<u>Original Article</u> Study of Prevalence and Associated Risk Factors of *Eimeria sp.*, in Camelsin Turkestan Region

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Abstract

Coccidiosis is one of the most pathogenic intestinal diseases caused by different species of Eimeria spp. (Phylum:Apicomplexa), that cause important economic losses to the livestock industry. Given the importance of camel breeding and its products in the regional economy, in this study the risk factors of prevalence of camels Coccidiosis in the Turkestan region of Republic of Kazakhstan was investigated by oocyte excretion monitoring in the herd. The results revealed that the prevalence of Eimeria sp, was136 (42.5%); and three types of Eimeria spp. were identified: Eimeriabactriani, Eimeriacameli, Eimeria dromedarii. The statistical analysisdemonstrated that the prevalence and intensity of infection in camel <1-year-old were 65.5% and 149.2 respectively, and they were more likely to be infected with *Eimeria* spp. compared with adult camels (22.5%, 5.7), (*pvalue* \leq 0.05). Another probable risk factor is seasons of the year as it seems the high and low prevalence and infection intensity are observed in summer (60%, 102.1) and winter (20.6%, 21.25), respectively. Multivariate analysis of our data revealed that age and season were significant risk factors (p < 0.005) and adoption of hygienic measures and husbandry practice are needed among the high risk groups(in young camel and summer season) to minimize, control and prevent spreadof the infection.

Keywords: Camel, Eimeria sp., risk factor, prevalence, Turkestan

1. Introduction

Camel breeding is a traditional branch of animal husbandry in Kazakhstan. Due to the nutritional habit and resistance to environmental conditions and proper adaptation of this animal, breeding and maintenance has long been used in this area. In the 80s decade of the last century, in the desert, semi-desert and steppe zones of the republic, camel farms appeared as reserves for the production of meat, milk and wool. Camel breeding is currently developing in the southern, south-eastern and western regions of Kazakhstan.

Parasitic diseases have greatly affected the growth and productivity of livestock, commonly gastrointestinal parasites, especially Apicomplexa protozoa such as *Eimeria spp*.. The economic damage of them including the death of animals and reduction of livestock products is major, especially in young animals (1-3).

Coccidia, protozoan parasites are host-specific and their oocyst is shed in the feces of both affected symptomatic and carrier animals. The sporulated oocystare ingested by animals when they consume contaminated feed, water, thenparasite injures intestinal cells and resulting in the host having diarrhea and hematochezia. An important point in the epidemiology of this infection is the high resistance of oocysts to environmental conditions.

Based on studies on the prevalence of Eimeria infection in American (4-6), German (6), English (7), Hungarian (8, 9), Czech (10) and Kazakh (11) camels

are infected with six species of *Eimerisspp.*, including: *Eimeria bactriani*; *Eimeria cameli* (12); *Eimeria dromedarii*; *Eimeria pellerdy* (13); *Eimeria rajastani*(14); *Isosporaorlovi* (15).

Eimeriabactriani synonyms: Eimeriacameli, In oocysts are spherical, 32x25-27 µm in size, having a micropyle, with 5-7 µmwidth. The oocyst membrane is golden-yellow or yellow-brown (16). Sporocystssized 7.7-8.8 x 6.3-8.8 µm contain lemon-shaped sporozoites and spherical inclusions (6). According to Yakhchali and ATARI (17), schizogony occurs in the mucosa of the small intestine, extending to a distance of 2 m from pylorus to ileum. Schizonts measuring 10-16 µm contain 20-24 merozoites measuring 9 x 2 µm. Microgametes sizes are up to 19 µm in diameter with a small number of microgametes. Eimeriabactriani in Kazakhstan has beenfound in Almaty, Kyzylorda, East Kazakhstan, North Kazakhstan and West Kazakhstan regions.

Eimeria cameli (16) synonyms: *Globidiumcameli*, oocysts described by A.A. TSYGANKOV (15) are pear-shaped, yellow-green or dark brown in color. The shell is three-layered, smooth, 5-8.7 μ m thick, on average 6.84 μ m. The outer layer of the shell is transparent, the middle one is yellow-green or dark brown, and the inner layer is dark green. The sporocyst contains a residual body of an indefinite shape and two spherical inclusions with a diameter of 3.75–5.0 μ m. Schizogony occurs partially in the abomasum and in the distal part of the ileum.

In Kazakhstan, the prevalence of *E. cameli* was, initially observed by P.S. Ivanova-Gobzem (6) in Northern Kazakhstan, and reported 40.7%.

Eimeriadromedarii, oocysts are oval, two-layered, contains smooth shell of golden-yellow and brown color, with 0.8-1.4 μ m thickness. As the cytoplasm contracts, the oocyst membrane is exposed and becomes light-pink or -yellow. The size of the oocysts is 23.1-32.5 × 19.9-25.2 μ m, on average 27.7 × 23.2 μ m. Sporulation at a temperature of 10-12°C lasts 15-17 days. In disputes, two comma-shaped or scaphoidsporozoites develop.

This species was found in Kazakhstan in Almaty, Kyzylorda, East Kazakhstan, North Kazakhstan and West Kazakhstan regions. *Eimeriapellerdy* (13), oocysts are oval or ellipsoidal shape and 22.5-24.0 x 12.0-13.5 μ m in size. The oocyst membrane is two-layered, smooth, and colorless and has no micropyle and there is a residual body in the sporocyst.

Eimeriarajasthani (14), oocysts are ellipsoidal measuring 34-39 x 25-27 μ m in size (average 36 x 25 μ m). The oocyst membrane is two-layered; the walls are, yellow-green in color and sporulation time is approximately one week. Oocysts have no residual body or polar granules (18).

Isosporaorlovi (15), in camels in the Almaty region of the Republic of Kazakhstan described as a new species of *coccidia Isosporaorlovi*: oocysts shape varies from ellipsoidal to cylindrical in size of 15-20 x 27-35 μ m (average 19.2 × 30.4 μ m). The oocyst membrane is smooth, yellow-green or green, and 1 μ m thick. There is no micropyle, the sporozoites are 7-9 by 4-6 μ m in size (19). *Isosporaorlovi* oocysts are very similar to *Isosporalacazei* oocysts from sparrows. Coccidia of the latter could easily get into the faces of camels accidentally and penetrate (20).

The aim of this study is to investigate the prevalence of *Eimeria spp*. infection in camels in Turkestan region. Also, the role of risk factors such as age and season in the prevalence of infection are evaluated.

2. Materials and Methods

During the period of 2019, fecal samples were collected from 320 camels in four seasonin the Aldiyar production cooperative in the Dermen district in the Arys city, Turkestan region. Sampling of camels was done in four age categories: below one year, one to two years, two to three years and adults. The investigation of parasitic contamination of the samples was done based on Berkinbay (21). The feces samples (3 g) were taken from the camels and preserved in 2.5% potassium dichromate solution. The feces were thoroughly rubbed in a porcelain dish with 15-20 ml of water. The suspension then was filtered through a metal sieve or

gauze and centrifuged for 5 minutes at 1000-1500 rpm. Then the upper layer of the liquid was poured off, and zinc chloride solutions with a specific gravity of 1.598 were added to the precipitate. The precipitate was thoroughly mixed and centrifuged again for 1 minute at 1000 rpm. Then, the upper film was removed to a glass slide.

Oocyst of Eimeria sp. was detected by light microscopy. The species identification of Eimeria was determined on the basis of morphological features of oocysts (shape, size, color, thickness and structure of the shell, the presence of micropyle, polar cap, residual body and light-refractive bodies), sporocysts (shape, size, presence of residual body and Stieda's bodies), sporozoites (shape, size, presence of light-refractive bodies) and the time of sporulation of these oocysts. At the same time the data of Levine and Ivens (6), Svanbaev (11) and Pcllérdy (22) were also taken into account.

The infection intensity (II) in a group (farm) or the arithmetic mean number of the parasites per infected animal was determined by dividing the total number of parasites found in feces sample by the number of infected animals.

2.1. Statistical Analysis

Data were analyzed using Statistical package for social sciences (SPSS) version 20 for univariate and multivariate logistic. The findings obtained regarding prevalence and risk factors were compared with Chisquare test. The ratios were determined for assessing the degree of association of risk factors. In all measurements, P-value less than 0.05 was considered significant.

3. Results

In this study, the overall prevalence and infection intensity (II) of *Eimeria spp.* in camels were 42.5%

(136/320) and 74.02, respectively. Clinical findings of the study samples were reported normal and the feces were soft and formed (Table 1). In these samples, three types of Eimeria spp. were identified including: *Eimeriabactriani*, *Eimeriacameli*, and *Eimeriadromedarii*.

 Table 1. Infection of camels with Eimeria spp. in the Turkestan region

Number of camels examined	Number of infected camels	P*	II**
320	136	42,5	74,02

* P: Prevalnce, ** II: infection intensity.

The morphological characteristics of the *Eimeria spp*. found in camels at the Turkestan region are shown in table 2. These parameters coincide with those of the literature (6, 11, 22, 23).

Examination of camel infestation in the age groups of one year, one to two years, two to three years and adults showed that age is one of the important factors in the prevalence of coccidiosis. Our findings revealed that young animals are more infected than adult animals. The highest rates of prevalence (65.0%) and intensity of invasion (149.2 oocysts) in camels were recorded at the age of up to 1 year (Table 3). Camels aged between 1 to 2 years are infected 47.5%, with II of67.8. oocysts, camels aged 2 to 3 years are infected by 35.0%, with II of 34.88 oocysts, and finally adult camels'prevalence was reported 22, 5%, with II of 5.70 oocysts.

Another risk factor investigated in the prevalence of *Eimeria spp.* of camels were season of the year (Table 4). Mean prevalence of infection in the studied ages of camels in winter is 21.25% with II of20.60 oocysts, in spring 41.25 % with II of 57.20 oocysts, in summer 60.0 % with II of 102.10 oocysts, in autumn 47.5 % with II of 77.70 oocysts. As presented the highest prevalence and II are observed in summer.

Type of Eimeria	Oocyst, n=80			Spore, n=40		Sporozoite, n=30			Polar	Sporulation.
	a size, µm co		shell	size, µm	shape	size, µm	Shape	Micropyle	granule	days
E. bactriani	29,1±2,2x26, 6±2,3	yellowish, yellowish brown	two-layered, smooth	15±2,11x9 ±1,7	lemon- shaped	4,5±1,0x6, 5±1,2	pear- shaped	+	-	7±1,3
E. cameli	95,1±4,5x75, 1±4,5	yellow-green, dark brown	three-layered, smooth	44,4±4,6x 17,5±4,5	boat- shaped	14,1±1,3x 6,8±1,4	Cylindri cal	+	+	12±3
E. dromedarii	27,8±3,8x23, 4±2,9	golden yellow, brown	two-layered, smooth	9,6±1,5x7, 5±1,4	oval, round	4,3±1,1x6, 2±1,2	comma- shaped, scaphoi d	-	-	16±2

Table 2. Morphological characteristics of Eimeria spp. in camels

Table 3. The prevalence and infection intensity (II) of *Emeria spp.* infection in camels based on age ($p \le 0.05$)

Camel age	Number of camels examined	Number of infected camels	P*	II**
Young animals up to 1 year	80	52	65.0	149.2
Young animals from 1 to 2 years	80	38	47.5	67.80
Young animals from 2 to 3 years	80	28	35.0	34.88
Adult camels	80	18	22.5	5.70

* P: Prevalnce, ** II: infection intensity,

Table 4. Seasonal infection of prevalence and infection intensity of camels with *Eimeria spp.* of various ages ($p \le 0.05$)

Camel age	Winter		Spring		Summer		Autumn	
	P *	II**	Р	II	Р	II	Р	II
Young animals up to 1 year	35.0	42.3	65.0	122.8	85.0	270.2	75.0	161.5
Young animals from 1 to 2 years	25.0	22.4	45.0	66.8	70.0	78.7	50.0	103.2
Young animals from 2 to 3 years	15.0	15.7	35.0	34.9	50.0	49.5	40.0	39.4
Adult camels	10.0	2.0	20.0	4.3	35.0	9.9	25.0	6.6
Mean:	21.25	20.60	41.25	57.20	60.0	102.10	47.5	77.70

* P: Prevalence, ** II: infection intensity,

In young animals up to one year old the prevalence is low in winter (35.0 %), and in the other seasons of the year, the prevalence is high as it was measured 65.0%, 85.0%, and 75.0 %. in spring, summer and autumn, respectively. The highest II is observed in spring (122.8 oocysts) and in autumn (161.5 oocysts), the lowest in winter 42.3 oocysts. In young animals up to two years of age, the lowest prevalence is observed in winter 25.0% with II of 22.4 oocysts, while in summer, the infection rate of animals increases (70.0% with II of 78.7 oocysts).

Young animals up to 3 years old have a low infection rate in all seasons of the year. In summer, the infection rate reaches 50.0 % with II of 49.5 oocysts. Adult animals have a low infection rate in all seasons of the year. The lowest infection rate is observed in winter (10.0% with II of 2.0 oocysts), it then increases from season to season: in spring, the prevalence is 20.0% with II of 4.3 oocysts, in autumn the prevalence equals to 35.0% with II of 9.9 oocysts, in autumn it decreases to 25.0% with II of 6.6 oocysts (Figure 1).



Figure. 1 Prevalence and infection intensity (II) of camels with *Eimeria spp.* depending on age and season. $(pvalue \le 0.05)$

4. Discussion

Eimeria sp. is a parasite of the genus Apicemplexan that has many species and can cause coccidiosis in animals (e.g. cattle, poultry, dogs, catssheep and goats)(24). This parasite is an intracellular protozoan that grows mainly in the gastrointestinal tract and causes diarrhea, weakness, dehydration, and weight loss. Mortality has also been observed in some infected animals. Eimeria spp. needs one host to complete its life cycle. The life cycle of this parasite consists of an exogenous phase (sporogony) which includes a free life phase outside the host and a parasitic endogenous phase inside the host. However, in the host, both asexual and sexual reproduction cycles take partand animals excrete spore-free oocysts fromtheir gastrointestinal tract during defecation. The excreted oocyststurn into spores after 2-7 days due to environmental conditions. Appropriateoxygen level, temperature and humidity are required for sporulation to occur (19).

Studies have found three species of Eimeria in the Turkestan region in camels: *Eimeria bactriani*; *Eimeria cameli*; *Eimeria dromedarii* (25-27). In the currentstudy, the most common camel Eimeria sp. were *E. cameli*, *E. bactriani*, and *E. dromedarii*. Yakhchali and ATARI (17) also reported *E. bactriani* and *E. Dromedarii*, the most common prevalent species in the region. These results are similar with the literature (3, 27-30).

One of the most important risk factor of prevalence of infection incamels with Eimeria spp.is age of herd. Young animals are moreprone to infection than adults. The highest rate of prevalence and II in animals wasrecorded in the first year of life. However, as the the infection rate decreases. camelsgrow up. Consequently, young animals are more susceptible to infection than adult animals. This situation is might bedue to modification of the feeding regime. As the green grass of thecamels' diet increases, the risk of Eimeria sp. infection increases as well. During this period, in camels, coccidiosis quite often is symptomatic, and manifestedmainly as a digestive disorder. The low infection rate of adult camels compared to young onesis explained by the development of age-related immunity. Kaufmann (31) reported that young camels were much more susceptible to Eimeria sp. infection than adults.

This parasite severely affects the animal digestion and homeostasis by causing damage to intestinal tissue, even in the absence of clinical manifestations, with adverse effects on animal welfare and function leading to disease and presented in the animal's behavior. The animal's body reactrapidly by active immunity systems (species specific), both humoral and cellular ones, upon first contact with the antigen, and its severity depends on the number of swallowed oocytes (32).

temperature, which decreases the possibility of parasite growth with temperature drop. Therefore, if the weaning age of young camels take part in lowtemperature locations, the probability of parasitic infection of camels reduces and they are more likely to grow and survive.

Undoubtedly, with increasing of age in livestock, in addition to constant contacts of the parasitewith the gastrointestinal epithelium, the herdimmune system become stronger and the animal acquires the capabilityto control the infection.

Authors' Contribution

Study concept and design: G. U. Acquisition of data: O. B.

Analysis and interpretation of data: O. B.

Drafting of the manuscript: U. S. S.

Critical revision of the manuscript for important

intellectual content: G. U. and O. B.

Statistical analysis: A. T.

Administrative, technical, and material support: O. B.

Ethics

This publication was made within the framework of the initiative theme: Improvement of methods for the diagnosis and prevention of eimeriosis in productive animals, registered on January 25, 2019 No. 0119RKU0022 by the National Center for State Scientific and Technical Expertise of the Science Committee of the Ministry of Science and Education of the Republic of Kazakhstan (MES RK)

Conflict of Interest

The authors declare that they have no conflict of interest.

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