



# Prevalence of Gastrointestinal Parasites in Water Buffalo (*Bubalus bubalis*) Calves Raised with Cattle in Smallholder Farming System in the Northwest of Iran

## İran'ın Kuzeybatı Bölgesindeki Küçük Çaplı Yetiştiricilik Sisteminde Sığırlar ile Birlikte Yetiştirilen Manda (*Bubalus Bubalis*) Yavrularında Gastrointestinal Parazitlerin Prevalansı

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### Abstract

The objective of this study was to investigate the prevalence of gastrointestinal parasitic infection in buffalo calves in Northwest of Iran. Fecal samples of 317 buffalo calves under 9 months of age (155 male and 162 female) were collected from villages around Urmia, Northwest of Iran. Centrifugal flotation technique using saturated zinc sulphate solution and sedimentation methods was used to detect protozoa and helminthes infections. The modified Ziehl-Neelsen technique was used for determining *Cryptosporidium* spp., infection. Eight *Eimeria* species oocysts were identified in 112 (35.3%) out of the 317 buffalo calves. *Eimeria zuernii* had the highest prevalence (23.3%) followed by *E. bovis* (15.1%), *E. ellipsoidalis*

(7.3%), *E. bareillyi* (2.5%), *E. bukidnonensis* (2.2%), *E. ovoidalis* (1.3%), *E. cylindrical* (0.9%), *E. auburnensis* (0.6%). No cysts of *Giardia* spp. were detected. The oocysts of *Cryptosporidium* spp. were detected in 8 (2.5%) buffalo calves. Twenty-three buffalo calves (7.3%) were positive for *Toxocara vitulorum* eggs. No clinical finding was observed in infected buffalo. In conclusion, *Eimeria* spp., *Cryptosporidium* spp and *T. vitulorum* are relatively common in buffalo calves and buffalo should be considered as a potential source of infection to other animal species.

**Keywords:** *Eimeria*, *Cryptosporidium*, *Toxocara vitulorum*, Buffalo Calves

### Öz

Bu çalışmanın amacı, İran'ın kuzeybatı bölgesindeki manda yavrularında gastrointestinal parazitler enfeksiyon prevalansını araştırmaktır. 9 aylıktan küçük 317 manda yavrusuna (155 erkek ve 162 dişi) ait dışkı örnekleri, İran'ın kuzeybatısında bulunan Urmia bölgesi etrafındaki köylerden toplanmıştır. Protozoon ve helmint enfeksiyonlarını belirlemek için; doymuş çinko sülfat çözeltisinden yararlanılarak santrifüj flotasyon tekniği ve sedimentasyon yöntemleri kullanılmıştır. *Cryptosporidium* spp. enfeksiyonunu belirlemek için modifiye Ziehl-Neelsen tekniği uygulanmıştır. 317 manda yavrusundan 112'sinde (%35,3) 8 *Eimeria* türü ookist tespit edilmiştir. *Eimeria zuernii* en yüksek prevalansa (%23,3) sahipken; bunu *E. bovis* (%15,1),

*E. ellipsoidalis* (%7,3), *E. bareillyi* (%2,5), *E. bukidnonensis* (%2,2), *E. ovoidalis* (%1,3), *E. cylindrical* (%0,9), *E. auburnensis* (%0,6) takip etmiştir. Hiçbir *Giardia* spp. kisti tespit edilmemiştir. *Cryptosporidium* spp. ookistleri 8 (%2,5) manda yavrusunda tespit edilmiştir. 23 manda yavrusu (%7,3) *Toxocara vitulorum* yumurtaları yönünden pozitif bulunmuştur. Enfekte mandalarda hiçbir klinik bulgu gözlenmemiştir. Sonuç olarak, *Eimeria* spp., *Cryptosporidium* spp. ve *T. vitulorum*, manda yavrularında kısmen yaygındır ve mandalar, diğer hayvan türleri için potansiyel bir enfeksiyon kaynağı olarak düşünülmelidir.

**Anahtar kelimeler:** *Eimeria*, *Cryptosporidium*, *Toxocara vitulorum*, manda yavruları

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## Introduction

Parasitism is one of the major problems affecting ruminants (Gupta et al., 1978). However, regarding ruminants, most of the data on the prevalence and distribution of parasites are on cattle. In terms of economic loss, coccidiosis is a widespread, serious disease affecting pre-weaned and recently weaned animals, especially in insanitary, stressful and/or crowded conditions. The disease may result in death, especially in young animals and may cause considerable economic losses in farm animals worldwide (Soulsby, 1982; Levine, 1985). Apart from *Eimeria azerbaijanica* and *E. thianethi* which are specific to the buffalo, various cattle *Eimeria* species have also been reported to prevail in buffalo (Patnaik and Pande, 1965; Gundrans and More, 1999). Fatal cases of coccidiosis have been reported in 10 buffalo calves infected with *E. bareillyi*.

*Giardia* spp. and *Cryptosporidium* spp. are common intestinal protozoan parasites that infect mammals including humans (Thompson and Monis, 2004; Appelbee et al., 2005).

*Toxocara vitulorum* is the largest intestinal nematode of bovidae including buffalo (Soulsby, 1982). This parasite has been reported to cause heavy economic losses to the livestock industry (Baruah et al., 1979). In some countries, the heavy burden of adult *T. vitulorum* on young buffalo calves resulted in a high mortality rate and economic losses (Lau, 1987).

There is a paucity of information on the prevalence, diversity and abundance of parasites of buffalo calves. Little is known about the prevalence of gastrointestinal parasites (in particular on protozoan pathogens) of buffalo calves, the extent of shedding of these parasites into the environment and the role of buffalo in the transmission of infection to other species. This study was conducted to determine the prevalence of certain gastrointestinal parasites in buffalo calves and the identification of the diversity and abundance of *Eimeria* spp. in northwest Iran, an area which has the largest population of water buffalo in the country.

## Materials and Methods

Fecal samples were collected from 317 male (155) and female (162) buffalo calves (water buffalo under 9 months old). The buffalo calves were randomly selected from villages around Urmia, northwest Iran. The area of the study is one of the main buffalo raising regions and currently has the largest population of water buffalo in Iran. Approximately 808,300 water buffalo are raised in this region. In this area, buffalo are raised by farmers for milk, meat and hide production. The area of the study is semi-humid, with an average yearly rainfall of 350 mm, maximum monthly temperature of 28.3°C in August and minimum monthly temperature of

-5°C in January. The buffalo were allowed to graze in the local ranges or beside the orchard gardens from spring to mid-autumn during the day and kept indoors at nights. Calves are left to run with their mothers until weaned at 3 to 6 months old. Young and older buffalo were allowed to mix freely and sometimes with other farm livestock, in particular cattle, and occasionally, with sheep and goats. No supplementary feeding is provided and water is available from rivers, portable piping, wells and sometimes pools of rain-water on the ground or stagnant water. During the winter and cold seasons the buffalo are usually enclosed indoors (in roofed stables or barns) all day and night usually with cattle in groups of 5 to 20 and fed with straw, dried grasses and alfalfa. In this housing system, the animals are usually bedded with straw or dried cow dung which is usually cleaned and refreshed once a day or every other day. Only, rarely strategic and regular anti-parasitic medications are administered to the buffalo.

The samples were collected directly from the rectum into disposable plastic bags and transported in a cool box on ice to the laboratory. For each sample, the sampling date, the animals' identification, age, sex, health status and fecal consistency at the time of sampling were recorded.

All samples were immediately transferred to the laboratory and examined by the flotation method using a zinc sulfate solution and sedimentation methods at the parasitological diagnostic laboratory of the Faculty of Veterinary Medicine, Urmia University. Each observed egg or oocyst was identified by using their morphological characteristics described by Levine (1985) and Soulsby (1982). Identification of *Eimeria* species was carried out on the faecal samples and those with at least 50 oocyst per gram were used for further examination, after sporulation in 2.5% potassium dichromate at room temperature. They were identified on the basis of the morphology of oocyst and sporocyst, according to Levine (1985), the characteristics of the identified oocyst are shown in Table 1 and 2. Measurements were made using a calibrated ocular micrometer under  $\times 40$  magnification. Faecal samples were further examined for *Giardia* detection under oil immersion.

For diagnosis of *Cryptosporidium* spp., fecal smears were prepared on glass slides; air dried and stained using a modified Ziehl-Neelsen technique. Measurements were made using a calibrated ocular micrometer under  $\times 100$  magnification.

## Results

Out of a total of 317 sampled buffalo calves, 112 (35.3%) were found positive for coccidian oocysts. Identification of species showed 8 *Eimeria* species. *Eimeria zuernii* had the highest (23.3%) and *E. auburnensis* had the lowest (0.6 %)

**Table 1.** Morphology of *Eimeria* species oocyst identified from infected buffalo from northwest Iran

<i>Eimeria</i> Species	Size of Oocyst (µm)	Size of sporocyst (µm)	Valve	Shape
<i>E. zuernii</i>	12×10	7×11	–	Ovoid
<i>E. bovis</i>	23×17	13×5	–	Ovoid
<i>E. ellipsoidalis</i>	12×10	11×5	–	Ellipsoidal
<i>E. bareillyi</i>	24×15	15×6	–	Pear shaped
<i>E. bukidnonensis</i>	34×26	12×9	+	Radially striated
<i>E. ovoidalis</i>	32×20	14×8	+	Ovoid
<i>E. cylindrical</i>	16×12	12×4	–	Ellipsoidal
<i>E. auburnensis</i>	32×19	15×6	+	Ovoid

**Table 2.** The number of positive samples and relative frequency (%) of *Eimeria* spp, *Cryptosporidium* spp and *Toxocara vitulorum* infection in fecal samples of 317 buffalo valves (1 month old to 9 months old) in the Urmia district, Iran

Parasites	No. of positive samples (%)
<i>E. zuernii</i>	74 (23.3)
<i>E. bovis</i>	48 (15.1)
<i>E. ellipsoidalis</i>	23 (7.3)
<i>E. bareillyi</i>	8 (2.5)
<i>E. bukidnonensis</i>	7 (2.2)
<i>E. ovoidalis</i>	4 (1.3)
<i>E. cylindrical</i>	3 (0.9)
<i>E. auburnensis</i>	2 (0.6)
<i>Cryptosporidium</i> spp	8 (2.5)
<i>T. vitulorum</i>	23 (7.3)

relative frequency of infection among the sampled buffalo calves. The number and relative frequency of samples positive for various *Eimeria* species are given in Table 1. Sixty-two samples (16.7%) were infected with a single *Eimeria* species, 34 (10.7%), 13 (4.1%), and 3 (0.9%) of calves had mixed infections with 2, 3 and 4 species, respectively. The number of oocyst per gram (OPG) ranged from 1-900. Of 317 buffalo calves, 8 (2.5%) were found to be infected with *Cryptosporidium* spp. No cysts for *Giardia* spp. were detected in any of the sampled buffalo. Concerning *T. vitulorum*, 23 out of 317 (7.3%) samples were detected positive for this nematode. The results are summarized in Table 2.

## Discussion

In the present study, *E. zuernii*, *E. bovis* and *E. ellipsoidalis* were the most prevalent species. Similar findings have been reported from South Africa, Tanzania and Kenya (Munyua and

Ngotho, 1989; Chibunda et al., 1996; Matjila and Penzhorn, 2002). Six out of 17 of the recorded *Eimeria* species that infect the buffalo (*E. ankarensis*, *E. azerbaijanica*, *E. bareillyi*, *E. gokaki*, *E. ovoidalis*, and *E. thianethi*) throughout the world, are known to be specific to buffalo, and the remaining 11 species (*E. alabamensis*, *E. auburnensis*, *E. bovis*, *E. brasiliensis*, *E. bukidnonensis*, *E. canadensis*, *E. cylindrical*, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis*, and *E. zuernii*) are cattle-originated (Gundrans and More, 1999; Penzhorn, 2000; Ribeiro et al., 2000). In the present study, a total of 8 *Eimeria* species were found in buffalo calves, of which 2 were specific to the water buffalo (*E. ovoidalis* and *E. bareillyi*), and the remaining 6 were of cattle origin. Workers have studied the prevalence of infection to *Eimeria* species in cattle of Iran. Eslami and Hosseini (1989) reported the prevalence of coccidian species in adult cattle 18.6% and in young calves 20.5%. In a study performed on water buffalo in Turkey 124 out of 130 water buffalo (95.4%) were found to be infected with *Eimeria* species (Sayin, 1968). In another study from Turkey, the rate of infection to these parasite was only 2% (1 out of 50 water buffalo) (Sayin, 1973). Nalbantoglu et al. (2007) from Turkey identified 78 out of 104 water buffalo (75%) infected with 11 *Eimeria* species as follows: *E. zuernii*, *E. bovis*, *E. ellipsoidalis*, *E. bareillyi*, *E. cylindrical*, *E. canadensis*, *E. auburnensis*, *E. brasiliensis*, *E. ankarensis*, *E. subspherica*, *E. alabamensis*. High prevalence of *Eimeria* spp. in buffalo indicates that this parasite found suitable conditions in buffalo to complete their life cycle. Therefore, it appears that water buffalo and cattle may be considered as reservoirs of the parasite for each other, the point which should be addressed in any control program in particular in regions where the cattle and buffalo were raised in close proximity.

No clinical symptom related to coccidiosis was observed in our study in the infected buffalo calves. However, clinical signs and fatality have been reported from affected buffalo calves by other studies. In Maharashtra state, India, 10 buffalo calves died of coccidiosis. At necropsy, they were found to be infected with various stages of *E. bareillyi* (Shastri and Krishnamurthi, 1975).

In Iran studies have been conducted in cattle reporting on the prevalence of *Cryptosporidium* spp. from 3.8% to 42.8% (Radfar et al., 2006; Azami, 2007; Fotouhi Ardakani, 2008). In a study in Italy performed in 90 buffalo farms with 347 fecal samples of buffalo calves 24.4% were positive for *C. parvum* (Rinaldi et al., 2007). In Tanzania, 22.0% (Mtambo et al., 1997). In Brazil, 8.3% of diarrhoeic and 10.3% of non-diarrhoeic buffalo calves were infected with *C. parvum* (Ribeiro et al., 2000). A study by Santin et al. (2004) identified *C. parvum* in only 1% of the samples from post-weaned dairy calves. The reported prevalence of *Cryptosporidium* spp. infection in the above-mentioned studies were relatively low. In the

present study, 8 healthy buffalo calves (2.5%) were found to be shedding oocysts of *Cryptosporidium* spp in feces. *Cryptosporidium* spp. are common parasites in humans, domestic animals and wild vertebrates (Fayer, 2010). Cattle have been reported as the primary hosts for *Cryptosporidium* species and pre-weaned calves are major hosts of *C. parvum* (Santin et al., 2004). However, buffalo calves may also pose a risk of zoonotic transmission to humans and should be considered as a potential source of infection to other animal species and may represent a potential risk for the contamination of the surface waters.

There are very limited studies on the epidemiology of *Giardia* spp. in buffalo. The prevalence of *G. duodenalis* is quite high in young calves (O Handley, 1999; Huetink et al., 2001). Though, very few reports on the prevalence of this parasite in buffalo calves are available. In Italy, 18.1% of water buffalo calves had antigens of *G. duodenalis* in their fecal samples. In addition, in 30.0% of buffalo farms the presence of copro-antigens of *G. duodenalis* has been detected (Rinaldi et al., 2007). However, Rinaldi et al. (2007) used different diagnostic techniques (detection of copro-antigens rather than detection of *Giardia* cysts) and different epidemiological approaches (geographical information system) in their study and it is difficult to compare their findings with those reported by others. In another study using molecular analysis of the *Cryptosporidium* small subunit ribosomal DNA gene and of the *Giardia*  $\beta$ -giardin gene, 26.3% and 14.0% of fecal samples from buffalo calves were positive for *Giardia* spp. and *Cryptosporidium* spp., respectively (Caccio et al., 2007). A positive association between the positivity to *G. duodenalis* in buffalo calves and the presence of sheep on farms has been observed, indicating that transmission between sheep and water buffalo can occur (Rinaldi et al., 2007; Caccio et al., 2007). Workers in Italy have concluded that water buffalo can contribute to environmental contamination with oocysts of *Cryptosporidium* spp. and cysts of *Giardia* spp. (Caccio et al., 2007). In the region of the present study, Dalir-Naghadeh et al. (2008) found *Giardia* cysts in feces of 35.3% of diarrheic and 8% of non-diarrheic calves. However, we did not find *Giardia* cysts in the feces of buffalo calves. It seems that the buffalo is not a suitable host for *Giardia* spp. In the area of this study, usually buffalo use common ranges with cattle, and occasionally, with sheep and goats. Further studies are needed to investigate the status of infection to *Giardia* spp. in buffalo, cattle, sheep and goats on farms and ranges where a mixed population of livestock are kept or grazed.

*T. vitulorum* is found mostly in tropical and subtropical climates in very young calves and buffalo calves; however, the prevalence of this parasite has greatly increased recently in countries like the USA and emerged in countries like the

Netherlands (Davila et al., 2010; Borgsteede et al., 2012). Several studies were performed on the rate of *T. vitulorum* infection in buffalo calves. *T. vitulorum* eggs were detected in fecal samples of 23% of 99 buffalo calves (Samizadeh-Yazd et al., 1981). In another study, the rate of infection to *T. vitulorum* in buffalo calves was 15.8% (Tavassoli, 1999). Infection with this parasite has been reported in more than 75% of the calves examined in Bangladesh, Burma, Ceylon, India, and Nigeria, 66% in Egypt and 58% in Burundi and Thailand (Selim et al., 1966; Thienpont and Kedeyser, 1981; Srikitjakaran et al., 1987). In the present study, the prevalence of *T. vitulorum* infection (7.3%) was lower than those from earlier studies conducted in Iran and the above-mentioned countries. The increased usage of anthelmintic by farmers in recent years may be among the probable cause of this finding. Calves become infected by ingesting infected larvae from an infected cow's milk. The larvae are present in greatest numbers in the colostrum 2-5 d after calving. Mature worms are present in the intestine of ten-day-old calves and eggs are passed by 3 weeks of age. Adult worms are expelled by the age of 5 months (Radostits et al., 2007). Since *T. vitulorum* affects mainly water buffalo, to prevent further infections and dissemination of this parasite, introduction of buffalo, in particular pregnant or recently calved buffalo and calves less than 6 months of age, from endemic areas into other regions should be limited. Also, it is recommended that all young calves (less than 6 months of age) be treated with an effective anthelmintic to control this parasite.

In conclusion, the present study showed that various species of *Eimeria*, *Cryptosporidium* spp and *T. vitulorum* are relatively common in buffalo calves. Cross-species transmission of some parasites can occur between water buffalo and other animal species sharing the same ranges or housing. Therefore, buffalo can contaminate, pastures, rangelands and forage and they should be considered as a potential reservoir and a source of infection to other species, including humans. Further detailed studies are needed to evaluate the risk factors predisposing buffalo gastrointestinal parasitic infections.

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