

## SURVEY ON INTESTINAL PARASITES INFECTIONS IN WATER BUFFALO CALVES, IN CENTER ROMANIA

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### Abstract

A cross-sectional survey was carried-out to investigate the occurrence and associated risk factors for digestive parasite infections in water buffaloes (*Bubalus bubalis*) calves, in Center Romania. For this, individual fecal samples were collected, from a total of 63 calves and tested for protozoan and helminth infection by using standard qualitative (flotation) and/or quantitative (Mini-Flotac) copro-microscopic techniques. Overall, 77.8% [95% CI: 63.58-87.03] of the tested animals were positive for at least one parasite infections, either as single (53.9%; 95% CI: 38.03-65.35) or mixed (23.8.0%; 14.39-38.38) infections. Of the positive animals, 13.4% showed diarrhoea and all were with mixed infections. Among the parasites detected, the most frequent was *Eimeria* spp. (68.2%), followed by *Toxocara vitulorum* (15.9%), *Giardia duodenalis* (14.3%), and *Strongyloides papillosus* (4.2%). These findings showed the presence of parasites that may seriously impact the animal health, some of them with zoonotic potential. Altogether the results of this study emphasize the requirement for specific measures for control parasite infections in water buffalo farms.

**Key words:** water buffaloes; calves; intestinal parasites; Center Romania.

### INTRODUCTION

Due to their economic importance in the world, representing over 10% of the total of large bovines and the second providers of meat and milk, following the cows, water buffaloes (*Bubabuls bubalis*) receive an increasing interest over the last decades in many countries, (Borghese, 2011). Meat quality, low fat and low cholesterol, but also high quality milk suitable for production of mozzarella, keep this species among the preferences of breeders.

Additionally, water buffalo poses characteristics of high adaptation capacity to various conditions, including on marshy meadows and a better feed efficiency compared to cattle (Hamid et al., 2016; Han et al., 2012). Most populations of water buffaloes are found in Asia (96%) (India, Pakistan, China) but they are also breed in other regions of the world as Africa, South America, and Europe (Abeygunawardena & Abeyratne, 2001; Wanapat & Chanthaknoun, 2015). In Europe, larger populations of buffaloes are in Italy (where it is the livestock that registered the highest increase in number), Romania, and Bulgaria, and in reduce number in other countries, such as Greece, Germany, U.K., Macedonia (Borghese,

2011). In Romania, buffalo breeding has a long history as traditional activity; the local breed being approved more than 30 years ago as a Romanian breed, genetic divers from others European buffaloes. Its current population of under 100000 animals residing in buffaloes farms, but also in small number reared in households, mainly in Center and north-western Romania (Borghese & Mazzi, 2005). Parasite infections of water buffaloes are commonly reported with significant economic causing significant losses as a consequence of the reduced rates of weight gains, production losses, condemnation of infected organs after slaughter, and deaths of infected animals (Mitrea, 2011; Thapa Shrestha et al., 2020). Moreover, among the variety of parasite species infecting buffaloes, helminthes and protozoa, some of them have also potential zoonotic, such as the *Giardia* spp., and *Cryptosporidium* spp. (Giangaspero & Berrilli, 2007). Surveys on the parasite infections in animals are of great relevance for the local breeders, allowing developing appropriate, evidence-based parasite control program (Mitrea, 2002; Ionita et al., 2013; Andrei et al., 2020).

Buffalo breeding is an important economic resource for the livestock in the Land of Fagaras (Tara Fagarasului), Center Romania. However, very few reports are available on the parasitic infections in Romanian buffaloes. Therefore, the aim of the present study was to investigate the occurrence and associated risk factors for digestive parasite infections in calves of water buffaloes, residing in a farm, in Center Romania.

## MATERIALS AND METHODS

### Study area

The study was conducted in a water buffaloes farm located in Brasov County, from the Land of Fagaras, in Center Romania (Figure 1). The Land of Fagaras is located in Hartibaciu Plateau, bordered by Carpati Mountains and Olt River's Valley, an excellent habitat for water buffaloes, providing moderate temperatures and rivers. In this favourable environment, buffaloes are using local vegetation from this hilly relief, consisting in pastures and meadows alternating with clusters of beech and oak forests.



Figure 1. View from the study buffalo farm, Central Romania

### Animals and investigations

A number of sixty-three buffalo calves, were randomly selected for a copro-parasitological study. The calves were grouped in three age categories, as follows: 3-6 weeks ( $n = 19$ ); 7-12 weeks ( $n = 24$ ) and 13-20 weeks ( $n = 20$ ).

From individual calve, faecal samples were collected directly from the rectum; fecal samples were transported within 24 h to the Laboratory of Parasitology, Faculty of Veterinary Medicine of Bucharest for further parasitological analyses.

The faecal samples were processed for parasitological investigations; all samples were analysed for parasitic stages (helminth eggs, protozoan oocysts) by using standard coproscopical methods, as follows: a flotation technique (NaCl flotation method for detecting protozoa

oocysts and helminth eggs; the  $ZnSO_4$  flotation (1.2 sp. gravity) was used for confirming *Giardia* cysts (Ionita & Mitrea, 2013); and a quantitative method (Mini-Flotac technique) (Cringoli et al., 2017), used to count the *Eimeria* oocysts and helminth eggs (sensitvitit: 5 eggs or oocysts/gram of feces).

### Data analysis

The results were analysed using descriptive statistics (Quantitative Parasitology 3.0 free software; Rozsa et al., 2000). For mean prevalence values, the 95% confidence intervals (95% CI) were computed; for comparison of different variables,  $p < 0.05$  was considered significant statistically.

## RESULTS AND DISCUSSIONS

In order to assess parasites infection in water buffaloes calves, a coprological study was carried out in calves residing in the Research and Breeding Buffaloes Farm, Sercaia, Brasov County, the only of such type in Romania.

Subsequently, 77.8% (95% CI: 63.58-87.03) of the analysed calves tested positive for at least one parasitic infections, either as single infection, in 53.9% (95 % CI: 38.03-65.35) of animals, or mixed infections, in 23.8% (95% CI: 14.39-38.38).

Of the tested faecal samples, 49 (77.8%) were normal, while 14 (22.2%) were diarrheic, of which 42.8% were positive for mixed parasite infections (Table 1).

Table 1. Epidemiological data on intestinal parasite infections in water buffalo calves, in Center Romania

| Tested animal/<br>Group     | Nr /<br>% | Positive animals for<br>parasite infection |                 |                 |
|-----------------------------|-----------|--|-----------------|-----------------|
|                             |           | Total                                      | single          | mixed           |
| Total ( $n = 63$ )          | nr        | 49   | 34              | 15              |
|                             | %         | 77.8                                       | 53.9            | 23.8            |
|                             | 95%<br>CI | 63.58-<br>87.03                            | 38.03-<br>65.35 | 14.39-<br>38.38 |
| <i>Age group</i>            |           |  |                 |                 |
| 3-6 weeks<br>( $n = 19$ )   | nr        | 12   | 8               | 4               |
|                             | %         | 63.2                                       | 42.1            | 21.05           |
| 7-12 weeks<br>( $n = 24$ )  | nr        | 19   | 13              | 6               |
|                             | %         | 79.2                                       | 54.2            | 25.0            |
| 13-20 weeks<br>( $n = 20$ ) | nr        | 18   | 12              | 6               |
|                             | %         | 90.0                                       | 60.0            | 30.0            |
| Feces consistency           |           |  |                 |                 |
| Normal<br>( $n = 49$ )      | nr        | 43   | 34              | 9               |
|                             | %         | 87.7                                       | 69.4            | 18.4            |
| Diarrheic<br>( $n = 14$ )   | nr        | 6  | -               | 6               |
|                             | %         | 42.8                                       | -               | 42.8            |

The frequency and prevalence of type of parasitic infections shown in Table 1 and Figures 2-4.

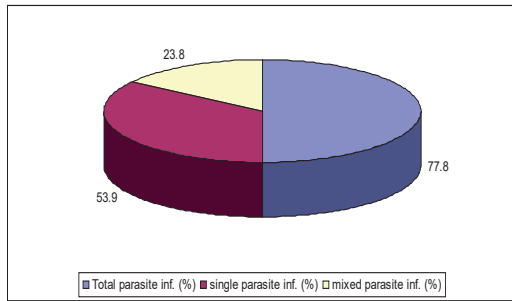


Figure 2. Prevalence of intestinal parasite infection (total, single and mixed infections) in water buffaloes calves in Center Romania

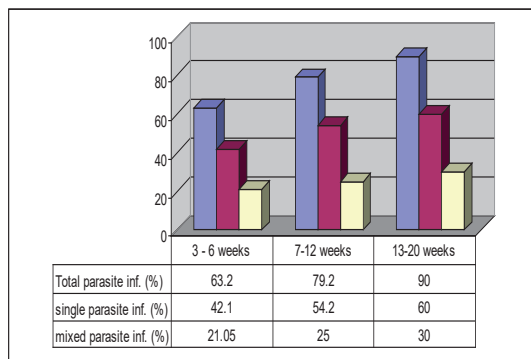


Figure 3. Prevalence of intestinal parasite infection, according to the age group, in water buffaloes calves in Center Romania

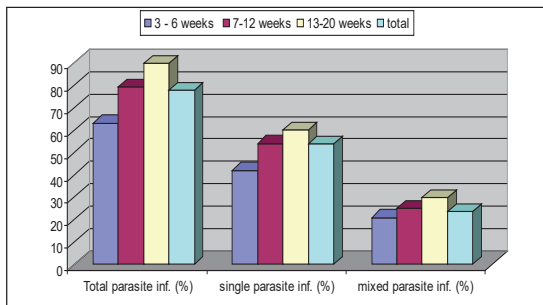


Figure 4. Distribution and type of intestinal parasite infection (single, mixed) according to the age group, in water buffaloes calves in Center Romania

The copro-parasitological examination revealed a diverse intestinal parasitofauna of buffalo calves, protozoa and nematode helminthes, as follows: *Eimeria* spp. (68.2%), *Giardia duodenalis* (14.3%), *Toxocara vitulorum* (15.9%), and *Strongyloides papillosus* (6.3%) (Table 2, Figure 5). For the *Eimeria*-positive samples, the mean opg was 2569.2 (SD:3071.92), while for the *Toxocara*-positive samples the mean EPG was 3507 (SD:2545.48).

Table 2. Composition and prevalence (%) of intestinal parasite community found in water buffalo calves, in Center Romania

| Variable             | Nr / % | Parasite species/genus (number; percentage) |                           |                           |                           |
|----------------------|--------|---|---------------------------|---------------------------|---------------------------|
|                      |        | <i>Eimeria</i> spp.                         | <i>Giardia duodenalis</i> | <i>Toxocara vitulorum</i> | <i>Strongyloides</i> spp. |
| Total (n = 63)       | nr     | 43  | 9                         | 10                        | 4                         |
|                      | %      | 68.2  | 14.3                      | 15.9                      | 6.3                       |
| <b>Age group</b>     |        |   |                           |                           |                           |
| 3-6 weeks (n = 19)   | nr     | 9   | 0                         | 7                         | 0                         |
|                      | %      | 47.4  | 0.0                       | 36.8                      | 0                         |
| 7-12 weeks (n = 24)  | nr     | 18  | 4                         | 3                         | 1                         |
|                      | %      | 75.0  | 16.7                      | 12.5                      | 4.2                       |
| 13-20 weeks (n = 20) | nr     | 16  | 5                         | 0                         | 3                         |
|                      | %      | 80.0  | 25.0                      | 0                         | 15.0                      |
| <b>Feces</b>         |        |   |                           |                           |                           |
| Normal               | nr     | 37  | 8                         | 6                         | 3                         |
|                      | %      | 86.0  | 88.9                      | 60.0                      | 75.0                      |
| Diarrheic            | nr     | 6   | 1                         | 4                         | 1                         |
|                      | %      | 14.0  | 11.1                      | 40.0                      | 25.0                      |

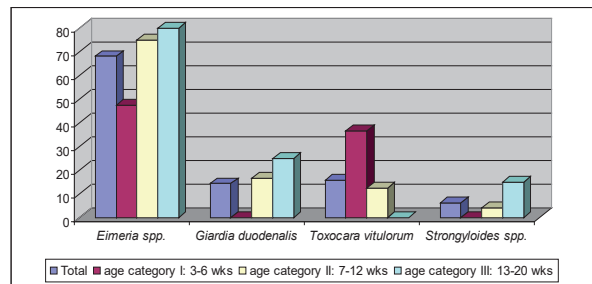


Figure 5. Prevalence of intestinal parasites, stratified by age group, in water buffaloes calves in Center Romania

In a recent study carried out in 38 buffalo calves aging 2-11 weeks, reared in households in north-western Romania (Salaj county), 87% were infected with at least one intestinal parasite, 42% as single, 44% as mixed infections (Barburas et al., 2022). Among the parasitic infections detected, the most prevalent parasites were *Eimeria* spp. (84%) and *T. vitulorum* (37%), followed by *S. papillosus* (16%), *Cryptosporidium* spp. (11%), and *G. duodenalis* (3%) (Barburas et al., 2022).

In our study, the most frequent parasitic infection detected in the examined calves was also with *Eimeria* spp., detected in all three age categories, with prevalence values increasing according to the age category from 47.5% in groups I, to 80.0% in group III ( $p < 0.05$ ). In six positive animals, feces were diarrheic, and all six were mixed infections. Eimeriosis is frequently reported in buffalo calves causing diarrhea and other general conditions, especially in sanitary and crowded conditions (Tavassoli et al., 2018).

Over eleven *Eimeria* species, originally described from cattle, have been reported in

buffalo. Additionally, *Eimeria bareillyi*, recognized as species buffalo-specific, is not transmissible to cattle. It is highly pathogenic to young calves and has been reported infecting buffalo worldwide including Brazil, Italy, Netherland, Turkey, Romania (Bastianetto et al., 2008; Ramirez et al., 2009; Dubey, 2018; Barburas et al., 2022). In our study, among the *Eimeria* oocysts, the following species were identified: *E. auburnensis*, *E. bovis*, *E. elipsoidalis*, *E. piriformis*, and *E. zuernii*; most of infections (86.0%) were subclinical and detected oocysts were in all age categories.

*Eimeria* infection was reported previously also with higher frequency in young animals up to 2 years, but also in adult animals (in 5.9-28.6%), emphasizing that adults are carriers and source of environmental contamination with oocysts that become infective for calves (Paltin et al., 2020).

The second more frequent parasite identified was *T. vitulorum*, with higher prevalence, of 36.8%, in calves of 3-6 wks, and no detected in calves of the 13-20 wks age category.

*T. vitulorum* is an ascarid nematode inhabiting the small intestine of bovines, including buffaloes, in tropical, sub-tropical, and temperate geographical areas, responsible of numerous economics losses. Calves become infected post-partum, early, by ingesting larvae which are excreted in the colostrum and milk of cows two - five days postcalving. Thereafter, within 3-4 weeks, the ingested larvae mature as adult nematode that produce large numbers of eggs eliminated in faeces at about the third weeks of age. Then, adult worms are expelled by the calves by the fifth month of age (Roberts, 1990; Roberts et al., 1990).

Despite that usually infections are subclinically, heavy infections results in severe disease, severe diarrhea, enteritis and high morbidity and mortality, especially in the age group of 1-3 months old buffaloes calves (Rast et al., 2013). Therefore, without an etiological diagnosis of diarrhea-causative agent - and adequate treatment management, high fatality rates cause serious economic losses (Roberts, 1990).

In our study, 60.0% of the *Toxocara*-positive calves aging 7-12 weeks had diarrheic feces, and were mixed infections with other parasites, while 40.0% of *Toxocara*-positive calves of 13-20 weeks age showed normal feces. These

findings are consistent with literature data reporting both subclinical and clinical infections in cattle and buffalo calves. For instance, Jones et al. (2009) found that most infected calves were appeared healthy, while the clinical signs in some of the infected calves were mild and may have been at least partially due to concurrent coccidiosis (Jones et al., 2009).

Barburas et al. (2022) reported infection with *T. vitulorum* in a similar prevalence range (37.0%) in buffalo calves reared in households in North-Western Romania.

Knowing that *T. vitulorum* undergoes a period of arrested development in the somatic tissues of adult cow, being transmitted to suckling calves galactogenic (by intramammary route), once the parasite is established in a herd, for its control it is required implementation of specific measures. As most *T. vitulorum* infected calves appear healthy, therefore is the potential to remain undetected in cattle or buffalo herds (Jones et al., 2009).

*G. duodenalis* cysts were detected in two of the three age categories with prevalence values ranging from 16.7% (7-12 wks of age) to 25% (13-20 wks of age), these findings emphasizing the potential risks for the public health due to its zoonotic potential (Giangaspero & Berrilli, 2007).

*Giardia* infection has also been reported in asymptomatic buffalo calves, aged from 1 to 9 weeks by using copro-antigen tests of *G. duodenalis* in 30% of farms and in 14.7% of animals, respectively. Co-infection was present but animals rarely presented diarrhea (Rinaldi et al., 2006). In our study, one animal with concurrent eimeriosis showed diarrhea.

Therefore, *G. duodenalis* could represent a problem to the water buffalo industry due to their economic cost and the risk of human exposure associated with cysts eliminated in the environment by infected hosts.

Additionally, in four samples collected from calves of age 7-12 weeks (n = 1) and 13-20 wks (n = 3) eggs of *Strongyloides* were detected, of 6.3%, and the frequency increased by age, from 4.2% to 15.0%; these findings are in line with a recent study in Romania, reporting an overall prevalence of 16.0% (Barburas et al., 2022).

Lower values, of 3.1% at the farm level (4/127 farms), were reported in water buffaloes

(adults, heifer/steers and calves) from Italy (Rinaldi et al., 2009).

The occurrence of *Strongyloides* in calves is also high clinical relevance. Although it infects animals of all age categories, clinical signs, including diarrhoea and malnutrition are frequently in young animals. Moreover, sudden death were reported in calves with high infection (EPG of 52000 - 411000) (Taira & Ura, 1991). Therefore, it is recommended that, taking into account the pathogenicity of *Strongyloides*, calves infected with this parasite shedding high egg number (epg/10000), should be treated (Thamsborg et al., 2017).

## CONCLUSIONS

The results of the present study showed the presence of parasites that may seriously impact the animal health, but also some of them with zoonotic potential. Additionally, the findings emphasize the requirement for regular monitoring of parasite community and specific measures to be taken for controlling parasite infections in water buffalo farms.

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