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Cryptosporidium spp. Infections in Livestock and Wild Animals in Azerbaijan Territory †

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Abstract: *Cryptosporidium* is an intracellular protozoan parasite and is increasingly gaining attention as a human and an animal pathogen, mainly due to its predominant involvement in worldwide waterborne outbreaks. This paper reviews the current knowledge and understanding of *Cryptosporidium* spp. in terrestrial and aquatic animals in Azerbaijan. The diagnosis of cryptosporidiosis relies on the identification of oocysts in faecal samples released by the infected host. Stool specimens were processed using the modified acid-fast staining method (Ziehl-Neelsen) and microscopically examined for *Cryptosporidium* oocysts. Thirteen species of *Cryptosporidium* (*C. fragile, C. ducismarci, C. serpentis, C. varani, C. baileyi, C. meleagridis, C. muris, C. parvum, C. ubiquitum, C. andersoni, C. bovis, C. hominis, C. suis*) from amphibians, reptiles, birds and mammals have been identified as a result of studies conducted between 1987 and 2019 on the structural features of *Cryptosporidium* oocysts in Azerbaijan territory.

Keywords: *Cryptosporidium*; microscopy; Azerbaijan

1. Introduction

Cryptosporidium is an intracellular protozoan parasite and is a major cause of gastroenteritis (cryptosporidiosis) worldwide. *Cryptosporidium* is a globally distributed genus of diarrheal pathogens capable of infecting various vertebrate species, including humans as well as domestic and wild animals [1]. To date, a total of 39 species and more than 70 genotypes of *Cryptosporidium* have been described, and 21 species and four genotypes out of these have been reported in humans [2,3]. Several *Cryptosporidium* species are known to be zoonotic with animals as major reservoirs [4]. Wild and livestock animals have the potential to act as a biological reservoir for harmful protozoan parasites—*Cryptosporidium* spp. [5].

Cryptosporidium completes its lifecycle in a single host and it is ubiquitous in nature. Oocysts can tolerate various environmental conditions and can survive in water and soil for many months because of the suitable moisture content and cool temperatures [6,7]. They can be transported long distances through air and also flushed quickly into the water sources because of the oocysts small size [8]. *Cryptosporidium* sparked great public health interest after the large human waterborne outbreak in Milwaukee in 1993 and rapidly was recognized as one of the most serious waterborne pathogens [9]. Outbreaks of cryptosporidiosis have been and continue to be reported in several countries [10–12]. Domestic animals, livestock, wildlife, and humans are potential reservoirs that

contribute to the contamination of food, surface waters and the environment by *Cryptosporidium* spp. oocysts, thereby transmitting the infection to other hosts via the fecal-oral route [13,14].

This paper aims to report on the main results of studies on cryptosporidiosis and *Cryptosporidium* among livestock and wild animals and birds in Azerbaijan territory and provide first-hand information about this important pathogen, taking into consideration veterinary and public health aspects of the Azerbaijan territory for the last 32-year period. In total, 13 species of *Cryptosporidium* from amphibians, reptiles, birds, and mammals have been identified and reported from studies conducted from 1987 to 2019 on structural features of *Cryptosporidium* oocysts in different regions of the Azerbaijan republic.

2. Material and Methods

This review is prepared based on the literature data. More than 70 publications describing the presence of *Cryptosporidium* in animals have been published in the last 32 years in Azerbaijan. The language of data collection was English, Russian and Azeri. The first report was in 1987 [15]. In the 32 years between 1987 and 2019, a total of 9408 samples from 57 studies on wild animals, birdsand common livestock, defined as cattle, sheep, goats, pigs, horses and buffaloes, were examined for *Cryptosporidium* infection. *Cryptosporidium* spp. is a widespread pathogen of many species of domestic animals. Most of the publications (70%) are about *Cryptosporidium* prevalence in domestic animals. Cryptosporidiosis in livestock is becoming a significant problem for animal health (both subclinically and clinically) and has resulted in economic losses due to increasing veterinary services and labor costs, increasing animal health-care costs, and a decrease in the growth rate and increase in mortality of severely infected animals.

In this period, stool specimens were randomly collected from 8668 livestock (pigs, horses, donkeys, goats, sheep, calves, buffalo, zebus, camels, and birds) and 740 wild animals (amphibians, reptiles, rodents, cats and dogs). The most studied livestock animals were pigs (n = 2857), sheep (n = 1823) and cows (n = 2595).

The diagnosis of cryptosporidiosis relies on the identification of oocysts in faecal samples. The diagnosis is established microscopically. Fine feces smears were fixed with methanol and were stained with carbol-fuchsin and methylene green by the Ziehl–Neelsen staining method or detection of *Cryptosporidium* oocysts according to the procedure described by Henriksen and Poplenz [16].

Microscopy is cheap, but requires a skilled parasitologist and/or related expert and the diagnostic yield is dependent on proper stool collection. *Cryptosporidium* spp. identification was based on conventional criteria, such as oocyst morphology and measurements. These criteria are in agreement with those used by Fayer et al. [17] and Morgan-Ryan et al. [18], who cited that the morphometric measurement of oocysts represents the cornerstone of *Cryptosporidium* taxonomy and is one of the requirements for establishing a new species.

3. Results

A total of 9408 samples from 57 studies on wild animals, birds and common livestock, defined as cattle, sheep, goats, pigs, horses and buffaloes, were examined for *Cryptosporidium* infection, with 2903 (30.86%) being positive for *Cryptosporidium* spp. using microscopy methods. Table 1 summarizes the prevalence and frequency of parasite-positive fecal samples from different groups of animals. *Cryptosporidium* prevalence is higher in wild animals (34.73%) than in farmed animals. Fecal samples were collected and tested from 8668 livestock animals living in farms in different regions of Azerbaijan; 30.55% of all tested samples were positive for *Cryptosporidium* oocysts. Fecal samples were collected and tested from 740 wild animals captured from different regions of Azerbaijan; 34.73% of all tested samples were positive for *Cryptosporidium* oocysts. The greatest prevalence of *Cryptosporidium* species shedding oocysts was observed in feces from testudines (55.77% positive), chickens (50.6% positive) and buffaloes (42.45% positive) [19–22]. Overall, feces from younger (immature) animals were more likely to test positive for *Cryptosporidium* spp. than feces from adult animals. Additionally, *Cryptosporidium* spp. prevalence was greater in feces from males than females [23].

Table 1. Prevalence of *Cryptosporidium* reported from animals by Microscopy.

Host Name	*n* Positive	*n* Total	%	References
Wild Animals				
Amphibians	30	87	34.48	[24]
Reptilia: Ordo: Testudines	58	104	55.77	[19,25,26]
Reptilia: Ordo: Squamata	26	114	22.81	
Rodents	112	325	34.46	[25,27,28]
Cats	17	54	31.5	[25]
Dogs	14	56	25	[25]
Subtotal	**257**	**740**	**34.73**	
Domestic (Farm) animals				
Birds	198	729	27.16	[20,25]
Domestic pigs	804	2857	28.14	[23,29]
Horse	2	4	50.00	[25]
Donkey	1	3	33.33	[25]
Domestic goat	25	127	19.69	[21]
Domestic sheep	623	1823	34.17	[21–23]
Cow	779	2595	30.02	[21–23]
Buffalo	135	318	42.45	[21,22]
Zebu (Indicine cattle)	14	30	46.67	[21]
Bactrian camel	65	182	35.71	[30]
Subtotal	**2646**	**8668**	**30.53**	
Total	**2903**	**9408**	**30.86**	

The prevalence of *Cryptosporidium* infection was higher in rural and urban areas of Azerbaijan than in mountainous areas [21,26]. Amphibians and reptiles, caught in the territory of Absheron and Gobustan, showed a high prevalence rate of *Cryptosporidium* infection. [26]. For the first time in Azerbaijan, *C. fragile* was identified as a parasite of Caucasian toads (*Bufo verrucomsissimus*) [19] and *C. ducismarci*as a parasite of Greek tortoises (*Testudo graeca*) [26].

The Caucasian agama (*Paralaudakia caucasia*) and Steppe-runner (*Eremias arguta*) was identified as the host of *C. serpentis*, and the Spotted whip snake (*Hemorrhois ravergieri*) and the Dice snake (*Natrix tessellate*) were found to be the hosts of *C. varani* [26].

Between 1989 and 2019, a total of 729 faecal samples from birds were collected from different regions in Azerbaijan.

According to the microscopy results using Ziehl–Neelsen staining, *Cryptosporidium* oocysts were present in 27.16% of the samples. The two *Cryptosporidium* species (*C. meleagridis* and *C. baileyi*) detected in bird fecal smears in the study areas from Azerbaijan were morphologically similar to the same species detected in birds in previous studies [31–33].

Mammals represent the largest group of animals known to be infected with *Cryptosporidium* spp. A total of 325 rodents of three species were examined for *Cryptosporidium* oocysts, 112 (34.46%) of

which were infected (Table 1). In Azerbaijan, three species of *Cryptosporidium*—*C. muris*, *C. parvum* and *C. ubiquitum* from rodents have been reported [27, 28]. After examination of 110 wild dogs and cats, caught in mountainous regions of Azerbaijan, *Cryptosporidium* oocysts were detected in 25% of dogs, 31.5% of cats (Table 1). The great majority of infections in mammals were reported in domestic animals of economic importance. The prevalence of *Cryptosporidium* in cattle, sheep, goats, buffaloes, and pigs were studied well in Azerbaijan. During the 32 years from 1987 to 2019, a total of 7750 (2857 pigs, 127 goats, 1823 sheep, 2595 cows, 318 buffalo, and 30 zebu) stool samples from domestic animals were examined for *Cryptosporidium* (Table 1). Three species of *Cryptosporidium* were found in large and small cattle and buffalo of Azerbaijan: *C. andersoni, C. bovis* and *C. hominis* [34]. In total, 182 Bactrian camels were investigated for the infection of *Cryptosporidium*; 65 (35.71%) of them were infected with *Cryptosporidium* oocysts (Table 1). In camels, two species of *Cryptosporidium*—*C. muris* and *C. andersoni*—were observed [30]. In the years from 2009 to 2015, a total of 2857 pigs were examined for the presence of *Cryptosporidium;* 804 (28.14%) were positive for *Cryptosporidium* oocysts (Table 1). It was noted that two species of *Cryptosporidium* were found in Azerbaijani pigs: *C. muris* and *C. suis* [29].

4. Discussion

The present review shows that there was a high prevalence of *Cryptosporidium* spp. (30.86%) in the areas studied in Azerbaijan. Studies of *Cryptosporidium* infection in animal livestock from different regions of Azerbaijan have revealed the following distribution pattern: the highest prevalence of infection was recorded in lowland areas and the lowest prevalence was recorded in mountainous regions The highest rate of infection with *Cryptosporidium* was found in reptiles and amphibians and other terrestrial animals from the Absheron peninsula and Gobustan reserve. The Absheron peninsula is the most urbanized territory of the Republic of Azerbaijan.

This review showed that *Cryptosporidium* spp. is a common parasite of animals in Azerbaijan. A total of 13 species of *Cryptosporidium* (*C. fragile*, *C. ducismarci*, *C. serpentis*, *C. varani*, *C. baileyi*, *C. meleagridis*, *C. muris*, *C. parvum*, *C. ubiquitum*, *C. andersoni*, *C. bovis*, *C. hominis*, *C. suis*) from amphibians reptiles, birds and mammals have been identified based on structural features of *Cryptosporidium* oocysts in studies conducted from 1987 to 2019 in Azerbaijan territories [34].

Species were determined based on a comparative analysis of morphometric parameters of the discovered oocysts with previously described known species of coccidia. Most researchers state that morphological features of *Cryptosporidium* oocysts are not sufficient criteria for *Cryptosporidium* species identification. Therefore, we refrain from species definition for the *Cryptosporidium* ssp. oocysts reported in this review until life cycle studies and DNA analyses are performed.

We intend to conduct cross-infection studies with the *Cryptosporidium* oocysts found by us, and to study their life cycles and then to conduct DNA analysis. Further studies are important to understand the epidemiology and transmission of *Cryptosporidium* in domestic and wild animals in Azerbaijan.

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