

Prevalence of Coccidian Species in the Water Buffalo (*Bubalus Bubalis*) in the Province of Afyon, Turkey

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Abstract

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The objective of this study was to identify the coccidian species present in water buffaloes in the vicinity of Afyon. Faecal samples were collected directly from the rectum of a total of 104 water buffaloes, and examined in the laboratory using Sheather's saturated sugar solution. Faecal samples found to contain *Eimeria* species were mixed with a solution of 2.5% potassium dichromate, left at room temperature in petri dishes until the oocysts sporulated, and then examined in order to identify the species.

Eleven different *Eimeria* species and one *Isospora* species were identified in 78 (75%) out of the 104 water buffaloes as follows: *E. zuernii* (55.1%), *E. auburnensis* (44.9%), *E. bovis* (44.9%), *E. ellipsoidalis* (28.2%), *E. ankarensis* (16.7%), *E. subspherica* (16.7%), *E. alabamensis* (11.5%), *E. cylindrica* (10.3%), *E. bareillyi* (5.1%), *E. canadensis* (5.1%), *E. brasiliensis* (3.8%), and *Isospora* spp. (46.2%). With this study, species causing coccidiosis in water buffaloes in the province of Afyon were identified for the first time, and *Isospora* spp. in the water buffalo is reported for the first time.

Water buffalo, coccidiosis, Eimeria, Isospora, Afyon, Turkey

Coccidiosis is an intestinal disease that affects several different animal species including cattle, sheep, goats, cats, water buffalo, dogs, rabbits, and poultry. Since the disease may result in death especially in young animals, it is the cause of a considerable economic loss in farm animals world-wide (Levine 1985; Mimioglu et al. 1969; Pellérdy 1974; Soulsby 1982). Coccidian species generally have host specificity, and in this respect the species of *E. ankarensis*, *E. azerbaijanica*, *E. bareillyi*, *E. gokaki*, *E. ovoidalis* and *E. thianethi* have been reported in water buffaloes. Moreover, species found in cattle, such as *Eimeria alabamensis*, *E. auburnensis*, *E. bovis*, *E. brasiliensis*, *E. bukidnonensis*, *E. canadensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis*, and *E. zuernii* have also been reported in the water buffalo (Levine 1985; Pellérdy 1974; Sayin 1968; Soulsby 1982).

Apart from *E. azerbaijanica* and *E. thianethi* which are specific to the water buffalo (Patnaik 1964; Patnaik 1965; Patnaik and Pande 1965; Pellérdy 1974; Yakimoff 1933), various cattle *Eimeria* species have been generally reported to prevail in the world (Gundran and More 1999; Patnaik 1964; Patnaik 1965; Patnaik and Pande 1965; Tubanguii 1931; Yakimoff 1933). In addition, the endogenous development of some *Eimeria* species in the small intestine (duodenum, jejunum and ileum) of the water buffalo has been defined histologically (Patnaik 1965; Patnaik and Pande 1965).

The population of water buffaloes was 104,965 in Turkey in 2005. The Afyon province

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has 3.4% (3,953) of the total population. Although this number seems to be low, the milk of water buffaloes is very important in the Afyon area. The Afyon province is the homeland of a special dessert made of water buffalo milk, known as “Afyon kaymagi” (Anonymous 2007; Anonymous 2007a).

There are very limited studies on the parasites of water buffaloes in Turkey. The studied parasites are mostly helminths. The prevalence of *Paramphistomum* spp. was 66.6% (Guralp 1981) and that of *Calicophoron daubneyi* was 9.75% (Tinar et al. 1992). Some helminth parasites were reported for the first time, such as *Monezia expensa*, *Avitellina centripunctata*, *Gonglyonema pulchrum*, *Artionema labiato-papillosa*, and *Paracooperia nodulosa* in water buffaloes (Merdivenci and Buyurman 1965; Tuzdil 1939; Guralp and Dogru 1968; Cetindag 1993). In the Samsun province, 100 water buffaloes were slaughtered and the parasites of the gastrointestinal system were investigated. Thirteen species (1 trematode and 12 nematoda) were found. *Coccidia* oocysts were found in 21% of the animals and also *Sarcocystis* macrocysts and microcysts were encountered in 13% of the oesophagus of the animals (Cetindag and Doganay 1996). Dundar and Ozer (1996) reported that *Sarcocystis* cysts were encountered in the oesophagus of water buffaloes as 39.2% macroscopically and 81.6% microscopically. No studies were done on the coccidian parasites in water buffaloes in the Afyon province previously. The prevalence of coccidian species in cattle is as follows: *Eimeria bovis* (34.55%), *E. auburnensis* (23.03%), *E. canadensis* (14.55%), *E. brasiliensis* (10.91%), *E. zuernii* (6.67%), *E. bukidnonensis* (3.03%), *E. cylindrica* (2.42%), *E. ellipsoidalis* (1.21%), *E. illinoisensis* (1.21%), *E. alabamensis* (1.21%) and *Isospora* spp. (1.21%) (Cicek et al. 2007).

The first study on coccidiosis in water buffaloes in Turkey, conducted by Sayin (1968), found only one water buffalo-specific *Eimeria* spp., *E. ankarensis*, alongside the cattle species *E. ellipsoidalis*, *E. zuernii*, *E. bovis*, *E. auburnensis*, *E. canadensis*, *E. subspherica*, *E. alabamensis*, *E. cylindrica*, *E. brasiliensis* and *E. wyomingensis* in water buffalo. *Eimeria ankarensis* was recorded as a new species in water buffaloes in this study, which also investigated the potential of *E. ankarensis*, *E. zuernii*, *E. auburnensis*, *E. ellipsoidalis* and *E. bovis* oocysts from the water buffalo to infect cattle calves, and found that with the exception of *E. ankarensis*, the other species could infect cattle. Therefore, *E. ankarensis* was classified as host-specific for the water buffalo. In another study on water buffaloes, Sayin (1973) reported the presence of *E. bareillyi* for the first time in Turkey.

No studies were done on the coccidian parasites in water buffaloes in the Afyon province previously. This study was conducted with the aim of identifying the coccidian species present in water buffaloes in the Afyon province and determining their prevalence.

Materials and Methods

This study was performed between 2002 and 2004 in the villages of Oren and Akcin in the Afyon province, where the raising of water buffaloes is common. The pastures where water buffaloes graze are also shared by cattle and sheep. The city has a continental climate: hot and dry summer, mild and rainy spring, cold and snowy winter.

Faecal samples were taken from the animals simultaneously. Faecal samples were collected directly from the rectum of a total of 104 water buffaloes, 40 of which were younger than 1 year of age, and the remaining 64 were older than 1 year; 54 of them were from Oren and 50 from Akcin villages, respectively. The samples were collected into plastic bags that were labelled and taken to the laboratory.

In the laboratory, after 3 washes, the sediment from each sample was mixed with Sheather's saturated sugar solution, centrifuged and examined under a microscope for the presence of protozoan oocysts. Samples containing coccidian oocysts were mixed with 2.5% potassium dichromate in petri dishes and left at room temperature for the sporulation of oocysts to take place. Species were then identified according to the size and morphological characteristics of the oocysts (the shape and colour of the oocysts; the thickness of the oocyst walls; the presence of micropyl, cap, polar granules, and oocyst or sporocyst deposits, the size and shape of the sporocysts, the shape of Stieda bodies and sporozoites, etc.). The identification of each species was made with reference to the measurements of 25 to 50 oocysts, from at least 5 hosts (or from the total number of hosts if less than 5), under a research microscope (Olympus) with a camera attachment.

Table 1. Coccidian species found in the faeces of water buffalo in Afyon and the infection rates

Species	Orenler Province (Number of infected animals)	Percentage of infection %	Akcin Province (Number of infected animals)	Percentage of infection %	Total (Number of infected animals)	Percentage of infection %
<i>E. ankarensis</i>	5	6.4	8	10.3	13	16.7
<i>E. bareillyi</i>	-	-	4	5.1	4	5.1
<i>E. zuernii</i>	20	25.6	23	29.5	43	55.1
<i>E. auburnensis</i>	17	21.8	18	23.1	35	44.9
<i>E. bovis</i>	16	20.5	19	24.4	35	44.9
<i>E. ellipsoidalis</i>	11	14.1	11	14.1	22	28.2
<i>E. subspherica</i>	9	11.6	4	5.1	13	16.7
<i>E. atabamensis</i>	6	7.7	3	3.8	9	11.5
<i>E. cylindrica</i>	4	5.1	4	5.1	8	10.2
<i>E. canadensis</i>	4	5.1	-	-	4	5.1
<i>E. brasiliensis</i>	3	3.8	-	-	3	3.8
<i>Isospora</i> spp.	27	34.6	9	11.6	36	46.2

x: number of infected animals
n: total number of infected animals (n = 78)

Statistical differences in the prevalence of coccidiosis between the age groups were determined using the Chi-square test.

Results

Seventy eight (75%) out of 104 water buffaloes were found to be infected with coccidian species. Faecal samples from 20 animals (25.6%) contained only 1 species; from 19 (24.4%), 2 species; from 14 (17.9%), 3 species; from 13 (16.7%), 4 species; from 3 (3.8%), 5; from 6 (7.7%), 6; from 2 (2.6%), 7 and from 1 animal (1.3%), 8 species. Infections with single species were found as follows: 13 animals were infected (65%) with *Isospora* spp., 2 animals (10%) with *E. auburnensis*, 5 (25%) animals with *E. ankarensis*, *E. bovis*, *E. ellipsoidalis*, *E. cylindrica* and *E. zuernii*, respectively.

Table 1 shows the coccidian species found and the rate of infection caused by each species. As seen in this table, a total of 11 *Eimeria* species and one *Isospora* spp. (Plate XI, Fig. 3) were found in water buffaloes. *Eimeria bareillyi* in the Orenler district and *E. canadensis* and *E. brasiliensis* in the Akcin district were not encountered. Two of the *Eimeria* species (*E. ankarensis* and *E. bareillyi*) (Plate XI, Fig. 1 and 2) were specific to the water buffalo, and the rest were cattle-originated species (*E. zuernii*, *E. auburnensis*, *E. bovis*, *E. ellipsoidalis*, *E. subspherica*, *E. alabamensis*, *E. cylindrica*, *E. canadensis*, and *E. brasiliensis*). The rate of infection caused by *E. ankarensis*, *E. zuernii*, *E. auburnensis*, and *E. bovis* was higher than that of the remaining species (Table 1).

Furthermore, 37 (92.5%) out of 40 water buffaloes less than 1-year-old and 41 (64.1%) out of 64 older than 1 year were found to be infected with various coccidian species. The difference between the rates of infection among the age groups was found to be statistically significant

Table 2. Distribution of coccidian species to the infected animals by age group

Species	Akcin Province				Orenler Province				Total			
	0-1 year old		Over 1 year old		0-1 year old		Over 1 year old		0-1 year old		Over 1 year old	
	Number of infected animals (x/n) n = 23	%	Number of infected animals (x/n) n = 27	%	Number of infected animals (x/n) n = 17	%	Number of infected animals (x/n) n = 37	%	Number of infected animals (x/n) n = 40	%	Number of infected animals (x/n) n = 64	%
<i>E. ankarensis</i>	7	30.4	1	3.7	4	23.5	1	2.7	11	27.5	2	3.1
<i>E. bareillyi</i>	2	8.7	2	7.4	-	-	-	-	2	5	2	3.1
<i>E. alabamensis</i>	3	13.0	-	-	3	17.6	3	8.1	6	15	3	4.7
<i>E. auburnensis</i>	14	60.9	4	14.8	8	47.1	9	24.3	22	55	13	20.3
<i>E. brasiliensis</i>	-	-	-	-	2	11.8	1	2.7	2	5	1	1.6
<i>E. bovis</i>	16	69.6	3	11.1	9	52.9	7	18.9	25	62.5	10	15.6
<i>E. canadensis</i>	-	-	-	-	1	5.9	3	8.1	1	2.5	3	4.7
<i>E. cylindrica</i>	3	13.0	1	3.7	1	5.9	3	8.1	4	10	4	6.3
<i>E. ellipsoidalis</i>	6	26.1	5	18.5	8	47.1	3	8.1	14	35	8	12.5
<i>E. subspherica</i>	3	13.0	1	3.7	6	35.3	3	8.1	9	22.5	4	6.3
<i>E. zuernii</i>	19	82.6	4	14.8	9	52.9	11	29.7	28	70	15	23.4
<i>Isospora</i> spp.	2	8.7	7	25.9	8	47.1	19	51.4	10	25	26	40.6

x: number of infected animals

n: 0-1-year-old animals

n_i: over 1-year-old animals

($P < 0.001$). The distribution of these species to the infected animals in each age group is given in Table 2. It is understood from this table (Table 2) that all coccidia species except *E. canadensis* are more prevalent in 0 to 1-year-old animals. *E. brasiliensis* and *E. canadensis* were not seen in 0-to-1-year-old water buffaloes in the Akcin district. *Eimeria alabamensis* was not seen in water buffaloes older than 1 year in the Akcin district. *Eimeria bareillyi* was not found in all of the water buffaloes in the Orenler district.

A high proportion (46.2%) of the water buffaloes was found to be infected with only one *Isospora* spp. in this study. The oocysts of this species measured 22.35×24.09 ($18.3 - 31.11 \times 18.3 - 31.84$) micrometres and were rounded, with double straight walls and no oocyst deposits. The sporocysts of the sporulated oocysts measured on average of 9.54×14.02 ($12.81 - 18.3 \times 7.32 - 10.98$) micrometres and were prominent, club-shaped, with button-shaped Stieda bodies, and deposits of sporocyst remains (Fig. 3).

Discussion

Six out of 17 of the recorded *Eimeria* species that infect the water buffalo (*E. ankarensis*, *E. azerbaijanica*, *E. bareillyi*, *E. gokaki*, *E. ovoidalis*, and *E. thianethi*) throughout the world are known to be specific to the water buffalo, while the remaining 11 species (*E. alabamensis*, *E. auburnensis*, *E. bovis*, *E. brasiliensis*, *E. bukidonensis*, *E. canadensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis*, and *E. zuernii*) are cattle-originated (Arslan 2001; Gundran and More 1999;

Patnaik 1964; Patnaik 1965; Patnaik and Pande 1965; Penzhorn 2000; Ribeiro et al. 2000; Sayin 1968; Tubangui 1931; Yakimoff 1933). In this study, a total of 11 *Eimeria* species were found in water buffaloes from the Afyon province, of which 2 were specific to the water buffalo (*E. ankarensis* and *E. bareillyi*), and the remaining 9 were of cattle origin (*E. alabamensis*, *E. auburnensis*, *E. bovis*, *E. brasiliensis*, *E. canadensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica*, and *E. zuernii*). Furthermore, one *Isospora* sp., the origin of which is open to discussion, was also identified, for the first time in the world, in a large number of water buffaloes (46.2%).

In one of the two other studies performed on water buffaloes in Turkey (Sayin 1968), (95.4%) 124 out of 130 water buffaloes in the vicinities of Ankara, Adapazari, Bolu, Corum, Kayseri and Samsun, and in the second study (Sayin 1973), (2%) 1 out of 50 water buffaloes from Adapazari, were found to be infected with *Eimeria* species. The identification of the species revealed that 2 were specific to the water buffalo (2% *E. bareillyi* and 9.9% *E. ankarensis*) and 10 were of cattle origin (53% *E. ellipsoidalis*, 48.8% *E. zuernii*, 43.8% *E. auburnensis*, 34.4% *E. bovis*, 20% *E. canadensis*, 15.3%, *E. subspherica*, 10.3% *E. alabamensis*, 4.6% *E. cylindrica*, 1.6% *E. brasiliensis*, and 0.7% *E. wyomingensis*). That research also identified and described *E. ankarensis* for the first time in the world, and reported the occurrence of *E. bareillyi* in the water buffalo for the first time in Turkey. In our study, 78 (75%) out of 104 water buffaloes from the province of Afyon were found to be infected with 11 different *Eimeria* species, 2 of which were recorded as specific to the water buffalo and the remainder of which originated from cattle. The presence of all these species, with the addition of *E. wyomingensis*, was reported in water buffaloes in Turkey in the two previous studies. In our study, *Isospora* spp. was reported for the first time in the water buffalo.

We found 8 *Eimeria* and *Isospora* species which were also found in a study (Cicek et al. 2007) carried out on cattle in the Afyon province, where we also performed our study. This shows that the species we found might be cattle-originated. In the same study it was noted that younger animals (27.23%) showed higher prevalence of coccidial infection than older animals (15.65%). In our study the infection rate was 92.5% in animals younger than 1 year and 64.1% in animals older than 1 year. Infection rates were compatible with the study of Cicek et al. (2007) regarding the effect of age on infection rates.

In conclusion, this study showed that 75% of water buffaloes in the Afyon province were infected with 11 different *Eimeria* species, 2 of which were host-specific to water buffaloes and 9 of which were of a cattle origin. A high number of the water buffaloes were found to be infected with a novel *Isospora* species which warrants further detailed studies.

Výskyt jednotlivých druhů kokciidií u buvolů indického (*Bubalus Bubalis*) v Turecku, provincie Afyon

Cílem této studie bylo identifikovat jednotlivé druhy kokciidií vyskytující se u buvolů indického v okolí Afyonu. Vzorky trusu byly odebrány přímo z rekta 104 buvolů indických a zkoumány v laboratoři s využitím Sheatherova cukerného roztoku. Vzorky, u kterých byla zjištěna přítomnost *Eimeria* spp. byly smíchány s roztokem dichromanu draselného o koncentraci 2,5% a ponechány při pokojové teplotě na petriho miskách, dokud oocysty nevyšperulovaly. Poté byly identifikovány jednotlivé druhy.

Bylo identifikováno jedenáct různých druhů *Eimeria* spp. a jeden *Isospora* spp. u 78 buvolů indických (75 %) ze 104 testovaných. Jednalo se o: *E. zuernii* (55,1 %), *E. auburnensis* (44,9 %), *E. bovis* (44,9 %), *E. ellipsoidalis* (28,2 %), *E. ankarensis* (16,7 %), *E. subspherica* (16,7 %), *E. alabamensis* (11,5 %), *E. cylindrica* (10,3 %), *E. bareillyi* (5,1 %), *E. canadensis* (5,1%), *E. brasiliensis* (3,8 %), a *Isospora* spp. (46,2 %). V rámci této studie byly poprvé identifikovány druhy způsobující kokciidiozu buvolů indických v oblasti Afyonu a byl u nich poprvé popsán výskyt *Isospora* spp.

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Fig. 1, 2 and 3. Sporulated oocysts of *E. ankarensis*, *E. bareillyi* and *Isospora* spp., respectively.
Bar = 10 μ