

A COPROLOGICAL STUDY ON GASTRO-INTESTINAL PARASITE COMMUNITY IN WATER BUFFALOES FROM A ROMANIAN FARM

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Abstract

*Parasite infections are one of the major constraints for profitable animal industry worldwide. Despite of the importance of water buffalo (*Bubalus bubalis*) in the overall economy of many countries, little is known about the occurrence and prevalence of parasitic species and its potential impact on the animal health and productivity. Therefore, the present study aimed to determine the endo-parasitofauna in a water buffalo farm in Romania. For this, during of April 2018, a coprological study was conducted, in which a total number of 105 buffaloes, allocated in four age category (< 6 months, n = 21; 6 months-2 years, n = 20; heifers, n = 30; milking, > 3 years, n = 34) were included. Fecal samples, collected from individual animals, were subjected for analysis by standard qualitative copro-parasitological methods to detect parasitic stages (protozoan oocysts, helminth eggs and larvae). Overall, 55.2% of samples were positive for parasite infection, of which, 36.2% as single parasite species and 18.9% with mixed infections of two (13.8%) and three (5.2%), respectively, parasite species. The endoparasite community included protozoa (*Eimeria* spp., *Giardia intestinalis*), trematodes (*Fasciola hepatica*, *Paramphistomum* spp., *Dicrocoelium dendriticum*), tapeworms (*Moniezia* spp.), and nematodes (lung-worms and digestive strongyles), with different prevalence value, according to the age category. These findings represent an important step for further epidemiological studies in order to develop sustainable control programs for Romanian water buffalo farms.*

Key words: internal parasite community, coproscopy, water buffaloes, Romania.

INTRODUCTION

Water buffaloes (*Bubalus bubalis*), large bovines (Artiodactyla: Bovidae), have a significant economic importance as providers of milk and meat in many countries (Borghese, 2005). They are ranked second in the world after the cow's milk, producing for over 12% of the world production of milk (CNIEL, 2002). Additionally, the importance of buffaloes must be understood by their added economic value related to the breeding and originating areas and to their capacity for expansion and adaptation to territories/regions to which cattle can not adapt and do not provide adequate productions (Borghese, 2011).

The global population of water buffaloes is about 172 million, of which 97% are found in Asia, 2.3% and 2.4% in Africa (Egypt) and South America, respectively, 0.3% in Europe and 0.02% in Australia. Of the approximately 500,000 water buffaloes from Europe, the

largest populations are in Bulgaria, Romania, and Italy (Borghese, 2011).

Buffalo milk is recognized and highly appreciated due to its nutrient content (Amarjit and Toshihiko, 2003). For instance, the Mediterranean buffalo breed produces high quality milk used for production of the famous „mozzarella” cheese (Romano et al., 2001).

In Romania, breeding of buffaloes is a traditional activity with a very long back history. The buffalo population was more than 200,000 animals in 1996, but afterwards its population decreased at about 100,000 in 2005 (Borghese and Mazzi, 2005).

Nevertheless, buffaloes are still used today on small private farms for draught and the goal of the selection process is to create a dual-purpose type of animal (milk and meat). Animals are housed and tied during the winter due to the unfavourable weather conditions and fed with hay, concentrates, silage, grazing on pasture in the warm season.

Currently, buffalo herds are spread in different counties especially from central and western Romania. Of these, an important breeding area is Țara Făgărașului (the Land of Fagaras), where, in direct competition with cattle, buffaloes have an increased efficiency in using low-quality vegetation. Buffaloes have been very well adapted to the ecological features of the local areas, contributing to the local economy. As production properties, buffaloes are mainly suitable for milk and meat productions. In addition to these characteristics of mixed production, this species is also used as a driving force in carrying out agricultural and transport activities (Borghese and Mazzi, 2005). There is well known that parasite infections represent major constraints for the economic profitability of breeding buffaloes, but also for the health and welfare of animals. Gastro-intestinal parasites are very common in animals using pasture and in farms using the extensive breeding management (Taylor, 2010). Therefore, for a better management of the both, economic part and animal health it is of high relevance the knowledge on the occurrence and prevalence of different gastro-intestinal parasites in a particular area (Mitrea, 2002). Limited approaches have been made in the last years on investigation parasitic infections in water buffaloes in Romania. Therefore, this study aimed to investigate the endo-parasite infections of water buffaloes from a Romanian farm.

MATERIALS AND METHODS

Study area

The study was conducted during of April 2018, in a buffaloes farm located in centre Romania, Brasov County (45°50'10.2"N 25°08'01.2"E), from the Land of Fagaras. This region is the main area for the distribution of buffaloes in Brasov County, where, in direct competition with cattle, they showed an increased efficiency in using of local vegetation.

The Land of Făgăraș, a historical region of Transylvania located in central Romania being surrounded by the Fagaras Mountains and Olt River's Valley, is a depression located along the middle course of the Olt River. It is characterized by two main types of relief: hilly relief, formed by the sub-mountain hills of

Persano-Făgăraș, with altitudes between 500 and 800 m, deeply fragmented by the groundwater action of the mountain rivers, and the Piedmont Plain of Făgăraș. With regards to the natural environment, this region belongs to the "slope lands" category (Cocean, 2011).

As climate, the territory is under the influence of the physical-geographical location (in the centre of the country), the presence and influence of the Southern Carpathians and the presence of the Olt River. Thus, the climate is cool (the annual average temperature is about 6-8°C), with thermal inversions in the cold season of the year, and rich rainfall (about 700-850 mm/year).

The local vegetation includes meadows and pastures that alternate with clusters of beech and oak forests. Gramineae have the highest spread in meadows, participating with up to 90% in the floristic structure of meadows, along with Leguminosae of which the most frequent are: *Trifolium pratensis*, *Trifolium repens*, *Lotus corniculatus*, *Onobrichis vicifolia* (<https://en.wikipedia.org/wiki/Fagaras>).

All these features characterize a favourable environment for raising animals in the Land of Făgăraș, including buffaloes.

Animals, samples, and investigations

A total number of 105 buffaloes, allocated in four age category groups, as follows: < 6 months (n = 21); from 6 months to 2 years (n = 20); heifers (n = 30); milking cows (> 3 years) (n = 34) was randomly selected for a copro-parasitological study.

Fresh faecal samples were collected from individual animals, and rapidly transported to the Laboratory of Parasitology, Faculty of Veterinary Medicine of Bucharest within 24 h, for parasitological analyses.

The faecal samples were processed and subjected for parasitological investigations by using standard qualitative coproscopical methods (Ionita and Mitrea, 2013), as follows:

- a flotation technique was used for demonstrating nematode and cestode helminth eggs and protozoan oocysts;
- a sedimentation technique for detecting trematode eggs.

The parasitic stages were microscopically identified based on their morphological characters.

Data analysis

The results were statistically analyzed by Quantitative Parasitology 3.0 free software (Rozsa et al., 2000). A p-values ≤ 0.05 was considered statistically significant. The 95% confidence intervals (CI) were computed.

RESULTS AND DISCUSSIONS

For assessing the gastro-intestinal parasite community in water buffaloes a parasitological study was conducted, in a buffalo farm in Centre Romania. For this, a total number of 105 animals randomly selected and allocated in four age categories (under 6 months; 6 months-2 years; between 2-3 years and >3 years) were subjected for coprological investigations. Subsequently, 55.2% (58/108; 95% CI: 45.22-64.96) of the tested animals were positive for at least one parasite infection, helminth and /or protozoan parasitic species. Overall, 11.4% (12/105) were single infection, while 43.8% (46/105) were mixed infections with two (25.7%;

27/105) and three (18.1%; 19/105) parasite species, respectively (Table 1).

The copro-parasitological investigations revealed a diverse gastro-intestinal parasite community, including protozoa (*Giardia duodenalis*, *Cryptosporidium parvum*, *Eimeria* spp.), trematode (*Fasciola hepatica*, *Paramphistomum cervi*, *Dicrocoelium dendriticum*), cestode (*Moniezia* spp.), and nematode (digestive strongyles) helminths.

The most prevalent parasite infection was represented by gastro-intestinal strongyles which were found in 41.9% of the tested animals, followed by the rumen fluke (*P. cervi*; 24.8%), the liver fluke *Fasciola hepatica* (21.0%), and *Eimeria* spp. (16.2%).

Other parasite infections were also detected, but in lower prevalence ranging from 1.9% to 4.8%, including the protozoa *G. duodenalis* and *C. parvum*, the lancet liver fluke *D. dendriticum*, and the tapeworm *Moniezia* spp. Details on the parasite species and prevalence by age category are presented in the Table 2 and Figure 1.

Table 1. Prevalence of endo-parasite community, as single and/or mixed infections, stratified by the age category, in a water-buffalo farm, Center Romania

Investigated animals/age group	Number (%) of positive animals for endoparasite infection			
	total positive	single infection	mixed infection (w2 spp.)	mixed infection (w3 spp.)
Total (n = 105)	58 (55.2)	12 (11.4)	27 (25.7)	19 (18.1)
<i>Age group</i>				
< 6 months (n = 21)	9 (42.9)	6 (28.6)	3 (14.3)	0
6 months-2 years (n = 20)	10 (50.0)	2 (10.0)	6 (30.0)	2 (10.0)
2-3 years (heifers) (n = 30)	22 (73.3)	2 (6.66)	14 (46.6)	6 (20.0)
> 3years (milking) (n = 34)	17 (50.0)	2 (5.8)	4 (11.8)	11 (32.3)

Table 2. Prevalence of gastro-intestinal parasite species in water buffaloes from a farm, Center Romania

Investigated animals/age group	Parasite species/genus: number and percentage (%) of positive animals							
	<i>Cryptosporidium</i>	<i>Giardia duodenalis</i>	<i>Eimeria</i>	<i>Fasciola hepatica</i>	<i>Paramphi-stomu</i>	<i>Dicrocoelium dendriticum</i>	<i>Moniezia</i>	<i>GI strongyles</i>
Total (n = 105)	4 (3.8)	5 (4.8)	17 (16.2)	22 (21.0)	26 (24.8)	4 (3.8)	2 (1.9)	44 (41.9)
<i>Age group</i>								
< 6 months (n = 21)	4 (19.0)	2 (9.5)	6 (28.6)	0	0	0	0	0
6 months-2 years (n = 20)	0	3 (15.0)	4 (20.0)	3 (15.0)	1 (5.0)	0	2 (10.0)	7 (35.0)
2-3 years (heifers) (n = 30)	0	0	5 (16.7)	8 (26.7)	14 (46.7)	1 (3.3)	0	20 (66.7)
> 3years (milking) (n = 34)	0	0	2 (5.9)	11 (32.4)	11 (32.4)	3 (8.8)	0	17 (50.0)

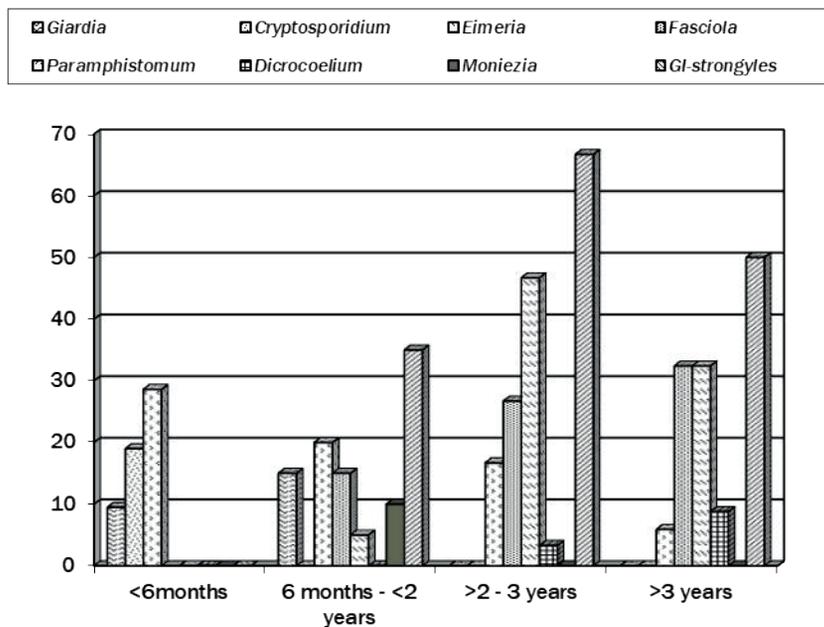


Figure 1. Prevalence of gastro-intestinal parasites, stratified by age category, in buffaloes from a farm in Center Romania

The coprological study undertaken to investigate the gastro-intestinal parasites in a buffalo farm in Center Romania showed a diverse parasite community, including protozoa and helminth species. Different prevalence values (statistically significant: $p < 0.0001$) according to the animal age category were registered. Thus, protozoa infections were more prevalent in animals aging under 6 months, while the helminth infections with gastro-intestinal strongyle and trematode helminths were more prevalent in heifers (animals aging between 2 and 3 years), followed by animals aging over 3 years (Figure 1). These findings confirm that grazing animals are exposed to parasite infection (Ionita et al., 2013; Buzatu et al., 2014). Animals under 6 months are more exposed to protozoa infections, while heifers and milking animals more likely to have helminth-infections.

The prevalence values relating to GI parasites in water buffaloes in this study are in line with similar studies in water buffaloes reared in grazing systems, such as in Poland, Italy, or India (Cringoli et al., 2009; Kobak and Pilarczyk, 2012; Marskole et al., 2016).

Therefore, strongyles are reported to be the most common parasites (up to 47.73%) in dairy water buffaloes (Swarnakar et al., 2015; Marskole et al., 2016). GI-strongyles are also reported as the most frequent helminths in water buffalo farms (33.1%) of central Italy,

but with lower individual level infection (5.4%), which could be due to the intensive buffalo breeding currently used (Rinaldi et al., 2009). Similarly, GI-strongyle infections were reported in water buffaloes from Poland, in a recent study, with the highest prevalence, of 22.22%, in May (Kobak and Pilarczyk, 2012). The liver (*F. hepatica*) and the rumen (*Paramphistomum* spp.) flukes were the second most frequent parasite infection in water buffaloes in our study, with very close prevalence values (21.0% and 24.8%, respectively), but higher than those reported by recent studies in European studies (7.1% and 1.3% and 2.1% at farm and individual, respectively level) (Rinaldi et al., 2009). These findings are consistent by the similar life cycle of these two trematode helminths requiring amphibious snails (*Galba truncatula*) as intermediate host, and therefore environmental factors from the study area are strong determinants (Mitrea, 2002).

However, flukes, *F. hepatica* and *P. cervi* were reported as the most prevalent GI-parasites in water buffaloes from a region in Poland (the Notecka forest region), with a mean prevalence of 32.0% and 11.0%, respectively, emphasizing that local climatic conditions favor mass infections of animals on pastures (Kobak and Pilaczyk, 2012).

On contrast, occurrence of the lancet liver fluke in the water buffaloes tested was reported with

a lower prevalence (3.8%), which could be also probably due to its life cycle involving earth snails (*Zebrina*, *Helicella*) and ants as intermediate hosts - thus, requiring different ecological conditions than those for the liver and rumen fluke (Mitrea, 2011). Similarly, it could be explained the lower occurrence (1.9%) of the tapeworm *Moniezia* spp., in line with those reported by other studies in Italian water buffaloes (2.4%) (Rinaldi et al., 2009); this is correlated with its dioxenous life cycle, requiring pasture oribatid mites to complete it.

Eimeria infection was more prevalent in young animals, aging up to 2 years (prevalence values over 20.0%, up to 28.2%), but *Eimeria* oocysts have been identified in animals from all age categories (prevalence values ranging from 5.9% up to 28.6%), suggesting the carrier-role of adults animals and their role for environmental contamination with oocysts infective for calves. Therefore, young animals should be kept off heavily contaminated pastures when they are most susceptible (Taylor, 2010; Mitrea, 2011).

Additionally, among the parasite community, there were also identified parasite species of zoonotic concern such as *G. duodenalis* and *C. parvum*, suggesting that water buffaloes can be involved in the spread and environmental contamination with cysts and oocysts potentially infectious to humans. Recent studies in Italian water buffaloes report infection with *G. duodenalis* (prevalence values ranging from 14.7% to 26.3%) and *C. parvum* (prevalence values up to 14.7%) (Rinaldi et al., 2007; Caccio et al., 2007). Additionally, by molecular analyses of the *Giardia* β -*giardin* and the *Cryptosporidium* small subunit ribosomal DNA gene, respectively, showed the presence of both zoonotic parasites, namely *G. duodenalis* assemblage A and *C. parvum*, respectively (Caccio et al., 2007).

Therefore, a proper disposal of animal feces and adequate management measures to clean the pasture are recommended, in order to avoid these risks (Giangaspero et al., 2007; El-Khodery and Osman, 2008).

It is known that the agroclimatic conditions, animal husbandry practice, and pasture management largely determine the occurrence, prevalence, and severity of various parasitic diseases in a particular geographical area.

Recent epidemiological studies in Romania have been reported on the occurrence and eco-epidemiology of the internal parasite community and horses, cattle and sheep, in different geographical areas (Mitrea et al., 2008; Ionita et al., 2013; Buzatu et al., 2014, 2016; Andrei et al., 2020). Therefore, update knowledge on the parasite community in water buffalo farms are of significant relevance for both animal health and welfare and productivity, will contribute to develop effective parasitological control programs.

CONCLUSIONS

The findings of the present study showed a diverse gastrointestinal parasite community in water buffaloes from a farm in Center Romania and emphasize on the epidemiologic pattern modulated by the ecological conditions that are favourable for evolving the life cycle of a large number of parasites, which may impact the animal health and their productions. Therefore, adequate parasitological control measures are suggested.

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