Intestinal parasites of buffalo calves from Romania: molecular characterisation of Cryptosporidium spp. and Giardia duodenalis, and the first report of Eimeria bareillyi

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Abstract: Buffaloes represent an important economic resource for several regions of the world including Romania. In the present study, we examined 104 faecal samples collected from 38 buffalo calves (2–11 weeks old) from household rearing systems in Romania for gastrointestinal parasites. All samples were tested using the saturated salt flotation, McMaster and modified Ziehl-Nielsen staining methods. PCR coupled with sequencing isolates were used to identify assemblages of Giardia lambia (Kunstler, 1882) and species of Cryptosporidium Tyzzer, 1907. Overall, 33 out of 38 examined buffalo calves were infected with different gastrointestinal parasites: 16 had single infections and 17 had mixed infections with two or three parasites. Species of Eimeria Schneider, 1875 (32/38; 84%) were the most prevalent parasites; eight species were identified according to the oocyst morphology, including the pathogenic E. bareillyi (Gill, Chhabra et Lall, 1963) which was detected for the first time in buffaloes from Romania. The nematodes Toxocara vitulorum (Goeze, 1782) (11/38; 37%) and Strongyloides papillosus (Wedl, 1856) (6/38; 16%) were also detected. Cryptosporidium spp. were found in four (11%) buffalo calves; two of them were molecularly identified as C. ryanae Fayer, Santin et Trout, 2008, and another one clustered in the same clade with C. ryanae, C. bovis Fayer, Santin et Xiao, 2005, and C. xoiu Fayer et Santin, 2009. Giardia duodenalis assemblage E was also molecularly detected in a single (2.6%) buffalo calf. The presence of other buffaloes in the same barn was identified as a risk factor for infection with T. vitulorum. Our results indicate extensive parasitic infections in buffalo calves from northwestern Romania and underline the necessity of prophylactic treatments for T. vitulorum and E. bareillyi.

Keywords: buffaloes, parasites, Toxocara vitulorum, Eimeria bareillyi, Cryptosporidium, Giardia

The worldwide population of water buffaloes Bubalus bubalis (Linnaeus) has been estimated as 206 million in 2018, with most populations being found in Asia (97%). Buffaloes represent a major contribution to the economy of these regions (FAO 2020). In Europe, buffaloes are mainly found in Italy, Romania, Bulgaria, Greece, Germany, United Kingdom, Macedonia, and Albania (Borghese 2010).

Parasitic infections can cause serious production losses in this animal species (Thapa Shrestha et al. 2020). Buffaloes are infected with several parasites and some of them have a zoonotic nature, such as ubiquitous coccidia of the genus Cryptosporidium Tyzzer, 1907, which cause serious disease in humans and animals. Three species of Cryptosporidium have been detected in buffaloes, namely C. parvum Tyzzer, 1912, C. ryanae Fayer, Santin et Trout, 2008 and C. bovis Fayer, Santin et Xiao, 2005 (see Helmy et al. 2013, Aquino et al. 2015, Ma et al. 2015, Ibrahim et al. 2016). Cryptosporidium parvum circulates among humans and different animals, and the infection is generated by ingestion of the oocysts which are dispersed widely in the environment and can resist harsh environmental conditions (Robertson et al. 2014). Molecular data have revealed that...
ruminants represent the major sources of infection with *C. parvum* for humans (Thompson 2011).

Another example is coccidiosis which is a major disease of livestock, including buffaloes, and is caused by species of the genus *Eimeria* Schneider, 1875. Although species of *Eimeria* are in general host-specific, cattle and buffaloes have been reported to host many species. *Eimeria bareillyi* (Gill, Chhabra et Lall, 1963) is specific to buffaloes and is also the most pathogenic species (Dubey 2020).

Reports on the parasitic infections in buffaloes in our country are few. Therefore, the objective of the present study was to determine the prevalence and species composition of different gastrointestinal (GIT) parasites infecting buffalo calves kept in household rearing systems in Romania. The new data may be useful for the implementation of effective surveillance and control programs.

**MATERIALS AND METHODS**

**Samples collection**

Rectal faecal samples from 38 buffalo calves were collected from March to June 2017. Faecal samples were labelled, stored in plastic bags and sent during the day of collection in a cooling box to the laboratory. In the laboratory, the samples were stored in the refrigerator until the next day when the samples were analysed. Two hundred grams from each faecal sample were stored in the freezer for PCR analysis.

Samples were taken from buffaloes reared in four villages (Românași, Păușa, Poarta Sălajului and Chichișa) from Sălaj County, northwestern Romania. One buffalo calf was sampled per household. During this period, animals remained in the barns.

Each buffalo calf was sampled ≤ 5 times. Sampling intervals were assessed by comparison to a molecular marker (O’GeneRuler® 100 bp DNA Ladder, Thermo Fisher Scientific Inc., USA), and their molecular weight was determined with Cell^F software (Bio-Rad, Hercules, USA), according to the manufacturer’s instructions. The harvested DNA was stored at -20 °C until further processing.

**Sample analysis**

Faecal samples were examined using the sodium chloride (specific gravity 1.28) flotation method. Cysts, oocysts and eggs found were identified according to their morphological characters (Mircean et al. 2011, El-Alfy et al. 2019, Dubey 2020), using an optical microscope (Olympus BX61). Positive samples were further tested using the McMaster technique to determine the number of oocysts/eggs per gram (OPG/EPG) of a faecal sample. Ziehl-Nielsen modified staining was used to confirm the identity of oocysts of *Cryptosporidium* spp. Microphotographs of oocysts were taken using an optical microscope (Olympus BX61) connected to a DP72 camera (Olympus Corporation, Tokyo, Japan), and the measurements were determined with Cell^F* software (Olympus Corporation, Japan). Different species of *Eimeria* were identified based on oocyst morphology, as described previously (El-Alfy et al. 2019, Dubey 2020).

**Molecular analysis**

All faecal samples were molecularly tested to detect *Giardia duodenalis* (Stiles, 1902) and *Cryptosporidium* spp. DNA, and positive samples were further sequenced to identify assemblages and species, respectively. DNA was extracted from each sample, using a commercial kit (Isolate II Faecal DNA Kit, Bioline, London, UK), according to the manufacturer’s instructions. The harvested DNA was stored at -20 °C until further processing.

*Giardia duodenalis* 432 bp fragment of the glutamate dehydrogenase gene (gdh) and *Cryptosporidium* 638 bp fragment of the SSU rRNA gene were amplified using nested PCR reactions (Lindergard et al. 2003, Read et al. 2004). The primers and reaction conditions are listed in Tables 1 and 2. Reactions were conducted using a C100 Thermal Cycler (Bio-Rad, Hercules, USA) in a final volume of 25 μl which consisted of 12.5 μl MyTaq Red HS Mix 2x master mix (Bioline, London, UK), 25 pmol of primers, and 4 μl of genomic DNA which was replaced in the second round by 4 μl of the first-round product.

PCR products were visualised by electrophoresis on 1.5% agarose gel stained with SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific Inc., Waltham, USA), and their molecular weight was assessed by comparison to a molecular marker (O’GeneRuler™ 100 bp DNA Ladder, Thermo Fisher Scientific Inc., USA).

PCR products were purified using a commercial kit ( Favor-Prep GEL/PCR Purification Mini Kit, Favorgen Biotech Corp., Ping-Tung, Taiwan) and commercially sequenced (Macrogen Europe B.V., Amsterdam, Netherlands). The revealed sequences were compared to other sequences available in the GenBank™ database, using the Basic Local Alignment Search Tool (BLAST) analysis. The phylogenetic analysis was conducted using MEGA X software, and the evolutionary history was inferred using the Maximum Likelihood method. The evolutionary distances were computed using the Jukes-Cantor model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1650)).

**Statistical analysis**

The frequency, prevalence and its 95% confidence interval (95% CI) were calculated for each identified parasite. These were performed overall, and according to age group. Five age groups

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Table 1. Primers used for the nested PCR

<table>
<thead>
<tr>
<th>Species</th>
<th>Oligonucleotide primer</th>
<th>Oligonucleotide sequence</th>
<th>Size of the amplified amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium spp.</td>
<td>CrSSU-1</td>
<td>GATTAAAGCCCATGCATGTCTAA</td>
<td>638</td>
</tr>
<tr>
<td></td>
<td>CrSSU-2</td>
<td>TCCATGCTGGAGTATTTCAAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CrSSU-3</td>
<td>CATTTAGTTTAATTTGATAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CrSSU-4</td>
<td>CTTGCTTTAAGCACTCTAATTTTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDHeF</td>
<td>TCAGTGAAATGATC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDHHF</td>
<td>GAGTTATAGTTTACTTGATAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDHRf</td>
<td>CAGTAC AAC TCY GYT TCC GT</td>
<td></td>
</tr>
<tr>
<td>Giardia duodenalis</td>
<td>GTRTC CCT GCA CAT CTC C</td>
<td></td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>GDHHF</td>
<td>CAGTAC AAC TCY GCT CTC GG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GDHRf</td>
<td>GTRTC CCT GCA CAT CTC C</td>
<td></td>
</tr>
</tbody>
</table>
were established: 2–3 weeks, 4–5 weeks, 6–7 weeks, 8–9 weeks, and 10–11 weeks. The average and standard error were calculated for OPG and EPG. Before further processing, the distribution data was assessed by D’Agostino-Pearson’s normality test. If not normally distributed, the data were transformed by logarithm in base 10. The ANOVA repeated measures analysis of variance test was used to identify statistically significant differences among age groups.

Risk factors, such as the presence of young and adult buffaloes, and the presence of cattle in the same barn were evaluated by chi-square test. A value of \( p < 0.05 \) was considered statistically significant. Statistical analysis was performed with EpilInfo 3.5.1 (CDC, USA) and MedCalc Statistical Software 19.0.4. (MedCalc Software Ltd., Ostend, Belgium).

### RESULTS

Thirty-three (87%) out of 38 examined buffalo calves were infected with at least one GIT parasite. Single (42%), dual (42%), and triple (3%) infections were noted. Parasites of five genera were detected; species of *Eimeria* were most prevalent (32/38; 84%) followed by *Toxocara vitulorum* (Goeze, 1782) (11/38; 37%), *Strongyloides papillosus* (Wedl, 1856) (6/38; 16%), *Cryptosporidium* spp. (4/38; 11%), and *Giardia duodenalis* (1/38; 3%) (Fig. 1, Table 3).

Eight species of *Eimeria* were identified: *E. bareillyi*, *E. zuernii* Rivolta, 1878, *E. cylindrica* Wilson, 1931, *E. ellipsoidalis* Becker et Frye, 1929, *E. auburnensis* Christensen et Porter, 1939, *E. canadensis* Bruce, 1921, *E. subspheraica* Christensen, 1941, and *E. bovis* (Fig. 2). Oocysts of all but one (*E. subspheraica*) were found in faeces of 2–3 weeks old buffaloes.
calves (56% were infected) and the prevalence increased with the age to 85% in 10–11 weeks old calves. *Eimeria bareillyi* was highly prevalent in 2–3 (36%) and 6–7 (76%) weeks age groups (Table 3). Estimated intensity of infection was $8.7 \times 10^4$–$1.5 \times 10^7$ OPG, being the highest in 2–5 weeks old calves and decreased with the age (Table 4).

Eggs of *Toxocara vitulorum* were found in all age groups with a prevalence ranging from 11% in 2–3 weeks old buffaloes to 23% in 10–11 weeks old buffalo calves (Table 3). The EPG value decreased with the age from $7 \times 10^5$ to $6.4 \times 10^4$ (Table 4), and the presence of adult buffaloes in the same barn with buffalo calves was identified as a risk factor for infection with *T. vitulorum* (Table 5).

Eggs of *Strongyloides papillosus* were detected since 4–5 weeks of age and the prevalence increased with the age from 4 to 23% (Table 3), with an estimated EPG of 444 on average.

Oocysts of *Cryptosporidium* spp. were detected in the faeces of four calves (3–4 weeks old). DNA of three isolates was successfully amplified and sequenced; two isolates were identified, according to the BLAST search, as *C. ryanae* (GenBank database Acc. nos. MW289064 and MW289065) and clustered with various *C. ryanae* isolates from dairy calves worldwide, particularly those from Asia (China and India). The third isolate (MW289066) grouped in the clade with *C. ryanae*, *C. bovis*, and *C. xiaoi* (Fayer et Santin, 2009), but as a separate sequence (Fig. 3).

*Giardia duodenalis* was molecularly identified in the faeces of a 9-week old buffalo calf. The revealed nucleotide sequence was identical to that of the isolate AB692776.

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Table 3. Frequency and prevalence of gastrointestinal (GIT) parasites found in buffalo calves of different age groups.

<table>
<thead>
<tr>
<th>Age Group</th>
<th><em>Giardia duodenalis</em></th>
<th><em>Cryptosporidium</em> spp.</th>
<th>Total Eimeria spp.</th>
<th><em>Eimeria bareillyi</em></th>
<th><em>E. zuernii</em></th>
<th><em>E. cylindrica</em></th>
<th><em>E. ellipsoidalis</em></th>
<th><em>E. auburnensis</em></th>
<th><em>E. canadensis</em></th>
<th><em>E. subsppherica</em></th>
<th><em>E. bovis</em></th>
<th><em>Toxocara vitulorum</em></th>
<th><em>Strongyloides papillosus</em></th>
<th>Single infection</th>
<th>Mixed infection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–3 weeks</td>
<td>0/18</td>
<td>1/18 (6%)</td>
<td>10/18 (55.6)</td>
<td>10/18 (55.6)</td>
<td>16/61 (26)</td>
<td>12/61 (20)</td>
<td>3/61 (4.9)</td>
<td>5/61 (8.2)</td>
<td>1/61 (1.6)</td>
<td>0/61</td>
<td>2/61 (3.3)</td>
<td>2/18 (11.1)</td>
<td>0/18</td>
<td>9/18 (50.0)</td>
<td>2/18 (11.1)</td>
<td>11/18</td>
</tr>
<tr>
<td>4–5 weeks</td>
<td>0/27</td>
<td>3/27 (11%)</td>
<td>23/27 (85.2)</td>
<td>23/27 (85.2)</td>
<td>16/37 (43)</td>
<td>7/37 (18.9)</td>
<td>12/37 (32.4)</td>
<td>1/37 (2.7)</td>
<td>1/37 (2.7)</td>
<td>0/37</td>
<td>2/37 (5.4)</td>
<td>4/27 (14.8)</td>
<td>1/27 (3.7)</td>
<td>15/27 (55.6)</td>
<td>8/27 (29.6)</td>
<td>11/18</td>
</tr>
<tr>
<td>6–7 weeks</td>
<td>0/24</td>
<td>4/37 (11%)</td>
<td>29/37 (76.3)</td>
<td>29/37 (76.3)</td>
<td>13/38 (34)</td>
<td>7/37 (18.9)</td>
<td>12/37 (32.4)</td>
<td>1/37 (2.7)</td>
<td>1/37 (2.7)</td>
<td>0/37</td>
<td>0/37</td>
<td>4/27 (14.8)</td>
<td>1/27 (3.7)</td>
<td>15/27 (55.6)</td>
<td>8/27 (29.6)</td>
<td>11/18</td>
</tr>
<tr>
<td>8–9 weeks</td>
<td>1/18 (5.6)</td>
<td>7/18 (38.9)</td>
<td>18/18 (100)</td>
<td>22/24 (92)</td>
<td>8/35 (22.9)</td>
<td>6/35 (17.1)</td>
<td>1/38 (2.6)</td>
<td>7/35 (20.0)</td>
<td>6/35 (17.1)</td>
<td>0/35</td>
<td>0/35</td>
<td>4/27 (14.8)</td>
<td>1/27 (3.7)</td>
<td>15/27 (55.6)</td>
<td>8/27 (29.6)</td>
<td>11/18</td>
</tr>
<tr>
<td>10–11 weeks</td>
<td>0/13</td>
<td>14/13 (106.2)</td>
<td>11/13 (85)</td>
<td>11/13 (85)</td>
<td>11/13 (85)</td>
<td>7/13 (53.8)</td>
<td>6/13 (46.2)</td>
<td>16/13 (123.1)</td>
<td>5/13 (38.5)</td>
<td>2/13 (15.4)</td>
<td>2/13 (15.4)</td>
<td>4/18 (22.2)</td>
<td>1/18 (5.6)</td>
<td>17/13 (130.8)</td>
<td>5/13 (38.5)</td>
<td>12/13</td>
</tr>
<tr>
<td>Total</td>
<td>1/38 (2.6)</td>
<td>4/38 (10.5)</td>
<td>32/38 (84.2)**</td>
<td>32/38 (84.2)**</td>
<td>52/38 (22.4)</td>
<td>34/38 (14.7)</td>
<td>33/38 (14.2)**</td>
<td>19/38 (50.0)</td>
<td>9/38 (23.7)</td>
<td>6/38 (15.8)</td>
<td>6/38 (15.8)</td>
<td>11/38 (36.8)</td>
<td>11/38 (36.8)</td>
<td>16/38 (42.1)</td>
<td>17/38 (44.7)</td>
<td>33/38</td>
</tr>
</tbody>
</table>
DISCUSSION

During the present study, the prevalence of intestinal parasites was evaluated in buffalo calves 2–11 weeks old from northwestern Romania. Three genera of parasitic protists (species of *Eimeria* and *Cryptosporidium*, and *Giardia duodenalis*) and two nematodes (*Toxocara vitulorum* and *Strongyloides papillosus*) were found. These GIT parasites are the most common found in buffalo calves worldwide (Ribeiro et al. 2000, Naag et al. 2015).

In the present study, coccidia of the genus *Eimeria* were the most prevalent GIT parasites and they were detected in 84% of buffalo calves. This ubiquitous parasitic protist has a worldwide distribution in water buffaloes which could be infected with at least 12 species of *Eimeria*, 11 of them being of cattle origin (El-Alfy et al. 2019). So far, *Eimeria bareillyi* is the only buffalo-specific species, non-transmissible to cattle, and is highly pathogenic to young calves (Dubey 2018). This is the first report of *E. bareillyi* oocysts in buffalo calves from Romania. However, infections were subclinical and the oocysts were detected in all age groups. Infections with *E. bareillyi* have been reported worldwide (Dubey 2018) including three reports from Europe, i.e., in Italy (Fusco et al. 1997, Guarino 1997) and the Netherlands (Dubey et al. 2008). The prepatent period is 12–15 days after experimental infection with 350,000–15,000,000 oocysts of *E. bareillyi* (see Dubey 2018). In natural infections, oocysts of *E. bareillyi* were detected at 13 days of age (Bastianetto et al. 2008), whereas oocysts of other species, e.g., *Eimeria bovis*, *E. ellipsoidalis*, *E. auburnensis* and *E. zuernii*, were noticed as early as 2–7 days of age (Bastianetto et al. 2007). Therefore, a study on buffalo calves from *E. bareillyi*-infected farm and involving daily examination of faecal samples from the first day of birth is required to detect whether oocysts of *E. bareillyi* are excreted in the first week of life.

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Table 4. Values of OPG/EPG (mean ± standard error) values for gastrointestinal parasites found in buffalo calves of different age groups

<table>
<thead>
<tr>
<th></th>
<th>2–3 weeks</th>
<th>4–5 weeks</th>
<th>6–7 weeks</th>
<th>8–9 weeks</th>
<th>10–11 weeks</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eimeria</em> spp.</td>
<td>83,914 ± 48,411</td>
<td>1,439,941 ± 1,350,781</td>
<td>149,988 ± 96,959</td>
<td>8,715 ± 3,289</td>
<td>81,214 ± 59,937</td>
<td>417,156 ± 338,143</td>
</tr>
<tr>
<td><em>Toxocara vitulorum</em></td>
<td>70,415 ± 21,503</td>
<td>26,678 ± 6,420</td>
<td>23,192 ± 13,348</td>
<td>9,186 ± 3,590</td>
<td>6,359 ± 2,934</td>
<td>26,510 ± 7,085</td>
</tr>
<tr>
<td><em>Strongyloides papillosus</em></td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>444 ± 82</td>
<td>444 ± 82</td>
</tr>
</tbody>
</table>

Table 5. Risk factors for infection with *Toxocara vitulorum* in buffalo calves

<table>
<thead>
<tr>
<th>Frequency (prevalence)</th>
<th>Odds Ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young buffaloes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 animals (n = 24)</td>
<td>2 (12.5)</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>3–4 animals (n = 14)</td>
<td>9 (91.7)</td>
<td>19.80</td>
<td>3.22–121.47</td>
</tr>
<tr>
<td><strong>Adult buffaloes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 animals (n = 22)</td>
<td>2 (13.6)</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>3–5 animals (n = 16)</td>
<td>9 (68.8)</td>
<td>12.86</td>
<td>2.22–74.54</td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n = 29)</td>
<td>10 (44.8)</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>Positive (n = 9)</td>
<td>1 (11.1)</td>
<td>0.24</td>
<td>0.03–2.18</td>
</tr>
</tbody>
</table>

Fig. 3. Maximum Likelihood analysis. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Outgroup taxon analysis.
third week of life. Adult worms are then expelled from the intestine by the fifth month of age (Roberts 1990, Roberts et al. 1990). In Europe, this nematode parasite has been reported at farms of cattle (Scala et al. 2001, Diakou and Papadopoulos 2002, Jones et al. 2009, Borgsteede et al. 2012, Chihai et al. 2012, Venjakob et al. 2017) and bisons (Goossens et al. 2007). In western Romania, the parasite was detected in 0.3% of 303 faecal samples from cattle (Sirbu et al. 2020).

Additionally, eggs of S. papillosus were found in 16% of the examined buffalo-calves in the present study, and the prevalence increased with the age. A lower prevalence (3%) was detected in water buffaloes (adults, heifer/steers and calves) from Italy (Rinaldi et al. 2009). However, higher values have been reported worldwide: 29% in India (Jyoti et al. 2014); 59% in Mexico (Ojeda-Robertos et al. 2017), and 87% in 3 months old calves in Sri Lanka (Roberts and Fernando 1990, Jyoti et al. 2014, Ojeda-Robertos et al. 2017).

Although species of Strongyloides Grassi, 1879 infect all age groups, clinical signs such as diarrhea and malnutrition are frequently seen only in young animals (Thamsborg et al. 2017). Sudden deaths were reported in naturally and experimentally infected calves and EPG values in dead calves varied from 52,000 to 411,000 (Taira and Ura 1991). Strongyloides-infected calves from our study presented low EPG values (under 1,000). Considering the high pathogenicity of the parasite, calves with high EPG (>10,000) should be treated (Thamsborg et al. 2017).

Our study demonstrated infection with cryptosporidia in 4 (11%) buffalo calves; this prevalence is comparable to earlier studies worldwide (Helmy et al. 2013, Ma et al. 2015, Ibrahim et al. 2016). However, our prevalence is much lower than that from buffalo calves (2–4 months) from Egypt (40%) and Brazil (54%) (Aquino et al. 2015, Aboelsoud et al. 2020). Compared to cattle (25–29%) from the country of study (Romania), the obtained prevalence in buffalo calves is lower (Bejan et al. 2008, Imre et al. 2011). DNA from three of the four Cryptosporidium isolates was successfully amplified and sequenced, and the obtained sequences revealed similarity with C. ryanae in two samples, whereas the third isolate was unidentified but related genetically to the bovine C. ryanae and C. bovis, as well as the ovine C. xiaoii. Cryptosporidium ryanae is the most common species of Cryptosporidium reported worldwide in buffaloes (Martins et al. 2018, Russell et al. 2020). In Romania, Cryptosporidium parvum was reported in calf and as the predominant species in lambs (Imre et al. 2011, 2013, Vieira et al. 2015). Our results cannot deny the role of buffaloes in the transmission cycle of C. parvum due to the limited number of tested samples and sequenced isolates.

A single sample was positive for infection with G. duodenalis and sequence analysis revealed it was the assemble A. This assemble is non-zoonotic and commonly circulates among different farm animals, including cattle, sheep, goat and horses (Heyworth 2016), and has been detected together with the assemble A in buffaloes from Italy (Caccio et al. 2007), Australia (Caccio et al. 2007, Abeywardena et al. 2013, Helmy et al. 2013), and Egypt (Helmy et al. 2013).

Generally, buffaloes are considered robust animals and therefore more resistant to diseases compared with cattle. However, this study pointed out that pre-weaned buffalo calves are exposed to early infection with pathogenic E. bareillyi, and other species of Eimeria, and to the infection with T. vitulorum. Moreover, the infection risk for T. vitulorum increased when more than two buffaloes are kept in the same barn. Multiple infections and simultaneous occurrence in the same region of the intestine can adversely affect the growth of buffaloes. These data pointed out the need for early coccidiostatic and anthelmintic treatments in buffalo calves, but they do not seem to be a source of zoonotic C. parvum and G. duodenalis in our area.

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Bährbrun et al.: Intestinal parasites of buffalo calves


