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DETECTION OF ANTIBODIES AGAINST *Leptospira* spp. AND *Listeria monocytogenes*, AND INTESTINAL PARASITE EGGS IN KANGAL DOGS*

Detección de anticuerpos contra *Leptospira* spp. y *Listeria monocytogenes*, y huevos de parásitos intestinal en perros de Raza Kangal, Turquía

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ABSTRACT

The Kangal dog is a giant shepherd dog breed from Kangal, Sivas-Turkey. It has been bred and kept as a herding dog for nearly a thousand years in that town. The Kangal dog is one of the important gene source and detailed investigations are needed in this breed. Several bacterial infections and intestinal parasitic infestations are known to cause important problems in dogs. In the present study, Kangal dogs were investigated for the presence of antibodies against *Leptospira* spp. and *Listeria monocytogenes* and for intestinal parasite eggs. In the study, 50 clinically healthy Kangal dogs aged 2 to 10 years old were randomly selected from various breeding farms in Kangal District. Haematological and routine biochemical parameters were evaluated in all dogs. Positive titers (≥ 1/100) against *Listeria monocytogenes* "O" antibodies were determined in 26 dogs (52%) through the Osebold method. Two of these dogs (4%) were also seropositive for *Leptospira canicola* as determined by a microscopic agglutination test. Faecal examination revealed *Toxocara canis* eggs in three dogs (6%; one of these was also positive for *Listeria*) and *Taenia* spp. eggs in one dog (2%). None of these investigated diseases was detected in 21 dogs. Topical selamectin and subcutaneous praziquantel were administered to the infested dogs. In conclusion, determination of leptospiral and listerial antibodies and intestinal parasites shows that these agents are present in the farms where Kangal dogs reside and further preventive measures should be taken to maintain the health of these dogs.

Key words: Kangal dog, leptospirosis, listeriosis, intestinal parasites.

* This study was produced from a doctorate thesis.

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RESUMEN

El perro Kangal es un gigante de la raza del perro pastor de Kangal, Sivas, Turquía. Han sido criados y mantenidos como perro pastor o guardian de ganado (El perro Kangal: El pastor de Anatolia) durante casi mil años en esta ciudad. El perro de Kangal es una de las importantes fuentes genéticas e investigaciones detalladas son necesarias en esta raza. Algunas infecciones bacterianas y parasitosis intestinales son conocidas por causar problemas importantes en los perros. En el presente estudio, los perros Kangal fueron investigados para detectar la presencia de anticuerpos contra *Leptospira* spp. y *Listeria monocytogenes* y de huevos de parásitos intestinales. En el estudio, 50 perros Kangal clínicamente sanos, de 2 a 10 años de edad fueron seleccionados al azar de varias granjas de cría en el distrito de Kangal. Parámetros bioquímicos y hematológicos de rutina fueron evaluados en todos los perros. Títulos positivos (≥ 1 / 100) contra la *listeria monocytogenes* "O" anticuerpos fueron determinados en 26 perros (52%) por el método de Osebold. Dos de estos perros (4%) también fueron seropositivos a *Leptospira canicola* según lo determinado por la prueba de aglutinación microscópica. El examen fecal reveló huevos de *Toxocara canis* en tres perros (6%, una de ellas fue también positivo para *Listeria*) y huevos de *Taenia* spp. en un perro (2%). Ninguna de estas enfermedades investigadas se detectó en 21 perros. Selamectina tópica y praziquantel vía subcutánea fueron administrados a los perros infectados. En conclusión, la determinación de anticuerpos contra leptospiras y listeria y los parásitos intestinales demuestra que estos agentes están presentes en las granjas donde los perros Kangal residen y se deben tomar otras medidas de prevención para mantener la salud de estos perros.

Palabras clave: Perro Kangal, leptospirosis, listeriosis, parásitos intestinales.

INTRODUCTION

The Kangal dog (*Canis familiaris domesticus*) is a giant dog of mastiff ancestry reared in the town of Kangal, in Sivas-Turkey. It is a breed that is grown and kept widely as a shepherd dog. It has also been used for guarding, fighting and hunting for nearly a thousand years in this town. The breed originated from dogs was brought from middle Asia to Anatolia by Turks during their immigration [9, 12]. Therefore, the Kangal dog breed is one of the important gene sources in Turkey. Protection of this ancient gene source requires a detailed investigation of the health status, feeding and breeding of Kangal dogs. Several important diseases have been detected in Kangal dogs in the region and these need to be further investigated.

A number of bacterial infections and intestinal parasitic infestations are known to cause important problems in the dogs. One disease is leptospirosis, a spirochaetal infectious disease seen in animals, humans and rodents. In dogs, the disease is caused by *Leptospira canicola, L. icterohemorrhagiae, L. grippotyphosa, L. autumnalis*, *L. pomona, L. bratislava* and *L. hardjo* [6, 8, 26]. The disease has a wide spectrum of clinical symptoms, varying from mild anicteric infection to a severe haemolytic syndrome. Diagnosis can be performed through one of several methods including a microscopic agglutination test (MAT), an enzyme linked immunosorbent assay (ELISA), dark-field microscopy of urine, pathogen culture, or polymerase chain reaction (PCR) [6, 8, 13].

Another common zoonotic disease is listeriosis, caused by *Listeria monocytogenes*. Its clinical manifestations are mostly associated with the nervous system and puppies are more prone to this disease than are adults [14]. The diagnosis can be made by a combination of both serological and clinical findings. The modified Osebold method is one of the serological methods used to detect *L. monocytogenes* “O” antibodies [18]. Both leptospirosis and listeriosis can be cured by specific antibiotics.

Intestinal parasites of dogs include *Toxocara canis, Dipylidium caninum, Taenia hydatigena*, and *Echinococcus granulosus* and *multilocularis*. Adult dogs typically serve as paratenic hosts, harbouring the larvae of *T. canis*, but puppies affected by roundworm can have a severe form of the disease [7, 23]. *Taenia hydatigena* is a huge tapeworm of carnivores that infects dogs when they consume undercooked livestock or venison [10]. *Echinococcus granulosus* parasitizes the small intestine of carnivores as an adult and has intermediate hosts such as livestock and humans, where it causes hydatid disease [10, 24]. All of these parasites can be eliminated using effective anthelmintic drugs after a confirmed diagnosis.

The purpose of this study was to determine some of the infectious and parasitic diseases (leptospirosis, listeriosis and intestinal parasites) in 50 breeding stock Kangal dogs from the region of Kangal, Sivas-Turkey.

MATERIALS AND METHODS

Fifty adult breeding stock Kangal dogs, aged 2-10 years old were used in this study. Twenty-one dogs were obtained from local villages, eight were obtained from breeding farms in the District of Kangal and twenty one were supplied from Sivas-Ulas centres for agricultural management. After obtaining clinical examination findings, Leptospiral and Listerial antibodies in sera samples and intestinal parasites in faecal samples were analysed in these dogs. Hematologic and biochemical analyses were also performed in blood and sera samples.

Body temperature, respiration and heart rate of each dog were recorded and physical examinations were conducted. Antibodies against *Leptospira* spp. were examined by MAT in a reference laboratory of the Etlik Veterinary Control and Research Institute, Ankara [5, 19]. Antibodies against *L. monocytogenes* were determined by the Osebold method in a reference laboratory of the Refik Saydam Hygiene and Sanitation Center, Ankara [18]. Complete blood counts (leucocytes (WBC), erythrocytes (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), neutrophil, lymphocyte, monocyte, eosinophile, basophile) in the blood samples with EDTA were performed with a blood counter (Beckman Coulter Gen-S Hematology Analyzer, USA). Biochemical parameters of sera samples (blood urea nitrogen (BUN), creatinine, total protein, albumin, amylase, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK), gamma-glutamyltransferase (GGT), total bilirubin, direct bilirubin (conjugated), indirect bilirubin (unconjugated), chlorine (Cl), sodium (Na), potassium (K), phosphorus (P) and calcium (Ca)) were analyzed with an automatic analyzer (ILAB Tm 900/1800, Instrumentation Laboratory, Italy).

Topical selamectin (Stronghold, Pfizer, USA) at a single dose of 6 mg/kg was administered on the area between shoulders of the dogs that were diagnosed with toxocariosis. Praziquantel (Cestodan, Konig Provet, Turkey) at a single dose of 5 mg/kg was injected subcutaneously to the dogs with taeniasis.

The data obtained from haematology, biochemistry and faecal examinations were statistically analyzed using one way ANOVA (Analysis of variance) in SPSS (Statistical Package for the Social Sciences) software programme for windows [11]. Since one dog had both listeriosis and parasitic infestation and two dogs had both listeriosis and leptospirosis, the number of animals in the Tables has been shown as 53, while the actual total number was 50. Hypothesis testing, as a confirmatory data analysis was used to evaluate the compatibility of the disease-free cases and *L. monocytogenes* “O” antibodies with reference values as means of the population.

RESULTS AND DISCUSSION

Evaluation of all dogs in this study revealed that 58 per cent of them had at least one disease and 42 per cent of them had no disease. Agglutination (≥ 1/100) against *Listeria monocytogenes* "O" antibodies were determined in 26 sera samples (52%). Seropositivity against *Leptospira canicola* was detected in two dogs (4%) (1/400 and 1/800 titers). Intestinal parasite eggs of *Toxocara canis* were determined in three dogs (6%) and *Taenia* spp. in one dog (2%). Two of the dogs showed both leptospirosis and listeriosis. One dog showed both listeriosis and intestinal parasitic infestation.

The values for rectal temperature, heart rate and respiration per minute were within normal ranges in all dogs (TABLE I).

Monocyte values in the disease-free and listeriosis cases were outside of the normal values based on hypothesis testing ($P<0.05$). Comparison of the haematological parameters between listeriosis cases and cases with intestinal parasite eggs revealed no statistically significant data ($P>0.05$). All other haematological values were within normal ranges. Haematological findings are summarised in TABLE II.

Lower mean values of total protein, K and Ca were found in the cases with intestinal parasite egg than in the disease-free cases. Lower mean values of Ca were also measured in the cases with intestinal parasite eggs than in cases with listeriosis. The mean values of Na and Cl in cases with listeriosis were outside of the normal ranges in hypothesis testing ($P< 0.05$). All other biochemical values were within normal limits. The results of biochemical analyses are shown in TABLE III.

The diseases of dogs are various and depend on the relationship between host and type of the infection in the region where the animal lives. The Kangal dog is used mainly as a shepherd dog in livestock herds. Most of the bacterial and parasitic diseases are already known to occur in both dogs and livestock. Listeriosis, leptospirosis and intestinal parasites are common problems in dogs. In this study, these diseases were investigated in Kangal dogs in their breeding region.

Listeriosis is widespread throughout the world and is seen in many species including dogs, cats (*Felis catus*), cattle (*Bos taurus*), sheep (*Ovis aries*), horses (*Equus caballus*), pigs (*Sus scrofa domestica*) and avians [4, 15]. In one study, 90% of the 30 dogs living in unhealthy conditions were determined to have antibodies against listeriosis [25]. Oni *et al.* [17] detected listeriosis in 20% of the dogs in Nigeria. Titers of *L. monocytogenes* "O" antibody showing agglutination of 1/100 and above were found in 26 Kangal dogs (52%) in the present study. The varied prevalence of listeriosis in different regions of the world may be related to the living conditions and risks of contaminations in these different regions. The breed of dog might also be a factor because Kangal dogs are mostly used as shepherd dogs in sheep herds in most regions of Turkey. Borku et al. [4] found that goats, horses and dogs in the same herd showed listeriosis and 46 per cent of the goats (*Capra*

TABLE I
THE MEAN VALUES AND STANDARD DEVIATIONS OF CLINICAL PARAMETERS IN KANGAL DOGS.

Parameters	L. monocytogenes (n = 26)	L. canicola (n = 2)	Fecal egg (n = 4)	Free (n = 21)
Rectal Temp. (°C)	38.4 ± 0.1	38.5 ± 1.13	38.2 ± 0.16	38.3 ± 0.1
Heart Rate (bpm)	90.4 ± 2.5	99 ± 15.6	90 ± 2.6	86.5 ± 2.6
Respiration (rpm)	27.08 ± 1.16	25.5 ± 6.36	24.0 ± 2.12	27.3 ± 1

TABLE II
THE MEAN VALUES AND STANDARD DEVIATIONS OF HAEMATOLOGICAL PARAMETERS IN KANGAL DOGS.

Parameters	L. monocytogenes (n = 26)	L. canicola (n = 2)	Fecal egg (n = 4)	Free (n = 21)
WBC (10^3/ml)	11.98 ± 1.1	15.6 ± 3.57	11.74 ± 1.5	9.81 ± 0.3
RBC (10^6/ml)	6.91 ± 0.2	6.4 ± 1.56	6.32 ± 0.4	10.08 ± 2.7
Hb (mg/dL)	15.57 ± 0.4	14.6 ± 3.68	14.75 ± 0.8	16.30 ± 0.4
HCT (%)	47.09 ± 1.2	43.9 ± 8.98	45.07 ± 2.5	49.13 ± 1.2
MCV (fL)	68.20 ± 0.7	68.6 ± 2.69	71.65 ± 2.9	67.30 ± 0.1
MCHC (g/dL)	33.05 ± 0.1	33.1 ± 1.56	32.77 ± 0.4	33.20 ± 0.1
Neutrophil (10^3/ml)	4.4 ± 0.6	4 ± 0.86	4.28 ± 1.2	2.67 ± 0.3
Lymphocyte (10^3/ml)	2.13 ± 0.2	2.9 ± 1.15	2.45 ± 0.5	2.35 ± 0.3
Monocyte (10^3/ml)	3.34 ± 0.5	2.1 ± 1.92	2.38 ± 0.6	3.02 ± 0.4
Eosinophile (10^3/ml)	1.69 ± 0.4	1 ± 0.82	2.41 ± 0.7	1.36 ± 0.2

TABLE III
THE MEAN VALUES AND STANDARD DEVIATIONS OF BIOCHEMICAL PARAMETERS IN KANGAL DOGS.

Parameters	L. monocytogenes (n = 26)	L. canicola (n = 2)	Fecal egg (n = 4)	Free (n = 21)
BUN (mg/dL)	13.19 ± 1.79	23 ± 2.82	15.25 ± 4.60	12.38 ± 1.67
Creatinine (mg/dL)	0.93 ± 0.07	0.85 ± 0.49	0.70 ± 0.057	1.03 ± 0.084
T. Protein (g/dL)	5.16 ± 0.28	4.55 ± 0.91	4.0 ± 0.24	5.73 ± 0.22
Albumin (g/dL)	2.49 ± 0.14	1.8 ± 0.42	2.22 ± 0.25	2.81 ± 0.13
Globulin (g/dL)	2.67 ± 0.21	2.75 ± 0.56	1.78 ± 0.24	2.92 ± 0.18
Amylase (U/L)	644.8 ± 56.4	556 ± 185.3	339 ± 13.2	692.4 ± 56.8
ALP (U/L)	70.30 ± 8.81	46.5 ± 16.26	35.50 ± 7.23	74.95 ± 8.82
ALT (U/L)	42.80 ± 16.9	244.5 ± 299	17.75 ± 4.51	27.80 ± 4.36
AST (U/L)	21.80 ± 1.71	33 ± 21.21	21 ± 2.27	25.33 ± 1.35
CK (U/L)	100.26 ± 8.5	114.5 ± 61.5	119.25 ± 18	109.76 ± 8.6
GGT (U/L)	2.61 ± 0.22	3 ± 0	3 ± 0.7	3.61 ± 0.34
T.Bilirubin (mg/dL)	0.22 ± 0.02	0.3 ± 0.14	0.17 ± 0.025	0.22 ± 0.023
D.Bilirubin(mg/dL)	0.10 ± 0.079	0.2 ± 0.14	0.075 ± 0.02	0.095 ± 0.06
I. Bilirubin (mg/dL)	0.11 ± 0.078	0.1 ± 0	0.10 ± 0.002	0.12 ± 0.014
Cl (mmol/L)	86.23 ± 3.39	68 ± 4.24	75.50 ± 4.57	92.76 ± 2.85
Na (mmol/L)	119 ± 4.57	99 ± 4.24	99.75 ± 4.51	127.09 ± 4.2
K (mmol/L)	3.87 ± 0.15	3.5 ± 0.42	3.22 ± 0.15	4.19 ± 0.13
P (mg/dL)	3.65 ± 0.23	3.6 ± 0.7	2.85 ± 0.68	4.35 ± 0.31
Ca (mg/dL)	8.29 ± 0.36	6.8 ± 0.56	6.27 ± 0.34	8.98 ± 0.29

hircus) in the province of Ankara were seropositive against listeriosis. Aktas *et al*. [1] detected listeriosis in 26.3% of dogs in the province of Erzurum.

Leptospirosis has been detected in both livestock and carnivores [13, 21]. *Leptospira canicola* has frequently been detected in dogs, although there are many serotypes of leptospirosis seen in dogs. Antibody titers of the spirochetal bacteria of leptospirae determined by MAT can be used to diagnose canine leptospirosis [6, 8, 26, 27]. In total, two of the 50 Kangal dogs (4%) in the present study showed seropositivity against *L. canicola*. Aktas et al. [1] found that 26.3% of the stray dogs in the province of Erzurum had the antibodies for leptospirosis. Ozdemir [19] reported that seropositivity against leptospirosis around the country was 26.9%, based on 286 sera samples from dogs. Bacterin immunizations against *L. canicola* and *L. icterohaemorrhagie* are administered widely in dogs via routine annual vaccination by veterinarians and breeders. Thus, compared to previous reports, one of the reasons for a low prevalence in the Kangal dogs presented here can be the use of routine vaccinations in dogs for almost two decades around the country. The most common leptospirosis serotypes were *canicola, grippotyphosa, icterohaemorrhagiae,* and *autumnalis*. These serotypes were also common in different countries around the world, *icterohaemorrhagiae, autumnalis,* and *canicola* in Japan [8], *canicola* and *icterohaemorrhagiae* in Canada [22], *autumnalis, grippotyphosa, bratislava, canicola, icterohaemorrhagiae,* and *pomona* in the USA [6, 26] and *icterohaemorrhagiae* in India [28]. Affected dogs are considered to be natural carriers of leptospirosis [26]. The positive dogs in the present study showed a clinically normal appearance.

Different rates of helminth infestation have been reported in dogs around the county [3, 29]. *Toxacara canis* infestation was found in 46 per cent of dogs in Sivas Province and in 4.2 per cent of dogs in Kayseri Province [3, 29]. In the present study, toxocariosis was found in six per cent of the dogs. This result may be related to the ages of the dogs because the infestation is rarely seen in adults and antiparasitic drugs are routinely used in the breeding farms. In this study, both selamectin and praziquantel were effective at curing toxocariosis and taeniasis, respectively, in agreement with previous reports [2, 16, 20, 24].

Monocytosis can be seen in acute and chronic inflammations, immune mediated diseases, disorders of tissue damage, monocytic leukaemia and geriatric ages. Monocytes increase during tumour defence and in some disease such as listeriosis, brucellosis, paratuberculosis and tuberculosis. The slight increase in monocytes, above the reference range, observed in the present study can be explained by latent, chronic or immune mediated diseases that can be detected in dogs. Other parameters were in normal ranges.

Evaluation of the biochemical and haematological parameters measured in this study needed to be accompanied by clinical findings and feeding information. Although the clinical findings were normal, some of the laboratory findings such as protein, K, Ca, Na and Cl were statistically different from the reference ranges. Some of the dogs were known to be fed a traditional food –called 'yal'– which only consists of barley flour. This supports the idea that malnutrition may cause metabolic changes. Furthermore, an underlying latent disease not detected in this study is another possible secondary reason for a normal appearance and some sort of metabolic changes. Further studies that examine other health problems and the feeding and breeding in Kangal dogs may therefore be planned.

CONCLUSION

In conclusion, determination of leptospiral and listerial antibodies and detection of intestinal parasite eggs in Kangal region shows that these agents are present in dog breeding farms. Preventive measures should be taken against these diseases to preserve the health of breeding populations of Kangal dogs in Turkey.

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