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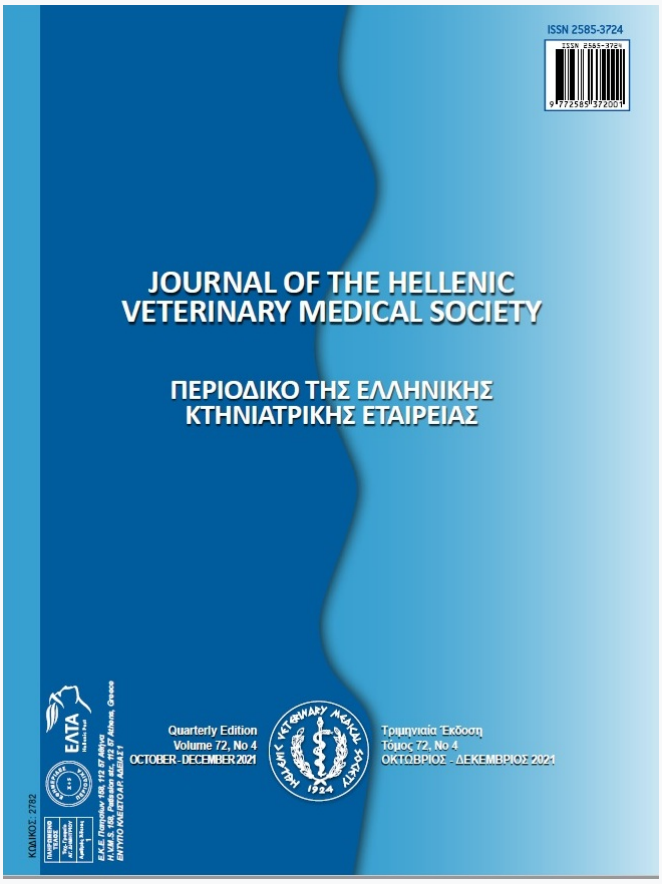


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*DA BĂRBURAȘ, A GYORKE, LM POP, R BĂRBURAȘ, V MIRCEAN, V COZMA*

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Research article
Ερευνητικό άρθρο

# Epidemiology of digestive parasites in buffaloes from Romania

**D.A. Bărburaș[a], A. Györke[a], L.M. Pop[a,*], R. Bărburaș[b], V. Mircean[a], V. Cozma[a]**

[a]*Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Cluj, Romania*

[b]*S.C. MedivetBărburaș S.R.L, Românași, Salaj, Romania*

**ABSTRACT:** Digestive parasites are a constraint on buffalo productivity. Data regarding the prevalence of parasitic diseases in buffaloes are reported in developed countries, but in Romania, they are missing. The aim of this study was to evaluate the prevalence of internal parasites in buffaloes raised in households and farms from Romania. Faeces samples were collected from 180 buffaloes of different ages (calves: 0-6 months; juveniles: 6-30 months; adults:> 30 months). The faecal samples were processed by coproparasitological methods: flotation, sedimentation, and McMaster. Digestive parasites were detected in 57.2% of buffaloes. Most buffaloes had single species infection(42.2%) ($p$<0.001), and only 15.0% had infection with 2 (14.5%) or 3 (0.6%) parasites. The prevalence of digestive parasites was higher in calves (68.8%) and juveniles (76.9%) compared with adults (19.6%) ($p$<0.001). *Eimeria* spp. (43.3%), *Buxtonella sulcata*(1.7%), *Fasciola hepatica*(4.4%), *Paramphistomum cervi*(2.8%), *Moniezia* spp. (0.6%), *Toxocara vitulorum* (11.7%), digestive strongyles (3.3%), *Strongyloides papillosus* (5.0%), and *Capillaria* spp. (0.6%) were identified. *Eimeria* spp. and *T. vitulorum*werethe most prevalent parasitesin calves and juveniles($p$<0.001). *F. hepatica* was the most prevalent parasite in adults.*Eimeria* spp. and *S. papillosus* were the only infections diagnosed in farmed buffaloes, with a higher prevalence (p>0.05) than in household buffaloes. *B. sulcata, Moniezia* spp., *T. vitulorum* and *S. Papillosus* were diagnosed only in young buffaloes, while *Capillaria* spp. only in adults. The OPG of*Eimeria* spp. was higher in young buffaloes compared with adults ($p$<0.001). Age was the risk factor identified for infections with *Eimeria* spp., *T. vitulorum* and *F. hepatica.*

***Keywords:*** digestive parasites, buffaloes, coproparasitological examination, OPG.

*Corresponding Author:*
Loredena-Maria Pop, Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania
E-mail address: loredana-maria.pop@usamvcluj.ro

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## INTRODUCTION

The buffalo farming is continuously increasing worldwide, including in Western-European countries (Bodnar, 2017). Buffaloes are the second biggest milk-producing species in the world (Guo, 2010). Buffalo milk has a higher fat and protein content than cow's milk and it is used in the dairy industry to produce mozzarella, cream, yogurt or butter (Coroian, 2012). Romanian buffaloes are raised traditionally for milk and meat production, and to a lesser extent for draught. Romania has the second highest proportion of milk recorded buffaloes from the Mediterranean area, after Italy (Borghese, 2010). Romanian buffalo population was ranked 2nd in Europe in 2004 with 100,000 heads, but gradually decreased up to 25,000 heads in 2013 (Vidu et al.,2008; Borghese, 2013). Around 97% of buffaloesare raised in centre and north-western regions of Romania (Coroian, 2009).

Internal parasites are a constraint on productivity (Bodnar, 2017). Parasitic diseases are responsible of decreased weight gains and milk production, capricious appetite, anaemia, mortality, leading to significant economic losses (Fikruet al., 2006). Maintenance and climatic conditions are extrinsic factors that determine the presence of internal parasites (Sargison, 2016), but the intraspecific susceptibility is based on factors such as genetics, physiological, nutritional status, age and feeding mode (Bhutto et al., 2002).

Extensive data regarding parasite epidemiology is required in order to control parasitic diseases.Regular monitoring of parasites in animalsis necessary to detect the early stages of infections (Gunathilaka et al., 2018).

Data on the epidemiology of internal parasites in buffaloes are reported in developed countries, howeverthe information is scarcein developing countries (Mamun et al., 2011). The prevalence of parasites in buffaloes from Romania has not been studied so far.

Taking into consideration the importance of buffalo rearing in Transylvania region, the purpose of this study was to evaluate the prevalence of internal parasites in buffaloes raised in households and farmsfrom the North-West of Romania.

## MATERIALS AND METHODS

### Study area

Romania is a Southeastern European country thathas a temperate continental climate, with four distinct seasons (spring, summer, autumn, and winter). The Carpathian Mountains split the territory into two groups: intra-Carpathian regions and extra-Carpathian regions. The Transylvania Depression and the western part of Romania is included in the first group and it is dominated by western moist air masses. Precipitation decreases on average from West to East and increases with altitude(A.N.M., 2008). However, in the last years, there is an increasing trend inextreme precipitation indices in north-western regions of Romania (Croitoru et al., 2016).

### Animals

One-hundred-eighty buffaloes were sampled in this study. Their age varied from 2 weeks to 24.0 years with an average of 45.1 (±5.3) months (3.8 years). The buffaloes were divided into three age groupsas follows: calves (*n*=101) from 0 to 6 months old; juveniles (*n*=28) older than 6 months to 30 months (2.5 years) old; and adults (*n*=58) older than 2.5 years (> 30 months) (Table 1). The buffaloes came from five counties: Cluj, Sălaj, Bihor, Bistriţa-Năsăud and Maramureş (Fig. 1). Most of the animals were raised in household system (160/180), while the rest of the animals originated from a farm in Cluj County.

The buffaloes from households were grazingon pastures nearby forests. These pastures were used by several species of animals, and all age groups. In the cold season, the animals were kept in small shelters. Milking was done manually; calves and young buffaloes were living in the same shelter as adults.

**Table 1.** Distribution of the study samples by age, gender, and origin

| | Number | Age [mean (±sem)] | | Gender | | Origin | |
|---|---|---|---|---|---|---|---|
| | | Months | Years | Females | Males | Household | Farm |
| **Calves** | 77 | 4.0 (0.2) | - | 44 | 33 | 67 | 10 |
| **Juveniles** | 52 | 11.7 (1.0) | - | 32 | 20 | 42 | 10 |
| **Adults** | 51 | 141.4 (9.8) | 11.8 (0.8) | 46 | 5 | 51 | 0 |
| **Total** | 180 | 45.1 (±5.3) | 3.8 (0.5) | 122 | 58 | 160 | 20 |


**Figure 1.**

### Samples and sample analysis

Single fresh faeces samples (*n*=180) were randomly collected from individual buffaloes of different ages.

The faecal samples were collected in sterile plastic containers, each labelled with reference number,transported,and stored at 4 °C in the Laboratory of Parasitology and Parasitic Diseases of The University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca until further processing.

The faecal samples were processed by coproparasitological methods:salt flotation technique(specific gravity 1.28) and sedimentation, followed by examination using optical microscopy. The parasitic elements were identifiedbased on morphological aspects (Zajac and Conboy, 2012).Ninety-nine out of 180 faeces samples were also examined by the McMaster method to determine the number of oocysts or eggs/gram of faeces (Zajac and Conboy, 2012).

### Statistical analysis

Cross-sectional measurements were performed at the individual level.The diagnostic methods used to identify the positive cases wereapplied once.The studied parameters were not time related.

Frequency, prevalence, and its confidence interval (95% CI) were calculated online in Epitools (Sergeant, 2018) for each identified parasitic infection, overall and for single or mixed infection. All these parameters were calculated overallas well as for each age group (calves, juveniles, and adults), gender (females vs.males) andorigin (household vs. farm) (Table 1). The differences recorded between groups were analysed through the chi-square test in MedCalc software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019).

Risk factors were evaluated for those parasites with significant results in univariate analysis in MedCalc. Logistic regression model was used forage category, gender, and origin as independent variables. An independent variable was removed if *p*value was >0.1 and the model was considered if the overall model fit was significant.

The arithmetic mean and standard error of the mean were calculated for OPG and EPG. The normal distribution of data was checked and then, the differences in OPG and EPG values among different groups were evaluated using the non-parametric test Kruskal-Wallis (MedCalc software version 19.1.3).

A value of $p<0.05$ was considered statistically significant.

## RESULTS

### The prevalence of digestive parasites in buffaloes from North-West of Romania

Following coproparasitological examination, parasitic infections were recorded in 57.2% of buffaloes. The following parasitic elements were identified: *Eimeria* spp. oocysts (43.3%), *Buxtonella sulcata* cysts (1.7%), *Fasciola hepatica* eggs (4.4%), *Paramphistomum cervi* eggs (2.8%), *Moniezia* spp. eggs (0.6%), *Toxocara vitulorum* eggs (11.7%), strongyle-type eggs (3.3%), *Strongyloides papillosus* eggs (5.0%) and *Capillaria* spp. eggs (0.6%). The infections with *Eimeria* spp. ($p = 0.0001$) and *T. vitulorum* ($p = 0.00006$) were significantly more prevalent (Table 2) (Fig. 2).

**Table 2.** Frequency, prevalence and its 95% confidence interval for identified parasites in buffaloes (*n*=180) from North-West of Romania

| Infections | Frequency | Prevalence (%) | 95% CI | Age Mean in months (95% CI) |
|---|---|---|---|---|
| ***Eimeria* spp.** | 78 | 43.3*** | 36.3-50.6 | 11.7 (3.9-19.6) |
| ***B. sulcata*** | 3 | 1.7 | 0.6-4.8 | 5.3 (1.5-9.1) |
| ***F. hepatica*** | 8 | 4.4 | 2.3-8.5 | 93.0 (18.8-167.2) |
| ***P. cervi*** | 5 | 2.8 | 1.2-6.3 | 52.8 (22.4-83.2) |
| ***Moniezia* spp.** | 1 | 0.6 | 0.1-3.1 | 2 |
| ***T. vitulorum*** | 21 | 11.7 | 7.8-17.2 | 5.7 (4.3-7.1) |
| **Strongyles** | 6 | 3.3 | 1.5-7.1 | 15.0 (-8.2-38.2) |
| ***S. papillosus*** | 9 | 5.0 | 2.7-9.2 | 6.8 (4.1-9.4) |
| ***Capillaria* spp.** | 1 | 0.6 | 0.1-3.1 | 60 |
| **Total** | 103 | 57.2 | 49.9-64.2 | - |

Chi-square *$p$< 0.05, **$p$< 0.01 and ***$p$< 0.001

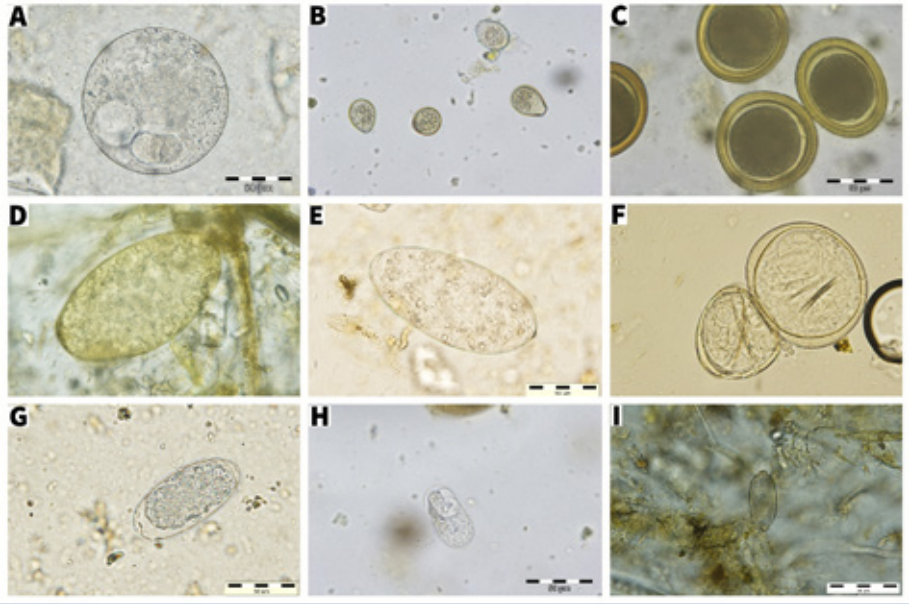


**Figure 2.**

The calves had a much higher prevalence of parasitic infections, than juvenile and adult buffaloes.Moreover, juveniles had a higher prevalence than adults.The infection with *Eimeria* spp. was the most prevalent in juveniles (59.6%) and in calves (57.2%) ($p$ = 0.0001). In adults, the infection with *F. hepatica* had the highest prevalence (9.8 %) ($p$ = 0.004) (Table 3).

The infection with *Eimeria* spp. was significantly more prevalent in males (56.9%) ($p$ = 0.02) than in females (36.9%) (Table 3).

In both farm and household system, the infection with *Eimeria* spp. had the greatest prevalence, but without statistical significance ($p$>0.05). Only the infectionswith *Eimeria* spp. and *S.papillosus* were detected in samples from buffaloes that were raised on farm (Table 3).

Single speciesinfectionswith *Eimeria* spp., *F. hepatica*, *P. cervi*, *Moniezia* spp., *T. vitulorum*, strongyles and *S. papillosus* were identified. The infection with *Eimeria* spp. was more prevalent than the other single infections ($p$ = 0.0001) (Table 4).

The most frequent parasitic association was that of *Eimeria* spp. and *T. vitulorum* (7.2%; 95% CI 3.9-12.03). Mixed infection with *Eimeria* spp. and *S. Papillosus* was observed in 6 (3.3%) buffaloes. Mixed infection with *Eimeria* spp. and *F. hepatica* was detected in 2 (1.1%) buffaloes. Other parasitic associations identified were *Eimeria* spp. with digestive strongyles, *Eimeria* spp. with *Buxtonella sulcata*, *Eimeria* spp. with *Capillaria* spp. and *Eimeria* spp., *B. Sulcata* and *T. vitulorum*, each in a single buffalo (0.6%).

**Table 3.** Frequency and prevalence [*n*(%)] of parasitic infections in buffaloes from North-West of Romania by age, gender and origin.

| Infections | Age category | | | Gender | | Origin | |
|---|---|---|---|---|---|---|---|
| | Calves *N*=77 | Juveniles *N* = 52 | Adults *N* = 51 | Females *N* =122 | Males *N* = 58 | Household *N* =160 | Farm *N* =20 |
| ***Eimeria* spp.** | 44 (57.2)*** | 31 (59.6)*** | 3 (5.9) | 45 (36.9) | 33 (56.9)* | 66 (41.3) | 12 (60.0) |
| ***B. sulcata*** | 2 (2.6) | 1 (1.9) | 0 | 2 (1.6) | 1 (1.7) | 3 (1.9) | 0 |
| ***F. hepatica*** | 0 | 3(5.8) | 5 (9.8)** | 6 (4.9) | 2 (3.4) | 8 (5.0) | 0 |
| ***P. cervi*** | 0 | 2 (3.9) | 3 (5.9)* | 4 (3.3) | 1 (1.7) | 5 (3.1) | 0 |
| ***Moniezia* spp.** | 1 (1.3) | 0 | 0 | 1 (0.8) | 0 | 1 (0.6) | 0 |
| ***T. vitulorum*** | 14 (18.2)** | 7 (13.5)** | 0 | 13(10.7) | 8 (13.8) | 21 (13.1) | 0 |
| **Strongyles** | 3 (3.9) | 2 (3.9) | 1 (2.0) | 5 (4.1) | 1 (1.7) | 6 (3.8) | 0 |
| ***S. papillosus*** | 4 (5.2) | 5 (9.6) | 0 | 7(5.7) | 2 (3.4) | 7 (4.4) | 2 (10.0) |
| ***Capillaria* spp.** | 0 | 0 | 1 (1.96) | 0 | 1 (2.0) | 1 (0.6) | 0 |
| **Total** | 53 (68.8)*** | 40 (76.9)*** | 10 (19.6) | 65 (53.3) | 38 (65.5) | 91 (56.9) | 12 (60.0) |

Chi-square *$p$< 0.05,**$p$< 0.01 and ***$p$< 0.001

**Table 4.** Frequency and prevalence of single and mixed parasitic infections in buffaloes from North-West of Romania.

| | Frequency | Prevalence (%) | 95% CI |
|---|---|---|---|
| **Single infections** | **76** | **42.2** | **35.2-49.5** |
| *Eimeria spp.* | 53 | 29.44 | 22.9-36.68 |
| *F. hepatica* | 5 | 2.78 | 0.91-6.36 |
| *P. cervi* | 4 | 2.22 | 0.61-5.59 |
| *Moniezia spp.* | 1 | 0.56 | 0.01-3.06 |
| *T. vitulorum* | 5 | 2.78 | 0.91-6.36 |
| Strongyles | 5 | 2.78 | 0.91-6.36 |
| S. papillosus | 3 | 1.67 | 0.35-4.79 |
| **Mixed infections** | **27** | **15.0** | **10.5-20.9** |
| *Eimeria* spp. + *B. sulcata* | 1 | 0.56 | 0.01-3.06 |
| *Eimeria* spp. + *Fasciola* spp. | 2 | 1.11 | 0.13-3.96 |
| *Eimeria* spp. + *Capillaria* spp. | 1 | 0.56 | 0.01-3.06 |
| *Eimeria* spp. + *T. vitulorum* | 13 | 7.22 | 3.9-12.03 |
| *Eimeria* spp. + *S. papillosus* | 6 | 3.33 | 1.23-7.11 |
| *Eimeria* spp. + digestive strongyles | 1 | 0.56 | 0.01-3.06 |
| *B.coli* + *T. vitulorum* | 1 | 0.56 | 0.01-3.06 |
| *F. hepatica*+ *P. cervi* | 1 | 0.56 | 0.01-3.06 |
| *Eimeria* spp. + *B. coli* + *T. vitulorum* | 1 | 0.56 | 0.01-3.06 |

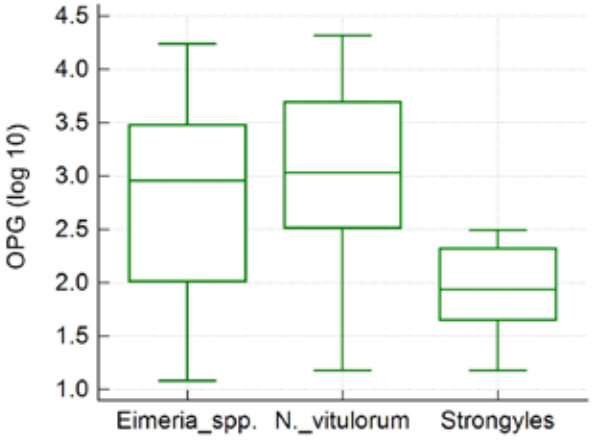


**Figure 3.**

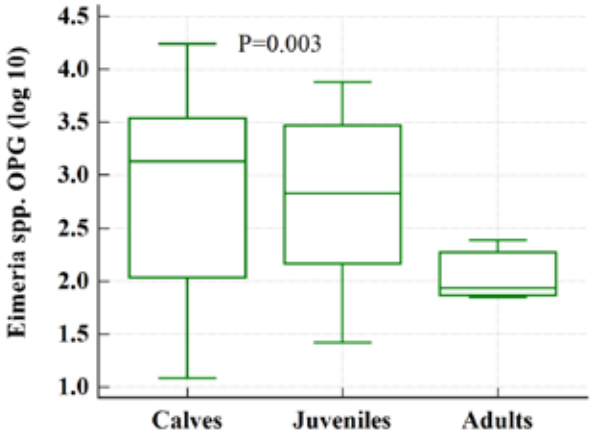


**Figure 4.**

**OPG/EPG**

The faecal egg and oocysts count per gram of faeces (EPG and OPG) are presented in Figure 3 and Figure 4: average (SE) *Eimeria* spp. 2176.6±395.1; *N. vitulorum* 4348.2 (±1643.9); strongyles 126.3 (±45.8). High values of *T. vitulorum* EPG and of *Eimeria* spp. OPG were recorded. *Eimeria* spp. OPG was significantly higher in calves (2679.8±631.8) and juveniles (1677.1±386.4) compared with adult buffaloes (132.7±54.9) ($p< 0.01$) (Fig. 3).

**Risk factors**

The age was identified as a risk factor for infection with digestive parasites (Table 3). The overall infection in young buffaloes was 2.7 (1.8-4.1) times more likely than in adult buffaloes. The same risk factor was identified for the infection with *Eimeria* spp. (young buffaloes OR 2.8; 95% CI: 1.8-4.3), *T. vitulorum* (young buffaloes OR 3.1; 95% CI: 1.5-6.5) and *F. hepatica* (adult buffaloes OR 3.8; 95% CI: 1.1-12.5).

**DISCUSSION**

In buffaloes, the parasites have a major economic impact through the high morbidity and mortality they cause. Thus, in order to design and apply effective surveillance programs, the prevalence of parasitic infections must be known (Sreedevi and Hafeez., 2014).

In the present study, the prevalence of internal parasites in buffaloes from North-Western Romania was 57.2%. In the study of Alam et al. (2016), 85.0% of examined animals were diagnosed with parasitic infections. Biswas et al. (2014) obtained a similar prevalence (84.9%). Jyoti et al. (2012) identified a prevalence of 73.6%, with coccidiosis the most prevalent infection detected.

Global prevalence of coccidiosis varies between 30 and 60%. The highest prevalence was reported in Iran (100%), Italy (100%) and Brazil (100%) (Bahrami and Alborzi, 2013; Fusco et al., 1997; Cringoli et al. 1998; Barbosa et al., 1992). In the present study, in buffaloes from North-West of Romania, the infection with *Eimeria* spp. had the highest prevalence (43.3%) from all detected infections, with significantly higher values in juveniles (59.6%) and calves (57.2%). In Brazil, a similar prevalence of coccidiosis was obtained (43.6%) (Rebouças et al., 1994).Coccidiosis mostly occurs in subclinical forms in bovines and its OPG value can vary considerably in different animals. An OPG greater than 1000 is considered as moderate infection, whilst in high infections the cattle shed more than 1 x $10^4$oocysts per gram of faeces (Koutny, 2012). In the present study the OPG of buffaloes was greater than 2 x $10^3$, suggesting a moderate infection that can have an impact on health and productivity.

The data are scarce with respect to the infection with *B. sulcata*. Prevalence of *B. sulcata* infection was reported in Nepal (27.0%) and India (35.0%) (Adhikari et al., 2013; Kumar et al., 2017).In the present study *B.sulcata* was detected only in 1.7% of samples.

The prevalences of fasciolosis (4.4%) and paramphistomosis (2.8%) obtained in the present study are similar to other results obtained in Pakistan (4%), India (2.0%) and Italy (2.1%) (Bhutto et al., 2002; Condoleo et al., 2007; Sreedevi and Haffez, 2014).

Similar results in terms of prevalence of infection with *Capillaria* spp. and *Moniezia* spp. were obtained in studies conducted in Bangladesh (*Capillaria* spp.:0.4%; *Moniezia* spp. :0.6%) (Biswas et al., 2014), Italy (*Moniezia* spp.: 0.2%) (Condoleo et al., 2007) and India (*Moniezia* spp.: 0.6%) (Sreedevi and Haffez, 2014).

Global prevalence of *T. vitulorum* infection varies between 1.4% and 33.0%. The highest prevalence was reported in Laos (25.5%), Egypt (28.4%) and Pakistan (33.0%) (Rast et al., 2012; Abdel-Rahman and El-Ashmawy, 2013; Bhutto et al., 2002), but the prevalence value in the present study (11.7%) was much lower than these values. A faecal egg count greater than 1000 is considered highly infectivefor livestock (Leahy, 2017). In the present study, the buffaloes shed more than 4000 *T. vitulorum* eggs per gram, suggesting that this parasitic infection was highly damaging. Moreover, the zoonotic potential of *T. vitulorum* remains to be clarified, as *Toxocara canis* and *Toxocara cati,* parasites of carnivores, are responsible for visceral larva migrans in humans (Leahy, 2017).

Literature data on infestation with *S. papillosus* and digestive strongyles are numerous and different prevalence have been reported depending on the age category studied and geographical area. The prevalence of *S.papillosus* infection from the present study (5.0%) is similar to that obtained in Pakistan (5.9%) (Azam et al., 2002).The prevalence of infectionwith digestive strongyles (3.3%) is similar with that obtained in India (5.0%) (Das et al., 2017).

In the present study, calves had a much higher infectionrate than juveniles and adults. Gunathilaka

found also that adults were less susceptible to parasitic infections, but the yearlings were more susceptible than calves (Gunathilaka et al., 2018).

The parasitism was higher in males than in females buffalo in the present study, but statistically significant only for the infection with *Eimeria* spp. Gunathilaka et al. observed a higher prevalence of gastro-intestinal parasites in males compared to females.According to these authors, this might be due to the attitude of farmers that focus more on female's health as they provide milk (Gunathilaka et al., 2018).

The prevalence of internal parasites was higher in buffaloes raised in household system because there is a permanent source of contamination (Radostits et al., 1994). Raising buffaloes on farm could be a method of prophylaxis that could lead to a decrease in the number of contaminated animals, and special attention should also be paid to deworming.

## CONCLUSIONS

Internal parasites were detected in 57.2% of the samples of buffaloes from North-West of Romania. *Eimeria* spp., *B. sulcata*, *F. hepatica*, *P.cervi*, *Moniezia* spp., *T. vitulorum*, digestive strongyles, *S. papillosus*,and*Capillaria* spp. were the parasitic infections identified. The highest prevalence was recorded for *Eimeria* spp. and *T. vitulorum*. The buffaloes from households had greater infectionrates. The calves had higher parasitic infectionprevalence than juveniles and adults. Single infections were more prevalent than mixed infections.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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