

## Prevalence of round worm (*Toxocara vitulorum*) in Balinese cattle calves in the western region of Timor-Leste

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### Introduction

*Toxocara/Neosascaris vitulorum* is a large, creamy-white round worm up to 30 cm long and 5mm in diameter. *Toxocara vitulorum* is widely distributed, serious parasites of young cattle, especially in tropical countries where the climate is favorable. The prevalence of this parasite reported in bovine calves were over 30 % (Devi *et al.*, 2000, 817-819). The mortality rates of calves ranged from 21% to 50% (Makundi *et al.*, 1996, 109- 112). The prevalence of this parasite in Timor Leste was first reported by Amaral (2003, 38). In the pilot study conducted by Amaral (2003, 38), it was found that the prevalence of *T. vitulorum* was 54%. However, the study sites for the pilot study were few and it was not conducted based on sound epidemiological knowledge. Therefore the purpose of this survey was to measure the prevalence of *T. vitulorum* in Western Region of Timor Leste. This survey was conducted following the recommendation made by Amaral (2003, 38) to do a national survey on *T. vitulorum* with adequate sample size based on sound epidemiological knowledge.

### Materials and methods

#### *Site selection*

Administratively, Timor Leste is divided into, 13 districts, 65 sub districts, 442 Sucos and 2225 aldeias (Hamlets)(NSD and UNFPA, 2010). For this survey, the division of administrative area is based on the recommendation by Amaral (2003, 41), who divides Timor Leste into three regions. The regions are 1) East Region (composed of the districts of Baucau, Viqueque and Lautem), 2) Central region (composed of Dili, Liquica, Alleu, Ermera and Manatuto districts), 3). West region (composed of Bobonaro, Ainaro, Manufahi and Covalima districts). The site selection for this survey was done for districts in Western region. Out of districts in West region, two districts were randomly selected, and then of the selected districts, one sub district was randomly selected for each district and lastly of the selected sub districts, two villages/sucos were randomly selected for faeces sample collection for each district. Of the selected sucos, at least half of the numbers of aldeias were covered. The survey was conducted from 16 - 18 April 2014 in Bobonaro district and 11 - 13 April 2014 in Covalima districts.

#### *Design prevalence and sample size*

The sample size of this survey was based on the design prevalence of 50%. This assumption of design prevalence was based on the prevalence of *T. vitulorum* reported by Amaral (2003), which was 54% (32.8% - 74.4%, 95%CI). According to Cannon & Roe (1982, 21), if the design prevalence is 50%, then the sample size is 96.

#### *Collection of samples*

Based on the sample size determined, 96 faeces samples were collected evenly from each selected sucos based on non-random sampling (sample collectors work from house to house and farm to farm asking if farmers have calves aged under one-year-old. Samples were obtained from calves of Bali cattle aged under one-year-old. Fresh faecal samples were collected from calves of Bali cattle aged under one-year-old whose faeces were just dropped at the time of sample collection or collected directly from the rectum of calves. Each sample was placed in an individually labelled plastic bag with some drop of formalin for preservation of the faeces. They were then analysed for faecal egg counts in National Veterinary Diagnostic Laboratory of Timor Leste using the McMaster technique.

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The number of eggs of *T. vitulorum* in fresh collected faeces were expressed as eggs per gram faeces (EPG) (Bryan and Kerr, 1989a,315).

#### Faecal examination

Saturated salt solution was made by dissolving salt (sodium chloride) in a container of water until some remained in the bottom. One gram of faeces was weighed (W) and placed in the bottom of a plastic cup. Fourteen ml of saturated salt solution was added to the cup to make up 15 mL of solution (V). This was then mixed and 1 ml or less was then sucked up using a Pasteur pipette and 0.3 ml (E) placed into two chambers of a McMaster faecal egg counting slide. After five minutes, the slide was examined with a microscope under low power. *Toxocara vitulorum* egg was then recorded.

Eggs per gram faeces calculation:

Weight of faeces= 1 g (W)

Volume of solution= 15 ml (V)

Volume examined= 0.3 ml (E)

1 gram faeces in 15 ml fluid (V/W) = X

Proportion examined= 1/(X/E) = 1/(15/0.3)= 1/50, therefore, eggs observed multiplied by 50 = EPG

#### Prevalence calculation and its 95% Confidence Intervals (95%CI)

Prevalence was calculated based the number of positive samples divided with total samples tested. The total sample tested was 192 (at region level), 96 (at district level) and 48 at village level. The 95% confidence interval (95%CI) was calculated using the exact binomial method (Daly, 1992, 351).

## Results and discussion

### Results - Prevalence based on sites

**Table 1** - Prevalence of *T. vitulorum* in each sites

District	Suco	Lab. Test		Total	Prevalence (95% CI)
		Negative	Positive		
Bobonaro	Manapa	38	10	48	20.83% (10.5%-35.0%)
	Meligo	45	3	48	6.25% (1.3%-17.2%)
	<b>Total</b>	<b>83</b>	<b>13</b>	<b>96</b>	<b>13.54%</b> <b>(7.41% - 22.04%)</b>
Covalima	Beiseuk	45	3	48	6.25% (1.3%-17.2%)
	Maudemu	44	4	48	8.33% (2.3%-20.0%)
	<b>Total</b>	<b>89</b>	<b>7</b>	<b>96</b>	<b>7.29%</b> (2.98%- <b>14.45%</b> )
Total Bobonaro and Covalima (West region)		<b>172</b>	<b>20</b>	<b>192</b>	<b>10.42%</b> <b>(6.48% - 15.63%)</b>

**Table 2** - Prevalence based on farming system

Districts	Suco	Farming system	Laboratory Test		Total	Prevalence (95% CI)
			Negative	Positive		
Bobonaro	Manapa	Free range	18	5	23	21.74%
		Tied up	4	3	7	42.86%
		Locked	16	2	18	11.11%
		<b>Total</b>	<b>38</b>	<b>10</b>	<b>48</b>	<b>20.83%</b> (10.5% -35.0%)
	Meligo	Tied up	3	0	3	0.00%
		Locked	42	3	45	6.67%
<b>Total</b>		<b>45</b>	<b>3</b>	<b>48</b>	<b>6.25%</b> (1.3%-17.2%)	
Total Bobonaro			<b>83</b>	<b>13</b>	<b>96</b>	<b>13.54%</b> (7.41% - 22.04%)
Covalima	Beiseuc	Free range	4	0	4	0.00%
		Tied up	1	0	1	0.00%
		Locked	40	3	43	6.98%
		<b>Total</b>	<b>45</b>	<b>3</b>	<b>48</b>	<b>6.25%</b> (1.3%-17.2%)
	Maudemo	Free range	2	0	2	0.00%
		Tied up	4	2	6	33.33%
		Locked	38	2	40	5.00%
		<b>Total</b>	<b>44</b>	<b>4</b>	<b>48</b>	<b>8.33%</b> (2.3%-20.0%)
Total Covalima			<b>89</b>	<b>7</b>	<b>96</b>	<b>7.29%</b> (2.98%-14.45%)
Total Bobonaro & Covalima			<b>172</b>	<b>20</b>	<b>192</b>	<b>10.42%</b> (6.48% - 15.63%)

**Table 3**- Eggs per gram faeces (EPG) in each site of Bobonaro and Covalima district

District	EPG	Suco		Total
		Manapa	Meligo	
Bobonaro	0	38	45	83
	50	5	2	7
	100	2	1	3
	150	1	0	1
	300	1	0	1
	400	1	0	1
	<b>Total</b>	<b>48</b>	<b>48</b>	<b>96</b>
Covalima	EPG	Beiseuc	Maudemo	Total
	0	45	44	89
	50	0	1	1
	100	1	0	1

	150	1	1	2
	400	0	1	1
	2150	1	0	1
	10100	0	1	1
	<b>Total</b>	<b>48</b>	<b>48</b>	<b>96</b>

## Discussion

*Transmission:* *Toxocara vitulorum* is transmitted to calves through the milk (transcolostral infection) and to both cows and calves through ingestion of contaminated pasture (Hansen and Bryan, 1994). This is because adult females lay eggs in the small intestine of the host (*Bos taurus*, *Bubalus bubalis* and *Bos indicus*) that are shed with the faeces. Adult cattle become infected after swallowing embryonated eggs. Larvae then emerge from the eggs in the intestine, penetrate the intestine and migrate via bloodstream to liver, lungs, trachea, mouth, oesophagus and back to intestine, where they will adults and produce eggs.

*Mortality rate:* Once the host is infected, it can cause high mortality rate. The mortality rate of Bali cattle calves due to this parasite in this region is not known, but the mortality rate reported in other countries is high. Mortality rates vary from 11 to 50% among countries. In Thailand for instance, it was reported that the mortality rate was 32% (Srikitjakarn *et al.*, 1987). In Bangladesh it was reported that almost all buffalo calves were infected with *T. vitulorum* and it was a major cause of calf mortality (Mia *et al.*, 1975, 153). During a 3 year observation (1988 to 1991) it was found that the mortality rates of calves ranged from 21% to 50 % respectively (Makundi *et al.*, 1996, 109- 112). Mortality rate of buffalo calves was 11% (n=90) (Srivastava and Sharma, 1981, 160-162). The high mortality rate in young calves results in huge economic losses (Shanker *et al.*, 1998, 598) mainly due to dead of calves and weight loss (Enyenihi, 1969, 171).

*Toxocara vitulorum* can infect several species. The species affected by *T. vitulorum* are *Bos taurus*, *Bubalus bubalis* and *Bos indicus* (Keith, 1951, 129; Patnaik and Pande, 1963, 128; Rao *et al.*, 2000, 79). Of the species affected *T. vitulorum* can infect both sexes. However, the life cycle will only be completed if it infects female animals, and the cows become pregnant (Akyol, 1993, 73). Worm burdens differ between buffalo and cattle in that worm burdens of buffalo calves are higher compared with cattle calves (Pholpark and Srikitjakarn, 1989, 243). The prevalence rate is also different between buffalo calves and bovine calves. In Bihar India, for example the prevalence of *T. vitulorum* in buffalo calves was higher (53.17%) compared to bovine calves (34.14%) (Devi *et al.*, 2000, 817). The overall infection rate for both species was 42.98% (n=542, 290 bovine and 252 bubaline).

*Eggs production:* Animals infected with *T. vitulorum* under bush conditions ranges from 10,000 to 30,000 per gram of faeces (Lee, 1955, 146). An experiment in Ghana showed that infected calves aged 2 to 41 days produced over 18,000 EPG (Agyei, 1991, 134), this value of EPG is between the range of what Lee (1955) reported. The EPG in western Region of Timor Leste in this study ranged from 50 to 10,200 EPG (Table 3). The EPG found in this study is also within the range of EPG reported by (Lee, 1955, 146-149).

*Prevalence:* The prevalence of *T. vitulorum* differs from one country to another. In Turkey for instance, it was reported that the average prevalence of this parasite was 5.1% (in calves younger than 6 months) (Akyol, 1993), whereas in India it was reported that the prevalence of *T. vitulorum* was 43% (Devi *et al.*, 2000, 817). The prevalence of this parasite in Timor Leste according to a pilot study conducted by Amaral (2003, 38) was 54%. The prevalence of *T. vitulorum* in Western region of Timor Leste according to this present study (Table 1 and 2) was 10.42% (6.48% - 15.63%, 95% CI). Of the 10.42% prevalence, 3.65% (1.48% - 7.37%, 95% CI) was found in Covalima and 6.77% (3.65% - 11.30%, 95%CI) was found in Bobonaro. At district level, the lowest prevalence of *T. vitulorum* was found in the district of Covalima 7.29% (2.98%-14.45%, 95%CI) and the highest was found in the district of Bobonaro 13.54% (7.41% - 22.04%, 95%CI). At the village level, the lowest

prevalence [6.25% (1.31% - 17.20%, 95%CI)] was found in village/suco of Meligo in sub district of Cailaco, Bobonaro district and village/Suco Beiseuc in Subdistrict of Tilomar, district of Covalima and the highest prevalence at village level 20.83% (10.47% - 34.99%, 95%CI) was found in the village of Manapa, at Bobonaro district.

*Prevalence based on the farming system:* The total samples collected (n=192), most of them (76%) are taken from calves that are locked followed by free range (15%) and tied up (9%). Of the total samples collected (192), 20 were positive of *T. vitulorum*. Of these 20 samples, 25% was from free range, another 25% from those calves that are tied up and 50% from calves that are locked (Table 2). The high percentage of positive sample from those calves that are locked could mean that calves under this management system has high risk of having *T. vitulorum* but also can be due to the fact that most samples collected were from this management system.

*Age at risk and species affected by T. vitulorum:* The prevalence of *T. vitulorum* in West region of Timor Leste **10.42%** (6.48% - 15.63%) is actually lower than the prevalence of bovine calves (34.14%) reported in Devi *et al.*, (2000, 817-819) & Makundi *et al.*, (1996, 109). This is probably due to the fact that from 192 samples collected, the age of Bali cattle between one and 3 months is only 18%, the rest 80 are from the age of 4 to 6 month and 2% from the age of 7 to 11 months old. The prevalence and intensity of *T. vitulorum* infection has been reported to vary according to the age of calves. The prevalence was found to be highest (70-75%) in 2 month old calves, intermediate in 1 - 1.5 month old calves (45 %) and was lowest in calves older than 3 months (10 %) (Makundi *et al.*, 1996, 109).

## Conclusion

From this finding it is clear that *T. vitulorum* is present in West Region of Timor Leste. *Toxocara vitulorum* prevalence in Western region of Timor Leste was 10.42% (6.48% - 15.63%). This prevalence rate was actually lower than the prevalence rate reported by Amaral (2003, 38) and prevalence reported by other countries but this could be due to the fact that the age of calves in this study mostly (80%) aged between 4 to 6 months.

## Recommendations

- It is recommended to MAF to do treatment targeting young calves younger than 6 months to prevent high mortality in young calves
- It is recommended to identify some traditional medicine that has been used by some farmers to see their efficacy in worm treatment

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