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A survey of caseous lymphadenitis in small ruminant farms from two districts in Perak, Malaysia – Kinta and Hilir Perak

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Abstract. A survey of Caseous Lymphadenitis (CLA), a bacterial infection in sheep and goats was conducted on small ruminant farms in two districts in Perak, namely Kinta and Hilir Perak. The objective of this survey is to determine the status of CLA infection in small ruminants. A total of 8 farms were screened, involving a total of 579 animals. Agar Gel Precipitation Test (AGPT) and Enzyme Linked Immuno Absorbent Assay (ELISA) were conducted on serum samples obtained from the animals. Results show that 8.5% of the animals had a positive reaction for AGPT test. It was found that 36 samples (17%) were found positive using both AGPT and ELISA methods, 9 samples (4%) were found positive only using AGPT method, 14 samples (6%)were found positive only using ELISA and 157 samples (73%) were found negative using both methods. Since there is no available data on the prevalence of the disease in the country, further epidemiological studies as well as reliable diagnostic detection methods need to be assessed for aiding in control and eradication programmes for this disease.

INTRODUCTION

The small ruminant population in Malaysia has been steadily increasing over the past ten years according to the census by the Department of Veterinary Services, Malaysia (http://agrolink.moa.my/jph). In 2006, the total population of sheep and goats in Malaysia was 116,697 head and 333,962 head respectively (Department of Veterinary Services, http://agrolink.moa.my/jph). There has been concerted efforts by the government to encourage small ruminant farming among smallholders as it can be a lucrative venture aimed to idealize Malaysia as the global centre for halal products and to make the agriculture sector the third pillar of economic growth (Ministry of Information, http://epu.jpm.my, Ministry of Agriculture http://www.moa.gov.my).

However, the livestock industry is plagued by diseases, which are the main causes of morbidity and mortality, reducing productivity and incurring losses for farmers. One such important disease is Caseous Lymphadenitis (CLA), caused by *Corynebacterium pseudotuberculosis*, CP, a gram-positive facultatively anaerobic rod resembling a coccus which can cause economic loss and occasionally debility and death in sheep and goats (Williamson, 2001).

CLA is prevalent in all the major goat and sheep-rearing areas of the world. In countries with large numbers of sheep such as Australia, the disease causes considerable financial losses through the condemnation and down-grading of affected carcasses at meat inspection (Paton, 1990). An additional loss comes through a syndrome called "thin ewe syndrome" which is associated with visceral CLA lesions. This syndrome is associated with low reproductive rates in the United States (Gates *et al.*, 1977) but the syndrome is not recognised as such in Australia (Paton, 1990). Although this syndrome is not typically associated with

death, this form of CLA leads to wasting, poor milk production and decreased wool production (Paton *et al.*, 1994). The prevalence of infection in adult sheep sent to Australian abattoirs is between 26% and 50% (Middleton *et al.*, 1991; Batey, 1986) and estimates from USA exceed 40% (Stoops *et al.*, 1984). This is a great loss for abattoir.

In Malaysia, *C. pseudotuberculosis* was first isolated at the Veterinary Research Institute, Ipoh in 1970 from a goat and in 1971 from a sheep. In the early sixties however, the condition was detected in imported sheep during meat inspection at the Johor Bahru abattoir (http://agrolink.moa.my/jph). This chronic contagious disease has not been studied in detail in Malaysia. Information on its epidemiology and prevalence is scarce (Paton, 1990). Thus, the objective of this survey is to report the occurrence of *C. pseudotuberculsis* infection in small ruminants located in Kinta and Hilir Perak Districts, Perak.

MATERIALS AND METHODS

Eight small ruminant farms (Table 1) were selected randomly, as suggested by the District Veterinary Office, in Kinta and Hilir Perak Districts.

Four farms situated in Kinta District and 4 farms situated in Hilir Perak District were chosen for this survey. Management and the farm animal population is shown in Table 1. Seven farms practiced semi-intensive management, where the animals were allowed to graze during the day for 4-8 hours and housed in a raised floor, wooden shed at night. One farm practiced intensive management where the animals were housed permanently and grass was supplied to the sheds. The population of the animals in the farms ranged from 31 to 121.

Blood samples were collected from 10-15% of the animals in each farm, making it a total of 579 samples available for testing. Sera were subjected to Agar Gel Precipitation Test (AGPT) and Enzyme Linked Immuno Absorbent Assay (ELISA) as described by (Sutherland *et al.*, 1987) and (Schreuder *et al.*, 1994; Dercksen *et al.*, 2000) respectively. However, not all the sera could be used for testing either due to the insufficient serum or the serum was already haemolysed. The results obtained using both methods were compared.

RESULTS & DISCUSSIONS

Table 2 illustrates the results of positive cases of CLA detected using AGPT method. A total of 579 serum samples were tested for CLA using the AGPT method. A total of 49 samples (8.5%) were positive using AGPT method. Based on the table, it was found that PR04 recorded the highest percentage of positive cases, i.e. 32% followed by BG01 (30%). With the exclusion of farm PR01, it was found that all farms recorded positive results using AGPT method. The lowest percentage of positive case was in GP02, with only 2%.

Table 1: General information on the 8 small ruminant farms involved in the study

District	Farm Code	Type of animal	Farm Management	Farm animal population
Kinta	BG01	Goat	Semi-Intensive	108
	GP01	Goat	Semi-Intensive	87
	GP02	Goat	Semi-Intensive	100
	GP03	Goat	Semi-Intensive	121
Hilir Perak	PR01	Goat and sheep	Intensive	31
	PR02	Goat	Semi-Intensive	80
	PR03	Goat	Semi-Intensive	92
	PR04	Goat	Semi-Intensive	72

Table 2: Number of positive sera detected with CLA using AGPT method

Farms code	Number of samples	Number of positive samples with AGPT	Percentage Positive (%)
BG01	30	9	30
GP01	87	2	2
PR01	31	ND	ND
GP02	46	1	2
GP03	141	4	3
PR02	80	4	5
PR03	92	6	7
PR04	72	23	32
TOTAL	579	49	8.46 (8.5)

Note: ND – Not done as sera was haemolysed

Table 3 illustrates the results for a total of 216 sera tested for CLA using ELISA method, and the comparison between the results between AGPT and ELISA method (at 0.250 O.D). It was found that 36 samples (17%) were found positive using AGPT and ELISA method, 9 samples (4%) were found positive only in AGPT method, 14 samples (6%) were positive only in ELISA and 157 samples (73%) were found negative in both methods.

Table 3: Comparison of serum samples tested for CLA using AGPT and ELISA method

For cut off 0.250 OD		
ELISA	AGPT	Total no. of serum tested
+	+	36
–	+	9
+	–	14
–	–	157
Total serum		216

Confirmation of *C. pseudotuberculosis* infection requires bacterial culture and identification. This is the gold standard for detecting positive CLA. The type of specimens collected for bacteriological examination is pus from abscesses (Paton *et al.*, 1994). However, due to the insidious and subclinical nature of CLA infections, it is difficult to acquire pus specimens for bacterial culture and identification.

Therefore, other methods of detection are being assessed for routine diagnostic use. For gel diffusion method, the sensitivity and specificity are 50-60% and 60-70% respectively (data obtained from serology unit, VRI). While sensitivity and specificity value for CLA tested using ELISA method are 91.8% and 80% respectively based on the AGPT result using formula given (http://www.poems.msu.edu/InfoMastery/Diagnosis/SensSpec.htm)

Based on Table 3, it was found that 8.5% (49/579) of the serum samples examined by gel diffusion method had evidence of CLA. The prevalence of infection ranged from 2 to 32 percent amongst the 8 farms having positive reactors. Two farms; BG01 and PR04 were found to have high (^{3} 30%) prevalence. The high prevalence in the semi-intensive farms may be due to the high stocking rate, leading to overcrowding which in turn allows for easy transmission of contagious diseases. Route of infection for CLA are various; *C. pseudotuberculosis* can enter its host through cuts in the skin after shearing and also from contamination of the environment including shearing sheds, holding pens, dip wash and in faeces (Seddon *et al.*, 1929). Besides that, the disease can be transmitted by physical transfer of CLA pus from

abscesses discharging at shearing and also by direct contact with other goat or sheep through abrasions from head-butting, ear biting and browsing (Nagy, 1971). Lung abscesses also play a major role in the spread of CLA in commercial sheep flocks. By culturing *C. pseudotuberculosis* from the tracheae of sheep with lung abscesses, it has been observed that many lung abscesses in sheep discharge into airways (Robertson 1980; Ellis *et al.*, 1987). When this occurs these sheep may be capable of spreading CLA to a large number of sheep at one shearing by aerosol contamination of skin cuts on uninfected sheep (Paton, 1997). Therefore, once CLA is detected in an animal, it becomes endemic in a farm or locality.

A critical part of raising goat is establishing correct stocking rates. Goat stocking rates must be based upon controlling internal parasites and avoiding over-crowding (www.tennesseemeatgoats.com). The presence of large number of positive reactors was perhaps, the result of congregation of large number of animals in a defined area. Some of these farms have imported goats of improved breeds and these animals are suspected to be more susceptible to CLA.

The infection seemed to proliferate on introduction of an infected animal to a farm, particularly, the semi-intensive farm where there were large number of susceptible animals in a given area. The organism is known to survive for long periods of time in the soil (Augustine & Renshaw, 1986). Infection of abrasions and wounds with pus containing the organisms or with contaminated soil would lead to infection of the animal. Various infection routes would require different management changes to minimise CLA spread at shearing. Control of the spread of caseous lymphadenitis is of utmost importance to ensure healthy marketable animals.

Since affected animals serve as reservoirs of infection, the most practical method of control of CLA in sheep and goat flocks, apart from the use of an effective vaccine, is to cull all animals that have palpable lesions (Renshaw *et al.*, 1979) especially as mentioned earlier lung abscesses in sheep is discharged into airways. In Malaysia, the effective way of controlling CLA is by culling infected animals. By looking at the route of transmission it is very important to practise good hygiene in shearing and handling of animals in minimizing infection within animals.

Culling policy, however is not popular among small farmers due to the high value of the animals. Animals which develop abscesses should be isolated from the flock and the abscesses lanced and flushed with an iodine solution. CLA is extremely resistant to antibiotic therapy because the thick caseous pus is contained in a tough fibrous capsule which antibiotics cannot penetrate. CLA vaccines formulated from concentrated, formalin-inactivated CP culture supernatants containing *pld* have considerable efficacy (Eggleton *et al.*, 1991). In South Africa, inactivated whole-cell vaccines, with an aluminium hydroxide adjuvant containing saponin and devoid of exotoxin, have provided better protection in sheep (Cameron & Bester, 1984). Live vaccines are no better than inactivated vaccines and the local reaction they cause at the injection site is unacceptable (Cameron & Bester, 1984). The antigenic purity of many of the early toxoid vaccines has been questioned, as numerous other soluble antigens are present in vaccines made from *C. pseudotuberculosis* culture filtrates (Muckle *et al.*, 1992). Later, carefully planned vaccination programmes with the toxoid have been shown to be effective in decreasing the disease to a low level (Paton, 1997). However, further studies on the efficacy of the vaccine need to be carried out to optimise its effect towards the control of CLA.

During the farm visit, about 2% of the animals showed clinical signs of abcesses at the lymph nodes around the neck and other parts of the body. Most animals did not show clinical signs, thus making a diagnosis challenging. Therefore, a culling policy should be effective.

The incidence of the disease in local goats has long been realized but not many studies have been carried out. The disease,

being insidious and subclinical makes it difficult to distinguish between infected and non-infected animals during the long incubation period of the disease (Paton, 1990). Due to this reason, it attracted little attention compared to other more important animal diseases like brucellosis and tuberculosis. Gel diffusion test may be a useful procedure for screening animals before introducing them into a clean farm or for monitoring the infection in an area. However, serological testing may not be accurate due to the presence of antibodies in previously exposed non-diseased or due to chronically infected animals becoming serologically negative or cross-reactivity of diagnostic antigens with antibodies against other bacteria. Since there were positive samples detected in this survey, there is a need for the survey to be expanded, especially survey for lesions at abattoirs in the future. This is to ensure that proper action can be quickly taken to control the disease in the country. The control of zoonotic diseases is of utmost importance for the progress of the nation's livestock industry.

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REFERENCES

Augustine, J.L. & Renshaw, H.W. (1986). Survival of *Corynebacterium pseudotuberculosis* in axenic purulent exudate on common barnyard fomites. *American Journal of Veterinary Research* **47:** 713–715.

Batey, R.G. (1986). *Frequency and consequence of caseous lymphadenitis in sheep and lambs slaughtered at a Western Australian abattoir. American Journal of Veterinary Research* **47:** 482.

Cameron, C.M. & Bester, F.J. (1984). An improved *Corynebacterium pseudotuberculosis* vaccine for sheep. *Onderstepoort Journal Of Veterinary Research* **51:** 263 – 267.

Department of Veterinary Services (http://agrolink.moa.my/jph/).

Dercksen, D.P., Brinkhof, J.M.A., Dekker-Nooren, T., van Maanen, K., Bode, C.F., Baird, G. & Kamp, E.M. (2000). A comparison of four serological tests for the diagnosis of caseous lymphadenitis in sheep and goats. *Veterinary Microbiology* **75:** 167–175.

Eggleton, D.G., Middleton, H.D., Doidge, C.V. & Minty, D.W. (1991). Immunisation against ovine caseous lymphadenitis: comparison of *Corynebacterium pseudotuberculosis* vaccines with and without bacterial cells. *Australian Veterinary Journal* **68:** 317-319.

Ellis, T.M., Sutherland, S.S., Wilkinson, F.C., Mercy, A.R. & Paton, M.W. (1987). The role of *Corynebacterium pseudotuberculosis* lung lesions in the transmission of this bacterium to other sheep. *Australian Veterinary Journal* **64:** 261.

Gates, N.L., Everson, D.O. & Hulet, C.V. (1977). *Effects of Thin Ewe Syndrome on reproductive efficiency. Journal of the American Veterinary Medical Association* **171:** 1266-1267.

Middleton, M.J., Epstein, V.M. & Gregory, G.G. (1991). *Caseous lymphadenitis on Flinders Island: prevalence and management surveys. Australian Veterinary Journal* **68:** 311.

Ministry of Agriculture (http://www.moa.gov.my).

Ministry of Information (http://epu.jpm.my).

Muckle, C.A., Menzies, P.I., Li, Y., Hwang, T. & Van Wesenbeeck, M. (1992). Analysis of the immunodominant antigens of *Corynebacterium pseudotuberculosis. Veterinary Microbiology* **30:** 47-58.

Nagy, G. (1971). Ticks and caseous lymphadenitis in sheep: preliminary observations. *Journal of the South African Veterinary Medical Association* **42:** 227-232.

Paton, M. (1990). *Caseous lymphadenitis. University of Sydney Post-graduate Committee in Veterinary Science, Proceedings* No **141:** 149.

Paton, M. Caseous Lymphadenitis a Global Sheep Disease. (up.ac.za/academic/lhpg/CD info/Papers/CLA/).

Paton, M.W., Rose, I.R., Hart, R.A., Sutherland, S.S., Mercy, A.R., Ellis, T.M. & Dhaliwal, J.A. (1994). New infection with *Corynebacterium pseudo-tuberculosis* reduces wool production. *Australian Veterinary Journal* **71:** 47–49.

Paton, M.W. (1997). Caseous Lymphadenitis. *Proceedings of the Fourth International Congress for Sheep Veterinarians*, Armidale NSW Australia, 121.

Renshaw, H.W., Graff, V.P. & Gates, N.L. (1979). Visceral caseous lymphadenitis in thin ewe syndrome; isolation of *Corynebacterium*, *Staphylococcus and Moraxella* spp. from internal abscess in emaciated ewes. *American Journal of Veterinary Research* **40**: 1110-1114.

Robertson, J.P. (1980). Studies on diagnosis, epidemiology and immunity of *Corynebacterium pseudotuberculosis* infection in sheep. *MPhil thesis, Murdoch University, Western Australia.*

Schreuder, B.E., Ter, L.E.A. & Dercksen, D.P. (1994). Eradication of caseous lymphadenitis in sheep with the help of a newly developed ELISA technique. *The Veterinary Record* **135:** 174-176.

Seddon, H.R., Belschner, H.G., Rose, A.L. & Blumer, C. (1929). Further observations on the method of infection in caseous lymphadenitis of sheep. *Australian Veterinary Journal* **5:** 139-148.

Sensitivity and specificity calculations. (http://www.poems.msu.edu/InfoMastery/Diagnosis/SensSpec.htm).

Stoops, S.G., Renshaw, H.W. & Thilsted, J.P. (1984). *Ovine caseous lymphadenitis: Disease prevalence, lesion distribution, and thoracic manifestations in a population of mature culled sheep from western United States. American Journal Veterinary Research* **45:** 557-561.

Sutherland, S.S., Eillis, T.M., Mercy, A.R., Paton, M.W. & Middleton, H. (1987). Evaluation of an enzyme-linked immunosorbent assay for the detection of *Corynebacterium pseudotuberculosis* infection in sheep. *Australian Veterinary Journal* **64**(9): 263-266.

Suzanne, W.G. Onion Creek Ranch; Meat Goats and Tennessee meat goats. (www.tennesseemeatgoats.com).

Williamson, L.H. (2001). Caseous lymphadenitis in small ruminants. *The Veterinary Clinics of North America: Food Animal Practice* **17**(2): 359-371.