

# Prevalence and Associated Risk Factors of *Toxocara vitulorum* Infections in Buffalo and Cattle Calves in Three Provinces of Central Cambodia

Pierre Dorny<sup>1,2,\*</sup>, Brecht Devleesschauwer<sup>1</sup>, Valérie Stolaroff<sup>1</sup>, Meas Sothy<sup>3</sup>, Rortana Chea<sup>3</sup>, Bunthon Chea<sup>3</sup>, Hor Sourloing<sup>3</sup>, Sum Samuth<sup>3</sup>, Seth Kong<sup>3</sup>, Koemseang Nguong<sup>3</sup>, San Sorn<sup>4</sup>, Davun Holl<sup>4</sup>, Jozef Vercruysse<sup>1</sup>

<sup>1</sup>Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium; <sup>2</sup>Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; <sup>3</sup>Division of Research and Extension, Royal University of Agriculture, Phnom Penh, Cambodia; <sup>4</sup>National Veterinary Research Institute, Department of Animal Health and Production, Phnom Penh, Cambodia

**Abstract:** The prevalence and associated risk factors of *Toxocara vitulorum* infection in buffalo and cattle calves was studied in 3 provinces in central Cambodia. Fecal samples were collected from 517 calves between the age of 1-15 weeks and processed for nematode egg counts by a modified McMaster method. A total of 64 calves were found to excrete *T. vitulorum* eggs in their feces (12.4%; 95% exact CI: 9.7-15.5). The mean fecal egg count was 2,798 EPG (SD=16,351; range=0-224,400). A multivariable generalized linear mixed model showed higher odds of *T. vitulorum* infection for buffalo versus cattle, for animals aged 4-8 weeks versus younger and older ones, and for animals with strongyle infection. There was no association with fecal consistency. Farmers should be aware of the potential impact of *T. vitulorum*, and treat their calves at the age of 2-3 weeks with anthelmintics such as benzimidazoles or pyrantel.

**Key words:** *Toxocara vitulorum*, cattle, buffalo, prevalence, risk factor, Cambodia

## INTRODUCTION

*Toxocara vitulorum* is an intestinal ascarid parasite of cattle and water buffaloes (*Bubalus bubalis*). Although the parasite may occur worldwide, it is of particular economic importance in tropical and subtropical regions, mainly due to lacking or inefficient control [1]. *T. vitulorum* causes morbidity and mortality in calves, which typically become infected early post-partum by ingesting larvae excreted in the colostrum and milk [2]. After a prepatent period of 3-4 weeks, the ingested larvae have matured to adult worms in the calf's duodenum that produce a large number of eggs, during a patent period of about 4 weeks. At the age of 8 weeks, most infected calves are able to clear the parasite due to strengthened and acquired immunity [3].

Cambodia, a Southeast Asian country, has a tropical climate characterized by distinct rainy and dry seasons. Agriculture is

the major work sector in Cambodia, and within this sector, livestock is the third largest subsector, behind crop production and fisheries [4]. Cattle (mainly *Bos indicus*) and water buffaloes (*Bubalus bubalis*) provide draught power, manure used as fertilizer or biogas, and increasingly, animal protein and income from trade [5]. Nevertheless, the ruminant sector is characterized by smallholder farmers rearing limited numbers of animals in traditional production systems. As a result, the animals are highly susceptible to endemic diseases, including *T. vitulorum* and other gastrointestinal parasites [6], resulting in substandard agricultural output.

The aim of this study was to investigate the prevalence and associated risk factors of *T. vitulorum* infection in buffalo and cattle calves in Cambodia.

## MATERIALS AND METHODS

### Study design

The study was conducted in 3 provinces, i.e., Pursat, Kampong Chhnang, and Kampong Cham, located in central Cambodia from June till October 2011. In each province, 6 communes were randomly selected and local farmers were asked for their cooperation. Fecal samples were collected from calves

•Received 25 November 2014, revised 21 January 2015, accepted 12 March 2015.

\*Corresponding author (pdorny@itg.be)

© 2015, Korean Society for Parasitology and Tropical Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

between the age of 1 and 15 weeks. The age of the animals was registered as either  $\leq 28$  days, 28-56 days, or  $> 56$  days.

### Fecal examination

Each fecal sample was examined for the presence of eggs of *T. vitulorum* and strongyle eggs and *Eimeria* spp. oocysts by the McMaster technique with a sensitivity of 50 eggs per gram of feces (EPG) [7]. The consistency of the fecal sample was scored as being either normal, or soft, or watery.

### Statistical analysis

*T. vitulorum* prevalence and corresponding exact 95% confidence intervals (CI) were calculated for the entire sample and for the different provinces, species, age groups, fecal consistencies, and co-infection categories. Additionally, the results of the *T. vitulorum* fecal egg counts were summarized by their arithmetic mean and SD.

Association of *T. vitulorum* prevalence with species, age group, fecal consistency, and co-infection category was assessed with a generalized linear mixed model for binary data, using adaptive Gaussian quadrature with 25 quadrature points. Commune was included as a random effect. A final multivariable model was obtained through a backwards selection procedure, at a significance level for removal of 5%. The

analysis was performed in R 3.0.2 using the lme4 package [8,9].

## RESULTS

A total of 517 animals were subjected in this study. Of these, 64 were found to excrete *T. vitulorum* eggs in their feces (12.4%; 95% exact CI: 9.7-15.5). The mean fecal egg count was 2,798 EPG (SD= 16,351; range= 0-224,400). Table 1 shows descriptive statistics by the province, species, age group, fecal consistency, and co-infection category.

The final multivariable generalized linear mixed model showed a higher odds of *T. vitulorum* infection for buffalo versus cattle, for animals aged 4-8 weeks versus younger and older ones, and for animals with strongyle infection (Table 2). There was no association with fecal consistency.

## DISCUSSION

To our knowledge, this is the first report on *T. vitulorum* infections in buffalo and cattle calves from Cambodia. Prevalence estimates of *T. vitulorum* in neighboring countries also appear to be rare. Compared to those countries, the prevalence observed in our study is relatively low (12.4%). In Thailand,

Table 1. Descriptive statistics of *Toxocara vitulorum* infection in Cambodian calves per province, host species, age group, faecal consistency, and co-infection category

Variable	Sample size (%)	Positive (%; 95% exact CI)	Mean EPG (SD)
Province			
Pursat	239 (46.2)	34 (14.2; 10.1-19.3)	3,638 (17,942)
Kampong Chhnang	155 (30.0)	14 (9.0; 5.0-14.7)	1,154 (7,148)
Kampong Cham	123 (23.8)	16 (13.0; 7.6-20.3)	3,237 (20,815)
Species			
Buffalo	169 (27.8)	34 (20.1; 14.4-27.0)	6,053 (22,038)
Cattle	348 (72.1)	30 (8.6; 5.9-12.1)	1,218 (12,440)
Age (days)			
$\leq 28$	59 (11.4)	7 (11.9; 5.0-22.9)	1,943 (10,778)
28-56	143 (27.7)	32 (22.4; 15.8-30.1)	7,756 (28,499)
$> 56$	315 (60.9)	25 (8.0; 5.2-11.5)	708 (5,906)
Faecal consistency			
Normal	374 (72.5)	44 (11.8; 8.7-15.5)	2,757 (14,883)
Soft	117 (22.7)	18 (15.4; 9.4-23.2)	2,953 (21,306)
Watery	25 (4.8)	2 (8.0; 1.0-26.0)	2,804 (10,530)
Strongyle infection			
No	182 (35.2)	39 (21.4; 15.7-28.1)	5,846 (25,234)
Yes	335 (64.8)	25 (7.5; 4.9-10.8)	1,142 (7,755)
<i>Eimeria</i> infection			
No	272 (52.6)	35 (12.9; 9.1-17.4)	3,453 (19,398)
Yes	245 (47.4)	29 (11.8; 8.1-16.6)	2,071 (12,102)

CI, confidence interval; SD, standard deviation.

Table 2. Estimates of generalized linear mixed model

Variable	Estimate (SE)	OR (95% Wald CI)	P-value
Species			
Buffalo	reference	-	-
Cattle	-1.295 (0.3127)	0.273 (0.148-0.504)	<0.001
Age (days)			
≤28	-0.874 (0.4919)	0.417 (0.159-1.094)	0.076
28-56	reference	-	-
>56	-1.150 (0.3203)	0.317 (0.169-0.593)	<0.001
Strongyle infection			
No	reference	-	-
Yes	-1.225 (0.3059)	0.294 (0.161-0.535)	<0.001

SE, standard error; OR, odds ratio; CI, confidence interval.

Srikitjakarn et al. [10] found a *T. vitulorum* prevalence of 58% in calves during their first 3 months of life. More recently, Holland et al. [11] observed a prevalence of 8% in Vietnamese calves aged 1-2 months, and Rast et al. [1] found a prevalence of 22.6% in buffalo and cattle calves aged <3 months in northern Lao PDR.

In our study, the main risk factors for *T. vitulorum* infection appeared to be host species and, not surprisingly, age. The apparent association with strongyle prevalence might be explained as an artefact due to the collinearity between strongyle infection and age (results not shown). Indeed, strongyle infections tend to be more common in older animals.

Roberts [12] reported clinical signs in toxocariasis, especially in buffalo calves. They included, poor hair coat, eczema, stools resembling white scour and having a foul smell, inappetency with intermittent colic and bloat [12]. More recent literature reports that calves with toxocariasis could have either pale colored or black diarrhea, or could be asymptomatic [13,14]. In our study, there was no association between *T. vitulorum* infection and fecal consistency. Further studies are needed to determine the contribution of *T. vitulorum* towards the overall clinical impact of disorders in calves of less than 3 months.

In conclusion, *T. vitulorum* is important in Cambodia, especially in buffalo cattle and animals between 4-8 weeks of age. Farmers should be aware of the potential impact of this parasite, and treat their animals appropriately. Anthelmintic treatment with either pyrantel or benzimidazoles at the age of 2-3 weeks has proved to be very effective in eliminating *T. vitulorum* both in cattle and buffalo calves in Cambodia (data not shown) and should be recommended to livestock owners in order to prevent potential pathologies caused by this parasite.

## ACKNOWLEDGMENTS

This study was carried out within the framework of the Parasitology Project in Cambodia funded by the Flemish Interuniversity Council (VLIR) and implemented at the Royal University of Agriculture (RUA) and the National Veterinary Research Institute (NaVRI) in Phnom Penh. The authors would like to express their gratitude to the cattle owners for their kind cooperation, as well as to the technicians and students from RUA and NaVRI for their assistance during the field sampling and the laboratory analysis.

## CONFLICT OF INTEREST

The authors report no conflict of interest related to this work.

## REFERENCES

1. Rast L, Lee S, Nampanya S, Toribio JA, Khounsy S, Windsor PA. Prevalence and clinical impact of *Toxocara vitulorum* in cattle and buffalo calves in northern Lao PDR. *Trop Anim Health Prod* 2013; 45: 539-546.
2. Roberts JA, Fernando ST, Sivanathan S. *Toxocara vitulorum* in the milk of buffalo (*Bubalus bubalis*) cows. *Res Vet Sci* 1990; 49: 289-291.
3. Roberts JA. The life cycle of *Toxocara vitulorum* in Asian buffalo (*Bubalus bubalis*). *Int J Parasitol* 1990; 20: 833-840.
4. Young JR, O'Reilly RA, Ashley K, Suon S, Leoung IV, Windsor PA, Bush R.D. Impacts on rural livelihoods in Cambodia following adoption of best practice health and husbandry interventions by smallholder cattle farmers. *Transbound Emerg Dis* 2014; 61 (suppl 1): 11-24.
5. Nampanya S, Suon S, Rast L, Windsor PA. Improvement in smallholder farmer knowledge of cattle production, health and

- biosecurity in Southern Cambodia between 2008 and 2010. *Transbound Emerg Dis* 2012; 59: 117-127.
6. Dorny P, Stolaroff V, Charlier J, Meas S, Sorn S, Chea B, Holl D, Van Aken D, Vercruysse J. Infections with gastrointestinal nematodes, *Fasciola* and *Paramphistomum* in cattle in Cambodia and their association with morbidity parameters. *Vet Parasitol* 2011; 175: 293-299.
  7. Thienpont D, Rochette F, Vanparijs OFJ. Diagnosing helminthiasis by coprological examination. Beerse, Belgium. Janssen Research Foundation. 1986, p 205.
  8. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014. <http://www.R-project.org/>.
  9. Bates D, Maechler M, Bolker B, Walker S. lme4: linear mixed-effects models using Eigen and S4. R package version 1.0-6. 2014. <http://CRAN.R-project.org/package=lme4>.
  10. Srikitjakarn L, Löhr KE, Leidl K, Hörchner F. Metaphylactic deworming program for buffalo calves (*Bubalis bubalis*) in North-East Thailand. *Trop Med Parasitol* 1987; 38: 191-193.
  11. Holland WC, Luong TT, Nguyen LA, Do TT, Vercruysse J. The epidemiology of nematode and fluke infections in cattle in the Red River Delta in Vietnam. *Vet Parasitol* 2000; 93: 141-147.
  12. Roberts JA. *Toxocara vitulorum* in ruminants. *Vet Bulletin* 1993; 63: 545-568.
  13. Jones JR, Mitchell ESE, Redman E, Gilleard JS. *Toxocara vitulorum* infection in a cattle herd in the UK. *Vet Rec* 2009; 164: 171-172.
  14. Starke-Buzetti WA. *Toxocara vitulorum* in livestock. In: Holland CV, Smith HV, eds., *Toxocara: The Enigmatic Parasite*. Wallingford, UK. CAB International. 2006, p 260-277.