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Seroprevalence of caseous lymphadenitis in Kangal Akkaraman sheep

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Abstract : In this study, the seroprevalence of *Corynebacterium pseudotuberculosis*, the causative agent of caseous lymphadenitis (CLA) infection, in Kangal Akkaraman sheep flocks was examined. For this purpose, blood samples were collected from 25 different sheep flocks (n = 379). According to the ELISA results, the numbers of CLA-positive and CLA-negative samples were 138 (36.4%) and 241 (63.6%), respectively. CLA positivity was observed in 35.7% of the females (n = 241), 37.7% of the males (n = 138), 40.2% of the sheep with clinical symptoms (n = 184), and 32.8% of the sheep with no clinical symptoms (n = 195) (P > 0.05). We concluded that *C. pseudotuberculosis* is a disease observed widely in the Kangal Akkaraman sheep breed in Turkey. To the best of our knowledge, this is the first study reporting the seroprevalence of CLA in Kangal Akkaraman sheep, a breed whose first official herdbook was only recently established.

Key words: *Corynebacterium pseudotuberculosis*, prevalence, sheep, Kangal Akkaraman

Kangal Akkaraman is a dual-purpose (bred for both meat and milk), stout, and heavy breed with a fat tail. It is usually bred to adapt to poor plant cover in areas with continental climate. The herdbook of the Kangal Akkaraman sheep breed was first established in 2012 (1), and there are currently no epidemiological studies that report the prevalence of caseous lymphadenitis (CLA) in this breed.

CLA is a bacterial and contagious infection caused by *Corynebacterium pseudotuberculosis* and characterized by formation of abscesses in lymph nodes and internal organs in sheep and goats (2). It has been reported that *C. pseudotuberculosis* causes infections with different symptoms in other animal and human (3,4).

The economic losses of CLA in the sheep industry are associated with early slaughter of agent-positive animals and downgrading of carcass, wool, and skin quality and yield (4,5). In addition, ewes with CLA may exhibit reproduction-related symptoms such as reduced fertility, abortions, stillbirths, and neonatal infections (6). In Australia, annual losses of $12–15 million were reported from CLA due to reduced yields of meat and wool (7).

In spite of being the golden standard, isolation and identification of *C. pseudotuberculosis* is not always practical and advantageous in the diagnosis of CLA. One of the reasons for this is that the skin and the surroundings of the animal pose a risk for infection and contamination for other animals during the puncture of the abscesses. Furthermore, fibrosis forms in ruptured external lesions, and in addition there is little pus and few live microorganisms. Moreover, animals with the visceral form of CLA may not have lesions that are suitable for sampling, though they still continue to be a source of infection for other animals (4). Therefore, serological tests have been developed, whereby humoral response towards the phospholipase D (PLD) exotoxin is assessed. Some of these serological tests include antihemolysis inhibition, indirect hemagglutination, hemolysis inhibition, immunodiffusion, and enzyme-linked immunosorbent assay (ELISA). Another test suggested for the identification of CLA is polymerase chain reaction, which is used to identify the bacteria isolated from abscesses (4,8). Previous studies proved that the ELISA test is an effective method in the control and eradication of CLA (3,9). In particular, ELISA methods based on the PLD antigen obtained by recombinant technology have been reported to be specific and sensitive in field diagnosis (4). Therefore, in this study, we chose to isolate the causing agent using an ELISA test based on the PLD antigen.

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There seems to be a paucity of serological studies reporting the epidemiology of the disease at regional and breed levels in Turkey. The majority of the work conducted to reveal the prevalence of the disease in Turkey has used slaughterhouse materials. The disease is widespread in Turkey due to many factors, the most important of which include the nature of the farms, with small-scale family businesses dominating the industry; intense animal movements between flocks; and farmers' lack of awareness of biosecurity measures and prevention methods.

It appears that no studies have previously reported the presence and prevalence of CLA in Kangal Akkaraman sheep. Thus, this study aims to determine the seroprevalence of CLA in the Kangal Akkaraman sheep bred commonly in Sivas Province in Turkey.

The material of this study consisted of 379 Kangal Akkaraman sheep (241 females, 138 males), randomly collected from 25 flocks from Altınyayla (n = 84, from 7 different flocks), Gemerek (n = 110, from 4 different flocks), Kangal (n = 31, from 6 different flocks), Şarkışla (n = 65, from 6 different flocks), Ulaş TİGEM (n = 80), and Hafik (n = 9). Sampled animals were aged between 1 and 4 years old and had no previous history of immunization against CLA. Blood samples were collected from the jugular vein using serum collection tubes coated with silicone during January and February 2015. After collecting the blood samples, animals were clinically examined for the presence of abscesses. Sera were centrifuged at 1500 × *g* for 15 min at room temperature and stored at –20 °C until analysis.

The ELITEST CLA # CK105A kit (HYPHEN BioMed, France) was used in order to determine the CLA antibodies in serum samples. Results were evaluated using an ELISA reader (BioTek EL × 808, United States) according to the manufacturer's instructions. For the direct ELISA method, we used the recombinant form of PLD as the *C. pseudotuberculosis* virulence factor to measure the anti-PLD antibodies in sera of sheep with CLA. Values above and below an optical density (OD) of 400 were considered to be positive and negative, respectively. A nonspecific reaction was identified when the OD with the negative antigen was more than 50% of the maximum OD of the positive control.

Pearson's chi-square (χ^2) test was performed to calculate the impact of sex and the presence of abscesses on CLA positivity. The risk of CLA positivity was expressed by computing the odds ratio. The data were analyzed using SPSS 14.0.1 (serial 9869264, SPSS Inc., USA). Statistical significance was defined as $P < 0.05$.

No clinical symptoms other than the presence of abscesses were determined. The ELISA results are summarized in Table 1. Mean ELISA results in CLA-positive and -negative sheep in six regions are presented in Table 2.

The CLA prevalences in different districts of Sivas Province were calculated as 25% in Altınyayla, 30% in Gemerek, 25.8% in Kangal, 50.76% in Şarkışla, 47.5% in Ulaş TİGEM, and 55.55% in Hafik (Figure).

The difference between the prevalence of CLA in females and males was not statistically significant ($P > 0.05$), as 35.7% and 37.7% of females and males were CLA-positive, respectively. These results suggest that the disease is observed in males 1.09 times more frequently than females (Table 3).

Table 3 shows that 32.8% and 40.2% of the animals without and with abscesses, respectively, were CLA-positive. CLA was seen 1.37 times more often in animals with abscesses than those without abscesses. No statistical differences were found between the two groups with and without abscesses ($P > 0.05$) (Table 3).

CLA is a prevalent, chronic, and subclinical infectious disease with substantial economic implications for sheep farms. The frequency of observance of the disease in Australia was reported as 26% (7). Similarly, the CLA prevalence in sheep was found as 42.4% in a study conducted in the western United States (10), and 12.6% from bacteriological analysis and 20.08% from

Table 1. ELISA test findings of CLA-positive and -negative sheep with and without abscesses.

	CLA	N	ELISA	Minimum	Maximum	95% confidence interval for mean	
			Mean ± std. deviation			Lower	Upper
Abscess (+)	Negative (-)	110	0.234 ± 0.146	0.037	0.602	0.206	0.261
	Positive (+)	74	1189.0 ± 1190.6	0.627	3562.0	913	1464
Abscess (-)	Negative (-)	131	0.219 ± 0.157	0.027	0.568	0.191	0.246
	Positive (+)	64	1499.9 ± 1255.7	0.616	3719	1186	1813
Total	Negative (-)	241	0.226 ± 0.153	0.027	0.602	0.206	0.245
	Positive (+)	138	1333.1 ± 1226.7	0.616	3719	1126	1539

Table 2. Mean ELISA results in CLA-positive and -negative sheep in 6 regions.

Region	CLA	N	Mean ± std. deviation	Minimum	Maximum
Altınyayla	Negative (-)	63	0.219 ± 0.167	0.03	0.57
	Positive (+)	21	1204.1 ± 1128.9	0.63	3276
Gemerek	Negative (-)	77	0.199 ± 0.143	0.03	0.6
	Positive (+)	33	1729.1 ± 1342.8	0.62	3719
Kangal	Negative (-)	23	0.218 ± 0.147	0.04	0.49
	Positive (+)	8	1677.9 ± 1299.4	0.65	3460
Şarkışla	Negative (-)	32	0.205 ± 0.145	0.05	0.54
	Positive (+)	33	954.2 ± 1126.9	0.66	3001
Ulaş	Negative (-)	42	0.298 ± 0.139	0.07	0.55
	Positive (+)	38	1403.8 ± 1202.7	0.63	3439
Hafik	Negative (-)	4	0.299 ± 0.142	0.17	0.49
	Positive (+)	5	675.3 ± 923.6	0.71	1692

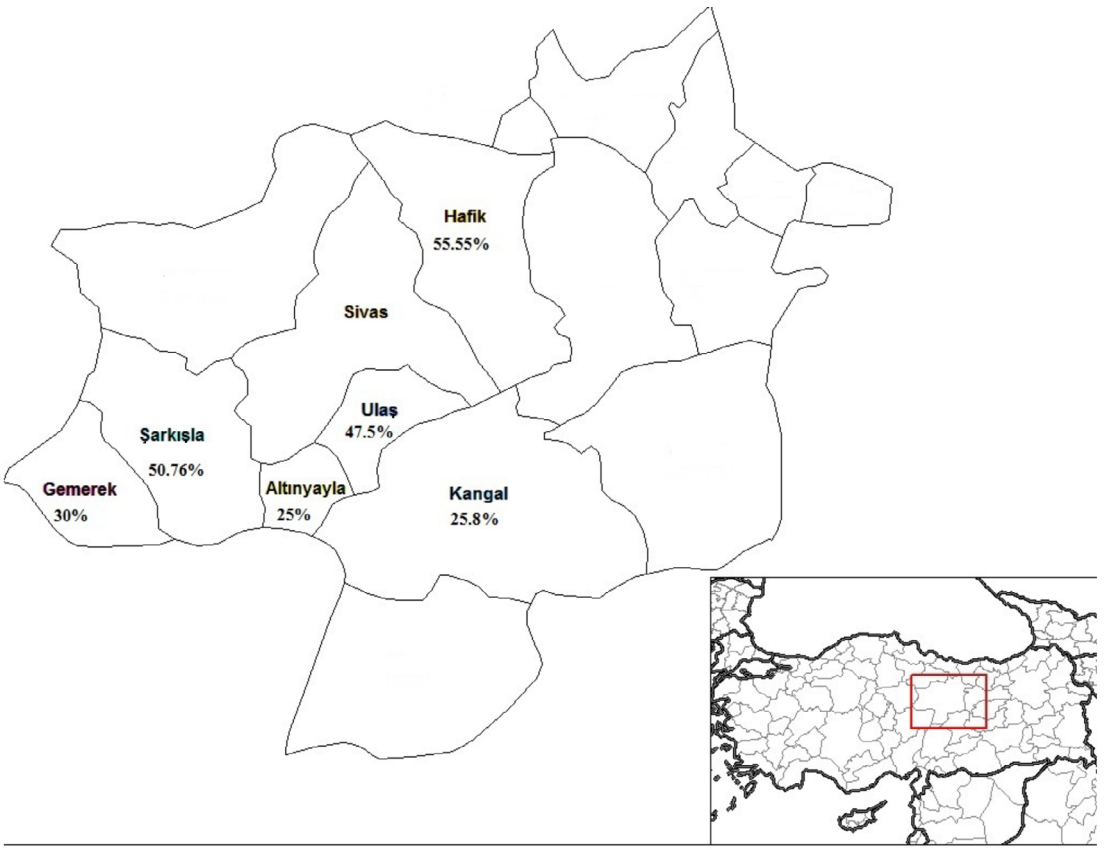

Figure. The CLA prevalence of sampled animals in different districts of Sivas Province.

histopathological analysis in Tabriz (Iran). While the frequencies of CLA observation in sheep and goats were 23.33% and 11.04%, respectively, according to clinical examination, these frequencies were reduced by 22.10% and 7.77% in sheep and goats, respectively, if the agent was determined through bacteriological methods (11). CLA positivity in sheep was identified as 71% in a study using an indirect ELISA method in Brazil (9). In

Table 3. The CLA prevalence according to sex and clinical symptoms.

		CLA		Statistical significance
		Negative (-)	Positive (+)	
Sex	Female (n = 241)	155 (64.3%)	86 (35.7%)	$\chi^2 = 0.151$ (P > 0.05) OR (Male / Female): 1.09 (0.71–1.68)
	Male (n = 138)	86 (62.3%)	52 (37.7%)	
Abscess	- (n = 195)	131 (67.2%)	64 (32.8%)	$\chi^2 = 0.151$ (P > 0.05) OR (Abscess (+ / -): 1.37 (0.91–2.09)
	+ (n = 184)	110 (59.8%)	74 (40.2%)	
Total (n = 379)		241 (63.6%)	138 (36.4%)	

χ^2 : Pearson chi-square values; OR: odds ratio.

Turkey, on the other hand, Çetinkaya et al. (12) sampled slaughterhouse materials and found 3.5% CLA positivity in Elazığ Province. Aydın (13) isolated 77 CLA-positive samples from a total of 552 sheep samples (12.6%) in Turkey. Similarly, Erganiş et al. (14) recorded a 16% *C. pseudotuberculosis* isolation rate from 100 sheep with abscessed lymph nodes. In another research carried out in Elazığ and its surroundings, 32.7% of the total samples (e.g., lymph nodes with abscesses) were CLA-positive (15). İlhan et al. (8) serologically tested 245 randomly selected sheep and found that 74 (30.2%) were CLA-positive and 171 (39.7%) were CLA-negative. In the present study, CLA prevalence in Kangal Akkaraman sheep was found as 36.4%. The differences between CLA prevalences in different studies may be attributed to breeding techniques, regional differences, breed sensitivity, material selection, and/or methods used to determine CLA.

The epidemiology of the disease may differ in extensive and intensive breeding systems. For example, in extensive breeding systems, CLA is more widespread in adults than lambs, as opposed to intensive breeding systems, where CLA's prognosis in lambs is mild and it has the tendency to become chronic in adults (16). Given that the present study materials were collected from semiintensive systems, these systems are envisaged to be of importance for the prevalence of the disease.

While the CLA prevalence in Kangal Akkaraman sheep, a recently registered breed, was determined as 36.4% in the current study, it was indicated that Merino sheep in Australia had a higher prevalence rate compared to other breeds (16). Similarly, Pépin (17) stated that some dairy breeds in France had higher CLA prevalence than other breeds, as well. In a study published by Ribeiro et al. (9) in Brazil, Santa Ines breed sheep exhibited the disease with 57% frequency in comparison with the Suffolk breed with 5% CLA prevalence.

In this study, although no statistically significant differences in prevalence were found between the two sexes (P > 0.05), CLA infection was observed in males 1.09 times more frequently than females. Higher disease prevalence in males than females may be due to the higher risk of pushing on and bursting abscesses during the mating season, when males interact physically more than females (e.g., mounting). Experimental studies where sheep were infected with *C. pseudotuberculosis* intravaginally have been reported (2). Even though the infection in rams may lead to orchitis and epididymitis (18), there are no detailed studies on the roles of rams in transmitting the pathogen. Studies carried out with sheep illustrated higher CLA prevalence in females than males (96% and 4%, respectively) (9). A similar result was published by Al-Gaabary et al. (19), who presented a 19.67% CLA occurrence in females in comparison with a lower frequency in males (12.42%). Batey et al.'s study (20), supporting the results of the current study, reported a higher prevalence in males than females in goats. Results published by Ribeiro et al. (9) argued for a higher prevalence of CLA in females than males. The differences could be explained by factors such as breed, region, and material selection and need further discussion.

The CLA lesions in sheep are progressive and chronic. Lesions do not show any clear symptoms unless the they are considerably large in size and quantity, affecting the organ's functionality. Pervasive abscesses in various volumes and structures may be located even in healthy-looking animals' internal organs. Livestock with abscesses localized in the lungs may cough or wheeze when breathing. Livestock with a large number of abscesses

in internal organs may experience chronic weight loss and unthriftiness, also known as "thin ewe syndrome" (21). Some of the important means by which the disease is transmitted are pastures, pens, and management tools contaminated by ruptured abscesses localized usually in superficial lymph nodes or in the bronchus (22). Another important transmission path is the drainage of abscesses in lungs into mucus and saliva (22,23). Bacterial cultures of trachea contents in sheep indicated that lung abscesses drain into the trachea (23).

The infection rate of 32.8% in animals without clinical symptoms in the current study indicates that subclinical infection has a major role in CLA prevalence. In this study, a prevalence of 40.2% and 32.8% of *C. pseudotuberculosis* was found using the ELISA method in animals with and without abscesses, respectively. While the detection of CLA in asymptomatic animals may reflect the presence of infection in the visceral form, the absence of CLA pathogens in animals with clinical symptoms may be due to diseases accompanied by suppurative lymphadenitis irrelevant to *C. pseudotuberculosis*.

This latter point is supported by İzgür et al. (24), who conducted a bacteriological study where they investigated the abscesses localized in lymph nodes of sheep brought to a slaughterhouse. They concluded that even though *C. pseudotuberculosis* (46.3%) was the dominate agent isolated, the following pathogens were also determined from the sampled materials: *Micrococcus* spp. (19.5%), *P. aeruginosa* (7.3%), *S. aureus* (7.3%), and *S. epidermidis* (4.8%). It should be noted that other bacteria causing suppurative lymphadenopathy, e.g., *Actinobacillus licheniformis*, *Arcanobacterium pyogenes*, and, in some countries, *Staphylococcus aureus* subsp. *anaerobius* (Morel's disease), should be taken into consideration for differential diagnosis (4,25).

In conclusion, it was determined that *C. pseudotuberculosis*, the etiologic agent of CLA, was frequently observed in Kangal Akkaraman breed sheep flocks in Turkey, highlighting the importance of control and eradication programs. Further studies are required to determine the role of males in transmission, breed sensitivity, the economic importance of disease, and the prevalence of Morel's disease for differential diagnosis in the near future.

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