UNIVERSITI PUTRA MALAYSIA

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

SITI NUR ATIKAH BINTI ABDUL LATIF

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SITI NUR ATIKAH BINTI ABDUL LATIF

A project paper submitted to the

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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 IgM Antibody Detection" by Siti Nur Atikah Binti Abdul Latif, and in our opinion it is satisfactory as partial fulfilment of the requirement for the course VPD 4999 - Final Year Project.

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DEDICATIONS

This project paper is dedicated to my parents,

Siti Ammarah Binti Haji Ahmad and Abdul Latif Bin Abdul Manan,

who have brought me up into this world,

and to my crazy siblings,

Zuenatic, Azudora, Raff and especially the youngest, Chakiechan, who successfully got married with the love of her life in between the course of this project,

without their love and craziness I would never finish or even start writing this thesis on time.

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LIST OF ABBREBIATIONS

%	: Percent
ºC	: Degree Celcius
ml	: Milliliter
µl	: Microliter
kbp	: Kilo-base pair
nm	: Nanometer
OD	: Optical Density
DNA	: Deoxyribonucleic Acid
ELISA	: Enzyme-linked Immunosorbent Assay
IgM	: Immunoglobulin M (antibody)
RPM	: Revolutions per Minute
UPM	: Universiti Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar UPM untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir

PREVALENSI SERUM INFEKSI VIRUS ORF DALAM KALANGAN RUMINAN KECIL DI BAWAH PROGRAM LADANG ANGKAT UPM BERDASARKAN PENGESANAN ANTIBODI IGM

Oleh:

Atikah Latif

2017

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

Penyelia bersama: Prof. Madya Dr. Faez Firdaus Jesse Abdullah

Orf adalah penyakit berjangkit dalam biri-biri dan kambing yang memberi kesan kepada kulit dan produktiviti mereka. Kajian ini bertujuan untuk menentukan status semasa penyakit orf dalam kalangan ruminant kecil di negeri Selangor. Antibodi IgM digunakan sebagai petunjuk untuk jangkitan semasa dalam kajian ini dan faktor-faktor risiko jangkitan semasa orf juga telah dianalisis. Sampel serum daripada 90 kambing dan 90 biri-biri telah dikumpul daripada ladang angkat di bawah UPM.

Kaedah enzyme-linked immunosorbent assay (ELISA) telah digunakan untuk mengesan antibody IgM, diikuti oleh analisa faktor-faktor risiko menggunakan ujian Chi-Square. Keputusan menunjukkan bahawa 33 ekor kambing (36.7%) dan 7 ekor biri-biri (7.8%) mempunyai antibody IgM terhadap virus orf, membuktikan bahawa infeksi semasa orf lebih lazim dalam kalangan kambing berbanding biri-biri. Ujian chisquare menunjukkan bahawa faktor-faktor risiko yang mempengaruhi jangkitan semasa orf adalah berbeza untuk kambing dan biri-biri. Faktor-faktor risiko untuk kambing adalah spesis, baka, lokasi ladang dan sejarah infeksi orf, manakala untuk biri-biri faktor-faktor risiko adalah spesis, baka, umur, jantina, kewujudan tanda-tanda klinikal orf, dan lokasi ladang. Kesimpulannya, kajian ini menunjukkan bahawa sebahagian besar populasi kambing mempunyai infeksi semasa orf walaupun faktor-faktor risiko mereka sedikit, tetapi infeksi semasa orf adalah rendah dalam populasi biri-biri walaupun faktor-faktor risiko mereka lebih banyak.

Kata kunci: Virus Orf, prevalensi serum, kambing, biri-biri, antibody IgM, ELISA

ABSTRACT

Abstract of the final year project presented to the Faculty of Veterinary Medicine UPM in requirement for the course VPD 4999 – Final Year Project

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

by:

Atikah Latif

2017

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

Co-supervisor: Associate Prof. Dr. Faez Firdaus Jesse Abdullah

Orf is an infectious disease in sheep and goats that affects their skin and general productivity. This study aimed to determine the current status of orf infection among small ruminants in the state of the Selangor. IgM antibodies, which were employed as indicators of recent infection, were detected in the study and associated risk factors were assessed. Serum samples were collected from 90 goats and 90 sheep obtained from UPM's foster farms. Qualitative enzyme-linked immunosorbent assay (ELISA) was used to measure IgM antibodies followed by chi-square test. Result showed that 33 goats (36.7%) and 7 sheep (7.8%) were positive for orf IgM antibodies, indicating that recent infection is more prevalent among goats compared to sheep. Chi-square test showed that the risk factors that may predispose them to orf infections were different among goat and sheep. For goats the risk factors are species, breed, farm location and history of orf, for sheep the risk factors are species, breed, age, gender, presence of clinical signs, and farm location. In conclusion, this study showed that a significant number of goat population already harbour active orf infection despite lower number of risk factors, however recent orf infection were less in sheep population, despite higher number of risk factors.

Key words: Orf virus, seroprevalence, sheep, goat, IgM antibody, ELISA

1.0 INTRODUCTION

Orf disease or contagious ecthyma is caused by Orf virus, a linear double-stranded DNA virus which belongs to the family *Poxviridae*, under the genus *Parapoxvirus* (Spyrou & Valiakos, 2015). Other names for this disease include scabby mouth, contagious pustular dermatitis, sore mouth, and "puru" (Ashwaq et.al, 2015). This disease can affect any ruminant species, however it mainly affects the sheep and goat species compared to other ruminants. The orf virus is also known to be zoonotic, transmitting to humans through direct contact with lesions on affected animals, however infection are often locally invasive and non-life threatening (Nandi et.al, 2011).

Orf virus infection usually starts with the formation of erythematous spots, followed by formation of papules, vesicles, pustules, and later on scabs once the vesicles or pustules rupture. The lesions are mainly observed around the mouth, nose, feet of animals, and teats of lactating does or ewes (Spyrou & Valiakos, 2015). This disease can affect all ages of production, however young animals are more severely affected that may lead to death (Ashwaq et.al, 2015). Transmission is through direct contact with infected animal, or contact with environment or material which harbors the virus, for example during grazing. The virus enters the host through breaks on the skin and replicates in the epidermal cells, leading to formation of skin lesions (Kumar et.al, 2015).

Infection with orf virus is usually benign and animals will recover spontaneously within three to five weeks (Kumar et.al, 2015). Nonetheless, it will greatly affect the production status when affected animals refuse to eat due to pain from the lesions around or inside the mouth. It will also reduce value of wool, hair and skin of sheep or goat kept for these trades. Besides that, infection with orf virus can be aggravated by secondary bacterial infections (Nandi et.al, 2011). Ruptured lesions might also attract flies, which could lead to cutaneous myiasis. Diagnosis of orf infection can be made through observation of scabby skin lesions around the mouth, legs or teats, histopathology of skin lesions, and serological testing for presence of antigen or antibody (Kumar et.al, 2015).

Orf virus may be a benign infection among small ruminant producers, however it has been a recurring problem among the farms in Malaysia, affecting the production of the farm in the long run. This is because during the course of the disease, even if the animal is eating well, the full nutrition that it receives is not 100 percent being used for body weight, movement and production, but will be utilized for the immune system for defensive mechanism against the orf infection. Therefore, to maximize productivity of the animals, it is better if the animals are free of orf virus infection. This can be achieved through prevention and control of the disease, and one of the ways to do this would be to screen the animals for any recent infection of Orf virus. Following the isolation and molecular identification of Malaysian orf virus from UPM's foster farms in the previous study by Ashwaq et.al (2015), there is further need to continue the investigation of the orf virus. Thus, this study is to:

1. To determine the recent infection rate of Orf virus among small ruminants (goats and sheep) in UPM's foster farms, Malaysia.

2. To identify the risk factors and its association towards the recent infection rate of Orf among small ruminants (goats and sheep) in UPM's foster farms, Malaysia.

The following hypotheses were proposed for this research:

1. The sheep and goats from UPM's foster farms, Malaysia have IgM antibodies to the Orf virus, indicating recent Orf virus infection.
2. The sheep and goats from UPM's foster farms, Malaysia have no IgM antibodies to the Orf virus, indicating no recent Orf virus infection.

2.0 LITERATURE REVIEW

2.1 Orf Disease

Orf disease or better known in Malaysia as '*puru*' is a disease of small ruminants that mainly affects the skin around the mouth and nose area. Other names for this disease includes contagious ecthyma, contagious pustular dermatitis, scabby mouth, and sore mouth (Ashwaq et.al, 2015). The disease primarily affect domestic sheep and goats, however other domestic and wild ruminants such as cattle, deer, camel, oxen, chamois, and mountain goats may be affected as well (Spyrou & Valiakos, 2015). Orf is a self-limiting benign disease, however it can have a significant impact on the animal's production in the long run. The disease is also known to be zoonotic to humans, and is an occupational hazard, but infections are locally invasive and non-life threatening (Kumar et.al, 2015).

2.1.1 Background and Current Status of Orf Disease

Orf disease have a worldwide distribution in countries that practice sheep and goat farming and is regarded as one of the top 20 most important viral disease affecting mostly the poor rural people in developing countries (McInnes, 2010). This disease was first described in sheep by Steeb in the year 1787, while in goat it was first described by Hansen in the year 1879 (Larson & Spickler, 2012). Orf is first described in humans in the year 1934 by Newson and Cross (Hoover et.al, 2016). In another reference by Kumar et.al (2015), the disease was initially reported in sheep and goats by Zellor in the year 1920 from South West Africa. Since then, the disease was reported from all various parts of the world, involving other species besides sheep and goats as well, such as cattle, dogs and camels, both wild and domestic. According to FAO/OIE/WHO in 1992 the disease has had its occurrence in Africa, America, Asia (including Malaysia), Europe, and Oceania. Outbreaks of orf have been reported from Norway, China, Indonesia, Iraq, Brazil, and Spain. Orf is now a notifiable disease in most of the prevalent countries (Kumar et.al, 2015).

2.1.2 Agent of disease

The disease is caused by the Orf virus, which belongs to the family of *Poxviridae*, from the genus of *Parapoxvirus*. The *Parapoxvirus* currently has four recognized species, which are the Orf virus, Bovine papular stomatitis virus, Pseudocowpox virus, and Parapoxvirus of red deer (Spyrou & Valiakos, 2015). Members of the *parapoxvirus* are cocoon-shaped, measuring 260 x 160nm in size, and consists of a linear double-stranded DNA, measuring around 130 – 160 kbp in size (Murphy et.al, 1999).

2.1.3 Clinical Signs

The incubation period for orf virus is 3-8 days (Mayr & Büttner, 1990). The clinical signs start with the formation of erythematous spots, progressing to papules, vesicles, or pustules, and later on crusty scabs when the vesicles rupture (Nandi et.al, 2011). Lesions are usually seen around the mouth and nose, and sometimes on the feet or teats. The disease is very contagious and can affect animal of any age, however younger animals are more severely affected (Ashwaq et.al, 2015). Secondary bacterial infections may follow after primary infection with the virus, and presence of flies could lead to cutaneous myiasis. In uncomplicated cases, infections are usually benign and animals will recover spontaneously within three to five weeks (Kumar et.al, 2015). In humans, infections have an incubation period of 2-4 days, and within 4-9 weeks localized lesions can be seen as macular, papular, nodular, and in some cases papillomatous in nature (Murphy et.al, 1999).

2.1.4 Pathogenesis

The virus is transmitted through direct contact with affected animals or through exposure to contaminated objects or environment (Murphy et.al, 1999). The virus can also be transmitted through aerosol route, and is common during handling of multiple animals, such as during vaccination, slaughter or shearing of wool (Mayr & Bϋttner, 1990). The virus enters the host through any breaks on the skin, for example small cuts at the sides of the mouth due to ingestion of sharp weeds or grasses (Nandi et.al, 2011). Once invade into the epithelium the virus multiplies, causing a primary viremia which results in changes to the skin and mucous membranes (Mayr & Bϋttner, 1990). The virus can remain in the environment for a long time, especially in dry climates (Spyrou & Valiakos, 2015).

2.1.5 Diagnosis

Diagnosis can be made through observation of typical lesions that usually occurs around the mouth, nose or feet, and it can be confirmed through virus isolation (Spyrou & Valiakos, 2015). Other methods of diagnosis includes haemagglutination test, complement fixation test, immunofluorescence test, PCR (polymerase chain reaction), ELISA (enzyme-linked immunosorbent assay), western blot, histopathology of skin lesion, and electron microscopy (Nandi et.al, 2011). Differential diagnosis for orf lesions include foot and mouth disease (FMD), sheep and goat pox, dermatophilosis, and mange infection.

2.1.5.1 ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is a method of diagnosis that involves the use of enzymes to link to an antibody or antigen present in a sample, which in turn is linked to an antigen or antibody that is affixed to the surface of the ELISA well. After a series of washing and incubation, the antibody-antigen-bound enzyme will then produce a colour change when a chromogenic substrate is added, which can be read via an ELISA plate reader using the measurement optical density (O.D.) (JoVE Science Education Database, 2017). There are currently four types of ELISA, which are the direct ELISA, indirect ELISA, sandwich ELISA, and competitive ELISA.

2.1.5.1.1 Sandwich ELISA

In sandwich ELISA, two layers of antibodies are used, which are the capture antibody and the detection antibody. The two antibodies will bind to an antigen, which is situated in the middle, hence the name 'sandwich' ELISA (Sino Biological Inc., 2014-2017).

2.1.6 Treatment, Control and Prevention

There is no definitive treatment for orf disease, and it is managed through supportive treatment, such as supplementation of vitamins, antibiotics for secondary bacterial infection, daily wound cleaning, and others. Control involves good hygiene practice, such as wearing gloves during handling, and separating sick animals from healthy animals (Kumar et.al, 2015; Spyrou & Valiakos, 2015). Prevention can be done through vaccination, proper disinfection and sanitary procedures, and quarantine of new animals (Nandi et.al, 2015; Onyango et.al, 2016).

2.2 Antibody

The immunoglobulins or antibodies is comprised of four glycosylated protein chains held together by disulphide bonds in a Y-shaped structure, and the protein chains consists of two heavy chains and two light chains. The heavy chain can be divided into five, which are alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ), while the light chain can be divided into two, which are kappa (κ) and lambda (λ). The antibodies are classified into five types based on the chains that they carry, and the classes are IgG, IgM, IgA, IgD, and IgE (Day & Schultz, 2011).

2.2.1 IgM Antibody

IgM is the largest of the immunoglobulins, and is mainly found in the circulation, thus it plays an important role during infections relating to the blood, for example during viremia. It is also important in primary immune response due to its ability to agglutinate multiple antigens (Day & Schultz, 2011). Therefore, it is expected that in recent infections, the IgM antibody level would be high.

2.3 Study of Orf in Malaysia

There are currently few research done on orf virus in Malaysia. The first study was conducted by Zamri-Saad et.al in 1992 regarding the effects of orf infection on body weight of lambs, and the most recent study done was by Ashwaq et.al in 2015 regarding the phylogenetics of orf virus. This might be because orf disease is considered to be less pathogenic by the farmers compared to other lethal diseases, and therefore it is seldom reported. There are currently no study done on the seroprevalence of Orf disease in Malaysia, hence this study was planned to detect infections of Orf virus in order to have better prevention and control against this disease.

3.0 MATERIALS AND METHOD

3.1 Sample Location

Samples were collected from the farms under UPM's foster farms, which were located around the area of Selangor, Malaysia.

3.2 Sample Size Calculation

The sample size was calculated using the standard epidemiological formula (Arya, et. al, 2012; Hinderson & Sundaresan, 1982), which is as follows:

$$n = \frac{Z^2pq}{L^2}$$

Where n = sample size

Z = Standard normal distribution at 95% confidence interval = 1.96

p = Prevalence in similar work

q = 1 – p

L = Allowable error, taken as 5% = 0.05

In this study, P =.7.32% (Gao et. al, 2016)

$$n = \frac{Z^2pq}{L^2}$$

$$= \frac{(1.96)^2 \text{ X } 0.0732 \text{ X } (1-0.0732)}{(0.05)^2}$$

$$= \frac{3.8416 \text{ X } 0.0732 \text{ X } 0.9268}{0.0025}$$

= 104.24 ≈ 100 samples

3.3 Blood Collection

Blood samples were collected from 100 sheep and 100 goats from UPM's foster farms using the jugular venipuncture method. The animals were manually restained and the head is then elevated at a 30° angle to expose the jugular vein. The vein was occluded, and after the alcohol swab a 21 gauge blood collection needle inserted inside a vacutainer was used to puncture the vein, and a plain blood tube was used to collect the blood. The blood tubes with the collected blood were then stored upright in an icebox for transportation.

3.4 Serum Sample Collection

The blood was processed as soon as possible by spinning at 3000 RPM for 5 minutes using a centrifuge machine. The collected serum was then harvested into 1.5 ml eppendorf tubes using a micropipette. The serum samples are then stored at -20°C until further use.

3.5 Procedure of ELISA Test Kit

The ELISA kit used for this study is the 'Contagious Pustular Dermatitis Virus Antibody IgM ELISA' kit for sheep and goat respectively, manufactured by Sunlong company. The ELISA kit is of the qualitative, indirect sandwich ELISA type. The plate has 96 wells in total, and 90 of the wells were used for analyzing the samples. Two wells were set as positive controls, two wells for negative control, one well for blank control, and one well was left empty as a divider. An image of the stripplates with the respective controls and samples are shown in diagram 1 below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive control	Positive control	Negative control	Negative control	Blank control	Empty well	S1	S2	S3	S4	S5	S6
B	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
C	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
D	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42
E	S43	S44	S45	S46	S47	S48	S49	S50	S51	S52	S53	S54
F	S55	S56	S57	S58	S59	S60	S61	S62	S63	S64	S65	S66
G	S67	S68	S69	S70	S71	S72	S73	S74	S75	S76	S77	S78
H	S79	S80	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90

Digram 1: an example of the stripplate with controls and samples

The procedures for both ELISA kits were the same. First, 50µl of negative and positive control reagent was added into the positive control and negative control wells respectively. Then, 40µl of sample dilution buffer was added to all the sample wells, followed by adding 10µl of serum sample into their respective wells. After mixing by gentle shaking of the plate, the plate was incubated at 37°C for 30 minutes.

After incubation, the plate was washed five times consecutively with the washing buffer, which was diluted with distilled water at 30 times for 96T. Then, 50µl of HRP-Conjugate reagent was added to each well except the blank control and empty well. The plate is then incubated again at 37°C for 30 minutes, followed by washing five times with the diluted washing buffer.

After the last washing, 50µl of Chromogen Solution A and 50µl of Chromogen Solution B was added to each well, then after gentle mixing the plate was incubated at 37°C for 15 minutes. Finally, 50µl of stop solution was added to each well, and the colour should change from blue to yellow.

3.6 Reading the Results

The ELISA stripplate was read using the Microtiter plate reader machine (WHYM201) at 450nm. The results are read based on the calculation of the critical value or cut off value, which can be obtained by adding the average value of the negative control reading with 0.15. If the value of the sample is less than the cut off value, then the result is negative, while if the value of the sample is more than the cut off value, then the result is positive.

3.7 Analyzing the Data

Data analysis was done using the IBM SPSS Statistics software. The Chi square test was conducted to analyze the results, with a significance value of 0.05. If the p-value is less than 0.05, the null hypothesis is rejected and we accept the alternative hypothesis. Conversely if the p-value is more than 0.05, we accept the null hypothesis and reject the alternative hypothesis. The null hypothesis is that there is no significance between the presence of recent orf infection and the risk factor calculated, while the alternative hypothesis is that there is significance between the presence of recent orf infection and the risk factor calculated.

4.0 RESULTS

In summary, 33 out of 90 goats (36.7%) and 7 out of 90 sheep (7.8%) tested were positive for orf IgM antibodies. Data analysis was conducted using the Chi-square test in the SPSS statistics software. The significance level was at $P<0.05$.

Table 1: Relationship of Presence of Orf IgM with Species of Animal

Risk Factors		Total Sample	Results of ELISA IgM test	
			Positive	Negative
Species	Sheep	90	7 (17.5%)	83 (59.3%)
	Goat	90	33 (82.5%)	57 (40.7%)
	Total	180	40 (22.2%)	140 (77.8%)
	P-Value		0.000	

The P-value for this risk factor is less than 0.05, indicating significant relation between presence of orf IgM with species.

Table 2: Relationship of Orf IgM with Demographic Parameters

Risk Factors		Total Sample	Results of ELISA IgM test			
			Goat		Sheep	
			Positive	Negative	Positive	Negative
Age	Young	33	2 (6.1%)	2 (3.5%)	7 (100%)	22 (26.5%)
	Adult	147	31 (93.9%)	55 (96.5%)	0 (0%)	61 (73.5%)
	Total	180	33 (36.7%)	57 (68.3%)	7 (7.8%)	83 (92.2%)
	P-Value		0.622		0.000	
Gender	Male	17	1(3%)	3 (5.3%)	4 (57.1%)	9(10.8%)
	Female	163	32(97%)	54 (94.7%)	3 (42.9%)	74(89.2%)
	Total	180	33(36.7%)	57 (63.3%)	7 (7.8%)	83(92.2%)
	P-Value		1.000		0.008	
Breed	Boer	1	0 (0%)	1 (1.8%)	-	-
	Saanen	38	9 (27.3%)	29 (50.9%)	-	-
	Jamnapari	5	1 (3%)	4 (7%)	-	-
	Gurun	4	0 (0%)	4 (7%)	-	-
	Kashmir	1	0 (0%)	1 (1.8%)	-	-
	Merino	39	-	-	1 (14.3%)	38(45.8%)
	Damara	47	-	-	4 (57.1%)	43 (51.8%)
	Cross breed	45	23 (69.7%)	18 (31.6%)	2 (28.6%)	2 (2.4%)
	Total	180	33 (36.7%)	57 (63.3%)	7 (7.8%)	83 (92.2%)

P-Value	0.019	0.003

The demographic parameters include age, gender and breed of the animals. The age is divided into young and adult, where animals less than one years old are categorized as young, while those more than one years old are categorized as adults. Out of 33 positive goats, 2 (6.1%) were from young animals, and 31 (93.9%) were from adult animals, while for sheep all 7 positives were from young animals (100%). The P-value for this risk factor was not significant for goat but is significant for sheep.

The gender is divided into two categories, male and female. 1 male (3%) and 32 female (97%) were positive for goats, while 4 male (57.1%) and 3 female (42.9%) were positive for sheep. The P-value for this risk factor was not significant for goats but is significant for sheep.

The goat breeds in this study includes Boer, Saanen, Jamnapari, Gurun, Kashmir, and cross breeds, while the sheep breeds include Merino, Damara and cross breeds. For the positive goats, 9 (27.3%) were Saanen, 1 (3%) was Jamnapari, and 23 (69.7%) were cross breeds, while for the positive sheep, 1 (14.3%) was Merino, 4 (57.1%) Damara, and 2 (28.6%) were cross breeds. The P-value for this risk factor was not significant for goats but was significant for sheep.

Table 3: Relationship of Orf IgM with Presence of Orf Lesions

Risk Factors		Total Sample	Results of ELISA IgM test			
			Goat		Sheep	
			Positive	Negative	Positive	Negative
Presence of clinical signs	None	175	33 (100%)	57 (100%)	5 (71.4%)	80 (96.4%)
	Present	5	0 (0%)	0 (0%)	2 (28.6%)	3 (1.2%)
	Total	180	33 (36.7%)	57 (63.3%)	7 (7.8%)	83 (92.2%)
	P-Value		Not Applicable		0.047	

All positive goats show no clinical signs, while for positive sheep 2 (28.6%) show clinical signs and 5 (71.4%) show no clinical signs. The P-value cannot be calculated for the goats, but it is significant for the sheep.

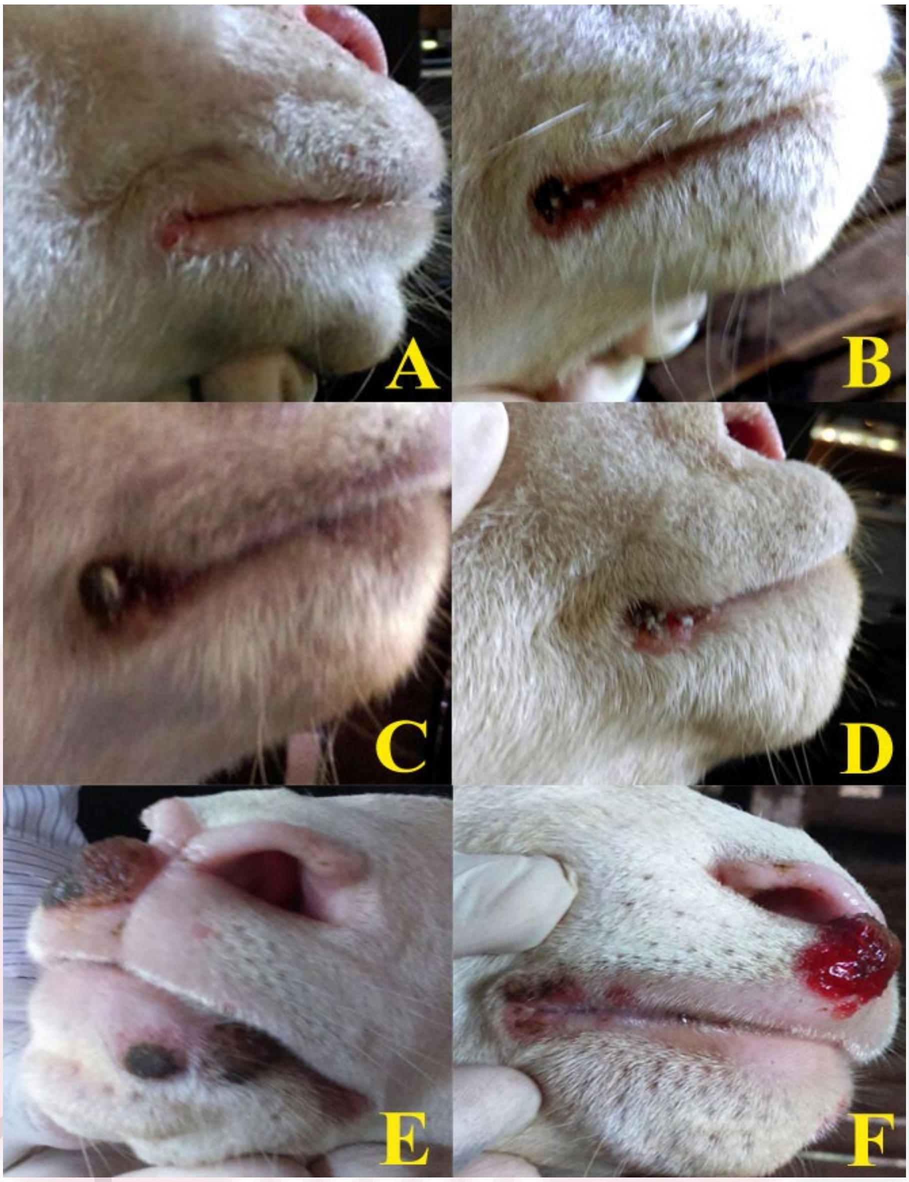


Figure 1: A collection of Orf lesions seen in some of the sampled animals. A) a small red spot at the side of the mouth with no scab yet. B), C), D) Black scabs have formed at the sides of the mouth, with mixture of small white crusts that look like small warts. E) Lesions have progressed to bigger round scabsaround 2x2 cm and 1x2 cm at the upper and lower lip, with some alopecia around the lip. F) Haemorrhagic orf lesion due to ruptured vesicle at the upper lip, with some mild lesions at the side of the mouth and also alopecia around the lips.

Table 4: Relationship of Orf IgM with Farm Characteristics

Risk Factors		Total Sample	Results of ELISA IgM test			
			Goat		Sheep	
			Positive	Negative	Positive	Negative
Farm location	Farm 1	53	5 (15.2%)	6 (10.5%)	1 (14.3%)	41 (49.4%)
	Farm 2	43	1 (3%)	11 (19.3%)	0 (0%)	31 (37.3%)
	Farm 3	43	16 (48.5%)	10 (17.5%)	6 (85.7%)	11 (13.3%)
	Farm 4	20	6 (18.2%)	14 (24.6%)	-	-
	Farm 5	21	5 (15.2%)	16 (28.1%)	-	-
	Total	180	33 (36.7%)	57 (63.3%)	7 (7.8%)	83 (92.2%)
	P-Value		0.010		0.000	
History of Orf	Yes	137	32 (97%)	46 (80.7%)	7 (100%)	52 (62.7%)
	No	43	1 (3%)	11 (19.3%)	0 (0%)	31 (37.3%)
	Total	180	33 (36.7%)	57 (63.3%)	7 (7.8%)	83 (92.2%)
	P-Value		0.050		0.091	
Vaccine against Orf	Yes	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	No	180	33 (100%)	57 (100%)	7 (100%)	83 (100%)
	Total	180	33 (36.7%)	57 (63.3%)	7 (7.8%)	83 (92.2%)
	P-Value		Not calculated		Not calculated	

In total, the animals were sampled from 5 different farms. For positive goats, 5 (15.2%) were from farm 1, 1 (3%) was from farm 2, 16 (48.5%) were from farm 3, 6 (18.2%) were from farm 4 and 5 (15.7%) were from farm 5. Out of 7 sheep positive, 1 (14.3%) was from farm 1 and 6 (85.7%) were from farm 3. The P-value was significant for both sheep and goat.

32 (97%) of the positive goats were from farm that had a previous history of orf, while only 1 (3%) were from farm with no history of orf. For sheep, all positive animals were from farm with history of orf (100%). P-value was significant for goats, but not significant for sheep.

P-value for vaccine status was not calculated, because none of the farms practice any vaccination against orf disease.

5.0 DISCUSSIONS

The ELISA results revealed that goats have a higher number of positives compared to sheep for orf IgM antibodies. Statistical analysis also shows that there is a significant relation between the prevalence and the species of animal. This finding tallies with the results in three other studies, where the prevalence of orf for sheep is 1.88% in England (Onyango et.al, 2016), and the prevalence for goats is 34.89% in China (Gao et.al, 2016) and 76.62% in Assam (Bora et.al, 2016). However, more clinical signs of orf (round, crusty scabs at the corners of the mouth) are seen in the sheep, and none were found in the goats that were sampled, even though most of the goats have a serological positive result. This finding is different from those of Zamri-Saad et.al (1994), where the study shows that goats are more likely to show severe orf lesions compared to sheep. This indicates that the goats are infected with orf, but shows no clinical signs of the disease probably due to the ability of the immune system to control the infection, or maybe the infection is very recent that clinical signs are yet to appear (Kumar et.al, 2015; Mayr & Büttner, 1990). Furthermore, the strain of the virus itself and the quantity of virus inoculated could also play a role in the different prevalence between these two species, since there are several strains of the virus in Malaysia (Ashwaq et.al, 2015).

Another risk factor taken into consideration is the age of the animal. In the analysis, age is shown to be significant for sheep but not significant for goats, which may indicate that goats are susceptible to orf infection regardless of age, however for sheep young animals are more susceptible to infections compared to adults. The result is in agreement with the findings of Onyango et.al (2014) who found that the disease is more prevalent in lambs, however the analysis for goats which shows that age was not significant differs from the results in the study by Bora et.al (2016), where they found that the disease is more prevalent in goats more than 8 months old. In general however, young animals are more prone to infection and more severe lesions, often leading to death, mainly because of their naïve immune system which has yet to become more developed (Spyrou & Valiakos, 2015).

The next risk factor analyzed was the gender. Gender was shown to be not significant for goats but is shown to be significant for sheep, whereby the sheep males show higher prevalence compared to females. This could be because of the small number of positive results in the sheep population, and since most of the positives were from the males, which are much lower in number compared to females, the data was shown to be significant. However, in most of the literatures regarding orf, there is little mention about the gender and prevalence of orf, so it is assumed that both genders are likely susceptible to infection (Nandi et.al, 2010; Kumar et.al, 2015; Spyrou & Valiakos, 2015).

Breed of the animal could possibly be a potential risk factor as well, as analysis shows that breed is significant for both sheep and goat. In goats the cross breeds have a higher number of seropositives, while in sheep the damara breed shows higher number of seropositives. In a study by Gao et.al (2016) and Bora et.al (2016), it was stated that certain breeds of goats are shown to have higher incidence of infection compared to other breeds sampled, and Kumar et.al (2015) also stated Boer goats are more affected by orf. However, for this study, more samples preferably of equal size for each breed needs to be obtained in order to get a more accurate result regarding which breed is more susceptible in Malaysia.

The next factor that was analyzed was the relationship between presence of orf clinical signs and its seroprevalence, where in the sheep it is shown to be significant, but in the goats it was not applicable, since none of the goats show any clinical signs and therefore there was inadequate data for analysis. This result shows that sheep are more prone to show clinical signs when infected with the virus, thereby allowing rapid identification of sick animals through observation alone, but in contrast, goats might harbor the infection but show no obvious clinical signs, therefore making identification of orf infection difficult in the field. The change in lesion severity between sheep and goat from the past years until now could be attributed to the ability of the virus to mutate, or because animals are learning to fight the infection as time passes (Zamri-Saad et.al, 1994; Kumar et.al, 2015). Besides that, five of the sheep shows clinical signs of orf, but only two were positive for orf IgM when tested with the ELISA kit, and this could maybe be due to the antibodies switching from IgM to other types of antibodies because of prolonged infection, or it a different infection from orf which has similar lesions, such as foot and mouth disease or sheep pox (Mayr & Büttner, 1990; Nandi et.al, 2010).

Another possible risk factor is the presence of cuts or abrasions on the skin, however it was not analyzed in this study because none of the animals had any visible cuts or wounds on their skin. Nevertheless, most literature states that the virus enters the host through cuts on the skin, especially during ingestion of grass, therefore this risk factor cannot be cast aside (Murphy et.al, 1999; Nandi et.al, 2010; Spyrou &

Valiakos, 2015). It is also not necessary for animals to have cuts on their skin for the virus to cause an infection, as the virus can possibly be transmitted through aerosol route (Mayr & Büttner, 1990). It could be that during our sampling, there were cuts on the skin of the affected animals, but the cuts are not visible to the naked eye, or that the cuts have healed after the virus successfully entered the skin, thereby showing no cuts when we sampled the animals.

Other than that, the farm location also has a significant relation with the prevalence of orf for both sheep and goats, where the highest number of positives were from animals sampled from farm 3. This shows that the disease might be endemic in certain farms but not all of the farms. All the farms do not have adequate biosecurity measures such foot or vehicle dips, even though they practice good animal husbandry practice, however they do differ in terms of farm management, either intensive or semi-intensive. This could be one of the reasons why certain farms have a higher prevalence of orf infection compared to other farms, as farms practicing intensive system is more prone to infection, due to increased contact between the animals, thereby providing rapid spread of virus (Gao et.al, 2016). Regardless, other factors might also play a role in the prevalence of orf in said farms, such as the level of nutrition provided, the origin of the supply of animals (country of import), frequency of visitors to the farm, and others (Onyango et.al, 2016; Kumar et.al, 2015; Mayr & Büttner, 1990).

The last factor analyzed was the history of orf in relation to prevalence of recent orf infection, which was shown to be significant for goats, but not significant for the sheep. This data indicates that if the farm had a previous history of orf infection, then there is a high probability of the disease occurring again, because the orf virus can remain dormant in the environment or in the fallen scabs of past infections for a very long time, especially in dry climates such as in Malaysia (Nandi et.al, 2010; Spyrou & Valiakos, 2015). Furthermore, immunity against the virus is short-lived, and animals previously infected with the disease can get re-infected again in future whenever they are under stress, for example during lambing or kidding season, where there is high chance of the mother transmitting infections to their young (Mayr & Bӧttner, 1990; Nandi et.al, 2010; Kumar et.al, 2015).

Finally, the relation between vaccination against orf and the prevalence of the disease was not calculated for both sheep and goats, as all of the animals sampled were unvaccinated against orf, and therefore no data is available for proper analysis. Nonetheless this factor can be studied further in future when orf vaccination is available and practiced in Malaysia, as vaccination against orf is proven to be helpful in countries that do practice orf vaccination, especially in the young animals (Onyango et.al, 2016; Kumar et.al, 2015).

6.0 CONCLUSION

As a conclusion, this study has shown that a significant number of the goat population are already harbouring active or recent orf infection, despite none of them showing any clinical signs and also having lower number of risk factors, where in contrast only a few of the sheep population has shown evidence of recent orf infection, despite them having higher number of risk factors involved.

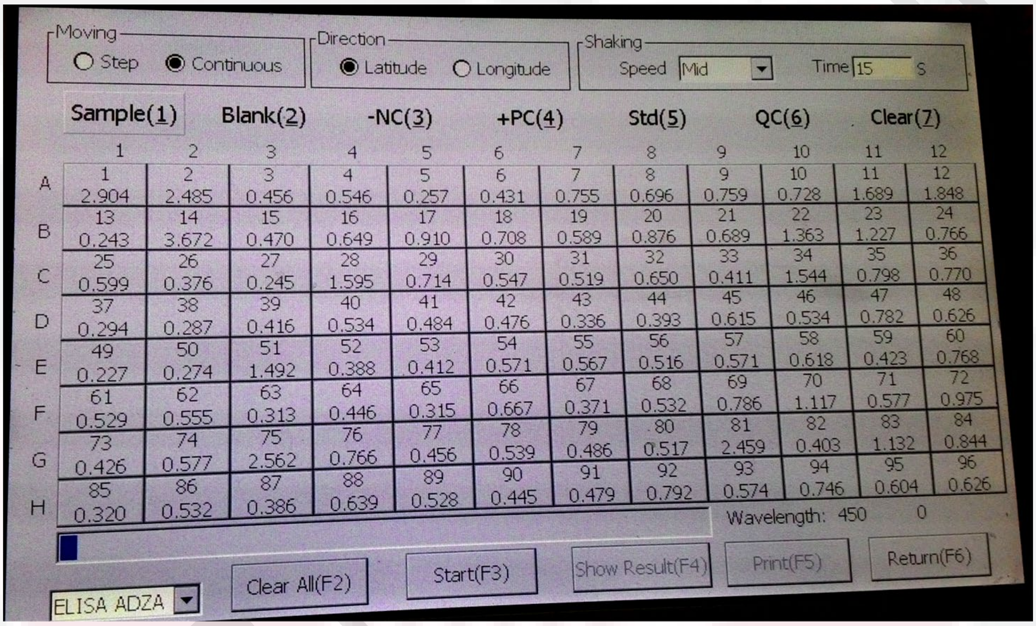
7.0 RECOMMENDATIONS

I would recommend the small ruminants in UPM's foster farms to be vaccinated against orf disease, especially the goat herd, once orf vaccine is available in Malaysia, since the disease is shown to be quite prevalent subclinically among the population. Other seroprevalence study on orf virus covering more states in Malaysia can also be carried out so that the prevalence of orf disease in Malaysia is better understood. I would also like to recommend the farmers to practice more strict biosecurity measures in their farm management so that spread of the disease among the small ruminants can be controlled, since the disease is shown to be endemic in some of the farms sampled.

8.0 APPENDICES

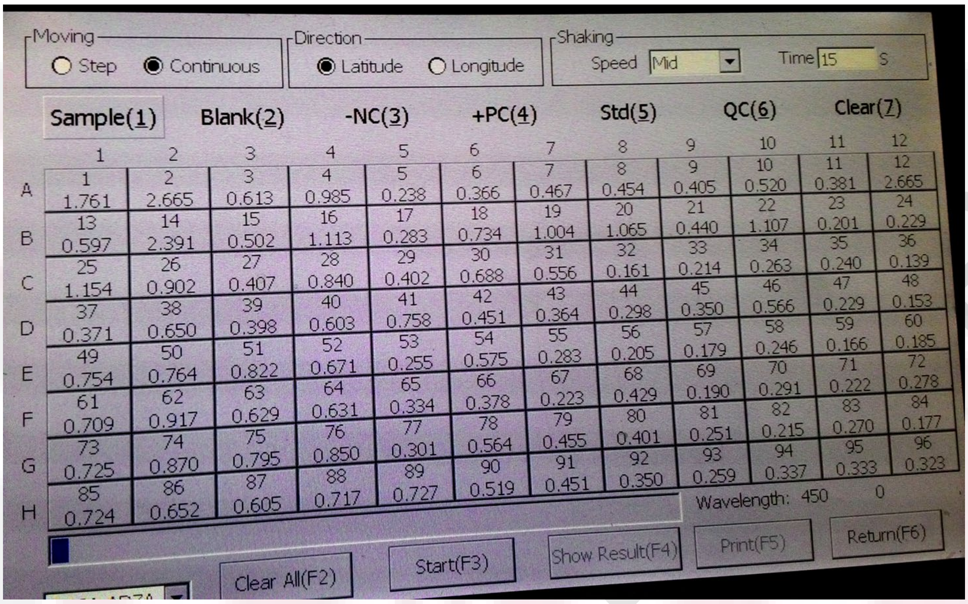
8.1 ELISA Results from the ELISA Microplate Reader

Figure 2: Goat ELISA results from microplate reader. Cut-off value is 0.651.



	1	2	3	4	5	6	7	8	9	10	11	12
A	1 2.904	2 2.485	3 0.456	4 0.546	5 0.257	6 0.431	7 0.755	8 0.696	9 0.759	10 0.728	11 1.689	12 1.848
B	13 0.243	14 3.672	15 0.470	16 0.649	17 0.910	18 0.708	19 0.589	20 0.876	21 0.689	22 1.363	23 1.227	24 0.766
C	25 0.599	26 0.376	27 0.245	28 1.595	29 0.714	30 0.547	31 0.519	32 0.650	33 0.411	34 1.544	35 0.798	36 0.770
D	37 0.294	38 0.287	39 0.416	40 0.534	41 0.484	42 0.476	43 0.336	44 0.393	45 0.615	46 0.534	47 0.782	48 0.626
E	49 0.227	50 0.274	51 1.492	52 0.388	53 0.412	54 0.571	55 0.567	56 0.516	57 0.571	58 0.618	59 0.423	60 0.768
F	61 0.529	62 0.555	63 0.313	64 0.446	65 0.315	66 0.667	67 0.371	68 0.532	69 0.786	70 1.117	71 0.577	72 0.975
G	73 0.426	74 0.577	75 2.562	76 0.766	77 0.456	78 0.539	79 0.486	80 0.517	81 2.459	82 0.403	83 1.132	84 0.844
H	85 0.320	86 0.532	87 0.386	88 0.639	89 0.528	90 0.445	91 0.479	92 0.792	93 0.574	94 0.746	95 0.604	96 0.626

Figure 3: Sheep ELISA results from microplate reader. Cut-off value is 0.949.



8.2 Questionaire of the Farm Profile

1	Name of farm	
2	Purpose of farm	
3	Management system	Intensive / Semi intensive / Extensive
4	Pasture area	
5	Total sheep & goats	
6	Type of housing	
7	Feed	Pellet / Grasses
8	History of diseases present in farm (past and present)	
9	Source of sheep & goats	Import / Local
10	Biosecurity	Footdip Vehicle dip Fencing HHP programme (vaccinations, deworming, etc)

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