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SEROPREVALENCE OF ORF VIRUS INFECTION AMONG SMALL RUMINANTS IN UPM'S FOSTER FARMS BASED ON IgG ANTIBODY DETECTION

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Seroprevalence of Orf Virus Infection Among Small Ruminants in UPM’s Foster Farms Based on IgG Antibody Detection”, by Lim Cheng Yi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project

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DEDICATION

This project paper is dedicated

To my parents,

For their never ending support and affection

To my sisters,

For their humor

To my late grandmother,

As a constant reminder that selfless acts of kindness matters

And finally,

To Google Scholar and the Microsoft Word experts,

For saving a computer illiterate's nerves.

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LIST OF ABBREVIATIONS

%	Percent
˚C	Celsius
kg	Kilogram
uL	Microlitre
ml	Milliliter
nm	Nanometer
O.D.	Optical density
rpm	revolutions per minute
ELISA	Enzyme-Linked Immunosorbent Assay
IgG	Immunoglobulin G
UPM	Universiti Putra Malaysia
et al.	*et alli* (and others)

ABSTRAK

Abstrak daripada kertas kerja yand dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Putra Malaysia untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Ilmiah Tahun Akhir.

KAJIAN SERUM JANGKITAN VIRUS ORF DALAM TERNAKAN RUMINAN KECIL DARI PROGRAM LADANG ANGKAT UPM BERASASKAN PENGESANAN ANTIBODI IgG

Oleh

Lim Cheng Yi

2017

Penyelia: Prof. Dato' Dr. Mohd Azmi Mohd Lila

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Penyakit ektima menular merupakan penyakit berjangkit yang disebabkan oleh virus Orf yang bercirikan luka berkeruping terutamanya di bahagian hidung dan mulut. Ia mendatangkan kerugian ekonomi yang besar akibat tumbesaran haiwan yang terlibat terbantut dan sejurusnya dilupuskan. Buat masa ini, Malaysia kekurangan maklumat mengenai status jangkitan jangka panjang Orf dalam negara. Tujuan kajian ini adalah untuk mengesan antibodi IgG terhadap virus Orf dalam kalangan ternakan biri-biri dan kambing dari Program Ladang Angkat UPM. Faktor risiko terlibat dalam jangkitan

Orf juga ditaksirkan. Sampel serum 90 biri-biri dan 90 kambing; bersama dengan perihal maklumat berkaitan diambil dari 5 ladang yang dipilih secara rawak. Sampel serum disimpan dalam suhu $-20^{\circ}C$ dan sejurusnya dijalankan ujian kualitatif Enzyme-Linked Immunosorbent Assay (ELISA) test. 12.22% populasi biri-biri dan 14.44% populasi kambing didapati sudah dijangkiti virus Orf. Bagi data biri-biri, analisis statistik menunjukkan bahawa terdapat perbezaan yang signifikan ($p<0.05$) dalam kadar prevalens menurut perbezaan jantina, umur dan ladang. Kadar prevalens bagi jantan lebih tinggi daripada betina. Haiwan muda menunjukkan kadar prevalens yang lebih tinggi daripada haiwan dewasa. Ladang yang dikendalikan secara tidak memuaskan menunjukkan kadar prevalens yang paling tinggi ketika dibandingkan dengan ladang lain. Kesimpulannya,, jangkitan jangka panjang Orf boleh didapati dalam ternakan biri-biri dan kambing dari Program Ladang Angkat UPM; di mana kadar prevalens Orf dalam populasi kambing adalah lebih tinggi daripada biri-biri.

Kata kunci*: Penyakit ektima menular, Orf, kadar prevalens, factor risiko, IgG, ELISA*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Final Year Project

SEROPREVALENCE OF ORF VIRUS INFECTION AMONG SMALL RUMINANTS IN UPM'S FOSTER FARMS BASED ON IgG ANTIBODY DETECTION

By

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2017

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Contagious ecthyma is an infectious disease caused by Orf virus; characterized by scabby lesions at the nostrils and mouth regions. It results in huge economic losses due to stunted growth or slaughter of the affected animals. There is inadequate information on the status of long-term Orf infection among small ruminants in Malaysia. This study aimed to detect the IgG antibodies against Orf virus infection in goats and sheep of selected UPM's Foster Farms. Associated risk factors of Orf infection were also assessed. Serum samples of 90 sheep and 90 goats, together with relevant historical information were obtained from 5 randomly selected farms. Serum samples were stored at -20˚C and subjected for qualitative Enzyme-Linked Immunosorbent Assay (ELISA) test. It was found that 12.22% sheep and 14.44% goat population were already infected by Orf. In sheep, statistical analysis indicated there was a significant difference ($p<0.05$) in prevalence rate among genders, ages and farms. The prevalence rate in males was higher than in females. Young animals showed higher prevalence than in adults. Poorly managed farm was the highest compared to other farms. In conclusion, Orf infection is present in sheep and goats from UPM's Foster Farms with prevalence rate in goats higher than in sheep.

Key words: *Contagious ecthyma, Orf, risk factor, prevalence rate, IgG, ELISA*

1.0 INTRODUCTION

Contagious ecthyma is an infectious skin disease caused by Orf virus distinctively characterized by scabby lesions at facial regions such as the nostrils and mouth. It is also known as contagious pustular dermatitis, scabby mouth, sore mouth or Orf (Fleming *et al.,* 2015). Orf should not be mistaken as Foot and Mouth Disease or Bluetongue Disease as symptoms appear similar (McInnes, 2010). It has a wide host range affecting sheep, goats and other wild animals such as alpacas, camels and reindeer.

The morbidity of the disease can reach up to 100% and mortality due to secondary causes may reach 15% (Ramesh *et al.*, 2008; Bora *et al.,* 2012). Secondary causes are such as lesions on the mouth in offspring interfering with suckling; leading to death due to starvation (Nandi, 2011). Weight loss have been reported in survived offspring. Marketability of sheep and goats for trading or slaughtering for meat purposes also declines due to nasty dermatological lesion.

It also has zoonotic concern as humans can also be infected through open wounds and cuts (Buttner & Rhiza, 2002). Lesions are caused by direct inoculation of infected material and usually develops locally at the hands. The occurrence is high among farm personnel during lambing, docking, shearing or slaughtering of positively infected animals (Nandi *et al.*, 2011).

According to Nandi *et al.*, vaccination is the only choice to control the Orf effectively. Along with strict sanitation practise, vaccination reduced the disease to none by 1969 in Egypt (El-Dahaby *et al.*, 1969). However, the use of vaccine is controversial as outbreaks still occurred in vaccinated animals (Kumar *et al.*, 2015).

A study was performed in Assam to determine the seroprevalence of contagious ecthyma in goats (Mousumi *et al.*, 2016) using traditional indirect Enzyme Linked Immunosorbent Assay (ELISA). Sandwich ELISA is proven to be a better alternative as samples do not have to be purified prior analysis. Currently, there has been increasing number of commercial ELISA pair sets built on sandwich ELISA.

There has been reported Orf disease outbreak throughout the years in Malaysia (Zamri *et al.*, 1992; Abdullah, 2015). However, there is inadequate information on seroprevalence for past infection of Orf among small ruminants in Malaysia. Immunoglobulin G; or better known as IgG can be used as a recognition tool to determine if animals have been exposed to the same antigen before; henceforth enabling detection of past infection.

For this research, the following hypotheses were proposed:

1. The goats and sheep in UPM's Foster Farms, Malaysia have previous antibody against Orf.
2. There are seroprevalence of Orf according to several risk factors among small ruminants in UPM's Foster Farms, Malaysia.

Therefore, the objectives of this study are:

1. To detect the presence of past IgG antibodies against Orf virus in goats and sheep in UPM's Foster Farms, Malaysia.
2. To identify the seroprevalence of Orf according to several risk factors among small ruminants in UPM's Foster Farms, Malaysia.

2.0 LITERATURE REVIEW

2.1 Orf Virus

Orf virus is a species of linear double stranded DNA virus taxonomically classified under the genus Parapoxvirus, in the family of Poxviridae. Similar to other members of the same genus, it is ovoid in shape with regular spiral arrangement of surface tubules. The virion has a complex structure with a core, lateral bodies, outer membrane and an envelope (Knowles, 2011). It is globally distributed with an incidence of up to 90% (Essbauer *et al.*, 2010). Genetic variability of Orf virus strains could be due to geographic locations and animal hosts (Li *et al.*, 2012). A study carried out in Malaysia showed that the Malaysian strain has close homology to the Indian and Chinese virus isolates (Abdullah *et al.*, 2015).

2.2 Contagious Ecthyma

Contagious ecthyma caused by Orf virus affects primarily in sheep and goats. There is an economic concern related to loss of young animals and weight loss (Hagis & Ginn, 2001). There has also been reports of cases of Human Orf in various parts of the world; making the disease a zoonotic concern (Georgiades *et al.*, 2005).

2.2.1 Clinical signs

Lesions are developed at the facial region especially at the lip commissure, oral mucosa and nostrils. In a span of time, the lesion progresses from small erythematous papules to larger merging papules which can be ulcerated (McElroy *et al.*, 2007). Ulceration can lead to exudation; thus creating pustules and scabs; giving it a scabby appearance (Nandi *et al.*, 2011). A secondary bacterial infection from opportunistic bacteria such as *Streptococci, Staphylococci*, or *Corynebacterium* (Haig, 2006) can lead to even complicated wounds. Mortality rate is usually low; but it can be high when secondary infection takes place (Gelaye *et al.,* 2016). Lesions are mild and healed rapidly when there are no secondary infections (Zamri *et al.*, 1993). Lesions can also be found in other body regions such as the teats, perineal region and feet.

2.2.2 Transmission & Pathogenesis

The entry of agent occurs when there's a breakthrough of skin via cuts, wounds or abrasion; hence establishing the infection. Virus penetrates in the mucosal layer of skin; leading to degeneration of spinose cells, hyperplasia of basal cells, oedematous and granulomatous dermatitis (Nandi *et al.,* 2011). Studies have shown that it can be due to spiky weeds in pastures (De La Concha-Bermejillo *et al.*, 2003), use of contaminated gavage feeding tube (Moore *et al.*, 1983), ear tagging (Housawi & Elzein, 1992) or aerosol transmission (Bowden *et al.*, 2008).

2.2.3 Epidemiology & Risk Factors

The etiological agent has a short incubation period with reported clinical cases 2 to 3 days after exposure sheep and goats. (The Center for Food Security & Public Health, 2015). Lambs and kids were found to have higher probability in contracting the disease compared to adults (Nandi *et al.,* 2011) as outbreaks are more common during lambing and kidding season. However, all age groups are still equally affected (Bouznach *et al.,* 2013). Dry environment is favourable for the virus and it can survive up to months and even years. However, lifespan is reduced in cold and damp conditions.

2.3 Immunoglobulin G (IgG)

2.3.1 Antibody

Antibody; in other words, immunoglobulin (Ig) is a large protein that bind to specific antigen on its antibody surface to induce an immune response. It is produced by a specialised group of white blood cells known as B lymphocytes. The Y structured protein consists of 4 polypeptide chains-2 identical light chains and 2 identical heavy chains linked by disulphide bonds. There are 2 regions of interest; namely the constant region and variable region. The constant region will regulate the mechanism used for antigen destruction. As for the variable region, it is further classified into hypervariable and framework region. Hypervariable region plays an important role in allowing specific antibody molecule to bind a specific antigen. There are 5 classes of antibody which can be found in the serum; namely IgG, IgM, IgA, IgD and IgE.

2.3.2 IgG and Past Infection

IgG can be split into 4 subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own biologic properties (Schroeder *et al.*, 2010). IgG molecules is made up of heavy chains known as γ-chains where it is found in blood and extracellular fluid The antibody controls infections in the body by binding to various pathogens such as virus, bacteria and fungi. It is one of the most abundant proteins in human serum, accounting for about 10–20% of plasma protein (Vidarsson *et al.*, 2014) and is mainly produced as a later response to a disease. IgG appears in both primary and secondary immune response; but higher titre is found in the latter.

2.4 Diagnostic Methods of Orf Virus

Infection in animals are commonly diagnosed according to history, clinical signs and post mortem lesions. Although clinical signs of Orf are distinct, further diagnosis is still required for confirmation and epidemiological investigations (Nandi *et al.*, 2011). Diagnosis can be confirmed via diagnostic tests which can be grouped into 3 categories; direct detection, virus isolation and serology.

Direct detection methods of Orf virus are electron microscopy of scabs, histopathology, virus isolation and Polymerase Chain Reaction (PCR). According to Tsai et al. (2009), a loop-mediated isothermal amplification (LAMP) assay approach allows easy, rapid, sensitive and specific detection of infection with Orf virus; making it suitable for large scale screening.

Virus isolation is performed on cell cultures or embryonated eggs; however uncommonly used due to difficult isolation and slow growth.

Serological tests used are serum neutralization, agar gel immunodiffusion (AGID), Enzyme-Linked Immunosorbent Assay (ELISA), complement fixation and agglutination.

2.5 ELISA

ELISA is an immunological assay technique that uses an enzyme linked to an antigen or antibody as a marker for the detection of a specific protein (Lequin, 2015).

It can be subdivided into four types; the direct ELISA, indirect ELISA, sandwich ELISA and competitive ELISA.

2.5.1 Sandwich ELISA

Sandwich ELISA; which could be direct or indirect in principle captures and detects antigen with 2 antigen-specific antibodies; making it highly flexible and specific. Unlike the traditional direct and indirect ELISA, it also has higher sensitivity as it overcomes problems arise from complex samples having containing additional proteins adsorbing to the well (Grange *et al.*, 2014). Hence, purification prior to measurement is unnecessary.

3.0 MATERIALS AND METHODS

3.1 Farm Data Collection

Information on basic management practices performed by the owners in each UPM's Foster Farms were obtained through a short interview session and observation by the interviewee.

3.2 Study Herd

90 sheep and 90 goats were selected via random sampling from 5 UPM's Foster Farms in a span period of two weeks.

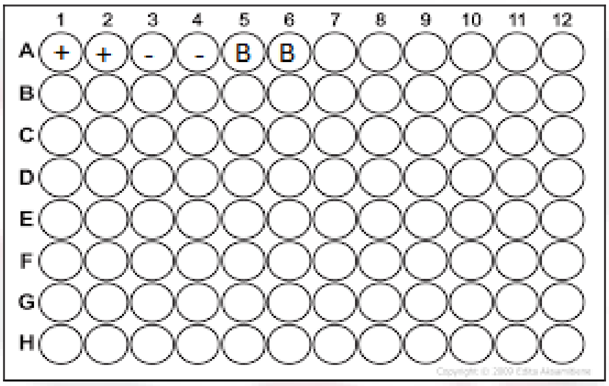
3.3 Sampling Technique

Whole blood samples were obtained via venipuncture from the jugular vein using 21G vacutainer and stored in red plain tubes. The blood filled tubes were stored in a transport cooler box.

3.4 Serum Extraction and Storage

The blood samples were taken to the laboratory and left to clot. It was then centrifuged at 10,000rpm for 5 minutes to separate the serum from the blood clot. Then, the serum was pipetted out with a clean pipette and filled into two 1.5ml Eppendorf tubes. The tube filled serum samples were kept at- 20˚C and -80˚C respectively until subjected to indirect Sandwich-ELISA test.

3.5 ELISA test



Legend
+: Positive control - : Negative control B: Blank control Remaining wells: Sample 1-90

Figure 1: 96-well Microtiter plate (SunLong Biotech Co., LTD)

The sheep Microelisa strip plate was prepared such as in Figure 1. 50µL of negative and positive control were added to the negative and positive control wells respectively. In the remaining 90 sample wells, 40µL of sample dilution buffer and 10µL serum sample were added. Each sample was mixed well with gentle shaking. After sealed with closure plate membrane, the Microelisa strip plate was incubated for 30 minutes at 37˚C.

While left incubated, the concentrated washing buffer was diluted with distilled water (30 times for 96T). The closure plate membrane was carefully peeled off. The wash solution was aspirated and refilled. The wash solution was discarded after resting for 30 seconds. The washing procedure was repeated for 5 times. 50µL of HRP-Conjugate reagent was added into each well except the blank control well. The incubation and washing procedure were repeated.

For colouring, 50µL of Chromogen Solution A and 50µL Chromogen Solution B were added into each well, mixed with gentle shaking in a dark room and incubated for 15 minutes at 37˚C. For termination of reaction, 50 µL of stop solution was added into each well. A colour change from blue to yellow was observed in the well. At 450nm, the absorbance O.D. was read using a Microtiter Plate Reader within 15 minutes after adding stop solution. The same method was performed on goat samples.

3.6 Result interpretation

The effectiveness of the test kit was confirmed as the average value of positive control was more than 1.00. The cut off value was obtained by the following formula:

Cut off (CO) value= the average value of 2 negative controls + 0.15

From the reading, OD values which were less than the CO value were interpreted as negative for presence of IgG; whereas OD values which were more than the CO value were interpreted as positive for presence of IgG. Similar method was performed on sheep samples.

3.7 Data analysis

The data collected was analysed using Chi Square test with IBM SPSS Statistics Version 22.

4.0 RESULTS

Side note: Prevalence rate in this study is based on the presence of IgG antibody in the samples of sheep & goat population.

4.1 Prevalence rates for past infection according to species

From the seroprevalence test performed, the sheep and goat group showed a prevalence rate of 12.22% and 14.44% respectively. The prevalence rate in goats is higher than sheep. The results are shown in Table 1.

Table 1: Prevalence rate according to species

Species	Sheep	Goats
Total samples	90	90
Total positive samples	11	13
Prevalence rate (%)	12.22	14.44

4.2 Prevalence rate based risk factors

i. Gender

In sheep, the prevalence rate in males (30.77%) is higher than in females (9.09%). On statistical analysis using Chi square, a significant difference ($p<0.05$) was detected. Hence, there is association between gender and prevalence rate. In goats, the prevalence rate in females (15.11%) is higher than in males (0%). No significant difference ($p>0.05$) is detected, Thus, there is no association between gender and prevalence rate.

Table 2: Prevalence rate based on gender in each species

Species	Sheep		Goats	
Gender	Male	Female	Male	Female
Total samples	13	77	4	86
Total positive samples	4	7	0	13
P value	0.027		0.258	
Prevalence rate (%)	30.77	9.09	0	15.11

ii. Age

In sheep, the prevalence rate in young (24.14%) is higher than adult (6.56%). When Chi square was used, a significant difference ($p<0.05$) was detected. Hence, there is association between age and prevalence rate. In goats, the prevalence rate in adult (15.11%) is higher than young (0%). No significant difference ($p>0.05$) was detected. Thus, there is no association between age and prevalence rate.

Table 3: Prevalence rate based on age in each species

Species	Sheep		Goats	
Age group	Young	Adult	Young	Adult
Total samples	29	61	4	86
Total positive samples	7	4	0	13
P value	0.017		0.258	
Prevalence rate (%)	24.14	6.56	0	15.11

iii. Clinical signs

In sheep, the prevalence rate in presence of clinical signs (33.33%) is higher than in absence of clinical signs (11.76%). Based on Chi square statistical analysis, no significant difference ($p>0.05$) was detected. Hence, there is no association between clinical signs and prevalence rate. In goats, the prevalence rate for absence of clinical signs (13.33%) is higher than in absence of clinical signs (0.00%). No significant difference ($p>0.05$) was detected. Thus, there is no association between clinical signs and prevalence rate.

Table 4: Prevalence rate based on clinical signs in each species

Species	Sheep		Goat	
Clinical signs	Absent	Present	Absent	Present
Total samples	85	5		90
Total positive samples	10	1	12	0
P value	0.487		0.377	
Prevalence rate (%)	11.76	33.33	13.33	0.00

iv. Farms

In sheep, the prevalence rate is the highest in Farm 3 (35.3%). Upon statistical analysis, a significant difference ($p<0.05$) was detected. Hence, there is association between farm and prevalence rate.

In goats, the prevalence rate is the highest in Farm 4 (25%). No significant difference (p>0.05) was detected. Thus, there is no association between farm and prevalence rate.

Table 5: Prevalence rate based on farms in each species

Species	Sheep					Goat				
Farms	1	2	3	4	5	1	2	3	4	5
Total samples	41	31	17	0	0	11	12	26	20	21
Total positive samples	2	3	6	0	0	1	0	6	5	1
P value	0.021					0.657				
Prevalence rate (%)	4.88	9.68	35.3	0	0	9.09	0	23.08	25	4.76

5.0 DISCUSSION

In general, there is presence of Orf infection among sample population; where the prevalence rate in goats is higher than in sheep. Within a social grouping, goats exhibit more aggression and compared to sheep. Hence, goats will demonstrate a social dominance within a social grouping. Unlike sheep, most goats are naturally horned (Sheep 101.*info*, 2015). When there is fight among goats especially in intensively managed production system (Orgeur *et al.*, 1990), the horn may cause a cut or abrasion leading to inoculation of an existing Orf virus from the environment. According to Popma et al. (2007), the incidence of aggressive behaviour increases with increasing number of animals in a pen. The natural inquisitive behaviour in goats can also increases risk of injury.

From the results, it is revealed that the prevalence rate based on gender, age and farm in sheep are significant. On the contrary, the prevalence rate based on clinical signs is not significant.

The prevalence rate in males appears to be higher than in females. This may be due to the aggression linked to higher testosterone levels (Archer, 1991); putting them in a risk of fighting trauma. Males are usually kept for reproduction purposes especially in a dairy farm for a few years until there is a decline in fertility. There is a risk of having previously infected with Orf virus; however, have resolved throughout the years.

The prevalence rate in young is higher than in adults. Lambs were found to have higher probability in contracting the disease compared to adults (Nandi *et al.*, 2011) as outbreaks are more common during lambing season. The immunity of offspring is still underdeveloped and rely mostly on maternal antibody for protection. Since there is no local vaccine currently available and vaccination is not practised, both the dam and young are not protected against an Orf infection.

There is no association between clinical signs and prevalence rate in sheep because the clinical signs are usually associated to a recent infection. According to Zamri *et al*. in 1994, goats will usually develop more severe lesions compared to sheep. In other words, scabby like lesions is less prominent or noticeable in sheep. Hence, clinical signs may be unreliable as a first line of diagnosis of an Orf infection in sheep. Apart from that, prevalence rate measured in this study is based on presence of IgG antibody; a parameter for detection of a past infection. Clinical signs are more commonly found in the first exposure compared to secondary or subsequent exposure. This is because primary immune response takes a longer time to establish immunity compared to secondary immune response.

The prevalence rate is the highest in Farm 3 as the farm had a recent outbreak of Orf infection; resulting from breach in biosecurity. Importation and trading of sheep of unreliable sources has been suggested to be the reason of outbreak. Hence, this shows that there is poor Herd Health Programme (HHP) compliance by the farmer. According to a survey by Jesse et al. in 2015, small ruminant farm personnel in Malaysia have inept knowledge and skill required for HHP. Cost constraint is also identified as a factor of incompliance. In future, education and proper recordkeeping should be done as it is crucial in monitoring program's progress (Mobini, 1999). Farm 3, an intensively managed farm can result in more contact between animals increasing the likelihood of contracting the disease. Orf that affects intensively managed herds can cause great loss (Mazur and Machado, 1989).

Based on the result, prevalence rate based on gender, age, clinical signs and farm in goats are not significant.; which can be due to the restriction in Chi Square test. The weakness of Chi Square test of independence is that no expected cases in the cells should be less than 1. There shouldn't be no more than 20% of the cells of the cross classification table should have less than 5 expected cases (McHugh & Mary L., 2013). The assumptions were unfortunately violated in the result of goats.

6.0 CONCLUSION

As a conclusion, there is presence of past IgG antibody against Orf in both sheep and goats in UPM's Foster Farms, Malaysia. It is also found that the prevalence rate in goats is slightly higher compared to sheep. 2. There are also seroprevalence of Orf according to several risk factors among small ruminants in this study. In sheep, gender, age and farm factor were found to have significant effect on prevalence rate for for past Orf infection. Lastly in sheep, clinical signs was found to have no significant effect on prevalence rate for past Orf infection.

7.0 RECOMMENDATIONS

Although it has been proven that there is a presence of past IgG antibody against Orf, more surveys should be done in other states to study its prevalence and economic impact in Malaysia. For detailed research, a correlation analysis should be done to measure the association between the risk factors and prevalence rate as Chi Square can only test for association. Apart from that, a local prototype vaccine for Malaysia Orf virus strains should be developed. Finally, it is highly recommended to encourage farmers to practice a good Herd Health Programme especially in disease awareness.

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APPENDICES

Appendix 1: Interview Form

1	Name of farm	
2	Purpose of farm	
3	Management system	
4	Pasture area	
5	Total sheep & goats	
6	Type of housing	
7	Feed	
8	Past/Recent outbreak in farm	
9	Source of sheep & goats	
10	Biosecurity	Footdip: Vehicle dip: Exposure to other animals: Vaccination:

Appendix 2: Sample Result Form for Sheep and Goats

No.	ID	Gender	Age	Clinical Signs	Farm	Presence of IgG

Appendix 3: Materials Provided in the ELISA Kits

Sheep & Goat Contagious Pustular Dermatitis Virus Antibody IgG (CPDV-IgG) ELISA Kit (SunLong Biotech Co., LTD)

No.	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	2-8 ˚C
4	Microelisa strip plate	1	2-8 ˚C
5	Negative control	0.5ml x 1 bottle	2-8 ˚C
6	Positiv control	0.5ml x 1 bottle	2-8 ˚C
7	HRP-Conjugate reagent	6ml x 1 bottle	2-8 ˚C
8	Sample diluent	6ml x 1 bottle	2-8 ˚C
9	Chromogen Solution A	6ml x 1 bottle	2-8 ˚C
10	Chromogen Solution B	6ml x 1 bottle	2-8 ˚C
11	Stop solution	6ml x 1 bottle	2-8 ˚C
12	Wash solution	20ml (30X) x 1 bottle	2-8 ˚C

Appendix 4: Chi Square analysis of prevalent rates based on gender in sheep

Gender * Result Crosstabulation

			Result		Total
			Positive	Negative	
Gender	Female	Count	7	70	77
		Expected Count	9.4	67.6	77.0
	Male	Count	4	9	13
		Expected Count	1.6	11.4	13.0
Total		Count	11	79	90
		Expected Count	11.0	79.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.872[a]	1	.027		
Continuity Correction[b]	3.061	1	.080		
Likelihood Ratio	3.877	1	.049		
Fisher's Exact Test				.050	.050
N of Valid Cases	90				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.59.

b. Computed only for a 2x2 table

Appendix 5: Chi Square analysis of prevalent rates based on gender in goat

Gender * Result Crosstabulation

			IgG		Total
			Positive	Negative	
Gender	Female	Count	13	73	86
		Expected Count	12.4	73.6	86.0
	Male	Count	0	4	4
		Expected Count	.6	3.4	4.0
Total		Count	13	77	90
		Expected Count	13.0	77.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.707[a]	1	.401		
Continuity Correction[b]	.013	1	.910		
Likelihood Ratio	1.279	1	.258		
Fisher's Exact Test				1.000	.530
N of Valid Cases	90				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .58.

b. Computed only for a 2x2 table

Appendix 6: Chi Square analysis of prevalent rates based on age in sheep

Age * Result Crosstabulation

			Result		Total
			Positive	Negative	
Age	Young	Count	7	22	29
		Expected Count	3.5	25.5	29.0
	Adult	Count	4	57	61
		Expected Count	7.5	53.5	61.0
Total		Count	11	79	90
		Expected Count	11.0	79.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.663[a]	1	.017		
Continuity Correction[b]	4.142	1	.042		
Likelihood Ratio	5.256	1	.022		
Fisher's Exact Test				.034	.024
N of Valid Cases	90				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.54.

b. Computed only for a 2x2 table

Appendix 7: Chi Square analysis of prevalent rates based on age in goat

Age * Result Crosstabulation

			Result		Total
			Positive	Negative	
Age	Young	Count	0	4	4
		Expected Count	.6	3.4	4.0
	Adult	Count	13	73	86
		Expected Count	12.4	73.6	86.0
Total		Count	13	77	90
		Expected Count	13.0	77.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.707[a]	1	.401		
Continuity Correction[b]	.013	1	.910		
Likelihood Ratio	1.279	1	.258		
Fisher's Exact Test				1.000	.530
N of Valid Cases	90				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .58.

b. Computed only for a 2x2 table

Appendix 8: Chi Square analysis of prevalent rates based on clinical signs in sheep

C. Signs * Result Crosstabulation

			Result		Total
			Positive	Ngative	
C. Signs	Present	Count	1	4	5
		Expected Count	.6	4.4	5.0
	Absent	Count	10	75	85
		Expected Count	10.4	74.6	85.0
Total		Count	11	79	90
		Expected Count	11.0	79.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.299[a]	1	.585		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.259	1	.610		
Fisher's Exact Test				.487	.487
N of Valid Cases	90				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .61.

b. Computed only for a 2x2 table

Appendix 9: Chi Square analysis of prevalent rates based on clinical signs in goat

C. Signs * Result Crosstabulation

			Result		Total
			Positive	Negative	
C. Signs	Present	Count	1	2	3
		Expected Count	.4	2.6	3.0
	Absent	Count	12	75	87
		Expected Count	12.6	74.4	87.0
Total		Count	13	77	90
		Expected Count	13.0	77.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.896[a]	1	.344		
Continuity Correction[b]	.012	1	.911		
Likelihood Ratio	.705	1	.401		
Fisher's Exact Test				.377	.377
N of Valid Cases	90				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .43.

b. Computed only for a 2x2 table

Appendix 10: Chi Square analysis of prevalent rates based on farm in sheep

Farm * Result Crosstabulation

			Result		Total
			Positive	Negative	
Farm	1	Count	2	40	42
		Expected Count	5.1	36.9	42.0
	2	Count	3	28	31
		Expected Count	3.8	27.2	31.0
	3	Count	6	11	17
		Expected Count	2.1	14.9	17.0
Total		Count	11	79	90
		Expected Count	11.0	79.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.801[a]	2	.005
Likelihood Ratio	8.971	2	.011
N of Valid Cases	90		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.08.

Appendix 11: Chi Square analysis of prevalent rates based on farm in goat

Farm * Result Crosstabulation

			Result		Total
			Positive	Negative	
Farm	1	Count	1	10	11
		Expected Count	1.6	9.4	11.0
	2	Count	0	12	12
		Expected Count	1.7	10.3	12.0
	3	Count	6	20	26
		Expected Count	3.8	22.2	26.0
	4	Count	5	15	20
		Expected Count	2.9	17.1	20.0
	5	Count	1	20	21
		Expected Count	3.0	18.0	21.0
Total		Count	13	77	90
		Expected Count	13.0	77.0	90.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.245[a]	4	.123
Likelihood Ratio	9.004	4	.061
N of Valid Cases	90		

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 1.59.