



**UNIVERSITI PUTRA MALAYSIA**

***SEROPREVALANCE OF SELECTED BACTERIAL AND VIRAL  
DISEASES OF SMALL RUMINANTS IN TERENGGANU, MALAYSIA***

**HASHI HAMZA ABDIRAHMAN**

**FPV 2020 21**



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OF SMALL RUMINANTS IN TERENGGANU, MALAYSIA**

By

**HASHI HAMZA ABDIRAHMAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of  
Master of Veterinary Science**

**July 2020**

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## DEDICATION

To my caring, loving parents, Family, friends and to my country, may Allah award you peace for ever. This thesis is dedicated to them.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

## **SEROPREVALANCE OF SELECTED BACTERIAL AND VIRAL DISEASES OF SMALL RUMINANTS IN TERENGGANU, MALAYSIA**

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**July 2020**

**Chairman : Professor Faez Firdaus Jesse bin Abdullah, PhD**  
**Faculty : Veterinary Medicine**

Small ruminant production is an important economic activity for many smallholder farmers in Malaysia. In the past decade, there has been increased demand for sheep and goat products. However, the development of regional livestock meat and milk trade is threatened by the continuous threat of many infectious diseases of bacterial and viral origin, which limits the productivity of smallholder flocks. This study investigated the seroprevalence and risk factors of selected bacterial and viral diseases of sheep and goats in Terengganu, Malaysia. A cross-section survey was conducted to collect blood samples and farm management data from the selected farms January and December 2018. Blood samples were collected from 180 sheep (n=60) and goats (n=120) from 8 smallholder farms in Terengganu. A structured questionnaire was completed by individual farmers to furnish management data for the analysis of possible risk factors associated with seropositivity to bacterial and viral diseases. In the laboratory, serum was separated from coagulated blood by centrifugation and preserved for specific ELISA assays at -20 °C. Seroprevalence of SBV was determined using commercial ID Vet® SBV multispecies cELISA test. Detection of anti-CAEV antibodies was done using Qayee-Bio caprine arthritis encephalitis virus (CAE) sandwich-ELISA test kit with a sensitivity of 100% and specificity of 99.6%. A commercial Sandwich-ELISA kit (Sunlong) with a sensitivity of 100% and a specificity of 99.6% was used for the direct detection of specific antibody against *Coxiella burnetii*, bluetongue and contagious ecthyma virus. Standard Agar gel precipitation test (AGPT) was used for the detection of antibodies against *Corynebacterium pseudotuberculosis*. The Rose Bengal rapid plate agglutination test was used for the detection of Brucella antibodies. The optical densities (OD) of the tests were measured at 450nm using ELISA Microplate Reader (Tecan Sunrise®, Switzerland) and the percentage inhibitions calculated as  $100 \times [1 - (\text{Sample optical density} / \text{Negative control OD})]$  were interpreted as follows: OD values  $\geq 40\%$  = positive, OD values  $\leq 30\%$  = negative and OD values 30%-40% = doubtful. The result of serological tests indicates 10.6% (95% CI=6.6-16.7) overall seroprevalence of SBV

with different ( $p < 0.05$ ) rates of infection between flocks, species, breed, gender, age, production system and management of small ruminants. The male had 5.26 times higher risk of exposure to SBV than females and young animals had 4.1 times higher risk of infection than adults. Serological analysis also revealed 27.2% (95% CI=22.8-37.3) overall prevalence of contagious ecthyma with different ( $p < 0.05$ ) rates of infection among the flocks, species, breed, gender, production, and management of small ruminants. The goats had 3.1 times more risk of orf infection than sheep while dairy animals had 2.82 times higher odds of orf than the mean and mixed production. The overall seroprevalence of q fever was 16.7% (95% CI=12.3-24.5) with different ( $p < 0.05$ ) rates of infection among flocks, species, breed, age, production, and management system of small ruminants. The species sheep (OR=21.36; 95% CI=2.29-198.41;  $p=0.007$ ), especially Barbados black belly (OR=24.35; 95% CI=2.77-213.76;  $p=0.004$ ) and Dorper sheep (OR=12.88; 95% CI=1.29-128.59;  $p=0.029$ ), and the young age (OR=5.04; 95% CI=1.66-15.31;  $p=0.004$ ) were risk the factors of q fever among small ruminants in the study area. The study further revealed 18.3% (95% CI=13.9-26.6) overall seroprevalence of bluetongue with different rates of infection among the flocks and breed of small ruminants. Flocks D (OR=5.29; 95% CI=1.16-24.08;  $p=0.031$ ), E (OR=8.22; 95% CI=1.46-46.27,  $p=0.017$ ) and G (OR=18.50; 95% CI=2.17-16.46;  $p=0.008$ ) were associated with higher risk of bluetongue in the study area. This study has also revealed 7.2% (95% CI=3.67-12.32) overall seroprevalence of CAEV with no difference ( $p > 0.05$ ) in infection rates among various categories of small ruminants. Our results also showed 5.6% (95% CI=2.3-10.0) overall seroprevalence of CLA with no difference ( $p > 0.05$ ) in infection rates among different categories of small ruminants. Serum antibodies against brucellosis were not detected in this study. This study reports the first occurrence of the novel and emerging transboundary Schmallenberg virus disease in Malaysia. The presence of anti-SBV antibodies in Malaysia indicates the potential effects of disease on small ruminant productivity. This study also revealed an alarming rate of re-emerging bluetongue and zoonotic q fever infections which need to be kept under control through a one health approach. We have also seen a high level of circulating antibodies to orf virus infection among the small ruminant population with a potential to cause a negative impact towards production. The absence of anti-brucella antibodies in small ruminants indicates the effectiveness of current control measures. In conclusion, this study highlights current seroprevalence status of selected bacterial and viral diseases of small ruminant in East Coast Malaysia and reports the first occurrence of novel and emerging SBV and also detected existing threats of re-emerging and zoonotic diseases, which requires a holistic herd health program and biosecurity implementation for effective control and prevention.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

## **SEROPREVELANSI PENYAKIT BAKTERIA DAN VIRUS TERPILIH BAGI RUMINAN KECIL DI TERENGGANU MALAYSIA**

Oleh

**HASHI HAMZA ABDIRAHMAN**

**Julai 2020**

**Pengerusi : Profesor Faez Firdaus Jesse bin Abdullah, PhD**  
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Produksi ruminan kecil adalah aktiviti ekonomi yang penting bagi kebanyakan peladang kecil di Malaysia. Dalam dekad yang lalu, terdapat peningkatan permintaan untuk produksi biri-biri dan kambing. Walau bagaimanapun, perkembangan perdagangan daging dan susu ternakan serantau diancam oleh ancaman berterusan dari banyak penyakit berjangkit yang berasal dari bakteria dan virus, yang membatasi produktiviti peladang kecil. Kajian ini meneliti seroprevalensi dan faktor risiko penyakit bakteria dan virus pada biri-biri dan kambing terpilih di Terengganu, Malaysia. Satu tinjauan telah dilakukan untuk mengumpulkan sampel darah dan data pengurusan ladang dari ladang terpilih pada Januari dan Disember 2018. Sampel darah diambil dari 180 biri-biri ( $n = 60$ ) dan kambing ( $n = 120$ ) dari 8 ladang pekebun kecil di Terengganu. Soal selidik berstruktur telah dilengkapkan oleh petani secara individu untuk menyediakan data pengurusan bagi menganalisa kemungkinan faktor risiko yang berkaitan dengan seroprevalensi terhadap penyakit bakteria dan virus. Di makmal, serum dipisahkan dari darah yang beku dengan cara emparan dan disimpan untuk ujian ELISA tertentu pada suhu  $-20\text{ }^{\circ}\text{C}$ . Seroprevalensi bagi SBV telah ditentukan menggunakan ujian komersil cELISA multispesies ID Vet® SBV. Pengesanan antibodi anti-CAEV dilakukan dengan menggunakan kit ujian sandwich-ELISA virus Qayee-Bio caprine arthritis encephalitis virus (CAE) dengan kepekaan 100% dan kecirian 99.6%. Kit Sandwich-ELISA komersial (Sunlong) dengan kepekaan 100% dan kecirian 99.6% digunakan untuk pengesanan langsung antibodi khusus terhadap *Coxiella burnetii*, bluetongue dan virus ecthyma berjangkit. Ujian piawai pemendakan gel Agar (AGPT) digunakan untuk mengesan antibodi terhadap *Corynebacterium pseudotuberculosis*. Ujian plat aglutinasi Rose Bengal digunakan untuk mengesan antibodi Brucella. Ujian ketumpatan optik (OD) diukur pada 450nm menggunakan ELISA Microplate Reader (Tecan Sunrise®, Switzerland) dan peratusan perencatan dikira sebagai  $100 \times [1 - (\text{Kepadatan optik sampel} / \text{OD kawalan negatif})]$  ditafsirkan seperti berikut: Nilai  $\text{OD} \geq 40\%$  = positif, nilai  $\text{OD} \leq 30\%$  = negatif dan nilai  $\text{OD} 30\% - 40\%$  = data tidak boleh dipercayai. Hasil ujian serologi

menunjukkan 10.6% (95% CI = 6.6-16.7) keseluruhan seroprevalensi SBV dengan kadar jangkitan ( $p < 0.05$ ) yang berbeza antara kawanan, spesies, baka, jantina, umur, sistem pengeluaran dan pengurusan ruminan kecil. Jantan mempunyai risiko pendedahan kepada SBV 5.26 kali lebih tinggi daripada betina dan haiwan muda mempunyai risiko jangkitan 4.1 kali lebih tinggi daripada haiwan tua. Analisis serologi juga menunjukkan 27.2% (95% CI = 22.8-37.3) keseluruhan prevalensi ecthyma berjangkit dengan kadar jangkitan ( $p < 0.05$ ) yang berbeza di antara kawanan, spesies, keturunan, jantina, pengeluaran, dan pengurusan ruminan kecil.

Kambing mempunyai 3.1 kali lebih banyak risiko jangkitan orf berbanding biri-biri sementara haiwan tenusu mempunyai kemungkinan 2.82 orf kali lebih tinggi berbanding purata dan campuran pengeluaran. Keseluruhan seroprevalensi q fever adalah 16.7% (95% CI = 12.3-24.5) dengan kadar jangkitan yang berbeza ( $p < 0.05$ ) di kalangan kawanan, spesies, keturunan, usia, pengeluaran, dan sistem pengurusan ruminan kecil. Spesies biri-biri (OR = 21.36; 95% CI = 2.29-198.41;  $p = 0.007$ ), terutamanya baka Barbados black belly (OR = 24.35; 95% CI = 2.77-213.76;  $p = 0.004$ ) dan biri-biri Dorper (OR = 12.88; 95% CI = 1.29-128.59;  $p = 0.029$ ), dan usia muda (OR = 5.04; 95% CI = 1.66-15.31;  $p = 0.004$ ) berisiko menjadi faktor q fever di kalangan ruminan kecil di kawasan kajian. Kajian ini selanjutnya menunjukkan 18.3% (95% CI = 13.9-26.6) keseluruhan seroprevalensi bluetongue dengan kadar jangkitan yang berbeza di antara kawanan dan keturunan ruminan kecil. Kawanan D (OR = 5.29; 95% CI = 1.16-24.08;  $p = 0.031$ ), E (OR = 8.22; 95% CI = 1.46-46.27,  $p = 0.017$ ) dan G (OR = 18.50; 95% CI = 2.17 -16.46;  $p = 0.008$ ) dikaitkan dengan risiko penyakit bluetongue yang lebih tinggi di kawasan kajian. Kajian ini juga telah menunjukkan 7.2% (95% CI = 3.67-12.32) keseluruhan seroprevalensi CAEV tanpa perbezaan ( $p > 0.05$ ) pada kadar jangkitan di antara pelbagai kategori ruminan kecil. Hasil kajian kami juga menunjukkan keseluruhan seroprevalensi CLA sebanyak 5,6% (95% CI = 2,3-10,0) tanpa perbezaan ( $p > 0,05$ ) pada kadar jangkitan antara kategori ruminan kecil yang berbeza. Antibodi serum terhadap brucellosis tidak dapat dikesan dalam kajian ini. Kajian ini melaporkan kejadian pertama novel dan penyakit virus transboundary Schmallenberg yang muncul di Malaysia. Kehadiran antibodi anti-SBV di Malaysia menunjukkan kemungkinan kesan penyakit pada produktiviti ruminan kecil.

Kajian ini juga memperlihatkan kadar yang membimbangkan mengenai jangkitan bluetongue dan zoonotik q fever yang perlu dikawal melalui pendekatan satu kesihatan. Kami juga telah melihat peredaran antibodi yang tinggi terhadap jangkitan virus orf di kalangan populasi ruminan kecil yang berpotensi menyebabkan kesan negatif terhadap pengeluaran. Ketiadaan antibodi anti-brucella pada ruminan kecil menunjukkan keberkesanan langkah-langkah kawalan semasa. Sebagai kesimpulan, kajian ini memfokuskan status seroprevalensi terkini penyakit bakteria dan virus ruminan kecil yang terpilih di Pantai Timur Malaysia dan melaporkan kejadian pertama dan kemunculan SBV yang muncul dan juga mengesan ancaman penyakit-penyakit semula jadi dan zoonosis yang ada, yang memerlukan program kawalan holistik kesihatan dan pelaksanaan biosekuriti untuk kawalan dan pencegahan yang berkesan.



## ACKNOWLEDGEMENTS

Firstly, I would like to thank Almighty Allah because this thesis would not have been completed without his spiritual guidance and for blessing me with all those wonderful people whom I have met.

I would like to express my special appreciation and gratitude to my supervisor, Prof. Dr. Faez Firdaus Jesse Bin Abdullah for his kindness, guidance, support, and understanding. I am also grateful to my co-supervisors, Prof. Dato Dr. Mohd Azmi Mohd Lila, Prof. Dr. Wahid Haron and Dr. Eric Lim Teik Chung for their guidance and total support to the success of my thesis.

I am also grateful to my fellow postgraduate students who have helped me in doing the research and interpretation of data in this study. I am most especially grateful to my Brothers, Mr Mohd Jefri Norsidin and Dr Paul Bura Thlama, for their support in the pursuit of this research objectives.

I am extremely grateful to my lovely parents, family and friends who were endlessly and tireless in supporting and helping me whenever I needed them. Finally, I am sincerely grateful to all those who showed their concern and encouraged me in accomplishing this project during my years of study in Universiti Putra Malaysia.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

DVS	Depart ment of Veterinary Services
GDP	Gross Domestic Production
Q-Fever	Query Fever
CAE	Caprine Arthritis Encephalitis
SBV	Schmallenberg Virus
CLA	Caseous Lymphadenitis
AKAV	Akabane Virus
RNA	Ribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
CNS	Central Nervous System
Rt-QPCR	Real time Quantitate Polymerase Chain Reaction
FLI	Friedrich Loeffler Institute
Rt-PCR	Real Time Polymerase Chain Reaction
VNT	Virus Neutralisation Test
BHK	Baby Hamster Kidney
DNA	Deoxyribonucleic Acid
PPV	Parapoxvirus
CE	Contagious Ecthyma
CPD	Contagious Pustular Dermatitis
CDC	Center For Disease Control
FMD	Foot and Mouth Disease
AGPT	Agar Gel precipitation Test
EM	Electron Microscopy

CFT	Complement Fixation Test
SNT	Serum Neutralisation Test
PCR	Polymerase Chain Reaction
RFLP	Restricted Fragments Length Polymorphism
OIE	International Epizootics Office
VP	Viral Proteins
DS	Double Stranded
BTV	Bluetongue Virus
mRNA	Messenger Ribonucleic Acid
NSP	Non-Structural Protein
MAP	Mitogen Activated Protein
TNF	Tumour Necrosis Factor
IL	Interleukin
IFN	Interferon
IgE	Immunoglobulin E
AA	Aedes Albopictus
CAEV	Caprine Arthritis Encephalitis Virus
OPP	Ovine Progressive Pneumonia
MV	Maedi-Visna
AGID	Agar Gel Immunodiffusion Test
WB	Western Blot
CLA	Caseous Lymphadenitis
rRNA	Ribosomal Ribonucleic Acid
NM1	Nine Miles 1

NM2	Nine Miles 2
OMPA	Outer Membrane protein A
CCV	<i>Coxiella</i> Contagious Vacuole
RAB	Ras-Related Protein
IFA	Indirect Fluorescence Antibody
EDTA	Ethylene Diamine Tetra Acetic acid
IFA	Immunofluorescence Assay
FAO	Food and Agricultural Organization
MRT	Milk Ring Test
RBT	Rose Bengal Test
MAT	Micro agglutination Test
RBPT	Rose Bengal Plate Test
SATT	Serum Agglutination Tube Test
UPM	Universiti Putra Malaysia
IACUC	Institutional Animal Care and Use Committee
PLD	Phospholipase D

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of the study

The population of small ruminant in Malaysia was formerly 227,800 sheep and 258,220 goats in 1998 (Chandrawathani, 1998) but currently has 368,080 goat and 133,478 sheep in 2017 (DVS, 2019). The predominant breed of goats in Malaysia includes indigenous Katjang and imported Jamnapari, Anglo Nubian, Alpine, Saanen, Toggenburg, Australian Feral and the Boer. The sheep breeds include the local Malin and exotic Dorset Horn, Wiltshire, Suffolk, Romney, Commercial Merino/Border Leicester crosses, Barbados Black Belly and Santa Ines (Chandrawathani, 1998). The Malaysian small ruminant industry is typically smallholder semi-intensive system in which individual flocks (5-50 animals) are kept in elevated houses at night and allowed limited grazing during the day and given feed supplements.

Due to the high local demand for mutton and goats' milk in Malaysia, the small ruminant production is fast becoming an attractive enterprise, making significant contributions to the gross domestic products (GDP) and livelihood of farmers in the country (Melissa et al., 2016). However, many issues are challenging small ruminant productivity in the country, among which disease is a significant factor. The significant economic diseases of small ruminants in Malaysia include pneumonic pasteurellosis, endoparasites, mange, contagious ecthyma, melioidosis, blue tongue and caseous lymphadenitis (Chandrawathani, 1998; Abdullah et al., 2013). Other diseases such as Q fever, Schmallenberg virus disease, caprine arthritis encephalitis virus and brucellosis are also emerging threats to small ruminant production in Malaysia (Noordin et al., 2010; Abdullah et al., 2018).

Brucellosis caused by *Brucella melitensis* in small ruminants causes abortion in females and orchitis, epididymitis and arthritis in males (Mantur et al., 2008) leading to infertility, high mortality in kids and lambs, mastitis, reduced milk production (Seifert, 1996; Quinn et al., 1999). Q fever is caused by *Coxiella burnetii*, which is an obligate intracellular bacterium with a Gram-negative reaction (Babudieri, 1959; Barlow et al., 2008). The disease is mostly asymptomatic (Kersh et al., 2013), but coxiellosis is associated with abortion and reproductive disorders in domestic animals (Agerholm et al., 2013). Caseous lymphadenitis is recognised as an economically significant chronic zoonotic disease of small ruminants caused by *Corynebacterium pseudotuberculosis* (Dercksen et al., 2000; Abdullah et al., 2013).

Contagious ecthyma is caused by orf virus in the family Poxviridae (Spyrou & Valiakos 2015). Clinical signs of the disease include the formation of erythematous spots, followed by papules, vesicles, pustules, and ultimately scab form around the mouth, nose, feet of animals, and teats of lactating females (Spyrou & Valiakos 2015). Bluetongue is an arthropod-borne viral disease (Maan et al., 2012) caused by

Bluetongue virus in the family Reoviridae, which has 27 serotypes worldwide (Maan et al., 2007; Bhanuprakash et al., 2009). Disease in small ruminants is associated with morbidity and mortality in some cases (Shoorijeh et al., 2010; Chatzopoulos et al., 2015). Caprine arthritis encephalitis (CAE) is a chronic disease of goats caused by a lentivirus (Plaza et al., 2009; Gregory et al., 2009). Infection shows primarily as painful arthritis and or encephalomyelitis (McGuire et al., 1984; de Cardona et al., 2016) and is characterised by significant economic loss due to decreased production in milk quality and quantity (Carneiro, 2011). Occasionally, synovitis, mastitis, pneumonia, reduced growth rates may be manifested in CAE virus infection (Greenwood et al., 1995). Schmallenberg virus (SBV) infection is an emerging arthropod-borne infectious disease of ruminants originated from Europe and spreading to other continents through livestock trade. The disease leads to stillbirth, or the birth of severely malformed offspring with congenital malformations such as scoliosis, torticollis, kyphosis, lordosis, hydrocephalus, arthrogryposis, ankylosis, brachygnathia and hypoplasia of the cerebellum (Kitano et al., 1994).

Clinical diagnosis of brucellosis is routinely achieved by isolation and identification using the phenotypic characteristics of the bacteria. Additionally, organisms can be detected using molecular, immunological, and serological tests (Quinn et al., 1994). The diagnosis of CLA relies on the presence of characteristic clinical symptoms, the isolation bacteria from skin abscess, and serological tests such as ELISA, complement fixation test, haemolysis inhibition test and immunodiffusion test (Cetinkaya et al., 2002; Jesse et al., 2011). Since there are no specific clinical signs of *Coxiella burnetii* infection, the only option is laboratory diagnosis. Because *Coxiella burnetii* is highly infectious, biosafety level 3 laboratory and experienced personnel are required to achieve diagnosis (Marrie et al., 1995). The diagnosis of CAE relies on clinical manifestation, post-mortem examination, histopathological findings, and serology, which are easy and efficient techniques (Waseem et al., 2015). The diagnosis of orf contagious ecthyma depends on the presence of scabby skin lesions around the mouth, legs or teats, histopathology of skin lesions, and serological testing for the presence of antigen or antibody and molecular characterisation of the virus (Kumar et al., 2015; Sadiq et al., 2017). The diagnosis of SBV infection is usually challenging to veterinarians because the infection is subclinical and often confused with other viral diseases such as bovine-herpes virus type 1, bluetongue virus and foot-and-mouth disease virus (Hoffmann et al., 2012).

## 1.2 Statement of the problem

Small ruminant meat and milk production is constrained by the prevalence of viral and bacterial diseases in Malaysia. Economic losses associated with morbidity, mortality, and cost of treatment of diseases significantly affect food security and the contributions of small ruminant production to the Agricultural GDP of Malaysia. Infectious diseases caused by bacteria, virus and parasites are common causes of morbidity and mortality (Jesse et al., 2013, 2018; Mohammed et al., 2016), reproductive failure and economic losses in small ruminant production in Malaysia (Chung et al., 2019). Outbreaks of diseases are the major factors threatening the sustainability of sheep and goat production in Malaysia. Previous studies in Malaysia

have documented the prevalence of orf, CAEV, bluetongue, q fever CLA and brucellosis among small ruminants and in some cases isolated the agents of these diseases. However, SBV is an emerging arthropod-borne pathogen of domestic and wild ruminants responsible for devastating economic losses in European countries with a potential for spreading across boundaries. The incidence of CAE is reported worldwide as a significant cause of morbidity and decreased milk production in dairy goats. Q fever also occurs worldwide and is recognised as an essential zoonosis affecting farmers, butchers, and veterinarians. These studies highlighted the prevalence of bacterial and viral diseases of small ruminants and their public health importance in Malaysia. However, there is no current published information on the seroprevalence status and risk factors of these diseases among smallholder sheep and goat flocks in Malaysia. Moreover, serological monitoring is a periodic exercise used to keep track of disease events in the national livestock population. Serological monitoring provides basic information about the level of herd immunity and suggests if there is an exposure to new infection in a population.

### **1.3 Objectives of the study**

The objectives of this study were to;

1. determine seroprevalence of common viral diseases of small ruminant in the state of Terengganu Malaysia.
2. determine seroprevalence of common bacterial diseases of small ruminant in the state of Terengganu Malaysia.
3. study the risk factors of the common viral and bacterial disease cause of small ruminant in the state of Terengganu Malaysia.

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