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

DHARSHINI A/P MASLAMANY

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. FaezFirdaus Jesse Bin Abdullah for his guidance, help and undivided attention while doing this project.

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

Sincere thanks to my groupmates (KalaiVaani, Hafizin), staffs of Large Animal Ward, UVH and Mr.MohdJefriNorSidin 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

TITLE……...i

CERTIFICATION……...ii

ACKNNOWLEDGEMENTS……....iv

CONTENTS…….…v

LIST OF TABLES AND FIGURES……...……..vii

LIST OF ABBREVIATIONS……...……...viii

ABSTRAK……..……ix

ABSTRACT……..……xii

1.0 INTRODUCTION……...……1

2.0 LITERATURE REVIEW……..……4

2.1 Pneumonic Pasteurellosis…….....4

2.2 *Mannheimiahaemolytica*……...5

2.3 Clinical Features of Pneumonia…..……...7

2.4 Pathology……..7

2.5 Vaccination……...…….…8

2.6 Role of Stress…….……...10

2.7 Heat Shock Protein…….……..……....11

2.7.1 Hsp70…….…….....11

3.0 MATERIALS AND METHODS….……14

3.1 Sample Population…….……..14

3.2 Lung Auscultation…….....14

3.3 Blood Sampling……....16

3.4 Serum Extraction……..16

3.5 Serological Testing……..16

3.5 Statistical Analysis…… 17

4.0 RESULTS……...…….. 18

4.1 Severity of pneumonia in vaccinated and non-vaccinated pneumonic goats……... 18

4.2 Results of HSP-70 concentration in vaccinated and non-vaccinated pneumonic goats…… 20

5.0 DISCUSSION…...…… 21

7.0 CONCLUSION AND RECOMMENDATION…….. 22

8.0 REFERENCES……..…… 24

9.0 APPENDIX……...…… 32

LIST OF TABLES AND FIGURES

		Page
Figure 1:	Proposed interaction between fever and heat shock response	13
Figure 2:	Mean of severity of pneumonia in pneumonic vaccinated and non-vaccinated goats	19
Figure 3:	Mean of concentration of HSP-70 in pneumonic vaccinated and non-vaccinated goats	20
Table 1:	Summary of sample population	14
Table 2:	Lung auscultation scoring	15
Diagram 1:	illustration of lung field for lung auscultation scoring	15

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 DIJANGKITI 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 ruminant kecil yang disebabkan oleh *Pasteurellamultocida* atau *Mannheimiahaemolytica* 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 pathogen dan meningkatkan rintangan haiwan kepada tekanan kimia. HSP-70 adalah pengadun molekul yang boleh menjadi biomarker penting dalam diagnosis penyakit bakteria dalam ruminant kecil. Kajian terdahulu mengenai pasteurella pneumonia tidak mengenalpasti keterukan pneumonia dan tindakbalas heat shock protein-70 dalam kambing pneumonia yang divaksin

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 lading ruminant 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. Walaubagaimanapun, analisis statistic 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 statistic 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-70CONCENTRATION IN VACCINATED AND NON-VACCINATED PNEUMONIC GOATS

By

DHARSHINI MASLAMANY

2018

Supervisor: Assoc. Prof. Dr. FaezFirdaus Jesse Abdullah

Co-Supervisor: Prof. Dr. Abd Wahid Haron

Pneumonic pasteurellosis is the second most important infectious disease in small ruminant caused by *Pasteurellamultocida* or *Mannheimiahaemolytica*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-70in 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-70analyses 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 *Pasteurellamultocida* or *Mannheimiahaemolytica* 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).

Mannheimiahaemolytica 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.

REFERENCES

Bahaman, A.R., Nurida, A.B., Sheikh-Omar, A.R. andZamri-Saad, M. (1991). Biotypes and serotypes of Pasteurellahaemolytica 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 Pasteurellahaemolytica 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 Pasteurellahaemolytica "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). Pasteurellahaemolytica 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 haemorrhagicsepticaemia 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 *Pasteurellahaemolytica.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 SchneiderM.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 *Pasteurellahaemolytica*. Journal of Medical Microbiology, 19, 25-34.

Gilmour N.J.L., Thompson D.A., Fraser J., The recovery of *Pasteurellahaemolytica* 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, ElodieRousset, 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 *Pasteurellatrehalosi*among ruminants in Egypt. New York Science Journal, 3(5): 135-141.

Konto&Sadiq, Muhammad Abubakar& Abba, Yusuf & Jesse A, FaezFirdaus&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, OstapchenkoValeriy G., Marques-Lopes Jose, Choy Wing-Yiu, Duennwald Martin L., Prado Vania F., Prado Marco A. M. (2017). TheHsp70/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 Mannheimiahaemolytica A1 leukotoxin 50 fusion protein. Infect Immun69: 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). Pasteurellamultodda and Pasteurellahaemolytica 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 Pasteurellamultocida and Pasteurellahaemo&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 Pasteurellahaemolytica. 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, 10thedn, 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 Pasteurellahaemolytica “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.