TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2018) 42: 277-284

doi:10.3906/vet-1710-103

Epidemiological survey and economic significance of bovine hypodermosis on the Kars Plateau in the Northeast Anatolia Region of Turkey

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Received: 31.10.2017 • **Accepted/Published Online:** 05.06.2018 • **Final Version:** 09.08.2018

Abstract: The aim of this study was to determine the prevalence and seroprevalence of hypodermosis and to calculate the economic loss associated with hide damage in the cattle on the Kars Plateau, Turkey. A total of 6563 randomly selected cattle were examined by skin palpation from 1 April to 31 May 2014. A subset of 465 serum samples was collected from October to November in 2014 and analyzed using a commercial ELISA kit. The overall prevalence of hypodermosis was 16.9% in the cattle examined by skin palpation. In respect of each of the risk factors assessed, the highest prevalence rates were recorded in 2-year-old (26.4%), Brown Swiss crossbred (17.9%), female (18.1%), and dark-colored (49.7%) cattle. A total of 15,349 warble larvae were detected under the skin of 1114 cattle. The larval count per animal ranged from 1 to 91, with a mean count of 13.8. Seroprevalence was found to be 73.8% (343/465), with the highest rates of infestation in 2-year-old (87.7%), dark-colored (81.1%), female (80.4%), and Brown Swiss crossbred (87.7%) cattle. The highest seroprevalence rate was 96.8% and the lowest was 49.5%. The economic loss caused by the disease was calculated as 18,615 Turkish lira in the survey areas.

Key words: *Hypoderma*, prevalence, ELISA, economic losse

1. Introduction

Hypodermosis is a parasitic disease caused by the development of *Hypoderma bovis* and *H. lineatum* (Diptera: Oestridae) larvae that develop under the dorsal skin of cattle (1–4). It is found in both developed and developing countries but is especially common in tropical and subtropical regions. The parasitic period of *Hypoderma* larvae in cattle is 9 to 10 months. Taking into account the development period of the pupa and adult fly in the environment, the lifecycle of the parasite is completed in 1 year. Adult flies deposit their eggs on the hair of the lower bodies of cattle. The first-stage larvae (L1) migrate in connective tissues of cattle from summer to autumn. The L1 molt into the second (L2) and third (L3) larval stages. The L3 stage occurs under the dorsal skin, producing swellings under the skin that are called warbles. The L3 fall to the ground and pupate. Approximately 5 weeks later, the adult flies emerge to complete their biological cycle. The adult flies live only 1–2 weeks. Irritation caused by the flies can result in poor feeding in cattle, which leads to lower weight gain and reduced meat or milk production, and may weaken the immune system. However, the most important economic loss arises due to the lesions in the skin caused by the larvae, which decrease the value of the hide. In addition, cases of paraplegia or death may occur in cattle as a result of the settlement or death of larvae around the spinal cord (1,4).

The diagnosis of hypodermosis in cattle is made by carcass examination in the slaughterhouse and by palpation of the dorsal skin and molecular-serological methods in live animals (3–16). Currently, ELISA methods are used to diagnose early infestation caused by the L1 larvae. These methods provide a simple, reliable, and accurate early diagnosis of the disease and therefore enable earlier treatment of cattle before the larvae can cause economic loss (5,14,17).

The Kars Plateau lies in the Northeast Anatolia Region of Turkey, an important area for extensive farming of livestock. Cattle graze on pasture on this plateau from April to October and are exposed to *Hypoderma* species. Research on hypodermosis, based on palpation, molecular, serological, and abattoir examinations in Turkey and throughout the world, found that the prevalence of infestation ranges from 3.6% to 84% in

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Turkey (4,7,14,16,18,19) and from 0% to 100% in the other countries (10,20–23). So far, only two studies have been carried out on hypodermosis in the Kars Plateau; one is about the treatment (24) and the other is about the diagnosis of the disease in live animals (25).

The aim of the study was to establish the prevalence and risk factors of hypodermosis in cattle by skin palpation in spring and the seroprevalence of infestation by ELISA in autumn in Kars Province. Another aim was to calculate the economic loss associated with hide damage caused by hypodermosis in the region.

2. Materials and methods

2.1. Study area

The present survey was carried out on the extensive pasture grounds of the Kars Plateau in the Northeast Anatolia Region, in an area that hosts large numbers of livestock and supplies a significant proportion of Turkey's demand for beef.

Eighteen villages were visited between 1 April and 31 May 2014 in the area where cattle breeding is common. The villages were randomly selected and allocated into four groups based on their altitude as follows:

1- Altitudes under 1700 m
2- Altitudes between 1700 and 1799 m
3- Altitudes between 1800 and 1999 m
4- Altitudes above 2000 m

2.2. Detection of hypodermosis by palpation and risk factors

Having obtained ethics approval from the Kafkas University Animal Experiments Local Ethics Committee (No: 2014-011) and the approval of the farmers, the cattle were examined by palpation for *Hypoderma* infestation in the 18 villages between 1 April and 31 May 2014. A total of 6563 cattle, from barns selected randomly in the villages, and which had not been treated with insecticides (as reported by the farmers), were subgrouped according to the following risk factors and assessed for variations among and within the groups: age (1, 2, and 3 or more years old), sex, breed (Simmental, Brown Swiss, Holstein, Charolais, indigenous breeds of Northeastern Anatolian Red and Black, and their crosses), and coat color (light, dark, and piebald).

2.3. Seroprevalence of hypodermosis

In order to detect the antibodies against L1 larvae of *Hypoderma* spp., considering the ages of the animals, 465 randomly selected blood samples were collected from four research areas between October and November 2014. The provided ELISA plates were precoated with purified extract antigens of L1 larvae of *Hypoderma lineatum*. Since the antigenic extract used in this kit does not discriminate antibodies to *H. bovis* from those of *H. lineatum*, *Hypoderma* spp. or hypodermosis was preferred in this text for terminology. The blood samples were transferred to the laboratory and centrifuged, and the serum samples were stored at –20 °C until the analysis. The sex, age, breed, and color (light, dark, and piebald) of the animals were recorded during sample collection. The serum samples were examined using a commercially available ELISA test kit according to the procedure for anti-*Hypoderma* antibodies (IDEXX Bovine Hypodermosis Antibody Test Kit-P06110), and the seroprevalence of the disease was determined.

2.4. Calculating the current economic loss caused by hypodermosis

In order to estimate the current economic loss caused by hypodermosis on the Kars Plateau, the difference in the local market price for healthy and damaged hides was correlated with the degree of prevalence of the infection found in the present study. During an interview with 3 merchants, it was learned that the price of healthy hide was 2 Turkish lira (TL) per kilogram while hide infested with *Hypoderma* larvae was 1 TL per kilogram in Kars. The mean weights of hide are 10 kg in 1-year-old animals, 15 kg in 2-year-old animals, and 20 kg in animals 3 or more years old.

2.5. Statistical analysis

The results of the positivity rates belonging to the characteristics (age, sex, breed, and coat color) obtained from this study were analyzed with Pearson's chi-square tests using SPSS 15.0 for Windows.

3. Results

3.1. Palpation findings

Infestation with hypodermosis was found in all 18 villages examined. The overall prevalence of hypodermosis was 16.9% (1114/6563) by skin palpation. Similar prevalence rates were detected among different altitudes and there was no significant relationship between the altitude and the rate of infestation determined by palpation ($P > 0.05$). The total larval count detected by palpation was 15,349 in 1114 infested cattle. The larval count per animal ranged from 1 to 91, with a mean larval count per animal of 13.8 by skin palpation. The relationships between palpation findings and characteristics of the animals are summarized in Table 1. The locations of the villages where hypodermosis was determined by palpation are shown in the Figure.

3.2. Serological findings

The rate of seroprevalence was found to be much higher in 73.8% (343/465) of the samples. The relationship between the serological findings and other characteristics is summarized in Table 2. The locations of the villages where hypodermosis was determined by ELISA are shown in the Figure.

Table 1. The relationship between palpation findings and the characteristics of animals in Kars Province.

Parameters			Palpation findings											
		Examined	Infested (%)	Altitude****								Larvae count	Mean larvae count
				under 1700 m		1700–1799 m		1800–1999 m		Above 2000 m			
				Examined	Infested	Examined	Infested	Examined	Infested	Examined	Infested		
Age*	1	1957	234 (11.9)	442	38	389	41	537	58	589	97	3762	16.1
	2	1003	265 (26.4)	170	40	196	68	292	75	345	82	5735	21.6
	≥3	3603	615 (17.1)	672	125	724	146	987	170	1220	174	5852	9.5
TOTAL		6563	1114 (16.9)	1284	203	1309	255	1816	303	2154	353	15349	13.8
Sex**	Male	1415	183 (12.9)	246	29	295	23	483	73	391	58	3664	20
	Female	5148	931 (18.1)	1038	174	1014	232	1333	230	1763	295	11685	12.6
TOTAL		6563	1114 (16.9)	1284	203	1309	255	1816	303	2154	353	15349	13.8
Breed***	Simmental	498	84 (16.9)	107	17	136	37	161	22	94	8	1035	12.3
	Simmental crossbred	2355	390 (16.5)	675	98	500	101	778	123	402	68	5873	15.1
	Brown Swiss	782	122 (15.6)	51	10	99	15	80	22	552	75	1416	11.6
	Brown Swiss Crossbred	2257	405 (17.9)	318	57	469	86	549	88	921	174	5498	13.6
	Others	671	113 (16.8)	133	21	105	16	248	48	185	28	1527	13.5
TOTAL		6563	1114 (16.9)	1284	203	1309	255	1816	303	2154	353	15349	13.8

*P < 0.001, **P = 0.001, ***P < 0.001, ****P = 0.554.

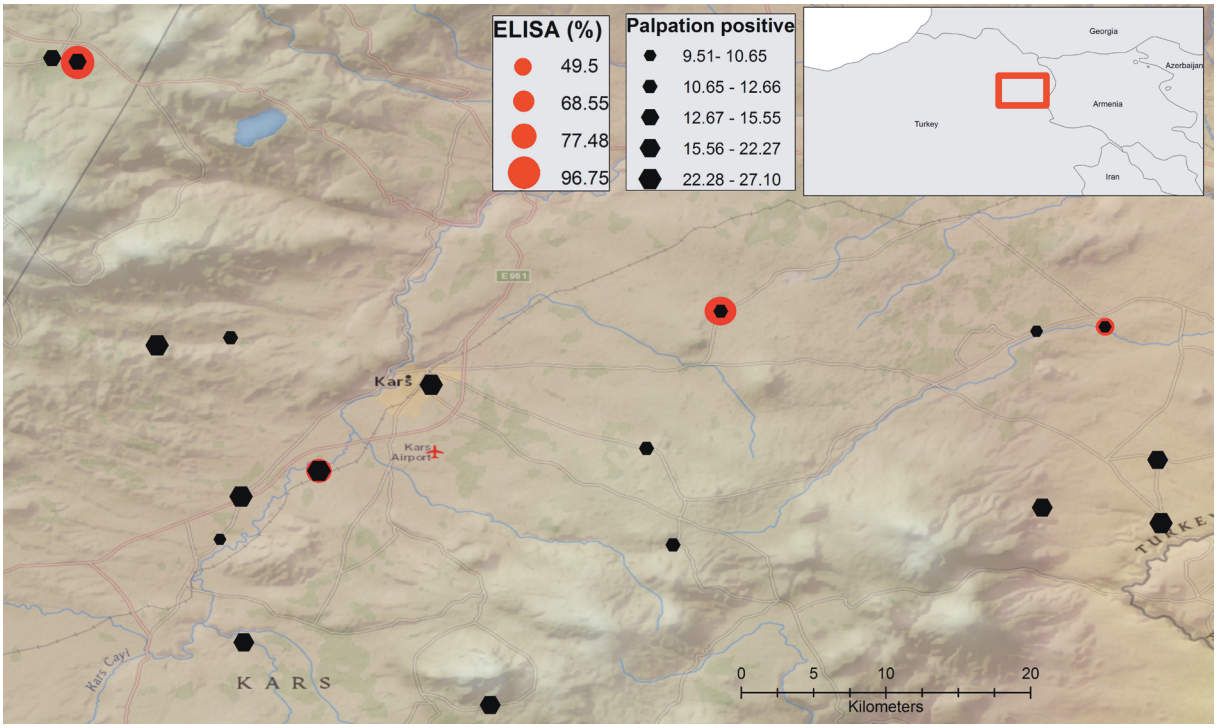

Figure. The location of the villages where hypodermosis was determined by palpation and ELISA.

3.3. Economic loss

In Kars, the price of healthy hide is 2 TL/kg, while the price of a hide infested with *Hypoderma* larvae is 1 TL/kg. The mean weights of hide are 10 kg in 1-year-old animals, 15 kg in 2-year-old animals, and 20 kg in animals 3 or more years old. In the animals studied here, the economic loss caused by the disease was calculated as 18,615 TL [(10 × 234) + (15 × 265) + (20 × 615)] (~$5170, based on a currency exchange rate of US $1 = ~3.6 TL in October 2017). When the prevalence rates determined for each age group were extrapolated to the total cattle population (132,419) on the Kars Plateau, the overall annual economic loss caused by hypodermosis was estimated as 407,500 TL [(3100 × 10) + (7100 × 15) + (13500 × 20)] ($113,000).

4. Discussion

Hypodermosis, an important disease in cattle, is prevalent in Turkey. *Hypoderma bovis* was first reported in Turkey by Kurtpınar (26), while *H. lineatum* was first reported in Turkey by Merdivenci (27). *Hypoderma bovis* is more prevalent than *H. lineatum* (28,29). Research on the prevalence of hypodermosis based on skin palpation, molecular, serological, and abattoir examinations was conducted in Turkey and elsewhere (4,7,10,14,16,18–23). To date, only two studies have been undertaken on hypodermosis on the Kars Plateau; one is about the treatment (24) and the other examines the prevalence of the disease in live animals (25). In our survey, the overall prevalence of hypodermosis was determined as 16.9% (1114/6563) by skin palpation. In our study, the prevalence of hypodermosis found in Kars was higher than that reported in some other parts of Turkey and other countries. This may be due to the fact that on the Kars Plateau animals are not treated with insecticides for hypodermosis (as reported by the farmers), and there is no control program for the disease.

The only previous study on the prevalence of the disease in Kars, conducted by Kara et al. (25), found a similar rate.

The combination of altitude, geographical features, and grazing type is known to affect the dissemination of hypodermosis. Kara et al. (25) found higher rates of infestation at lower altitudes. Ahmed et al. (21) reported that topography played an important role in the distribution of hypodermosis. In their study, the prevalence of hypodermosis was found to be 8.9% in lowland areas, 20.6% in hilly areas, and 30.8% in semihilly areas. Sayın et al. (7) reported that the rate of infestation with hypodermosis was 23.7% for cattle grazed in grassy areas, 25.5% in damp areas, 27.1% in mountainous regions, 31.2% in arid regions, 36.5% in forested areas, and 38.2% in bare areas. The risk of infestation in animals grazed on pasture is higher than that in animals kept and fed indoors (19,23,30). In our survey, the highest rate of infestation was found by palpation in a village at the lowest altitude (under 1700 m), where animals are kept and fed outdoors (Figure). All the animals examined in our study grazed

Table 2. The relationship between serological findings and other characteristics of animals in Kars Province.

Altitude	Infection rate % (I/E)	Serological Findings											
		Color*			Age*		Sex*			Breed*				
		Light (I/E)	Dark (I/E)	Piebald (I/E)	1 (I/E)	2 (I/E)	≥3 (I/E)	Male (I/E)	Female (I/E)	Simmental (I/E)	Simmental Crossbred (I/E)	Brown Swiss (I/E)	Brown Swiss Crossbred (I/E)	Others (I/E)
Under 1700 m	49.5 (53/107)	22/39	18/39	13/29	5/58	25/26	23/23	16/47	37/60	5/22	39/72	2/2	6/10	1/1
1700–1799 m	68.6 (85/124)	18/29	26/36	41/59	13/22	10/13	62/89	12/20	73/104	24/29	47/71	4/7	7/13	3/4
1800–1999 m	77.5 (86/111)	2/2	24/28	60/81	42/60	13/18	31/33	28/43	58/68	12/18	52/66	2/4	17/20	3/3
Above 2000 m	96.8 (119/123)	19/19	99/103	1/1	23/25	16/16	80/82	7/7	112/116	0/0	4/4	45/48	70/71	0/0
TOTAL	73.8 (343/465)	61/89	167/206	115/170	83/165	64/73	196/227	63/117	280/348	41/69	142/213	53/61	100/114	7/8

I: Infested, E: examined, *P < 0.001.

on pasture. Both grazing type and climatic conditions related to altitude may have influenced the reproductive environment for *Hypoderma* spp. The difference between foci was found to be statistically significant ($P < 0.005$). These results are consistent with other research (19,30).

Total and mean larval counts indicate the severity of infestation. In previous studies, the mean number of larvae varied between 5.1 and 12.1 per animal. In our survey, the mean number of larvae was 13.8, higher than that reported in other studies (7,25,31,32). This difference may be explained by the type of grazing practiced, by altitude, lack of specific treatment, and also the regions.

According to Saidani et al. (23), young animals (≤12 months) are more frequently infested than older animals. Infestation rates were reported to increase with age due to the fact that a degree of immunity to warble larvae develops in older animals (25), and because calves are born before the fly season both in Kars and around Turkey. In our survey, the rate of infestation was 11.9% (234/1957) in 1-year-old animals, 26.4% (265/1003) in 2-year-old animals, and 17.1% (615/3603) in animals 3 or more years old.

Skin color may be an important factor in determining the behavior of the warble flies. This behavior was evaluated only by one study, carried out by Kara et al. (25), and it was found that warble flies laid their eggs on dark-colored animals. Similarly, in our survey, we observed that the warble flies tended to lay their eggs on dark-colored animals (49.6%, 553/1114).

In contrast to the findings of Kara et al. (25), a higher infestation rate was detected in the females (18.1%, 931/5148) than in the males (12.9%, 183/1415). It was thought that the reason was the alteration of breeding type. In recent years, female cattle are bred more than males for their milk and their calves in this province.

In Algeria, it was found that Flechvieh cattle had a higher prevalence than other breeds (23). In recent years, purebred and crossbred animals have been preferred over native cattle in the Kars region because of their high yield. In our study, the rates of infestation among breeds were similar, with the highest rate of hypodermosis determined in Brown Swiss crossbred cattle (17.9%, 405/2257).

In our survey, the total larval count was 15,349 in 1114 infected cattle. The mean larval count per animal was 13.8. In respect of color, the mean larval count was higher in the piebald (3390/224) animals (mean 15.1) than in the light-colored (4408/337, mean 13.1) or the dark-colored (7551/553, mean 13.7) cattle ($P < 0.005$).

The mean larval count was 16.1 in 1-year-old cattle (3762/234), 21.6 in 2-year-old cattle (5735/265), and 9.5 in cattle 3 or more years old (5852/615) cattle. The differences in the mean larval counts and infestation rates were found to be statistically significant between the age groups ($P < 0.005$). The level of infestation was higher in the males (mean 20 larvae, 3664/183) than in the females (mean 12.5 larvae, 11685/931). In respect of breed, the level of infestation was the highest in Simmental crossbred cattle (mean 15) ($P < 0.005$).

Nowadays, ELISA methods are used to diagnose early infestation caused by the first-stage larvae. Serodiagnostic methods provide simple, reliable, and accurate early diagnosis of the disease, which enables the treatment of cattle before larvae can cause damage that would lead to economic loss (5,14,17). In a study in Şanlıurfa Province of Turkey, the seropositivity rate was found to be 38.6%. There was a statistically significant difference in seropositivity rates between pure and crossbred animals, but there was no significant difference in relation to sex (19). Karatepe et al. (33) reported seropositivity rates of 28.9% (53/183) using a commercial ELISA kit and 21.8% (40/183) by indirect ELISA. In another study, in the eastern and southeastern regions of Turkey, the seropositivity rate was found to be 23.3% in total; 31% in females and 14.1% in males; and 27.7% in local breeds, 26.8% in crossbred animals, and 19.7% in purebred animals (13). Balkaya et al. (14) reported 28.6% (223/778) seropositivity in female local breed cattle. In the present survey, the seroprevalence of hypodermosis on the Kars Plateau was found to be higher than in the other surveys, at 73.8% (343/465).

Higher seropositivity rates have been reported in animals over 1 year old than in younger animals. In line with these studies (13,19,34), in our survey the seroprevalence rates were 50.3% in 1-year-old animals (83/165), 87.7% in 2-year-old animals (64/73), and 86.3% in animals 3 or more years old (196/227). It was determined that the rate of seroconversion increases with age ($P < 0.005$). This may be explained by the fact that a degree of immunity to warble larvae develops with age, and that 1-year-old animals grazed on pasture for only one season and had less exposure to fly attacks.

Seropositivity was found to be higher in the dark-colored (81.1%, 167/206) cattle than in the light-colored (68.5%, 61/89) and the piebald (67.7%, 115/170) animals; in females (80.4%, 280/348) than in males (53.9%, 63/117); and in Brown Swiss crossbred cattle (87.7%, 100/114) than in other breeds (87.5%, 7/8) including Brown Swiss (86.9%, 53/61), Simmental crossbred (66.7%, 142/213), and purebred Simmental (59.4%, 41/69), respectively.

The seroprevalence rates of hypodermosis were determined as 42.3% in Spain (34), 43.3% in Italy (30), between 6% and 51.7% in China (17), 96% in Mongolia, and 0% in Japan (35). In Albania, the seropositivity rates were 38.7% and 41.3% in two sampling seasons (10). Our study found a higher rate of seroprevalence (73.8%) than that reported by some other researchers (10,13,14,17,19,30,33,34) and lower than that found in Mongolia by Boldbaatar et al. (35). The seroprevalence rate ranged from 49.5% to 96.8% among the villages that were

studied. The differences between the findings result from factors such as topography, temperature, wind, grazing pattern, breed, age, sex, skin color, fly activity, and, above all, the use of control programs.

As for the results according to sex, our results showed that the higher seroprevalence rates were in females (80.4%) and were similar to those in the study of Simsek et al. (13) and Balkaya et al. (14). However, considering the results according to breed, contrary to the studies of Simsek et al. (13) and Balkaya et al. (14), the higher seroprevalence rate in our survey was in Brown Swiss crossbred cattle (87.7%).

Consistent with the results of much research (2,11,13,25,30), in our study, the seroprevalence rate (73.8%) was much higher than the actual prevalence estimated in proportion to the *Hypoderma* larvae (16.9%) found under the skin. This might be due to the fact that developing protective immunity arrests and destroys the larvae before they can reach their final destination under the dorsal skin. This argument is also supported by the age-related decrease in prevalence. While the seroprevalence tends to increase with age, actual prevalence and the number of larvae under the dorsal skin decrease with age.

In a study conducted in Afyonkarahisar Province of Turkey (15), the annual economic loss due to hypodermosis was estimated to be 18,288 TL. In the present survey, in Kars, the price of healthy hide is 2 TL/kg, while the price of hide infested with *Hypoderma* larvae is 1 TL/kg. The approximate weight of the hide of an animal is 10 kg in 1-year-old animals, 15 kg in 2-year-old animals, and 20 kg in animals 3 or more years old. The economic loss caused by the disease was calculated as 18, 615 TL (~$5170) in the studied area and estimated to be 407,500 TL (~$113,000) for the Kars Plateau as a whole. These results are similar to those of Çiçek et al. (15).

For studies conducted about hypodermosis (7,11,13,14,16,21–23,25,33), including our survey, it can be said that some risk factors such as topography/altitude, season, temperature, grazing pattern, breeds, age, sex, and color may influence the prevalence of hypodermosis.

It is known that the biological development of *Hypoderma* species differs from country to country and from region to region due to seasonal conditions. Hence, the most suitable time for treatment, prevention, or control of hypodermosis is important (16,32). In Kars Province, warble fly larvae can be seen on animals in February, and larval counts peak in March and April (25). Therefore, according to our study and other studies (24,25) in Kars Province, animals should be treated by the middle of November.

In conclusion, this study, in conjunction with our previous research (25), indicates that hypodermosis is a prevalent infestation in cattle in the province of Kars, with a consequent economic loss, and requires an urgent implementation of control programs. Such programs were shown to be very successful in contributing to the eradication or control of hypodermosis in European countries such as Denmark, the Netherlands, Ireland, the United Kingdom, the Czech Republic, Germany, France, and Switzerland (2).

Acknowledgment

This study was financially supported by the Scientific Research Projects Coordination Unit of Kafkas University (Project No: 2015-TS-13). We thank the members of the Republic of Turkey Ministry of Food, Agriculture and Livestock; the Animal Health Department of Kars; and Assoc Prof Dr Pınar Demir (Kafkas University, Faculty of Veterinary Medicine) for their help.

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