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PREVALENCE OF CASEOUS LYMPHADENITIS IN GOATS

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BY

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AL FATIHAH (PEMBUKA)

Bismillahirrahmanirrahim
(Dengan Nama ALLAH Yang Maha Pemurah Lagi Penyayang)

Alhamdulillah hirrabbil 'alamin
(Segala Puji Bagi ALLAH, Tuhan Sekelian Alam)

Arrahma nirrahim
(Yang Maha Pemurah Lagi Penyayang)

Ma likiaumiddin
(Yang Memiliki Hari Pembalasan)

Iyya kanakbudu waiyya kanastain
(Hanya Pada Mu Kami Sembah dan Berserah)

Ihdinash sirathalmustaqim
(Tunjukkilah Kami Ke Jalan Yang Lurus)

Sirathallazi na an am ta'alaihim ghairi
maghdubialaihim walladha llin
(Ya itu Jalan Orang-orang Yang Engkau Beri Nikmat dan
Bukan Jalan Orang-orang Yang Tersesat).

Amin.

Alhamdulillah, syukur ke hadrat ALLAH kerana dengan limpah

kurnianya projek ini telah berjaya mencapai matlamatnya.

With special dedication to them, together and separately :

My family and future wife, Hani

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ABSTRACT

Seven percent (242/3484) of the goats examined from various farms in Peninsular Malaysia had evidence of caseous lymphadenitis as demonstrated by the gel diffusion technique. Three states, namely Kedah (10.8%), Negeri Sembilan (6.8%) and Selangor (6.7%) had high prevalence of infection. A higher prevalence was observed in the intensive farms (9.4%) as compared to the smallholders (1.0%). Pusat Ternakan Haiwan, Gajah Mati was shown to have the highest prevalence (15.8%) among all the farms. A study of the UPM Goat Units indicated a high prevalence (14.6%) of the infection amongst the animals. The management, breeds and age of the animals in relation to the prevalence of CLA are discussed.

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INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic contagious disease and has been recognised as a widespread and important bacterial disease of sheep and goats throughout the world (Benham et al, 1962). However, there are only a few reports available concerning the prevalence of the disease in goats. The history of the disease in goats has never been studied and is still not clearly understood (Ashfaq and Campbell, 1979). Thus, most of the information available regarding the disease were made from studies in sheep (Ayers, 1977).

The causal organism, Corynebacterium pseudotuberculosis was first recognised by Nocard in 1885 and named C. pseudotuberculosis Nocard. In 1891, Preisz and Guinard described the organism from a renal abscess of a sheep and gave the name Bacillus pseudotuberculosis ovis (Benham et al, 1962; Marsh, 1965).

The disease is characterized by a unilateral or bilateral enlargement and suppuration of the superficial lymph nodes (Jensen, 1982; Stoops et al, 1984). It may also involves the visceral lymph nodes and occasionally lungs, spleen, liver, kidneys (Jubb and Kennedy 1970; Monlux et al, 1965) and heart (Hamir, 1981). Affected lymph nodes were normally distended by a central mass of thick, greenish-white purulent material within a thick fibrous capsule (Ashfaq and Campbell, 1980). The organism has also been reported to cause infection in cattle, horse (Blood and henderson, 1983), camel, deer, mule, rhinoceros, pigs, rodents, monkeys, ducks (Benham et al, 1962) and man (King, 1980).

In Malaysia, the first isolation of C. pseudotuberculosis was made in 1970 by the Veterinary Research Institute, Ipoh (Zubaidah et al, 1985). The occurence of CLA in goats was not recognised

until first described by Omar et al (1983). Very little is known about the epidemiology of the disease in goats and sheep in this country.

The objective of this study is to examine the prevalence of CLA in goats in Malaysia. The study will also examine the:

i) prevalence of the infection in relation to places, states and the country as a whole.

ii) prevalence of the infection in intensive farms and smallholders.

iii) prevalence of CLA in two herds of goats of different breeds and age in UPM reared under different management system.

LITERATURE REVIEW

A) Prevalence of caseous lymphadenitis in sheep and goats

Caseous lymphadenitis has been reported in sheep and goats in many countries including Australia (Hein and Cargill, 1981; Nairn and Robertson, 1974), Egypt (Zaki, 1968), France, India, Iran, Italy, Kenya, Mexico, New Zealand (Benham et al, 1962; Jensen, 1982; Tadayon et al, 1980), Norway (Lund, 1982), Philipines, South Africa, South America and the United States (Ashfaq and Campbell, 1979; Jensen, 1982). Addo et al (1978) reported the prevalence of CLA in goats and sheep in Nigeria. Out of 664 sheep and goats from nine flocks in Sokoto state, 28 (4.2%) were clinically infected. In Kanuda state, 7.3% of 4734 goats at one centre and 3.5% of 256 sheep and goats at another centre were found infected.

In Malaysia, the disease was first recorded in a sheep and goat farms in 1980 (Zubaidah et al, 1985). Sheikh Omar and

Chulan (1980) reported the presence of caseous abscessation due to C. pseudotuberculosis in majority of the ovine lungs and lymph nodes obtained from an abattoir in Selangor in 1977. In a similar study CLA was regularly observed during meat inspection by Tham and Sheikh Omar (1980) and they found high prevalence of the infection occuring in sheep imported live from Australia. In their study, 49 sheep from a group of 150 animals slaughtered at the Shah Alam abattoir in Selangor had abscesses in the lungs, lymph nodes and liver. The disease however has not been recorded in local sheep. Omar et al (1983) were the first to describe the occurence of CLA in a herd of goats imported from the United States. Bahaman et al (1985) reported the isolation of C. pseudotuberculosis from 3 cases (18.7%) and 5 cases (7.7%) of abscesses in sheep and goats respectively, whilst Zubaidah et al (1985) encountered 2 (4%) serologically positive cases from a group of 50 sheep and 28 (1.3%) cases out of 2168 goat sera tested.

B) Distribution of CLA according to age and sex

Caseous lymphadenitis is a chronic disease of adult sheep particularly from 3 to 6 years of age (Marsh, 1965) and probably were exposed to infection through shearing (Blood and Henderson, 1983). Stoops et al (1984) reported a considerable numbers of matured, culled sheep infected with CLA in Western United States. However, many workers believed that lambs and kids were just as susceptable to the infection but prevalence is low (Marsh, 1965). Ashfaq and Campbell (1979) observed that, there is a relationship between age and prevalence in the distribution of the disease in goats. They reported a low prevalence of clinical CLA in kids less

than 6 months old and more than 4 years old. Lund[a] (1982), similarly reported a few cases of clinical CLA amongs kids less than 3 months of age.

Many workers agreed that sex has no relationship to the distribution of the disease. However, Ashfaq and Campbell (1979) observed a significant association between sex and the disease. They noticed that castrated males appeared to be infected less often (4.5%) than the uncastrated males (8.4%) or females (8.1%). They suggested that this could be due to changes in the social behaviour after castration as they tend to be less aggressive and therefore less prone to injuries.

C) Method of infection and distribution of lesions of CLA

In sheep, prescapular and prefemoral lymph nodes were the most frequently affected (Ashfaq and Campbell, 1979; Marsh, 1965; Monlux et al, 1965), while, mediastinal, bronchial and sublumbar lymph nodes were the most commonly affected visceral lymph nodes (Hamir, 1981; Marsh, 1965; Monlux et al, 1965). Nagy (1976) showed that there was a definite relationship between route of infection and distribution of lesions. Skin wound particularly due to shearing were the main route of entry and less commonly via navel, docking wounds, inhalation of infected dust, ingestion of contaminated material, venereal infection (Nagy, 1976), unbroken skin (Nairn and Robertson, 1974) and mucous membrane (Brogden et al, 1984). Ruptured abscesses and faeces play an important role in transmitting the organism (Nagy, 1976).

Lungs were the most commonly infected organ and result in pulmonary abscesses and pneumonia (Brogden et al, 1984; Hamir, 1981;

Monlux et al, 1965). The lungs could have contacted the infection either by haematogenous route or by inhalation. Brogden et al (1984) have experimentally induced CLA in lambs by intravenous inoculation and after 28 days multiple abscess were observed in the lungs and lymph nodes. The number of abscesses in the lungs had correlation with the inoculation dose. Pulmonary lesions may also occur as miliary foci or as one large single abscess (Monlux et al, 1965). Lesions have also been recorded in the diaphragm, eyes, brain, epididymis, scrotum, joint and bursae (Hamir, 1981; Nagy, 1976).

In goats, the mandibular lymph nodes were the most frequently infected followed by the parotid lymph nodes (Ashfaq and Campbell, 1979; King, 1980). Ashfaq and Campbell (1979) illustrated the body distribution of CLA in the goats (see APPENDIX II). Systemic caseation of lymph nodes is not common in goats (Ayers, 1977) but death from pneumonia due to C. pseudotuberculosis were more common in goats than sheep (King, 1980).

The distribution of abscesses in goats and sheep suggested different routes of entry of the organism. In goats, infection may be contracted not only through skin wounds, but also through ingestion of the organism with entry of the organism through the buccal mucosae (Ashfaq and Campbell, 1979). However, Ashfaq and Campbell (1980) have shown that the organism was not voided in the faeces or nasal secretion of infected goats in contrast with sheep. Common behaviour such as frequent licking and head and neck rubbing against fence posts or other structures (Burrell, 1981) and eating habit such as eating thorns and thistles explained the typical variation in the distribution of abscesses in goats (King, 1980); William, 1980). The lymphatic system may also play an important

role. More efficient lymphatic drainage in the oral cavity of goats not only cause early abscessation of mandibular lymph nodes, but also abscence of abscesses at the site of inoculation in the oral cavity. Efficient drainage of the lymph in the oral cavity may be attributed to the continuous movement of the jaws while eating and regurgitating (Ashfaq and Campbell, 1980).

D) Diagnostic test

Many serological methods such as the agglutination test and allergic test have been used in diagnosing C. pseudotuberculosis infection in animals (Zaki, 1968). However, most of the serodiagnostic tests that have been tried in the field were involved in the detection of serum antitoxin by the antitoxin's ability to neutralise the effect of the C. pseudotuberculosis exotoxin (Ayers, 1977). Zaki (1968) has described an in vitro test (anti-haemolysin inhibition-AHI) in which sera containing antibodies to C. pseudotuberculosis neutralised the inhibitory action of C. pseudotuberculosis upon Staphylococcal β-lysin. The test is claimed to be specific and good correlation was noted between the serological and actual isolation of C. pseudotuberculosis from infected animals. A comparative study of in vitro (AHI) and in vivo (mouse protection-MP) test was also done by Zaki (1974) and he concluded that MP was more valuable than AHI especially in endemic localities. Burrell[a] (1980) described a haemolysis inhibition test for detection of antibody to C. pseudotuberculosis exotoxin which was based on the inhibition, by immune serum, of the haemolytic activity of C. pseudotuberculosis exotoxin for sheep red cells. The test was also shown to be suitable for studies on perinatal immunity.

In another work, Burrell[b] (1980) described a double immunodiffusion technique for detection of C. pseudotuberculosis antitoxin.

MATERIALS AND METHODS

A) Sera

A total of 3484 goat sera from animals of various ages, sexes and breeds from 9 farms (mainly government farms) and 22 smallholders involving 8 states in Peninsular Malaysia were collected over a period extending from 16th. February 1984 to 4th. December 1985. All samples except from UPM Goat Units were received through the Makmal Diagnosa, Petaling Jaya.

Thirty six samples were collected from UPM Goat Unit A mainly of local and unknown-local crosses, whilst the other 19 samples were from UPM Goat Unit B in Puchong which were mainly Anglo Nubian-Jamnapari-local crosses.

B) Serological test

The gel diffusion technique (double immunodiffusion technique), developed by Robertson (1980) was used to screen the sera for CLA. The basis of the test is the inter-reaction between toxin and antitoxin to produce detectable precipitin line between the two wells in a semisolid Ion agar.

a. Preparation of exotoxin (C. pseudotuberculosis)

The basic medium consisted of bovine heart 100 grams (finely grinded), bovine liver 100 grams (finely grinded), pepsin 5 grams, 10 ml concentrated HCl and 1000 ml distilled water. The ingredients were mixed thoroughly before being macerated for 24 hours in a water bath at 56°C. After heating for 5 minutes on a steamer (approximately

90°C), the medium was left overnight at room temperature. The medium was then filtered through a fine filter paper, pH was adjusted to 7.6 and autoclaved at 121°C for 15 minutes and stored at 4 C until used. Each culture in 250 ml volumes of medium in 500 ml flask was seeded with 2 to 3 loopfuls of C. pseudotuberculosis colonies scraped from a 48 hours blood agar culture plate. The flasks were incubated aerobically for 7 days at 37°C in a slanting position. Only culture which developed pellicles were used. The bulk of the bacterial cells were removed by centrifugation at 1500 rpm for 30 minutes at 4°C, and the supernatant was sterilised by passing through a sterile cellulose membrane (0.2 μm). The supernatant was stored in 50 ml aliquots in sterile flask at 4°C. The sediment was check for purity by subculturing onto blood agar plates and the toxin, for sterility by incubating one of the flask at 37 C for 7 days. Presence of growth of C. pseudotuberculosis was indicated by granular turbidity and if this occured, the toxin has to be filtered and checked for sterility repeatedly.

b. Preparation of lysin (Staphylococcus β- aureus)

Lysin was used for titrating the toxin of C. pseudotuberculosis based on the principle that the C. pseudotuberculosis toxin can inhibit the activation of lysin.

The ingredients consisted of proteose peptone 20 grams, anhydrous potassium dihydrogen orthophosphate 1 gram, anhydrous dipotassium hydrogen orthophosphate 1 gram, heptahydrate magnesium sulphate 0.2 gram, dihydrate calcium chloride 0.1 gram and 4.3 ml of syrupi ammonium lactate.

The flask containing the medium was covered and boiled for 10 minutes with constant stirring and then filtered with a filter

paper and allowed to cool at room temperature. Approximately 200 ml of the solution was then dispensed to 500 ml flask and autoclaved at 121 C for 15 minutes. The medium was checked for sterility by incubating aerobically at 37 °C and then stored at 4 °C until being used. Each flask containing the medium was inoculated with 5 ml of 3 to 5 hours culture of S. β- aureus grown aerobically in pepton water or tryptose soy broth. The flasks with 80% CO_2 and 20% O_2 were incubated at 37 C for 48 hours. After incubation, the medium was centrifuged at 1500 rpm for 30 minutes, the supernatant was then filtered through a cellulose acetate membrane with average spore diameter (ASD) of 0.65 μm and stored at 4 °C until used.

c. Preparation of bovine erythrocytes

Bovine blood was collected aseptically in an equal volume of sterile modified Alserver's solution and stored at 4 °C for a maximum of a week. For titration, the cells have to be washed 3 times with saline and centrifuged at 1500 rpm for 10 minutes. Then, 3 ml of washed packed cells were suspended in 97 ml saline for titration.

d. Lysin titration (Minimal Haemolytic Dose)

Two-fold dilution of β- lysin were made in 0.5 volume of normal saline (eg. 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256). Saline control tube of 0.5 ml normal saline was also included. Then 0.5 ml of a 3% washed bovine red cells was added to each tube followed by 1 ml saline to all tubes and mixed. The tubes were incubated at 37 °C for an hour, then were gently mixed and placed at 4 C for a minimum 3 hours or overnight. The highest dilution of lysin showing complete haemolysis (end point) represent the haemolytic titre or is known as the Minimal Haemolytic Dose (MHD).

For toxin titration, 2 MDH were used.

e. Toxin titration

Two-fold dilution of the supernatant of C. pseudotuberculosis toxin were made in 0.5 ml normal saline (as in d.). A saline control tube containing 1 ml saline and 0.5 ml 3% bovine red cell, and a lysin control tube which contained 0.5 ml 2 MDH lysin, 0.5 ml 3% bovine red blood cells plus 0.5 ml normal saline were included. Then 0.5 ml saline was added to each tube followed by 0.5 ml 3% bovine red cells to all tubes. They were gently mixed and incubated for 4 hours at 37°C. Upon removal from incubator, presence of haemolysis was noted. A further, 0.5 ml of 2 MHD lysin was added to all tubes except to the control tube. Again, they were gently mixed and incubated for one hour and left overnight at 4°C. The end point is read as the highest dilution that showed complete agglutination.

The lysin control tube should show complete haemolysis and no haemolysis should occur in saline control. For gel diffusion test, the titre of the toxin must be 1/512 or greater and it should not be used if the titre less than 1/526.

f. Preparation of gel

Purified agar (OXOID) 5 grams, sodium chloride 8 grams and 2.5 ml of phenol were dissolved in 500 ml of distilled water. The mixture was allowed to stand at room temperature for half an hour for gel imbibition. Then, the solution was gently heated to boiling point until a clear solution is formed, followed by autoclaving at 121°C for 15 minutes. The gel was ready for plating after cooling in a water bath at 56°C.

g. Preparation of Ion agar plates

Steriled 80 mm diameter petri dishes were used to contain approximately 22 ml Ion agar. The plates were placed on a level surface at room temperature until the agar had set, after which they were stored at 4 C for a minimum of 24 hours before used. Just prior to use, wells of 7 mm diameter on 11 mm centres (see APPENDIX III) were cut out clearly and evenly using a template. The peripheral wells were in a standard hexagonal pattern surrounding a central well.

h. Marking system

Four sets of 6 test wells were cut into the gel. In this manner, 24 sera were tested per petri dish and 4 (one for each set) for the toxin. By placing upside down, the plates were marked as I, II, III and IV. In set I, one well was marked as dot at the upper side to indicate well number one (for serum) and numbered 1 to 6 in an anticlockwise direction. The same system were used for the rest of the sets but the number used was the continuation of the first set (see APPENDIX III). Control serum normally filled the 24th. well.

i. Experimental procedure

To each well, 0.1 ml test serum was deposited the outer wells in anticlockwise sequence as indicated by the marking surrounded the central well containing exotoxin of similar amount. For this purpose 100 μm micropipette and tips were used. The empty wells were filled with serum and marked to avoid confusion. The purpose was to avoid the empty wells to drain fluid from the gel, thus affect the diffusion of the toxin and antitoxin as the gel tend to dry faster. The petri dishes then were left on a level bench in a moist

atmosphere, at room temperature for 3 days.

C) Intepretation of the test

The plates were examine after 2 to 3 days of incubation for precipitin lines under a bright light source against black background. A positive serum was indicated by precipitin line formed between antitoxin and toxin. The precipitin varied depending on the concentration of exotoxin, individual response of the animal and a complex nature of the toxin. The low antitoxin concentration may precipitate close to the serum wells and the higher concentration precipitated closer to the central well. If the antitoxin level were the same for all the sera, line of identity would form.

RESULTS

This present survey disclosed that out of the 3484 sera from various age groups, sexes and breeds of goats from different geographic regions and under different management system, 242 (7%) were positive to the test. The prevalence of CLA in individual farms ranged from 0% to 31.6% (APPENDIX IV). The evidence of infection among goats in smallholders were very low. Seropositive samples from smallholders were only detected in two areas; Sabak Bernam and Rawang, with prevalence of 13.3% and 20% respectively (APPENDIX IV).

Among the farms, Pusat Ternakan Haiwan Gajah Mati showed the highest prevalence of CLA (15.8%), followed by UPM goat herds (14.6%), Pusat pembiakan Kambing Kampung Pah (8.7%), Pusat Ternakan Haiwan Pantai (8.3%) and Pusat Ternakan Haiwan Batu Arang (8.0%)-(TABLE 3). No cases were recorded in other farms. The prevalence of CLA in the farms from Kedah, Selangor and Negeri Sembilan is significant.

TABLE 1: CLA POSITIVE REACTORS: COMPARISON BETWEEN FARMS AND SMALLHOLDERS

	No. samples	No. positive	% positive
Farms	2464	232	9.42
Smallholders	1020	10	0.98
Total	3484	242	6.95

$P (X^2 > 3.841) = 0.05$
$P (X^2 > 6.635) = 0.01$

TABLE 2: DISTRIBUTION OF POSITIVE REACTORS TO CLA IN GOATS ACCORDING TO STATES

States	No. samples	No. positive	% positive
Kedah	574	62	10.80
Negeri Sembilan	1116	76	6.81
Selangor	1546	104	6.73
Pulau Pinang	98	-	0
Perlis	63	-	0
Perak	39	-	0
Kelantan	39	-	0
Johor	9	-	0
Total	3484	242	6.95

$P (X^2 > 14.067) = 0.05$
$P (X^2 > 18.475) = 0.01$

TABLE 3: DISTRIBUTION OF POSITIVE REACTORS TO CLA AMONGST INTENSIVE FARMS

Farms	No. samples	No. positive	% positive
Pusat Ternakan Haiwan Gajah Mati	392	62	15.82
Unit Kambing UPM (2)	55	8	14.55
Pusat Pembiakan Kambing Kampung Pah, Jelebu	425	37	8.71
Pusat Ternakan Haiwan Pantai	469	39	8.32
Pusat Ternakan Haiwan Batu Arang	1077	86	8.0
Ladang Ternakan Ijok	19	-	0
Universiti Malaya (IPT)	17	-	0
Institut Haiwan Kluang	9	-	0
Pusat Latihan Ternakan Sungai Siput	1	-	0
Total	2464	232	9.42

$P (X^2 > 15.507) = 0.05$
$P (X^2 > 20.090) = 0.01$

TABLE 4: CLA PREVALENCE IN THE TWO GOAT HERDS IN UPM

	No. samples	No. positive	% positive
UPM Goat Unit A	36	2	5.56
UPM Goat Unit B (Puchong)	19	6	31.58
Total	55	8	14.55

$P (X^2 > 3.841) = 0.05$
$P (X^2 < 6.635) = 0.01$

TABLE 1 summarized the prevalence of CLA between intensive farms and smallholders. There was a dependence relation of prevalence of CLA in goats between farms (9.4%) and smallholders (1.0%) at both 0.05 and 0.01 level of significance. There seems to be a significant distribution of the disease according to states in Peninsular Malaysia. Kedah has the highest percentage of positive reactors (10.8%) followed by Negeri Sembilan (6.8%) and Selangor (6.7%). Goats from other states appears to be free of CLA (TABLE 2).

TABLE 4 illustrates the prevalence of CLA in 2 goat herds in UPM. A significant correlation of prevalence of the disease between the two herds observed at 0.05 but not at 0.01 level of significance. Out of 36 (47.4%) goats from Unit A tested, only 2 (5.6%) animals showed positive reactions, compared to 6 (31.6%) goats out of 19 (90.5%) screened in the other unit (B). All positive reactors were goats of 3 to 7 years of age. Only one goat had clinical signs as described in APPENDIX VII.

Unit A consisted of local and unknown-local crosses goats from newborn up to 4 years of age. A total of 79 animals were housed in 4 raised huts with slatted floors. The animals were allowed restricted grazing periodically, three times a week from 10 am to 2 pm. They were fed with concentrate in the afternoon and water was available ad lib. On other days when the goats were housed for the whole day, cut grass and concentrate were supplied.

Unit B were mainly made up of experimental goats of Anglo Nubian-Jamnapari-local crosses ranged from newborn to 7 to 8 years of age. A total of 21 animals were housed in a slatted floor raised hut. Intensive system was practised in this herd where they were given cut grass and concentrate once a day with water ad lib.

In general, most animals in the population had poor body condition and skin problem.

DISCUSSION

Almost seven percent (242/3484) of the goats examined had evidence of infection to Corynebacterium pseudotuberculosis. Overall, the prevalence of the disease in this country is consistent with findings in other parts of the world. In a survey of CLA in United States, Ashfaq and Campbell (1979) recorded, 8.1% (324/4013) of the animals had abscesses, of which 70% were caused by C. pseudotuberculosis. In another study, in 3720 feral goats examined during routine meat inspection in Australia, Hein (1981) found that CLA was the most frequently encountered pathological condition. The prevalence of infection among 9 separate consignment of animals averaged 7.4% (ranged 0.3% to 18.8%).

Prevalence of the disease varied from place to place from a disease-free area to a high prevalence of 31.6%. This study reveals that the prevalence of the disease is significantly higher in intensive farms than the smallholders, amongs atates as well as places. It is obvious that the difference in the prevalence of the disease in the farms compared to smallholders is correlate to the types of management and breed.

In Malaysia, the goat population is about 352,300 of which 88.6% is found in Peninsular Malaysia. Out of that, Kambing Katjang contributed 84% of the goats (Mahyuddin, 1984) and most of them are kept by smallholders owning less than 2 hectars of land (Peter et al, 1984). In 1974, the first exotic breeds such as Anglo Nubian, British Alpine, Toggenburg, Saanen, Jamnapari or Etawah and German fawn goat

were brought to the country for upgrading of Kambing Katjang. Most of these improved animals were distributed to all over the country particularly major government farms (Mahyuddin, 1984). Eventhough no information is available about the distribution of the disease among the various breeds of goats in this country, based on records, the disease appeared to be due to introduction of goats from United States (Omar et al, 1983) where CLA is rampant in the Saanen, Toggenburg, Anglo Nubian and in crossbred goats (Ashfaq and Campbell, 1979). The low prevalence of the disease in goats from smallholders could due to the breed of goats. The incidence of the disease in local goats is still not known and has never been observed (Omar et al, 1981). Zubaidah et al (1985) reported a very low prevalence of the disease among the smallholder herds (1.3%) and positive cases were all from the crossbred animals.

Type of management system plays a major role in disease transmission. Most smallholders (69%) raised goats either under the semi-intensive or extensive system (Peter et al, 1984). This may reduce the chances of direct or indirect contact between infected and healthy animals. Intensive goat keeping is normally practised by government or institutional farms where the goats are totally confined to a shed and feeds are brought to them (Mahyuddin, 1984). In this system, frequent contact and contamination of feeds, water and place with pus from ruptured abscesses of diseased animals explain for the high prevalenve of the disease in the intensive farms.

The disease appears to be endemic only in three states namely Kedah, Negeri Sembilan and Selangor. No cases were detected in the other states. These figures could be correlated to the number of

samples that had been obtained from various places. For example, out of 9 sera from Institut Haiwan Kluang tested, none was positive, but Shamshad (1985-personnal communication) reported a rather high prevalence of clinical CLA in the herd.

Universiti Pertanian Malaysia Goat Unit was established in 1979 with animals imported from Flora, Indiana, United States, of the Toggenburg, Saanen and Anglo Nubian breeds (Omar et al, 1983). However, Herd A was re-established recently with mainly local and unknown-local crosses that were brought from few farmers in the state. A closer study on the two goat herds revealed the same observation as the prevalence of the disease significantly correlated to the breed and types of management system as previously discussed. Only one goat was observed with a clinical CLA. The goat was a female, 7 year-old Anglo Nubian-Jamnapari-local cross of poor body condition. On closer examination, the right mandibular lymph node was enlarged (about 3 to 4 cm diameter) firm, cold and painless. Upon incision, the abscess discharged an odourless, thick and greenish yellow pus. In such a case, William (1980) has ruled out several differential diagnosis including abscesses due to Corynebacterium pyogenes or Staphylococcus aureus, salivary cyst, cyst of the brachial cleft, bottle jaw due to severe anaemia, soft tissue abscess and thyroid goiter.

Many workers believe there is no sex distribution of the disease (Ashfaq and Campbell, 1981). In this study, all the positive reactors were from female animals. This does not indicate anything as the animals were predominantly females (95% of the samples tested).

All the serologically positive goats were from 3 to 7 years. This result was consistent with other findings that the incidence

is higher in older goats due to repeated exposure to infection. However, goats ranging from 3 months of age to 10 years have been reported to be infected (Ashfaq and Campbell, 1981; Lund[a], 1982). Lund[a] (1982) observed the increased in titre values from the first to the third year of age was probably the result of longstanding antigenic stimulation. He also observed a decreasing titre values in goats aged 4 to 7 years in endemic areas. This may be related to lower immune response to C. pseudotuberculosis possibly because less antigen is presented to immunocompetent lymphocytes in immune animals.

Lund[b] (1982) studied the colostral transfer of antibodies against C. pseudotuberculosis in goats and antibody status of kids during the first 10 months of life. Most kids have antihaemolysin against C. pseudotuberculosis up to 5 to 8 weeks of age (Burrell, 1980; Lund[b], 1982). The presence of antitoxin against CLA at very early age indicates that it is not due to infection but likely due to passive colostral transfer.

Gel diffusion test is relatively easy to perform except when preparing the wells. Vacuum sucker was not suitable to use due to soft (semi solid) nature of the gel. The use of 22 ml of gel per plate produced a rather shallow wells, thus, easy for the serum or toxin to overflow and mixed with each other. For that it is recommended to use 25 ml of gel per plate. The test is claimed to be sensitive and reliable in detecting positive reactors (Burrell, 1980).

There is a considerable difference of opinions about the economic importance of caseous lymphadenitis to the sheep and goats industries (Jensen, 1982). Many farmers were unaware of the presence of the disease as it seldom causes death. However, Burrell (1981) revealed that significant economic losses caused by the disease are

condemnation of the infected carcasses or parts of the carcasses at slaughter, euthanasia of severely infected goats, reduced milk production, unthriftiness, occasional death, jeopadisation of income from boarding or mating with outside stock, the appearence of goats at stud show and sale of valuable breeding stock to local and export market. Addo (1980) encountered abortion in ewes, weak and infected lambs at 41 and 55 days when the animals were experimentally infected with C. pseudotuberculosis.

Recently the disease caused concern particularly in countries exporting mutton (Ashfaq and Campbell, 1980). King (1980) estimated 1.7 million Aust. dollar loss in sheep carcasses in West Australia each year. Similar losses could be expected in goats.

In Malaysia the economic importance of the disease is still not assessed. Information about the economic losses resulting from condemnation of infected carcasses or parts of carcasses are not available. However, it is suspected that a significant loss may arise from occasional death, chronic emaciation and poor reproductive performance.

In conclusion, in view of the high prevalence of goats that were seropositive to C. pseudotuberculosis in this country, further studies should be carried out particularly the epidemiology of the infection. Further serological surveys are warranted to establish the prevalence of the disease in the other states. On economic and public health, there is a need to control and eradicate the infection.

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APPENDIX I

NOMENCLATURE, CHARACTERISTIC AND IDENTIFICATION OF

C. PSEUDOTUBERCULOSIS

Nomenclature:

Order : Eubacteriales

Family : Corynebacteriaceae

Genus : Corynebacterium

Species : ovis / pseudotuberculosis (Benham et al, 1962)

Characteristics:

- Gram positive
- small rod or coccobacilli (pleomorphic) which are slender (0.5 to 0.6 by 1.0 to 3.0 μm) and often appears as filamentous forms
- often arranged in pairs or bundles resembling Chinese letters or palisades with club-shaped (irregular staining) and frequently banded or beaded with metachromatic granules
- non motile
- non sporing or non capsulated
- generally aerobic, microaerophilic or facultative aerobic
- the organism are usually numerous in young lesions, but scanty in old lesions
- survive up to 18 weeks in moist shady positions and 1 week in sunlight
- intracellular parasite.

(Benham et al, 1962; Ayers, 1977; Soltys, 1979; King, 1980)

APPENDIX I (continuation)

Identification:

a) Culture

Pus should be cultured on blood agar and on Loeffler's medium and incubated for 48 hours at 37 C under increased CO_2 concentration.

Blood agar

It produces white to ivory-coloured opaque convex colonies with matt surface, often with a narrow zone haemolysis.

Loeffler's medium

The colonies are yellow in colour, slightly raised, moist and glistening. (Soltys, 1979)

b) Staining

Gram's method.

APPENDIX I (continuation)

c) Biochemical reaction

	Ashfaq and Campbell 1979	Soltys 1979	Omar 1983
Catalase	+	+	+
Urease	V	+	+
Haemolysis	+	V	
Liquefaction of coagulated serum			
Clear zone on 10% agar milk			
Pigment			
Arginine hydrolysis		+	
Glucose	acid	acid	acid
Sucrose	alkaline		acid
Arabinose			acid
Maltose	acid		acid
Lactose	alkaline		
Galactose	acid		
Mannose	acid		
Fructose	acid		
Mannitol			acid(t)
Rhamnose			acid(t)
Dulcitol			acid(t)
Raffinose			acid(t)
Inulin			acid(t)
Ornithine			acid
Salicin			acid
Litmus milk	No change		
Gelatin	No change		
Citrate			
Nitrate			

V - variable ; t - trace

d) Animal inoculation

Organisms inoculated into male guinea pigs intraperitoneally often causes bilateral orchitis after 3 to 7 days, or local lesions depending on the virulence of the organisms. (Soltys, 1979)

APPENDIX II

BODY DISTRIBUTION OF CASEOUS LYMPHADENITIS IN GOATS

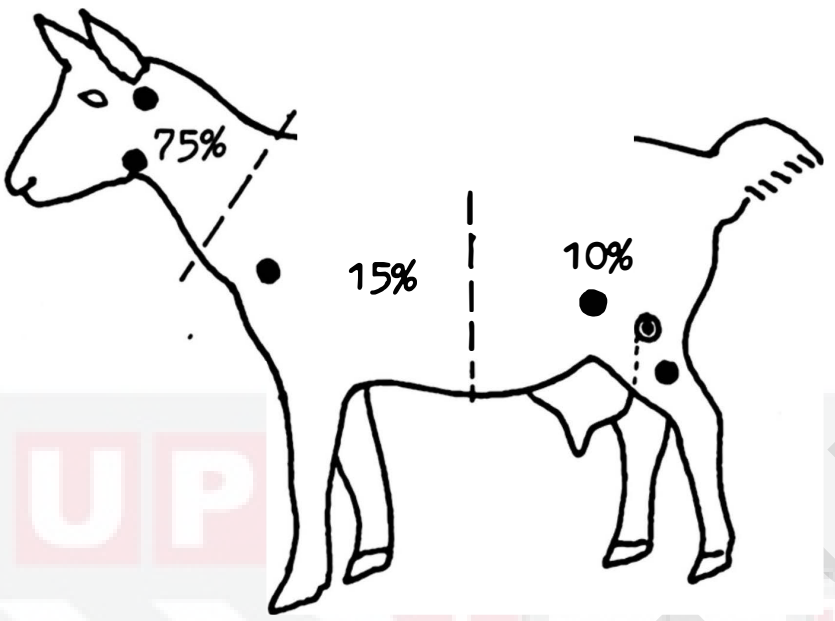

Fig. 1. The 'dot mark' indicate the location of superficial lymph nodes where abscesses commonly occur and the percent distribution is noted in the three areas of the goat's body (Ashfaq and Campbell, 1981

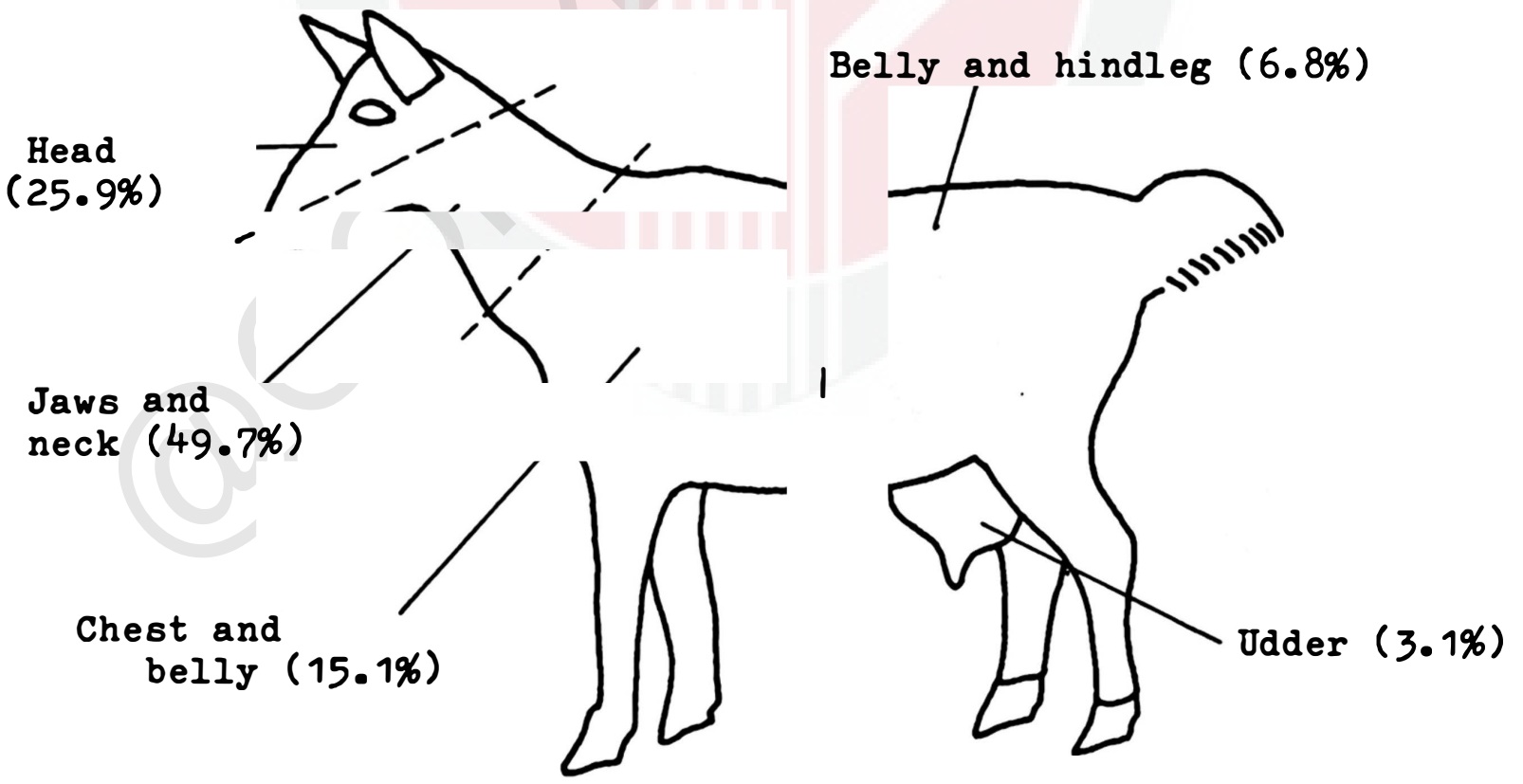

Fig. 2. Body distribution of superficial abscesses in 324 goats with caseous lymphadenitis (Ashfaq and Campbell, 1979)

APPENDIX III

MARKING SYSTEM

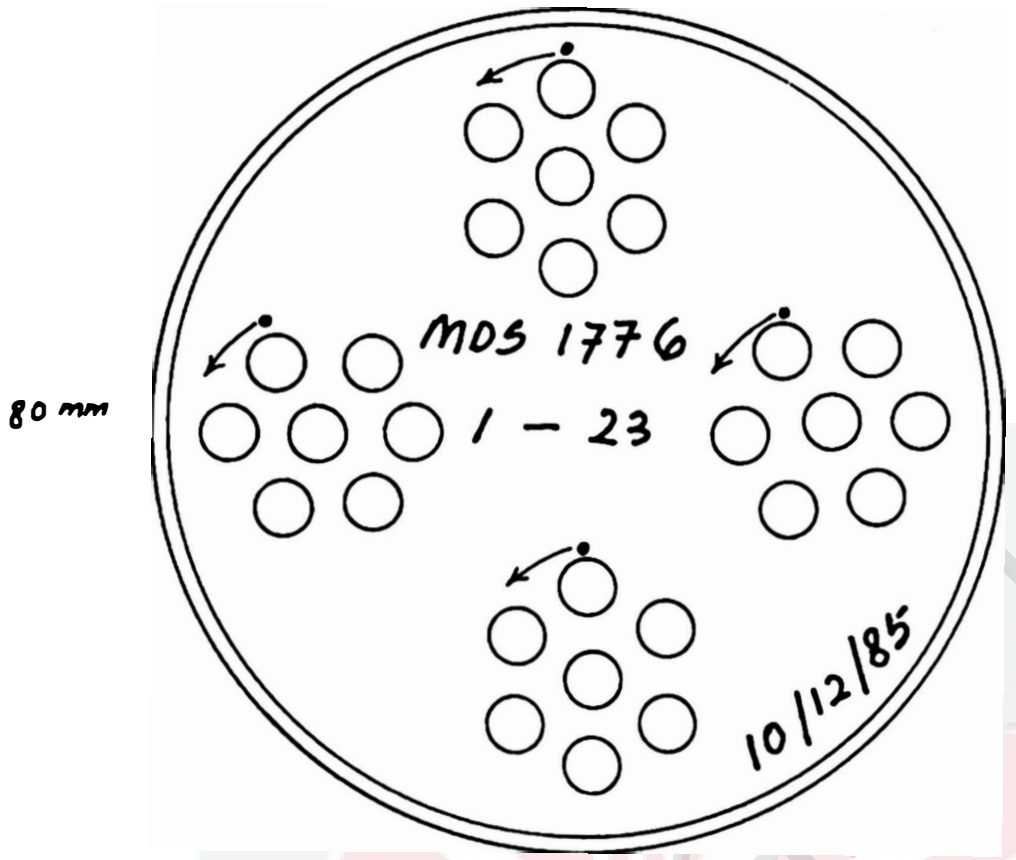

Fig. a. Top: Sample identification and date. Control serum should fill the 24th. well. The test should be read in an anticlockwise direction starting from the 'dot' of set I and so on.

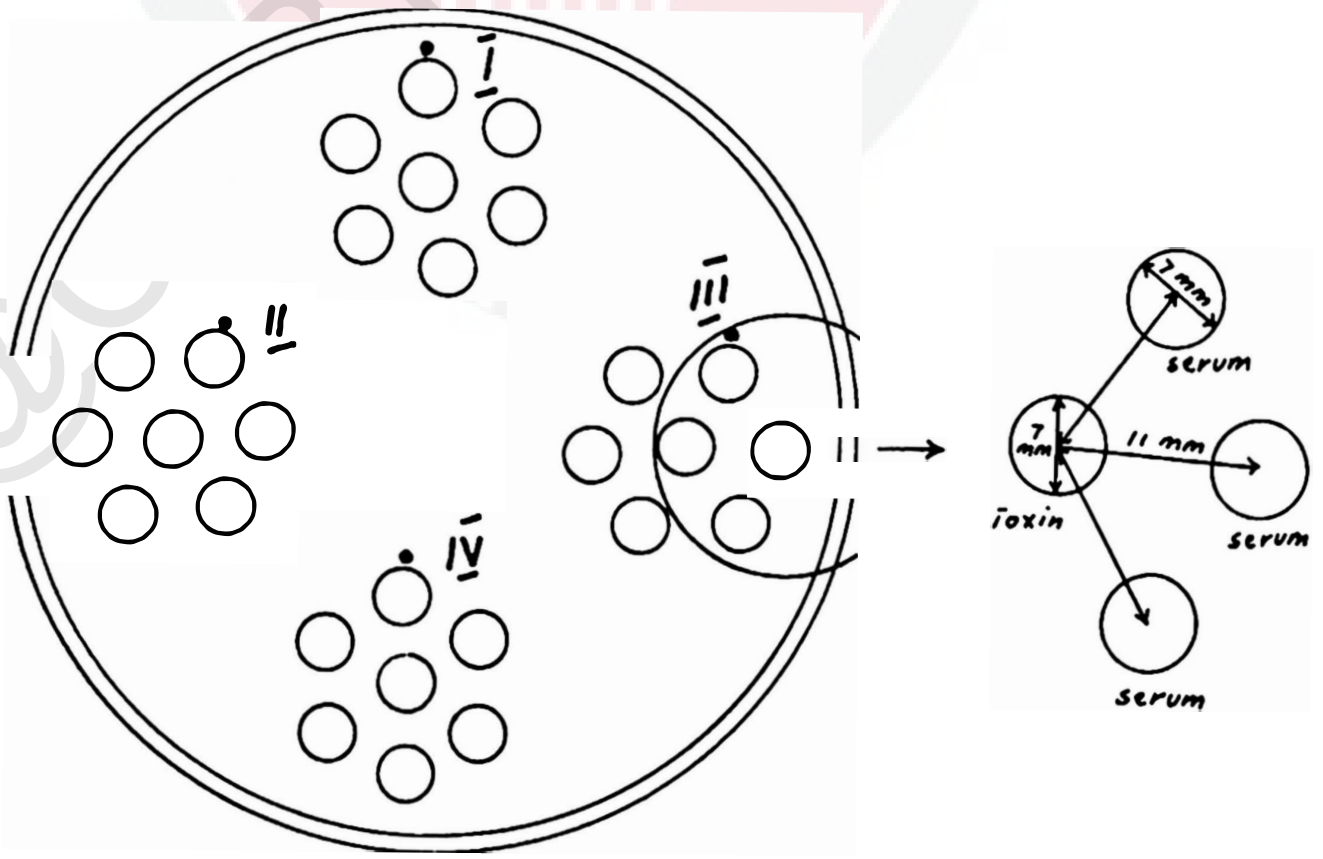

Fig. b. Bottom: The four sets of wells were marked as I, II, III and IV. The well marked 'dot' in set I represented sample '1' and well marked 'dot' in set II represented sample '7' and so on.

APPENDIX IV

DISTRIBUTION OF POSITIVE REACTORS TO CASEOUS LYMPHADENITIS FROM VARIOUS PLACES IN PENINSULAR MALAYSIA

Places	No. samples	No. positive	positive
A. NEGERI SEMBILAN			
i . FARMS (F)			
Pusat Ternakan Haiwan Pantai	469	39	8.32
Pusat Pembiakan Kambing Kampung Pah	425	37	8.71
Total (F)		76	8.50
ii. SMALLHOLDERS (SH)			
Pusat Haiwan Daerah Seremban	222		0
TOTAL	1116	76	6.81
B. SELANGOR			
i . FARMS			
Pusat Ternakan Haiwan Batu Arang	1077	86	7.99
Ladang Ternakan Ijok	19		0
Universiti Malaya (IPT)	17		0
Unit Kambing UPM (2)	55	8	14.55
Total	1168	94	8.05
ii. SMALLHOLDERS			
Penternak Daerah Kelang	90	-	0
Hulu Selangor	20		0
Penternak Daerah Semenyeh	26	-	0
Pejabat Haiwan Sabak Bernam	60	8	13.30
Ulu Langat	25		0
Pejabat Haiwan Daerah Kajang	25		0
Kuala Selangor	92		0
Pejabat Haiwan Gombak	25		0
Ulu Kelang	5		0
Rawang	10	2	20.0
Total	378	10	2.65
TOTAL	1546	104	6.73

APPENDIX IV (continuation)

Places	No. samples	No. positive	positive
C. KEDAH			
i . FARM			
Pusat Ternakan Haiwan Gajah Mati	392	62	15.82
ii. SMALLHOLDERS			
Pejabat Haiwan Jitra	30		0
Pejabat Haiwan Yan	31		0
Pejabat Haiwan Sik	30		0
Baling	30		0
Kota Setar/Pendang	30		0
Pejabat Haiwan Kuala Muda	31		0
Total	182		0
TOTAL	574	62	10.80
D. PULAU PINANG			
Makmal Diagnosa Bukit Tengah (SH)	98		0
E. PERLIS			
Pejabat Haiwan Perlis (SH)			0
F. PERAK			
i . FARM			
Pusat Latihan Ternakan Sungai Siput	1		0
ii. SMALLHOLDER			
Institut Penyelidikan Haiwan			0
TOTAL	39		0
G. KELANTAN			
Makmal Diagnosa Kelantan (SH)	39		0
H. JOHOR			
Institut Haiwan Kluang	9		0

APPENDIX V

DISTRIBUTION OF POSITIVE REACTORS TO CASEOUS LYMPHADENITIS AMONGST SMALLHOLDERS

Smallholders	No. samples	No. positive	positive
Pejabat Haiwan Daerah Seremban	222		0
Penternak Daerah Kelang	90		0
Hulu Selangor	20		0
Penternak Daerah Semenyeh	26		0
Pejabat Haiwan Sabak Bernam	60	8	13.3
Ulu Langat	25		0
Pejabat Haiwan Daerah Kajang	25		0
Kuala Selangor	92		0
Pejabat Haiwan Gombak	25		0
Ulu Kelang	5		0
Rawang	10	2	20.0
Pejabat Haiwan Jitra	30		0
Pejabat Haiwan Yan	31		0
Pejabat Haiwan Sik	30		0
Baling	30		0
Kota Setar/Pendang	30		0
Pejabat Haiwan Kuala Muda	31		0
Makmal Diagnosa Bukit Tengah	98		0
Pejabat Haiwan Perlis	63		0
Institut Penyelidikan Haiwan	38		0
Makmal Diagnosa Kelantan	39		0
TOTAL	1020	10	0.98

$P (X^2 > 31.410) = 0.05$ and $> 37.566 = 0.01$

(e) - (k) : $P (X^2 < 3.841) = 0.05$

APPENDIX VI

CLA: POSITIVE SERUM REACTORS IN UPM GOAT UNIT A

ID	TEST RESULT	CLINICAL SIGNS
3013	-	
122	-	
X	-	
3017	-	
3004		
6604		
3023		
6623		
3007	-	
3019	-	
6605		
6669	-	
6639	-	
6603	-	
3002		
3001	+	No clinical sign observed
6617	-	
6640	-	
3005	-	
6609	-	
6638	-	
6612	-	
3014	-	
3030	-	
4337		
4335	+	No clinical sign observed
4334	-	
3009		
3912		
6630		
6604	-	
3018		
6642	-	
3011		
3031		
3021		

Breeds: Local and unknown-local crosses

Total animals: 76 (Newborn to 4 years of age)

Total sample tested: 36

Percentage tested: 47.4%

Total positive: 2

Percentage positive: 5.56%

APPENDIX VII

CLA: POSITIVE SERUM REACTORS IN UPM GOAT UNIT B - PUCHONG

ID	TEST RESULT	CLINICAL SIGNS
1027		
1007 (M)	+	Generally poor body condition
1026		
1023		
1021		
1003		
1010		
1028		
1029		
1004	+	Emaciated and poor body condition
1005	+	Enlargement of right mandibular lymph node about 3 - 4 cm diameter and poor body condition
1001	+	Generally poor body condition
1006	+	- do -
1009		
1025	-	
1008		
1016		
1024	+	Emaciated and generally poor body condition
1014	-	

M - Male

Breeds: Anglo Nubian-Jamnapari-local cross

Total animals: 21 (Newborn to 7 - 8 years of age)

Total sample tested: 19

Percentage tested: 90.5%

Total positive: 6

Percentage positive: 31.58%