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Cryptosporidies (Cryptosporidiidae, Coccidia, Apicomplexa) of Sheep in Azerbaijan

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THIS article analyzes the parasitological situation of Cryptosporidiosis in sheep in Azerbaijan now. Fecal isolates of the studied animals were collected in private farms of the Absheron and Shamakhi districts. Fecal isolates were examined at the Laboratory of Biochemistry of Host Parasite Relations of the Institute of Zoology of the National Academy of Sciences of Azerbaijan. Total 120 sheep were examined for cryptosporidium infection. Experiments were carried out in January 2021 to November 2021. Cryptosporidium oocysts detected thin fecal smears were fixed with methyl blue and stained with Carbol-Fuchsin according to Ziehl-Neelsen. Based on research among 120 clinically suspected sheep were examined only 29 (24.2%) had infection – Cryptosporidium sp. In the present study, prevalence of Cryptosporidium infection in relation to season dynamics were also studies. Revealed that prevalence of Cryptosporidium sp. in sheep in Absheron and Shamakhi districts is much higher in autumn (45.7%), spring (20%), and in winter – 16.6 % than summer season (8%).

Keywords: Oocyst, Animal, Domestic ruminants, Infection.

Introduction

Apicomplexan protozoan parasites of the genus *Cryptosporidium* is an intracellular parasitic protozoan that infect the gastrointestinal tract and lungs of a wide variety of animals, including humans. It is widespread throughout the world. The parasite has a complex life cycle that includes both asexual and sexual stages. *Cryptosporidium* species are the causative agents of opportunistic diseases of the host. In immunodeficient or immunosuppressed individuals, diarrhea can result in significant morbidity and mortality, particularly in AIDS patients. The World Health Organization (WHO) as an HIV-associated disease classifies human cryptosporidiosis. The main transmission mechanism of cryptosporidiosis occurs by the faecal–oral route (person-to-person and animal to person). Infection of the hosts occurs when the only free-living stage of the life cycle of Cryptosporidium, the oocyst, enters the host organism.

C.hominis and *C.parvum* are the major causing agents of human cryptosporidiosis both in immunocompetent and in immunocompromised individuals [1]. *C.hominis* is more prevalent in North and South America, Australia, and Africa, whereas C. parvum causes more human infections in Europe, especially in the UK [3]. Particularly,

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C.meleagridis can be confirmed as an emerging human pathogen, being responsible for 1% of all infections in England [3] and about 10% in Peru, where its prevalence is as high as for *C. parvum* [4].

Cryptosporidiosis is currently a significant problem in the field of medicine and veterinary medicine. Thus, in the United States, $ 4.3 million is spent annually on the study of cryptosporidiosis, which is $ 300,000 [2, 20]. In some countries about massive outbreaks of human diseases with cryptosporidiosis, who were infected through tap water [9].

A recent important long-term typing overview reported the epidemiology of human cryptosporidiosis in the UK (England and Wales) Species *C. parvum* cases were younger, although *C. hominis* was more prevalent in infants under one year and in females aged 15 to 44 years [17, 5].

Cryptosporidium ubiquitum and *C. xiaoi* are often the most common species in older animals [19, 21] and are con-sidered as low-pathogenic species [8,7] and therefore are mostly reported in appar-ently healthy sheep [6].

In Azerbaijan, animal studies for infection with Cryptosporidium began in the late 1980s. The research of farm animals was carried out in individual and private farms of the Absheron Peninsula, some regions of eastern and northeastern Azerbaijan, the vicinity of Ganja and adjacent mountainous and foothill regions of Goygel and Dashkesan. Thanks to systematic research, it was found that domestic ruminants, cattle and small ruminants, buffaloes, camels, are largely infected with cryptosporidium. [11].

For 5 years (2009-13), were investigated the distribution of cryptosporidia of domestic ruminants in various livestock farms in Gobustan and Sheki-Zagatala region (eastern and western parts of the Greater Caucasus Mountains). The material for research was the feces of cows, sheep and buffaloes. Fecal isolates of the studied animals of different age and sex were collected seasonally in individual and farm households in Gobustan (2010-12) and Sheki-Zagatala region (2011-13) (Balakan, Gakh, Sheki, Zagatala districts) [15].

However, until now, the degree of infection of domestic ruminants with Cryptosporidia and the species composition of the latter in many regions of Azerbaijan remained unknown.

The purpose of this research is to identify the degree of infection of sheep with Cryptosporidium in Absheron and Shamakhi districts.

Methods

The material for the research was sheep feces. Fecal isolates of the studied animals were collected in private farms of the Absheron region (2021). Fecal isolates were examined at the Laboratory of Biochemistry of Host Parasite Relations of the Institute of Zoology of the National Academy of Sciences of Azerbaijan.

Cryptosporidium oocysts detected thin fecal smears fixed with methyl blue and stained with Carbol-Fuchsin according to Ziehl-Neelsen [12]. A small lump of fresh feces was applied to a glass slide, a thin smear was made, and it was thoroughly dried in air. Then it was fixed for five minutes in methyl alcohol, dried at room temperature and quickly carried out 3-5 times over the flame of the burner. The carbol-fuchsin solution was stained for 20 minutes. Composition solution: basic fuchsin - 2 g, 96% ethyl alcohol - 12 ml, phenol - 5 ml, distilled water up to 100 ml. Next, the smear was washed with tap water, discolored with water 10% sulfuric acid for 20-60 sec., Washed in water, washed again with a 5% solution of malachite green in 10% ethyl alcohol, washed in water and dried at room temperature and examined under a microscope x 100 (with immersion).

Data from the study were entered in Ms-Excel, for statistical processing the results used the statistical program IBM SPSS Statistics 20.

Results

In total, fecal isolates from 120 heads of sheep from private farms in Absheron and Shamakhi districts were examined for infection with cryptosporidia. Studied animals in the Absheron region showed an average degree of invasion by cryptosporidia in all seasons of the year. According to the results of studies infected sheep were observed more in autumn extensive invasion (EI) - 45.7% (35/16), in spring - 20% (30/6), in winter they were less -16.6% (30/5), and in summer -8% (25/2). Thus, of the 120 examined animals, oocysts of Cryptosporidium were found in 29 sheep, that is 24.2% (Table 1).

The intensity of invasion in all seasons in all animals in all the private farms we studied ranged from 1 to 7 oocysts, sometimes up to 8-9.

In sheep, we found round oocysts spherical in shape, with a diameter of 3.5±0.2 μm and 4.4 μm (FI = 1.04; n = 29).

TABLE 1. Average degree extensive of invasion by cryptosporidia of sheep in all seasons.

Seasons of the year	Number of sheep	Extensive of invasion of sheep (%)
Winter	30	16.6
Spring	30	20
Summer	25	8
Autumn	35	45.7

Discussion

It is known that in sheep in different countries such species as *C.ubiquitum, C.xiaoi, C.parvum* are widespread [10]. Other species *C.fayeri, C.homminis, C.suis, C.andersoni* and several genotypes were found only in adult animals. [18] *C.ubiquitum* is a common zoonotic pathogen. [13].

Gaibova et al. diagnosed the presence of only 2 types of cryptosporidia in domestic ruminants in Azerbaijan: *C.parvum* Tyzzer, 1912 and *C.andersoni* Lindsey et al., 2000. *C.parvum* has also been reported in humans [13]. In sheep found by Gaibova, oocysts were round, 4.2, 5.01, and 5.85 μm in diameter (IF = 1.0) and elongated oocysts 5.85-5.01 × 4.2-3.34 μm in size, on average 4.7 ± 0.04 × 4.7 ± 0.05 μm (IF = 1.04; n = 50). Also were larger, spherical, 6.7 and 5.85 in diameter (IF = 1.0) and elongated, 6.7-5.85 × 6.7-5.01 μm in size (IF = 1.0-1.2), on average 6.3 ± 0.07 × 6.08 ± 0.08 μm (IF = 1.03; n = 39) [22].

Spring peaks were due to *C.parvum*, while *C.hominis* was more prevalent during the late summer and early autumn as well as in patients reporting recent travel abroad [16].

The oocysts of Cryptosporidium found by us and described as Cryptosporidium sp. were smaller than those found and described by Gaibova [11] .

Our results on seasonal infection of sheep with cryptosporidium are coincide results by Gaibova et al. where it is reported that more infected sheep were observed in autumn (EI-58.1%), in winter they were less (EI-18.2%), in spring the EI slightly increased - 23.6%. In summer, sheep from the Gobustan semi-desert are driven to winter pastures; therefore, no data on the summer infection of sheep with cryptosporidia [22].

Conclusion

In conclusion, our analyses showed that cryptosporidium oocysts were found in 2 sheep from 25 (8%) in the Absheron and Shamakhi districts in the summer season. The intensity of sheep invasion was (1-2 oocysts), rarely in isolates of sheep feces were found to 10 oocysts. Although the goal of our research in the Absheron and Shamakhi districts of Azerbaijan was achieved, given the damage caused by cryptosporidiosis of domestic animals, we consider it expedient to further research to study the epizootic situation in this region.

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Conflict of interest

The authors has no conflict of interests to declare regarding the publication of this paper.

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