANKITI PNEUMONIA ANTARA YANG DIVAKSIN DAN TIDAK DIVAKSIN

Oleh

DHARSHINI MASLAMANY

2018

Penyelia: Prof. Madya Dr. Faez Firdaus Jesse Abdullah

Penyelia bersama: Prof. Dr. Abd Wahid Haron

Pasteurella pneumonia adalah penyakit berjangkit kedua yang paling penting dalam ruminan kecil yang disebabkan oleh *Pasteurella multocida* atau *Mannheimia haemolytica* jenis A2, A7 dan A9. Heat shock protein (HSP) seperti HSP-70 adalah protein utama yang ditimbulkan oleh tekanan yang memainkan peranan penting dalam pemusnahan patogen dan meningkatkan rintangan haiwan kepada tekanan kimia. HSP-70 adalah pengadun molekul yang boleh menjadi biomarker penting dalam diagnosis penyakit bakteria dalam ruminan kecil. Kajian terdahulu mengenai pasteurella pneumonia tidak mengenal pasti keterukan pnemonia dan tindak balas heat shock protein-70 dalam kambing pneumonia

yang divaksinas dan tidak divaksin. Oleh itu, kajian ini telah dirancang di mana sebanyak 76 ekor kambing (30 telah divaksin dan 46 tidak divaksin) dipilih daripada empat ladang ruminan kecil. Haiwan-haiwan itu dikelompokkan kepada tiga kumpulan iaitu kumpulan sihat yang divaksin dan tidak divaksin, kumpulan pneumonia yang divaksin dan kumpulan pneumonia yang tidak divaksin berdasarkan pemeriksaan klinikal. Keterukan pneumonia ditentukan berdasarkan pemarkahan auskultasi paru-paru dan keterukan dikategorikan sebagai ringan, sederhana dan parah. Sampel darah telah dikumpulkan daripada kambing-kambing ini dan sampel-sampel itu dianalisis untuk HSP-70 kambing menggunakan teknik ELISA. Keputusan menunjukkan bahawa kepekatan HSP-70 lebih tinggi dalam kambing pneumonia yang tidak divaksin berbanding dengan kambing pneumonia yang telah divaksin. Kepekatan HSP-70 meningkat sebanyak 25% dalam kambing pneumonia yang divaksin dan 45% dalam kambing pneumonia tidak divaksin berbanding kambing yang sihat. Walau bagaimanapun, analisis statistik menunjukkan bahawa tiada perbezaan yang signifikan ($P > 0.05$) dalam kepekatan HSP-70 antara kambing pneumonia yang divaksin dan tidak divaksin. Keterukan tanda-tanda klinikal menunjukkan bahawa kambing yang tidak divaksin mempunyai tanda klinikal 50% lebih parah dibandingkan dengan kambing pneumonia yang divaksin. Secara statistik terdapat perbezaan yang signifikan ($P < 0.05$) dalam keterukan tanda-tanda radang paru-paru antara kambing pneumonia yang divaksin dan tidak divaksin. Kesimpulannya, kajian ini menunjukkan bahawa kepekatan HSP-70 meningkat pada kambing pneumonia yang tidak divaksina berbanding dengan kambing pneumonia yang telah divaksin. Secara umum, tanda klinikal yang kurang teruk dan kepekatan rendah HSP-70 dalam kambing yang divaksin menunjukkan bahawa kambing yang divaksin mempunyai imuniti dan perlindungan yang lebih baik terhadap jangkitan pneumonia.

Kata kunci: *Pneumonia, Kambing, keterukan, tanda-tanda klinikal, Heat Shock Protein, HSP70, vaksin.*

ABSTRACT

An abstract of the paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Final Year Project.

DETERMINATION SEVERITY OF PNEUMONIA AND RESPONSES OF HEAT SHOCK PROTEIN-70 CONCENTRATION IN VACCINATED AND NON-VACCINATED PNEUMONIC GOATS

By

DHARSHINI MASLAMANY

2018

Supervisor: Assoc. Prof. Dr. Faez Firdaus Jesse Abdullah

Co-Supervisor: Prof. Dr. Abd Wahid Haron

Pneumonic pasteurellosis is the second most important infectious disease in small ruminant caused by *Pasteurella multocida* or *Mannheimia haemolytica* serotype A2, A7 and A9. Heat shock protein (HSP) such as HSP-70 is a major stressed-induced proteins that play a key role in destruction of pathogen and increase host resistance to chemical stresses. HSP-70 is a molecular chaperone that can be a vital biomarker in the diagnosis of bacterial diseases in small ruminant. Previous studies of pneumonic pasteurellosis have not dealt with the severity of pneumonia and responses of heat shock protein-70 in vaccinated and non-vaccinated pneumonic and non-pneumonic goat. Therefore, this study was designed where total of 76 goats (30 vaccinated and 46 non-vaccinated) were selected from four small ruminant farms. The animals were grouped into three groups namely normal vaccinated and non-vaccinated, vaccinated pneumonic group and non-vaccinated pneumonic group based on the clinical examination. Severity of pneumonia was determined based on the lung auscultation scoring and the severity was categorized as mild, moderate and severe. Blood samples were collected from these goats and the samples were subjected for goat HSP-70 analyses using ELISA technique. The findings showed that HSP-70 concentration is higher in non-vaccinated pneumonic goats than in vaccinated pneumonic goats. The HSP-70 concentration increased by 25% in vaccinated pneumonic goat and 45% in non-vaccinated pneumonic goats compared to the normal goats. However, the statistical analysis revealed that there is no significant difference ($P > 0.05$) in the concentration of HSP-70 between vaccinated and non-vaccinated pneumonic goats. The severity of clinical signs revealed that non-vaccinated goats showed 50 % more severe pneumonia clinical signs compared to vaccinated pneumonic goats. Statistically there was significant difference ($P < 0.05$) in the severity of clinical signs of pneumonia between vaccinated and non-vaccinated pneumonic goats. In conclusion, the present study highlights that HSP-70 concentration were elevated slightly in non-vaccinated pneumonic goats compared to the vaccinated pneumonic goats. In general, less severe clinical signs and low concentration of HSP-70 in vaccinated goats showed that vaccinated goats had a better immunity and protections against pneumonia infection.

Key word: *Pneumonia, Goats, severity, clinical signs, Heat Shock Protein, HSP70, vaccine.*

1.0 INTRODUCTION

Pneumonic pasteurellosis is an important infectious diseases of small ruminant industry with higher prevalence rate around the world including Malaysia (Gilmour *el al*., 1991). This disease produce an acute infection, severe fibrinous bronchopneumonia and septicaemic in sheep, goat and cattle (Mohammed and Abdelsalam, 2008). Pneumonic pasteurellosis characterized by inflammation of pulmonary parenchyma with bronchitis and often pleuritic (Radostits *et al.,* 2007). The causative agent for this disease are *Pasteurella multocida* or *Mannheimia haemolytica* serotype A2, A7 and A9. Host become susceptible to these bacteria due to stressful conditions for instance transportation, overcrowding, malnutrition, weaning and also following concurrent viral infection or other diseases (Zamri-saad *et al* 1994; Brogden *et al* 1998).

Mannheimia haemolytica is an endemic disease with mortality rate of 39% in small ruminant industry (Jesse *et al* 2015). This is an opportunistic bacterium which are non-motile gram-negative small rods that found in nasopharyngeal and oral regions of clinically healthy goats and are often isolated from asymptomatic carriers (Kaoud *et al* 2010). This disease clinically manifested by an increased in the respiratory rate, changes in the depth and character of respirations, coughing, abnormal breath sounds on auscultation and, in most bacterial pneumonias, evidence of toxemia (Radostits *et al*., 2007).

The diagnosis of pneumonia is primarily made on clinical signs and history (Donachie *et al* 1995). Moreover, lung auscultation is an important aid for diagnosis. It is helpful in determining the stage of development and identification of nature of the lesion in the lung field (Radostits *et al.,* 2007). Serological diagnosis and nasal swab to isolate *M.haemolytica* often unsuccessful. Confirmatory diagnosis is made at necropsy with the presence of acute inflammatory changes of thorax and the lung lesion showing hepatized and/or necrotic lung (Donachie *et al* 1995). Histological diagnosis using affected lung lesion to demonstrate oat cells will give further confirmation on the agent present (Donachie *et al* 1995).

Perhaps, the optimum control of pneumonic pasteurellosis can be achieved only through vaccination (Donachie *et al* 1995). Vaccine against pasteurellosis of goats and sheep are available commercially, including alum precipitate and oil adjuvant vaccine (Mosier, 1993; Chandrasekaran *et al.,* 1994). It contained locally isolated *P. haemolytica* type A7 and *P.multocida* types A and D (Chandrasekaran *et al.,* 1991). In addition, the recombinant vaccine for Mannheimiosis produced against *M. haemolytica* serotypes A2, A7 and A9 was also successfully reduced incidence of pneumonic pasteurellosis in a Boer goat farm in Sabah, Malaysia. (Bahaman *et al.,* 1991; Sabri *et al*., in 2013).

Heat shock proteins (Hsps) are molecular chaperones that involve in and required for cellular growth, function, and survival with proper folding, maturation, and breakdown of proteins (Ritossa, 1962). Heat shock proteins are classified according to their molecular weight and functions. Protein with molecular weight of approximately 27, 70, and 90 kiloDalton (kDa) are referred as Hsp27, Hsp70 and Hsp90 respectively (Lindquist, 1986). The Heat Shock Protein 70 (Hsp70) is used in this study as it is considered to be the most sensitive protein among HSPs, and plays a role in various bacteria (Valizadeh *et al.,* 2017). Briefly, a superficial interactions of the HSP to pathogen will lead to destruction of the pathogen because it activates the immune system of the host cell to counteract the pathogen (Knaust *et al*., 2007). HSP also induced during fever to increase the host resistance to the chemical stresses (Perdrizet, 1995).

To our knowledge, there is no study has been done to observe the response of Heat Shock Protein-70 in vaccinated and non-vaccinated pneumonic goats. Therefore, was designed to determine HSP-70 in group of goats with pneumonic signs from vaccinated and non-vaccinated groups.

2.0 LITERATURE REVIEW

2.1 Pneumonic pasteurellosis

Pneumonic pasteurellosis is an important infectious diseases of small ruminant industry with higher prevalence rate around the world including Malaysia. This disease affecting goat, sheep and cattle and responsible for high mortality rate among livestock (Gilmour *el al*., 1991; Links *et al*., 1992: Boudreaux, 2004). Pneumonic pasteurellosis account for about one-third deaths among cattle around the world (Boudreaux, 2004). Pneumonic pasteurellosis causes inflammation of pulmonary parenchyma which usually occur in combination with inflammation of the bronchioles (Radostits *et al.,* 2007). This disease causes acute infection, severe fibrinous bronchopneumonia and septicaemic in various animal (Mohammed and Abdelsalam, 2008).

This disease caused by a microorganism from genus *Pasteurella,* named after Louis Pasteur who first discovered this organism (Trevisan, 1887).The two major species frequently isolated are *Pasteurella multocida* and *Mannheimia haemolytica* or *Pasteurella haemolytica* which produce septicemic or the pneumonic infection in various animal species (Gilmour, 1993). Infection can be caused by *P. multocida* alone or mixed infection involving both *P. multocida* and *P. haemolytica* in goats (Loganathan & Chandrasekaran, 1992). *Pasteurella multocida* type B has been associated with septicaemic disease known as haemorrhagic septicaemia whereas *Pasteurella multocida* type A and D were associated with pneumonic pasteurellosis. Similarly, *Pasteurella* *haemolytica* A1 cause pneumonic pasteurellosis in cattle and *Pasteurella haemolytica* A2 cause pneumonic pasteurellosis in sheep and goats (Gilmour, 1993).

Pneumonic pasteurellosis affect sheep of all ages. Young animals less than 3 weeks old usually have hyperacute infection with generalized infection, while acute infection with pleurisy and pericarditis shown in animals between 3 to 12 weeks old (Gilmour, 1993). One of the predisposing factor that lead to this disease in small ruminant is stressful conditions for instance transportation, overcrowding, malnutrition, weaning and also following concurrent viral infection or other diseases, which could alter the normal homeostasis of the host and become susceptible to these bacteria (Brogden *et al*., 1989; Zamri-saad *et al*., 1994). In sheep and goats it is combination of compromised pulmonary defense mechanism and environmental stress (multifactorial) that causes the illness in contrast to calves mostly occurs in newly weaned animals that have been through markets and mixed together. (Gilmour, 1993; Mohammed and Abdelsalam, 2008).

2.2 *Mannheimia haemolytica*

Mannheimia haemolytica, which was known as *Pasteurella haemolytica* previously, is a gram-negative; facultative anaerobic coccobacillus bacteria. It is a member of the family *Pasteurellaceae*, genus *Mannheimia* (Rice *et al* 2007). *Mannheimia haemolytica* is the primary agent for the disease of pneumonic pasteurellosis in small ruminant. However, *Pasteurella multocida* also been involved in many acute outbreaks. The most frequently isolated serotypes of *Pasteurella haemolytica* from pneumonic pasteurellosis cases are A2, A7, and A9 (Davies *et al.,* 1982; Bahaman *et al.,* 1991). This species found in normal commensal of the upper respiratory tract in both sheep and goats (Gilmour et al., 1974).

M. haemolytica has various virulence factor that capable of promoting adhesion, colonization and proliferation of organism within the host. Fimbriae is one of the virulence factor. It is a smaller appendages present in the surface of M. haemolytica similar as in many Gram-negative bacteria. They are specific surface structures of the bacterial cell wall which permit adhesion onto the epithelial layer of host (Morck *et al,.* 1987; Mohammed and Abdelsalam, 2008). Moreover, the cell wall of M. haemolytica contains a lipopolysaccharide (LPS) endotoxin. It is one of the important virulence factor that involved in pathogenesis of pneumonic pasteurellosis (Mohammed and Abdelsalam, 2008). Some experimental evidence indicated that LPS endotoxin is directly toxic to endothelial cells and capable of altering leukocyte functions and causing lysis of blood platelets (Breider *et al.,* 1990). Apart from LPS, a protein known as cytotoxin associated with lytic activity specifically in macrophage, lymphocyte, neutrophil and platelets which accounts for its terminology leukotoxin (Clinkenbeard and Upton, 1991). M. haemolytica also has capsules that composed of polysaccharide (Gilmour *et al*., 1985). It protect the invading organism against cellular and humoral defense mechanisms of the host. The capsular material of M. haemolytica also interact with the pulmonary surfactant and thereby facilitating the adhesion of the organism to the respiratory tract epithelium of susceptible animals (Brogden *et al*., 1989).

2.3 Clinical features of pneumonia

The signs of acute pneumonia are dullness, anorexia, pyrexia of greater than 40.6^{0}C, varying degree of hyperpnoea or dyspnea and sudden death. There also will be rapid shallow respiration accompanied with profuse mucopurulent nasal and ocular discharges (Donachie *et al*., 1995). During outbreaks, the early signs noticed by farmer are sudden deaths and death of sick animals. Within few days acute cases disappear, and replaced by animals that show more obvious clinical signs of pneumonia (Gilmour, 1993). Goats that survive from acute stage will be chronically infected with severe weight loss and respiratory dyspnea and deaths may occur (Brogden, 1998). Lung auscultation is an important aid for diagnosis. It is helpful in determining the stage of development and identification of nature of the lesion in the lung field (Radostits *et al.,* 2007). At the early stage of pneumonia the breath sounds are increased over the antero-ventral aspects of the lungs. Crackling sounds may develop suggestive of increased exudate in airway, but in uncomplicated interstitial pneumonia, clear, harsh breath sounds are auscultated (Radostits *et al.,* 2007).

2.4 Pathology

In most case of pasteurellosis extensive ecchymotic hemorrhage in throat and over the ribs can be observed. Upon opening trachea, bronchi, and lung surfaces petechiation and frothy exudates coupled with straw-colored pericardial fluids present that confirms the pneumonic pasteurellosis case. In hyperacute cases, lungs are swollen, heavy and cyanotic with frothy hemorrhagic fluid on cut surface. Chronic cases develop consolidation of lung especially at cranial and ventral parts of lungs. In acute cases lung consolidation combined with hepatization of lung will present at necropsy (Jesse *et al.,* 2015). Histologically, Gram-negative coccibacilli fills in the alveoli and intense hyperemia with hemorrhages of lung tissue can be seen. The diagnosis using affected lung lesion to demonstrate 'oat cells' will give further confirmation on the agent present. 'Oat cell' is elongated cells filling the alveoli with basophilic spindle shaped nucleus (Donachie *et al* 1995).

2.5 Vaccination

The optimum control of pasteurellosis is achieved only through vaccination. During outbreak of pasteurellosis in goats, vaccination provide active immunity in the period when the passive immunity from colostrum has waned (Donachie *et al* 1995). Vaccines that have been developed for pasteurellosis are include alum precipitated and oil adjuvant vaccines (Chandrasekaran *et al*., 1994). These killed vaccines are widely used for disease prevention. It contained locally isolated *P. haemolytica* type A7 and *P.multocida* types A and D (Chandrasekaran *et al.,* 1991). Initially, the locally produced formalin-killed oil adjuvant vaccine, combining *Pasteurella haemolytica* and *Pasteurella multocida* of unknown serotypes was unsuccessful in controlling natural infection (Zamri-Saad *et al.,* 1989). However, this improved oil adjuvant vaccine incorporating known serotype successfully protected animal from experimental infection (Chandrasekaran *et al.,* 1991; Zamri-Saad *et al.,* 1993). In that study locally isolated *P.haemolytica* A7 and *P.multocida* types A and D in oil adjuvant significantly reduced the lung lesions in five vaccinated lambs compared to four non-vaccinated control lambs following experimental infection of *P.haemolytica* A7 (Chandrasekaran *et al.,*1991). One of the disadvantage of oil adjuvant vaccines are local lesions at the site of injection (Jamaluddin 1993). Since, the non-adjuvant vaccine do not produce strong immune response, a less-irritating aluminium hydroxide adjuvant vaccines can be used as an alternative (Gilmour and Gilmour 1989).

In fact, the recombinant vaccine for Mannheimiosis (RVM) also have been developed and efficacy of vaccine was evaluated for period of two years in a Boer goat farm in Sabah, Malaysia. Goats more than 6-months-old were given the vaccination and booster dose at 6 month interval (for two years) as a result, It was reported to substantially reduce the incidence of the pneumonic pasteurellosis in that Boer goat farm (Sabri *et al.,* 2013). Mortality rate and respiratory signs were dramatically lowered with the intranasal administration of RVM as it provide mucosal or humoral immunity in goats (Sabri *et al.,* 2010). Subsequently, less handling of the goats may needed to possibly reduce stress leading to better uptake of the vaccine and gives better protection against mannheimiosis (Sabri *et al*., 2013). Although injectable Mannheimia vaccine provide varying protection it is less practicable, time consuming, increases the labor cost and induce more stress to animal, therefore intranasal vaccination could be a better choice (Lee *et al.,* 2001; Sabri *et al.,* 2013).

2.6 Role of stress

Stress is one of the factor that increases susceptibility of animals to respiratory diseases. It is difficult to access physiological, psychological and physical environment, however, the stress level in animal can be measured through certain parameters. For instance, body temperature, plasma cortisol, heart rate, glucose level, ketone, urea and free fatty acids (Swanson, 1995). The most common environmental stressors that predispose animal to this disease are heat stress, overcrowding, inclement weather, poor ventilation with high humidity, transportation and handling (Knowles *et al*., 1995). Moreover, castration and docking, weaning and change in feed, exhaustion and hunger during transportation also increase stress level in animal (Cole, 1996).

Stress plays an important role in the natural incidence of pneumonic pasteurellosis and increased isolation rates of *P.haemolytica* (Thomson *et al.,* 1985; Zamri-Saad *et al.,* 1991). In healthy animals, the mucociliary ladder, the cellular and humoral defense mechanism of respiratory tract serve in eliminating this organism (Gilmour, 1993). While in stressed animal, there is breakdown of innate pulmonary immune barriers which increases susceptibility of animal to *Mannheimia haemolytica* (Martin, 1996; Brogden *et al*., 1998). The bacteria will travel along the trachea and enter the lung tissue, this lead to severe inflammatory reaction dominated by fibrinous exudate in lung Mohammed and Abdelsalam, 2008).

2.7 Heat shock protein

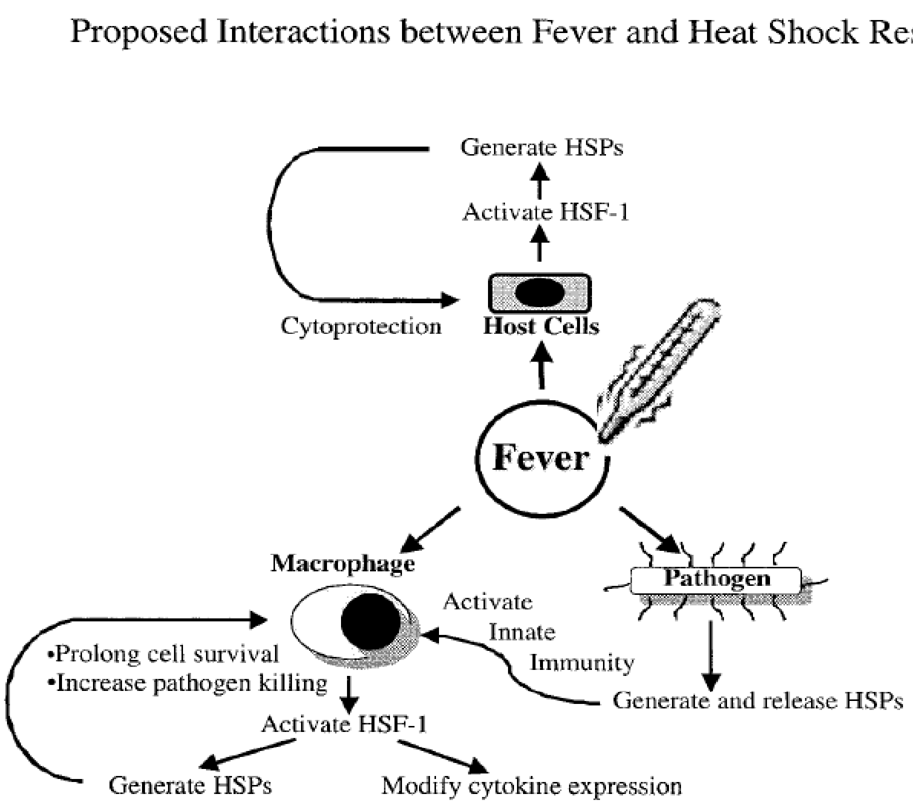
Heat shock proteins are molecular chaperones that involve in and required for cellular growth, function, and survival with proper folding, maturation, and breakdown of proteins (Ritossa, 1962). Because heat stress strongly induces the synthesis of these proteins, they are called HSP and also, chaperones (Valizadeh *et al.,* 2017). Heat shock proteins are classified according to their molecular weight and functions. Protein with molecular weight of approximately 27, 70, and 90 kiloDalton (kDa) are referred as Hsp27, Hsp70 and Hsp90 respectively (Lindquist, 1986). In this study we used Hsp70 as it is the most sensitive protein among HSPs, and plays a role in various bacteria (Valizadeh *et al.,* 2017).

2.7.1 HSP-70

Heat shock proteins are induced by stress which produced during shock transcription and translation process of cell (Jeffrey, 2000). The HSP-70 is one of the heat shock protein that highly available molecular chaperones that participate in many biological processes, such as protein folding and refolding or breakdown of aggregated peptide products (Lackie *et al.,* 2017). Synthesis of HSP-70 can elicit on exposure of various stressor include toxin, oxidants, viral and bacterial infection, fasting and fear (Zulkifli *et al.,* 2000). The HSP-70 are induced as an early response to stressors to protect cells, initiate recovery, and provide state of resistance for subsequent stress (Gerner and Schneider, 1975; Kregel, 2002). In addition, HSP-70 involve in protein quality control and helping the stressed animal to inhibit cellular apoptosis by enhancing the thermostolerance level in many cell type (Mishra *et al*., 2014). There are many studies on the role of HSP 70 in disease resistance and general heat tolerance in poultry has been done (Liew *et al.,* 2003; Zulkifli *et al.,* 2003). However, there is limited study conducted in goat.

The Heat Shock Protein 70 is considered to be the most sensitive protein among HSPs, and plays a role in various bacteria (Valizadeh *et al.,* 2017). Briefly, a superficial interactions of the HSP to pathogen will lead to destruction of the pathogen because it activates the immune system of the host cell to counteract the pathogen. These proteins cause pathogenic bacteria not to pass through the host body barriers (Knaust *et al*., 2007).

Moreover, fever is one of the response we could see in most of the infection in animals to accelerate pathogen clearance and shortening disease duration (Jeffrey, 2000). For instance, fever is one of the clinical sign observed in goat infected with pneumonic pasteurellosis (Donachie *et al*., 1995). One of the way fever protect host cells during infection is by inducing Hsp expression in host cells, thereby cells become resistant to chemical stressors produced by the infective organism (Perdrizet, 1995). To simplify, two possible mechanisms of fever that directly involve the heat shock in the regulation of immune defenses is shown in Fig 1 (retrieved from Cell Stress & Chaperones, 2000). Some studies by Feder and Hofmann (1999) and Ray (1999) suggested that induction of heat shock in mammals occur when the temperature rises >4°C above normal level. However, stress response can be activated by slight increase in temperature (Brown and Rush 1996). Additionally, when mechanism of recombinant HspB (member of HSP 60 family) was studied, it able diagnose caprine Q fever and detect a recent infection from reactivation of infection in goats (Isabelle *et al.,* 2009). While these statements clearly show that Hsps act as cytoprotective during infections, and there are high possibility that



elevation of HSP70 can be observed in pneumonic goat in this study.

Figure 1: Proposed interaction between fever and heat shock response

3.0 METHOD AND MATERIALS

3.1 Sample population

Four (4) goat farms consist of two (2) vaccinated and two (2) non-vaccinated farms were selected in this study. 15 clinically normal and 9 pneumonic goats from vaccinated farm; 15 clinically normal goats and 31 pneumonic goats from non-vaccinated farms were enrolled for this study as shown in Table 1. The age of the goats ranged from 1 to 3 years. The age was determined based on dentition.

Table 1: Summary of sample population

	VACCINATED GOATS	NON VACCINATED GOATS
NORMAL	15	15
PNEUMONIC	15	31

3.2 Lung auscultation

Clinically normal goats accessed based on history, general appearance, no nasal and ocular discharges and no abnormal findings on lung auscultation. On the other hand, pneumonic goats were determined based on the history, general appearance, clinical signs (nasal discharge, ocular discharge, rapid shallow respiration and etc.) and harsh and crackles sound on lung auscultation. Severity of pneumonia in each goat were scored using lung auscultation scoring. Refer to diagram 1: lung field on each side was divided into four quadrants. On each quadrant, 5 points were auscultated. Score was given according to the crackles or harsh sound heard at each points. So, one quadrant will give full score 5/5 and the total score for one side of the lung is 20/20. A complete auscultation on both side of the lung will give score of 40/40.

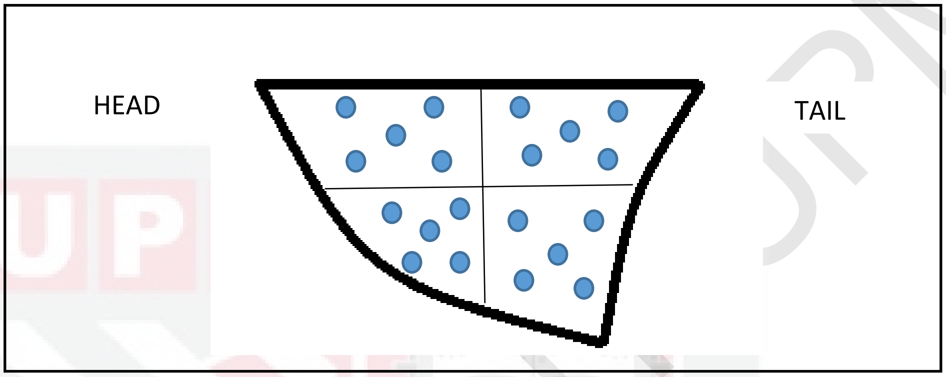


Diagram 1: illustration of lung field for lung auscultation scoring. Each ● indicating one point.

From the auscultation score findings, severity of pneumonia was categorized into three levels namely mild, moderate and severe (Table 2).

Table 2: Lung auscultation scoring

LUNG AUSCULTATION SCORE	STATUS
$\frac{1-15}{40}$	Mild
$\frac{16-24}{40}$	Moderate
$\frac{25-40}{40}$	Severe

3.3 Blood sampling

Blood was obtained from each goat via jugular venipuncture using 21 G needle into labelled plain tube to obtain the serum for serological testing. The blood tubes were immediately placed in an ice box for transportation to laboratory to be processed. This study was conducted with the approval of the Institutional Animal Care and Use Committee UPM/IACUC/AUP-U011/2018

3.4 Serum extraction

Blood collected in plain tubes were centrifuged at 5000 rpm for 3 minutes to harvest serum. At least 1 mL serum from each tube was transferred to labelled 1.5 mL micro-centrifuge tube. The micro-centrifuge tubes were then stored at -20^{0}C for serological evaluation.

3.5 Serological Testing

The serum samples to be subjected to analysis using Goat heat Shock Protein 70 (HSP-70) ELISA kit was kept in room temperature prior to use. Before running the ELISA, the conjugate solution was prepared by diluting at 1:19. The wash solution prepared by diluting 1 copy of 20X washing buffer plus 19 copies of the distilled water. Wells were set as blank wells, standard wells and test sample wells respectively:

(1) Blank well: leave blank

(2) Standard well: 50 µl of standard was added

(3) Test sample well: 40 µl of Special diluent and then 10 µl of sample added

(4) 50 µl of horseradish peroxide (HRP) added into each well, except blank well. Then, sealed the plate and gently shaked, then incubated for 60 minutes at 37^0C.

Discard Liquid excess, drying, fill each well with diluted washing liquid, mix and shake for 30 seconds, discard the washing liquid and tap the plate into absorbent paper to dry. This step repeated five times and then pat dry. 50 µl of chromogen solution A added to each well, and then, 50 µl of chromogen solution B into each well. Shake and incubated for 10 minutes at 37^0C. For final measurement, stop solution was added and within 15 minutes, optical density (OD) was measured at 450 nm wavelength.

3.6 Statistical Analysis

Mann-Whitney U test using IBM® SPSS® Statistics V23.0 was used to compare severity of vaccinated and non-vaccinated pneumonic goat. While, One-way ANOVA was used to compare significant association of HSP-70 concentration between vaccinated and non-vaccinated pneumonic goat.

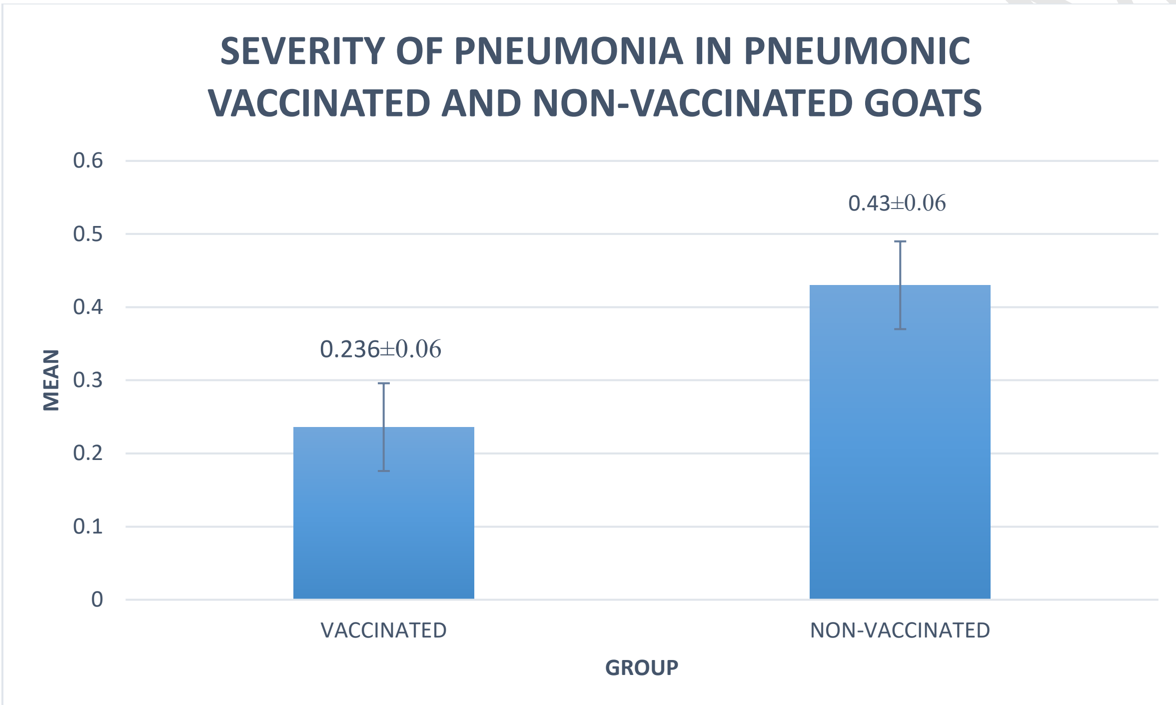
4.0 RESULTS

4.1 Severity of pneumonia in vaccinated and non-vaccinated pneumonic goats

Result of lung auscultation scoring revealed from 15 pneumonic goats from vaccinated group, 12 goats (80%) had mild pneumonia, 1 goat (7%) was in moderate pneumonia and 2 goats (13%) were categorized as severe pneumonia infection. In non-vaccinated group from 31 pneumonic goats, 10 goats (32%) had mild pneumonia, 4 goats (13%) had moderate pneumonia and 17 goats (55%) were categorized as severe pneumonia. Findings are tabulated (Appendix A and Appendix B). Auscultation score, 0.025-0.325 showed mild infection, 0.35-0.65 as moderate and 0.675-1 for severe pneumonia.

Based on the mean auscultation score, the vaccinated group showed mild pneumonia where the mean score was 0.236 where else, the non-vaccinated group showed moderate pneumonia with mean score of 0.43 (Figure 2).

Figure 2: Mean of severity of pneumonia in pneumonic vaccinated and non-vaccinated goats.

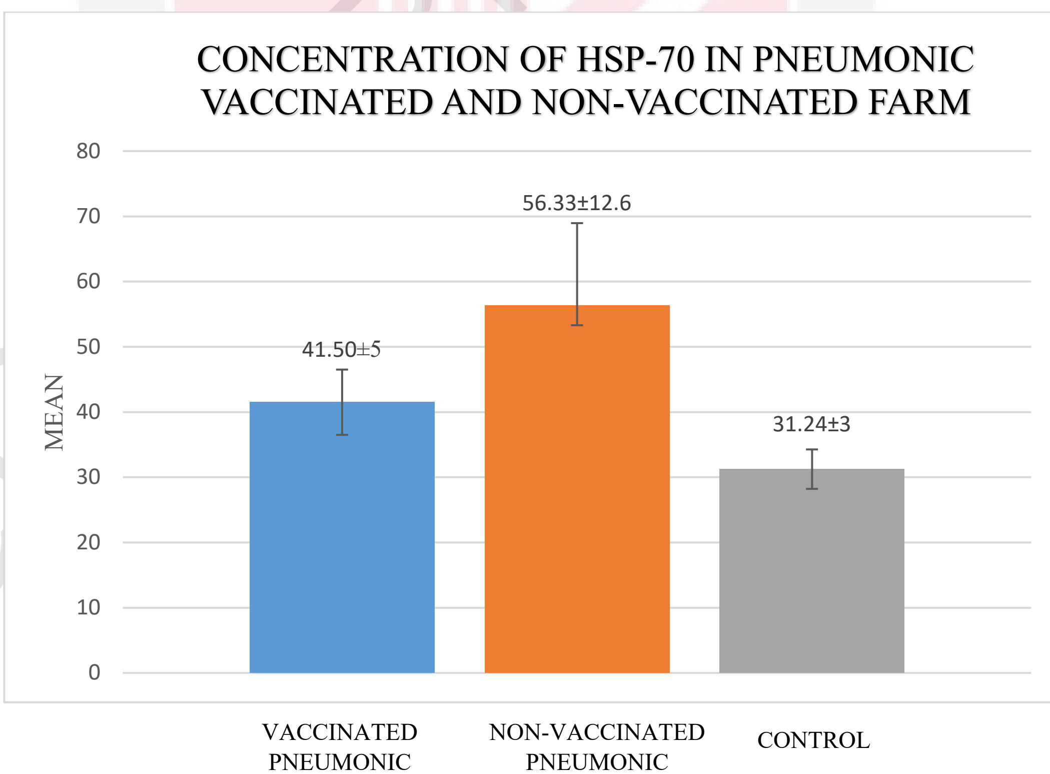


There were no significant difference in severity of pneumonia between vaccinated and non-vaccinated pneumonic goats. Therefore the alternative hypothesis was not accepted in this study.

4.2 Results of HSP-70 concentration in vaccinated and non-vaccinated pneumonic goats

The results showed there was higher concentration of HSP-70 in non-vaccinated pneumonic group, followed by vaccinated pneumonic goats and least concentration of HSP-70 in control group (Figure 3). The mean concentration of HSP-70 in non-vaccinated pneumonic group, vaccinated group and control group are 56.33, 41.5 and 31.24 respectively. Statistical analyses revealed there were no significant difference between the groups tested.

Figure 3: Mean of concentration of HSP-70 in pneumonic vaccinated and non-vaccinated goats



5.0 DISCUSSION

Data from this study showed, in pneumonic group 80% goats had mild pneumonia, 7% had moderate pneumonia and 13% had severe pneumonia from vaccinated group. For non-vaccinated group, 32% goats had mild pneumonia, 13% had moderate pneumonia and 55% had severe pneumonia from lung auscultation scores as mentioned by Radostits *et al.* (2007) states that lung auscultation is an important aid for diagnosis and helpful in determining the stage of development and identification of nature of the lesion in the lung field. The data of the mean auscultation score was computed, pneumonic goats from vaccinated farm mostly had mild infection compared to non-vaccinated pneumonic goats showed moderate infection. These findings were in agreement with Chandrasekaran *et al.* (1991) where there was significant reduction in lung lesions in five vaccinated lambs with locally isolated *P. haemolytica* A7 and *P. multocida* types A and D oil adjuvant vaccine compared to four non-vaccinated controls following experimental challenge with *P. haemolytica* A7. This concludes that vaccinated goats are more protected against pneumonic pasteurellosis whereas non-vaccinated farms have less protection especially goats that exposed to stressful conditions (Zamri-saad *et al.,* 1994). The alum precipitated pneumonia vaccine in these farms contributed to the improved level of immunity in goats to protect against pneumonia. It is recommended to practice vaccination in farms as the price of vaccine is cheap and affordable and give good protection against pneumonia disease.

The mean concentration of HSP-70 in non-vaccinated pneumonic goat higher than the vaccinated pneumonic goats and least concentration of HSP-70 in control group. Statistically there was no significant difference (P >0.05) in HSP-70 concentration in vaccinated and non-vaccinated pneumonic goats. Till date, there is no study has been conducted nor data available to compare with this study findings on the presence of HSP-70 in pneumonic goat. In spite of that, as HSP-70 is considered to be the most sensitive protein and plays a role in various bacteria (Valizadeh *et al.,* 2017), where it is assumed to be increased in pneumonic goat as per hypothesis of this study. Additionally in a study, recombinant HspB (member of HSP 60 family) was used as a potential antigen to diagnose caprine Q fever. HSP seems to be able to indicate a recent infection, as well as a reactivation of infection in experimentally and naturally infected goats (Isabelle *et al.,* 2009).

In this study, lung auscultation is the only diagnostic method used to identify the pneumonic goat and this method unable to detect carrier goats. The carrier animals are detected based on nasal swabs in the case of active carriers and culture of lymph nodes from slaughtered animals in the case of passive carriers (Gilmour, 1993). Nevertheless, the findings could be inconsistent due to small sample size, sampling was done at only one period of time and short duration of the study.

6.0 CONCLUSION AND RECOMMENNDATION

In conclusion, there is significant difference ($P < 0.05$) in severity of pneumonia between vaccinated and non-vaccinated pneumonic goats and low concentration of HSP-70 in vaccinated pneumonic goats. The less severe clinical signs and low concentration of HSP-70 in vaccinated goats showed that vaccination contributed to the reduction in severity of pneumonia cases and goats had a better immunity and protections against pneumonia infection.

However, it is recommended that the study conducted on larger scale involving greater number of animals and continues study for a period of time will provide truer picture on the efficacy of HSP-70 as biomarker for pneumonia in goats.

REFERENCES

Bahaman, A.R., Nurida, A.B., Sheikh-Omar, A.R. and Zamri-Saad, M. (1991). Biotypes and serotypes of Pasteurella haemolytica and their importance in the production of vaccines for pneumonic pasteurellosis in sheep. Vet Malaysia 3: 33-35.

Boudreaux, C. M. (2004). A novel strategy of controlling bovine pneumonic pasteurellosis: Transfecting the upper respiratory tract of cattle with a gene coding for the antimicrobial peptide cecropin B. M. Sc. Thesis, Louisiana State University,USA.

Breider, M. A., Kumar, S. & Corstivel, R. E. (1990). Bovine pulmonary endothelial cell damage mediated by Pasteurella haemolytica pathogenic factors. Infection and Immunity. 58: 1671−1677.

Brogden, K. A., Adlan, C., Lehmkuhl, H. D., Cutlip, R. C., Knights J. M. & Engen, R. L. (1989). Effect of Pasteurella haemolytica "A1" capsular polysaccharide on sheep lung in vivo and on pulmonary surfactant in vitro. American Journal of Veterinary Research. 50: 555−559.

Brogden, K. A., Lehmkuhl, H. D., & Cutlip, R. C. (1998). Pasteurella haemolytica complicated respiratory infections in sheep and goats. Veterinary Research, 29 (3-4): 233-254.

Brown, I. R., Rush, S.J. (1996). In vivo activation of neural heat shock transcription factor HSF1 by a physiologically relevant increase in body temperature. J Neurosci Res 44: 52–57.

Chandrasekaran, S., Kamal Hizat, A., Zamri-Saad, M., Johara, M.Y. and Yeap, P.C. (1991). Evaluation of combined pasteurella vaccines in control of sheep pneumonia. *Brit. Vet. J.* 147:437-443.

Chandrasekaran, S., Kennett, L., Yeap, P.C., Muniandy, N., Rani, B., Mukkur, and T.K.S. (1994) Characterization of immune response and duration of protection in buffaloes immunized with haemorrhagic septicaemia vaccine. Vet Microbiol. 41: 213-219.

Clinkenbeard, K.D. and Upton, M.L. (1991). Lysis of bovine platelets by *Pasteurella haemolytica* leukotoxin. *Am. J. Vet. Res.* 47: 1134-1138.

Cole N.A., Metabolic changes and nutrient repletion in lambs provided with electrolyte solutions before and after feed and water deprivation, J. Anim. Sci. 74 (1996) 287-294.

Davies, D.H. and Penwarden, R.A. (1981). The phagocytic cell response of the ovine lung to *Pasteurella haemolytica. Veterinary microbiology.* 6:183-189.

Donachie, W. (1993). Pneumonic pasteurellosis: An update. *Proceedings of 5th. VAM Congress,* pp. 51-52.

Feder, M.E. and Hofmann, G.E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol. 61: 243 282.

Jesse Abdullah, F.F., Tijjani, A., Adamu, L., Teik Chung, E.L., Abba, Y., Mohammed, K., Saharee, A.A. and Haron, A.W. (2015). Pneumonic pasteurellosis in a goat. Iranian Journal of Veterinary Medicine. 8:293-296.

Gerner, E.W. and Schneider M.J. (1975). Induced thermal resistance in HeLa cells. Mature, 256: 500- 502.

Gilmour, N.J.L., Angus, K. W. & Gilmour, J. S. (1991). Diseases of Sheep. pp. 133-9. ed W. B. Martin & I. D. Aitken. Blackwell Scientific Publications.

Gilmour, N, J, L. and Gilmour, J.S. 1989. In: Adlam, C.and Rutter, J.M. (eds) Pasteurella and pasteurellosis, Academic Press, London. 223-262.

Gilmour, N.J.L., Menzies, J.D., Donachie, W. and Fraser, J. 1985. Electronmicroscopy of the surface of *Pasteurella haemolytica*. Journal of Medical Microbiology, 19, 25-34.

Gilmour N.J.L., Thompson D.A., Fraser J., The recovery of *Pasteurella haemolytica* from the tonsils of adult sheep, Res. Vet. Sci. 17 (1974)413-114.

Gilmour, N.J.L. (1993). Pasteurellosis: The disease. *Pasteurellosis in Production Animals.* ACIAR Proceedings No43, pp79-82.

Isabelle Fernandes, Elodie Rousset, Philippe Dufour, Karim Sidi-Boumedine and Anny Cupo. Evaluation of the recombinant heat shock protein B (HspB of as a potential antigen for immunodiagnostic of Q fever in goats. Veterinary Microbiology, Elsevier, 2009, 134 (3-4), pp.300.

Jeffrey D. Hasday and Ishwar S. Singh. 2000. Fever and the heat shock response: distinct, partially overlapping processes. Cell Stress & Chaperones 5:471–480.

Kaoud H, El-Dahshan AR, Zaki MM, dan Nasr SA (2010). Occurrence of *Mannheimia haemolytica* and *Pasteurella trehalosi* among ruminants in Egypt. New York Science Journal, 3(5): 135-141.

Konto & Sadiq, Muhammad Abubakar & Abba, Yusuf & Jesse A, Faez Firdaus & Tijjani, Abdulnasir & Chung, Eric & Adamu, Lawan & Osman, Abdinasir & Mohd Lila, Mohd & Haron, Abd Wahid. (2015). Clinical Management of Pneumonic Pasteurellosis in Kids: A Case Report. International Journal of Livestock Research, 5(4), 100-104.

Knaust A, Weber MV, Hammerschmidt S, Bergmann S, Frosch M, Kurzai O. Cytosolic proteins contribute to surface plasminogen recruitment of Neisseria meningitidis. J Bacteriol. 2007; 189: 3246-3255.

Knowles T.G., Brown S.N., Warriss P.D., Phillips A.J., Dolan S.K., Hunt P., Ford J.E., Edwards J.E., Watkins P.E., Effects on sheep of transport by road for up to 24 hours, Vet. Rec. 136 (1995) 431-438.

Kregel, K.C., 2002. Heat shock proteins: modifying factors in physiology stress responses and acquired thermotolerance. Journal of Applied Physiology, 92: 2177-2186.

Lackie Rachel E., Maciejewski Andrzej, Ostapchenko Valeriy G., Marques-Lopes Jose, Choy Wing-Yiu, Duennwald Martin L., Prado Vania F., Prado Marco A. M. (2017). The Hsp70/Hsp90 Chaperone Machinery in Neurodegenerative Diseases. Frontiers in Neuroscience, 11:1-23. https://doi.org/10.3389/fnins.2017.00254

Lee RWH, Strommer J, Hodgins, Shewen PE, Niu Y, *et al*. (2001) Towards development of an edible vaccine against bovine pneumonic pasteurellosis using transgenic white clover expressing a Mannheimia haemolytica A1 leukotoxin 50 fusion protein. Infect Immun 69: 5786-5793.

Liew, P.K, I. Zulkiflli, M. Hair-Bejo, A.R. Omar and D.A. Israf, 2003. Effects of early age feed restriction and thermal conditioning in heterophil/lymphocyte ratio, heat shock protein 70 and body temperature of male broiler chickens subjected to acute heat stress. Poultry Science, 82: 1879-1885.

Lindquist, S. 1986. The heat shock response. Annual of Review in Biochemistry, 55:1151-1191.

Links, I.J., Searson, J. E., Godwin, J., Glastonbury, J. R., Philbey, A. P. & Matthews, L. M. (1992). Pasteurella multodda and Pasteurella haemolytica infections in ruminants and pigs in Southern New South Wales. In Pasteurellosis in Production

Animals. pp. 108-11. ed B. E. Patten, T. L. Spencer, R. B. Johnson, D. Hoffman & L. Lehane. ACIAR Proceedings No.43, pp108-111.

Loganathan, P. & Chandrasekaran, S. (1992). Clinicopathological changes in goats experimentally infected with Pasteurella multocida and Pasteurella haemo&tica. In Proceedings of the National IRPA Seminar; pp. 20-1. Ministry of Science, Technology and Environment, Malaysia.

Malazdrewich, C., P. Thumbikat & S. K. Maheswaran, 2004. Protective effect of dexamethasone in experimental bovine pneumonic mannheimiosis. Microbial Pathogenesis, 36, 227−236.

Maria, L. (2007) Bacterial Pneumonia in Goats. In: Alabama Cooperative Extension System online publication. www.aces.edu/urban. accessed 1/10/2017.

Martin, W. B., 1996. Respiratory infections of sheep. Comparative Immunology, Microbiology and Infectious Diseases, 19, 171−179.

Mohammed, R.A., Abdelsalam, E.B. (2008) A review on pneumonic pasteurellosis (respiratory mannheimiosis) with emphasis on pathogens virulence mechanisms and predisposing factors. Bulg J. Vet Med. 11: 139-160.

Mohammed, R. A., 2002. The effect of iron compounds and other factors on the pathogenesis of pneumonic pasteurellosis in Nubian goats. Ph.D. Thesis, Faculty of Veterinary Science, University of Khartoum, Sudan.

Morck, D. W., T. G. Raybould, S. D. Acres, A. Babiuk, J. Nelling & J. W. Costerton, 1987. Electron microscopic detection of glycocalyx and fimbriae on the surface of Pasteurella haemolytica. Canadian Journal of Veterinary Research, 51, 83−88.

Mosier, D.A. (1993). Prevention and control of pasteurellosis. In: *Pasteurellosis in Production Animals.* ACIAR Proceedings No43, pp121-134.

Parvathi Anilkumar, Vidya S. Krishnan, Raghava Varman Thampan, Goat endometrial heat shock protein-90 (Hsp-90): Development of an expedient method for its purification and observations on its intracellular movement, Protein Expression and Purification. (2010).Volume 71, Pages 49-53, ISSN 1046-5928, https://doi.org/10.1016/j.pep.2009.11.006. 53. Perdrizet GA. 1995. Heat shock and tissue protection. *New Horiz* 3: 312–320.

Radostits, M., O & C Gay, C & Hinchcliff, Kenneth & Constable, Peter. (2007). Veterinary Medicine. A textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th edn, W. B. Saunders.

Ray PK. 1999. Stress genes and species survival. Mol Cell Biochem 196: 117–123.

Rice, J.A., Carrasco-Medina, L., D. C. Hodgins and P. E. Shewen (2007). Mannheimia haemolytica and bovine respiratory disease. Animal Health Research Reviews 8(2); 117–128.

Ritossa, F. (1962). A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18, 571–573. doi: 10.1007/BF02172188

Sabri, M.Y., Shahrom-Salisi, M., Emikpe, B.O. (2013) Comparison prior and post vaccination of inactivated recombinant vaccine against mannheimiosis in Boer Goats Farm in Sabah. J Vaccines Vaccin. 4:1.

Sabri MY, Zamri-Saad M, Shalisi MS, Misri S (2010) The reduction of mortality in a goat breeding farm in Sabah by inactivated recombinant vaccine. Proceedings of BIT Life Sciences 2nd Annual World Vaccine Congress, Beijing, China, 301.

Schopf, Florian H., Biebl, Maximilian M., Buchner, Johannes (2017). The HSP90 chaperone machinery. Nature Reviews of Molecular Cell Biology, 18: 345–360. doi:10.1038/nrm.2017.20

Swanson J.C., Farm animal well-being and intensive productive systems, J. Anim. Sci. 73 (1995) 2744-2751.

Thomson, R. G., S. Chander, M. Savan & M. L. Fox, 1975. Investigation of factors of probable significance in the pathogenesis of pneumonic pasteurellosis in cattle. Canadian Journal of Comparative Medicine, 39,194−207.

Trevisan, V. (1887), *Rend. 1st. Lombrado (Ser 11)* 20:88-105. Cited by Mutters, R.R., Manheim, W. and Bisgaard, M. (1989). Taxonomy of the group. *In:* Pasteurella and Pasteurellosis, Adlam, C. and Putler, J.M. (eds). *Academic Press, New York.*

Valizadeh A, Pakzad IR and Khosravi A. Investigating the Role of Thermal Shock Protein (Dank) HSP70 in Bacteria. J Bacteriol Mycol. 2017; 4(3): 1055.

Zamri-Saad, M., Azri, A., Nurida, A.B., Sheikh-Omar, A.R. (1994) Experimental Respiratory Infection of Goats with Mycoplasma arginini and Pasteurella haemolytica A2. J Trop Agric Sci. 17:239-242.

Zamri-Saad. M., S. Jansi, A. B. Nurida & O. A. R. Sheikh, 1991. Experimental infection of dexamethasone treated goats with Pasteurella haemolytica "A2". British Veterinary Journal, 147, 565−568.

Zamri-Saad M., W.M.Kamil and A.R. Mutalib (1989). Inablity of an oil adjuvant vaccine to control naturally occurring pneumonic pasteurellosis of pure-bred sheep. *J. Vet. Mal.*1:91-92.

Zamri-Saad M., M.S. Ismail, A. Noraziah, A.R. Bahaman and A.R. Sheikh-Omar (1993). Evaluation of an oil adjuvant vaccine for control of pneumonic pasteurellosis in sheep. In: Pasteurellosis in Production Animalss. Pattern B.E., T.L. Spencer, R.B. Johnson, D. Hoffmann and L. Lahane (eds), ASCIAR Proceedings No43, pp177-179.

Zulkifli, I., M.T. Che Norma, D.A. Israf and A.R. Omar, 2002. The effort of early age food restriction on heat shock protein response in heat-stressed female broiler chickens. British Poultry Science, 43: 117-121.

Zulkiflli, I., P.K. Liew, D.A. Israf, A.R. Omar and M. Hair-Bejo, 2003. Effects of early age feed restriction and thermal conditioning in heterophil/lymphocyte ratio, heat shock protein 70 and body temperature of male broiler chickens subjected to acute heat stress. Journal of Thermal Biology, 28: 217-222.

8.0 APPENDIX

Appendix A: Lung auscultation score and infection status in vaccinated goat farm

ANIMAL ID	AUSCULTATION SCORE (x/40)	SEVERITY OF PNEUMONIA	STATUS
N208	0	0	NORMAL
A17	0	0	NORMAL
102	0	0	NORMAL
417	0	0	NORMAL
R08	0	0	NORMAL
NS201	0	0	NORMAL
322	0	0	NORMAL
320	0	0	NORMAL
V407	0	0	NORMAL
V007	0	0	NORMAL
R09	0	0	NORMAL
NS105	0	0	NORMAL
206	0	0	NORMAL
12562	0.3	MODERATE HARSH	PNEUMONIC
605	0.125	MILD HARSH	PNEUMONIC
617	0.175	MILD HARSH	PNEUMONIC
601	0	0	NORMAL
603	0.175	MILD HARSH	PNEUMONIC

Appendix A (cont.)

ANIMAL ID	AUSCULTATION SCORE (x/40)	SEVERITY OF PNEUMONIA	STATUS
R19	0.15	MILD HARSH	PNEUMONIC
316	0	0	NORMAL
R15	0.05	MILD HARSH	PNEUMONIC
415	0.15	MILD HARSH	PNEUMONIC
638	0.1	MILD HARSH	PNEUMONIC
624	0.075	MILD HARSH	PNEUMONIC
623	0.125	MILD HARSH	PNEUMONIC
686	0.225	MILD HARSH	PNEUMONIC
2D	0.225	MILD HARSH	PNEUMONIC
NS9	0.125	MILD HARSH	PNEUMONIC
A8178	0.7	SEVERE HARSH	PNEUMONIC
14576	0.8	SEVERE HARSH	PNEUMONIC

Appendix B: Lung auscultation score and infection status in Non-vaccinated goat farm

ANIMAL ID	AUSCULTATION SCORE (x/40)	SEVERITY OF PNEUMONIA	STATUS
8034	0.075	MODERATE HARSH	PNEMONIC
2B	0.2	MILD HARSH	PNEMONIC
3B	0.175	SEVERE HARSH	PNEMONIC
4B	0.5	MODERATE HARSH	PNEMONIC
5B	0	0	NORMAL
4207	0.175	SEVERE HARSH	PNEMONIC
7B	0.75	SEVERE HARSH	PNEMONIC
4206	0.775	SEVERE HARSH	PNEMONIC
9B	0.85	SEVERE HARSH	PNEMONIC
10B	0.175	MILD HARSH	PNEMONIC
18018	0.5	MODERATE HARSH	PNEMONIC
12B	0.225	MILD HARSH	PNEMONIC
13B	0.8	SEVERE HARSH	PNEMONIC
14B	0.8	SEVERE HARSH	PNEMONIC
15B	0.15	MILD HARSH	PNEMONIC
8017	0.125	MILD HARSH	PNEMONIC
17B	0.75	SEVERE HARSH	PNEMONIC
18B	0.125	MILD HARSH	PNEMONIC
19B	0.15	MILD HARSH	PNEMONIC
KOH4203	0.15	MILD HARSH	PNEMONIC
21B	0.85	SEVERE HARSH	PNEMONIC
22B	0.225	SEVERE HARSH	PNEMONIC
23B	0.9	SEVERE HARSH	PNEMONIC
24B	0	0	NORMAL

Appendix B (cont.)

ANIMAL ID	AUSCULTATION SCORE (x/40)	SEVERITY OF PNEUMONIA	STATUS
25B	0	0	NORMAL
26B	0.75	SEVERE HARSH	PNEMONIC
27B	0.75	SEVERE HARSH	PNEMONIC
28B	0	0	NORMAL
29B	0	0	NORMAL
30B	0.075	SEVERE HARSH	PNEMONIC
1C	0	0	NORMAL
0144	0	0	NORMAL
0778	0	0	NORMAL
9272	0.125	MILD HARSH	PNEUMONIC
5C	0	0	NORMAL
6C	0.225	SEVERE HARSH	PNEUMONIC
7C	0.75	SEVERE HARSH	PNEUMONIC
8C	0.225	SEVERE HARSH	PNEUMONIC
9C	0.9	MILD HARSH	PNEUMONIC
10C	0.05	MODERATE HARSH	PNEUMONIC
11C	0	0	NORMAL
12C	0	0	NORMAL
13C	0	0	NORMAL
14C	0	0	NORMAL
15C	0	0	NORMAL
16C	0	0	NORMAL