



Epidemiology and pathology of onchocercosis of cattle in Bangladesh

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Abstract

Onchocercosis is a parasitic disease caused by the different species of nematodes belonging to the genus *Ochocerca* affecting both humans and animals. *Ochocerca armillata*, *O. gutturosa* and *O. gibsoni* affect mainly cattle, and also buffaloes. Here, we studied epidemio-pathology and vector flies of onchocercosis in cattle in Bangladesh. Out of 310 animals examined, 163 (52.6%) were infected with different species of *Onchocerca*. Three species of *Onchocerca*, namely, *Onchocerca armillata* (24.8%), *Onchocerca gutturosa* (24.7%) and *Onchocerca gibsoni* (13.3%) were identified. We recovered *O. armillata* only from aorta, *O. gutturosa* from the *ligamentum nuchae* and *O. gibsoni* from the subcutaneous nodules. Nodular lesions were detected at the neck (49/20.9%), abdomen (5/2.1%), flank (8/3.4%), brisket (33/14.0%) and thigh/legs (32/13.6%) regions. We observed age as a potential diterminant in the occurrence of the infection. Prevalence of *O. armillata* and *O. gutturosa* was significantly higher in older animals (> 4 years) than yearlings (1-2 years). Sex, breed and rearing system did not significantly influence the prevalence of bovine onchocercosis. Our study revealed that seasons of the year was another important determinant for onchocercosis, and prevalence was the highest in the summer. Grossly, the affected aorta was thickened, dilated and with elevated intima. Histological examination revealed parasitic granuloma present in blood vessels characterized by the infiltration of eosinophils and neumerous sections of the parasites. We identified only *Culicoides* spp., but no *Simulium* spp., as a vector from the study area. Vector flies were the most abundant in the early morning and least at noon, and mostly at the umbilical region of cattle. *Culicoides* spp. were most abundant in the winter. Our results suggest that occurrence of onchocercosis is fairly high in cattle in Bangladesh irrespective to age, sex, breed and management.

Key words: Bovine onchocercosis, epidemiology, pathology, vector fly, cattle

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Introduction

Onchocercosis is a parasitic disease caused by different species of *Onchocerca* (Nematoda: Filaridae) and transmitted by the flies of the genera *Simulium* and *Culicoides* affecting humans and animals. Numerous species have been described within the genus *Onchocerca*, however, three of which are commonly found to infect cattle and buffaloes, such as *Onchocerca armillata*, *Onchocerca gutturosa* and *Onchocerca gibsoni* (Achukwi *et al* 2004, Soulsby 1982). *O. armillata* is commonly found in the wall of the aorta, where the

other species are found in fibrous connective tissues around the *ligamentum nuchae* and subcutaneous tissues, mainly in the brisket region. In subcutaneous tissues, they produce honey comb like nodules. Although the nodules are commonly found in brisket, but may also be distributed throughout the body. These parasites may cause damage to the skin of the affected animals. Besides, onchocercal infection predisposes humpsore in cattle which is one of the major limiting factors of the market value of hides. Thus, onchocercosis is associated with great

economic losses. On the other hand, *O. armillata* affects the aortic wall resulting fatal outcome (Soulsby 1982, Mtei et al 1990, Nmorsi et al 2002, Beytuket et al 2005). Furthermore, zoonotic significance of bovine onchocercosis has recently been reported (Takaoka et al 2004).

Onchocercosis has been studied in many countries of the world such as North, Central and South Africa, Sudan, Puerto Rico, Turkey, India and Australia. Seddon (1967) recorded that 65% of the slaughtered cattle in Northern Australia were infected with *O. gibsoni*. Hussain et al. (1975) found 68.5% and 89.2% prevalence of *O. gutturosa* and *O. armillata*, respectively in Sudanese cattle. Alibasoglu et al (1969) observed that 80% cattle in Turkey were infected with *O. armillata*. Bwangamoi (1969) recovered onchocercal parasites from cattle in African countries and considered the parasites as economically important. *O. gutturosa* and *O. lienalis* have been recovered from British cattle (Trees et al 1987). Onchocercal infection was detected about half a century ago in cattle in Bangladesh (Wolfe and Aslamkhan, 1970, Haq et al. 1977 and Nooruddin et al. 1985). However, epidemiology of onchocercal infection and the pathological changes caused by them in cattle in Bangladesh are yet to be studied which are essential for the planning and execution of a fruitful control program more effectively. The present work was designed to study epidemiology and pathology of bovine onchocercosis in Bangladesh.

Materials and Methods

Ante-mortem examination: To study the epidemiology and pathology of onchocercosis, we conducted multi-stage, slaughter house-based, ante-mortem and post-mortem examinations. We randomly selected and examined 310 cattle. Before examination, age, sex, breed and information about rearing were recorded. Then animals were examined by parting of hair against their natural direction, digital palpation and simultaneous close inspection to detect suspected subcutaneous nodules. Size, shape, and consistency of these nodules along with their distribution on the body surface of the affected animals were recorded carefully.

Postmortem examination: Following skinning of the slaughtered cattle, subcutaneous tissues, and muscles were examined and subcutaneous nodules were collected carefully. Collected nodules were thoroughly examined and the gross pathology was recorded. Suspected subcutaneous nodules were preserved in 10% buffered neutral formalin for histopathological study. Muscle, fasciae, connective tissues, heart, great vessels (especially, aorta) and *ligamentum nuchae* were collected and examined carefully to detect parasites and the pathological changes.

Collection and identification of parasites: Suspected nodules were excised and kept in a Petridish containing normal saline. Parasites were collected and preserved in glycerin alcohol after gentle washing with normal saline. *Onchocerca* species were identified according to the keys and descriptions as per Soulsby (1982) and Yamaguti (1961) by preparing temporary slides adding one drop of lactophenol (Cable 1957).

Histopathological study: Formalin fixed tissue were processed, embedded in paraffin and thin (5µm) sections were prepared. The sectioned tissues were stained with haematoxylin and eosin following the procedures described by Luna (1968).

Detection of Vector: Suspected flies were collected and identified according to the keys and descriptions given by Soulsby (1982) and Cable (1957) preparing permanent slides.

Statistical analysis: Data were presented as mean ± SEM. For multiple group comparisons one-way ANOVA followed by post-hoc Bonferroni analysis was used. For direct comparisons, unpaired 2-tailed student's *t* test was employed. A value of $P < 0.05$ was considered as significant. Odd ratio was calculated according to the formula given by Schlessman (1982).

Results and Discussion

Overall prevalence of onchocercosis in cattle is fairly high in Bangladesh: Out of 310 animals examined, 163 (52.58%) were infected with *Onchocerca* spp. (Table 1). We identified three species of *Onchocerca*, namely, *O. armillata*, *O.*

gutturosa and *O. gibsoni* from cattle (Figure 1). Of the species identified, prevalence of *O. gutturosa* was the highest (86/27.7%) followed by that of *O. armillata* (77/24.8%) and *O. gibsoni* (41/13.3%). The mean parasitic burden of *O. armillata* (2.02±0.18) was higher than *O. gutturosa* (1.91±0.19) showing overall parasitic burden per nodule 1.97±0.13 (Table 1). Our findings were lower than those of Takaoka *et al* (2004), Anosike *et al* (2001), Hussain *et al* (1975) and Seddon *et al* (1967) who recorded 46-92% onchocercosis in cattle. In contrast, the present findings were higher than the findings of Pazhanivel *et al* (2006) who recorded 4.62% prevalence of bovine onchocercosis.

Table 1. Overall prevalence of *Onchocerca* spp. infection in cattle

Parasite	Animal infected (%)	Parasitic burden	
		Range	Mean±SE
<i>O. gutturosa</i>	86 (24.7) ^a	1-9	1.91±0.19
<i>O. armillata</i>	77 (24.8) ^a	1-8	2.02±0.18
<i>O. gibsoni</i>	41(13.3%) ^b	1-6	1.8±0.9
Total	163 (52.6)	0-9	1.97±0.13

n=310, values with the same superscript in the same column are statistically insignificant but values with different superscripts are statistically significant (p<0.05).

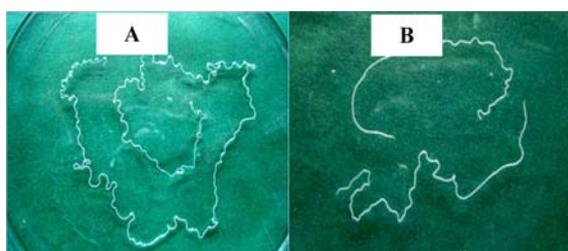


Figure 1. Parasites recovered from the subcutaneous nodules. Parasites were collected and preserved in glycerin alcohol after gentle washing with normal saline, and photographs were taken. (A) *O. gutturosa* (B) *O. armillata*.

The discrepancies among the present and previous studies might be due to variation in the geographical locations, climatic conditions of the experimental area, method of study and availability of vectors. Also, distribution of these three parasites were strictly organ specific, and we recovered *O. gutturosa* from *ligamentum nuchae*, *O. armillata*

from aorta and *O. gibsoni* from subcutaneous nodules.

Age significantly influences the prevalence of onchocercosis in cattle: We observed that age of the host had an effect on the prevalence of onchocercosis in cattle. In case of *O. gutturosa*, prevalence was significantly (p<0.01) higher in adult cattle (57.5%) than yearlings (40%). Calculated odds ratio implied that older cattle were 1.5 and 1.12 times more susceptible to *O. gutturosa* than yearlings and young, respectively (Table 2), which conformed to those of Anosike *et al* (2001). Anosike *et al* (2001) reported that prevalence of onchocercosis was significantly (p<0.05) higher in aged cattle (59.8%) than those in younger cattle (36.7%). Similarly, in case of *O. amillata*, prevalence was significantly (p<0.01) higher in adult cattle (55.2%) than yearlings (21.4%). Calculated odds ratio implied that older cattle were 4.51 and 1.08 times more susceptible to *O. amillata* than yearlings and young, respectively (Table 3). These findings conformed to the findings of Pazhanivel *et al* (2006), and they showed that cattle aged more than 2 years old were mostly affected (7.09%) with *O. armillata*. It is difficult to explain the exact mechanism how age of the animals influenced the prevalence of the parasites but it may be assumed that the exhausted immune system of the older animals had an effect in this regard.

Effect of sex of the animal on the occurrence of bovine onchocercosis is inconclusive: Effects of sex of the host on the prevalence of bovine onchocercosis caused by *O. gutturosa* were statistically insignificant. The present study revealed that prevalence of *O. gutturosa* infection was little bit higher in females (55.6%) than that in males (52.6%), and females were 1.12 times more susceptible to the infection (Table 2). This finding was supported by the findings of Anosike *et al* (2001) and Hussain *et al* (2000) who reported slightly higher prevalence of *O. gutturosa* infection in females. In contrast, prevalence of *O. armillata* was significantly (p<0.05) higher in females (56.1%) than that in males (44.9%), and females were 1.56 times more susceptible to *O. armillata* than male (Table 3). This finding was in agreement with the earlier findings of Pazhanivel *et al* (2006) who

reported significantly higher prevalence of *O. armillata* in females (6.57%) than males (0.69%). Also, Ebong *et al* (2007) reported the higher prevalence of onchocercosis in females (57.5%) than in males (42.5%). In the contrary, Okwa (2004) reported relatively higher prevalence of

onchocercosis in males (53.72%) than females (31.6%). However, Vivas *et al* (2000) reported almost similar prevalence of onchocercosis in males (42.5%) and females (41.5%). Therefore, it was difficult to consider sex of the animal as a leading determinant of the infection.

Table 2. Effect of age, sex and seasons on the prevalence of *O. gutturosa* infection in cattle

Parameters		Animal affected (%)	Parasitic burden	
			Mean± SE	Odds Ratio
Age	Yearling (1-2 yrs, n=15)	6 (40) ^a	0.87±0.35	Young vs yearlings= 1.8
	Young (>2- 4 yrs, n=97)	53 (54.6) ^b	2.14±0.24	Adult vs young=1.12
	Adult (>4yrs, n=47)	27 (57.5) ^b	2.13±0.32	Adult vs yearlings=1.5
Sex	Male (n=78)	41 (52.6) ^a	2.01±0.27	Female vs male=1.12
	Female (n=81)	45 (55.6) ^a	2.02±0.25	
Seasons	Rainy (n=60)	34 (56.7) ^a	1.95±0.27	Rainy vs winter=1.46
	Winter (n=72)	34 (47.2) ^b	1.72±0.26	Summer vs winter=2.26
	Summer (n=27)	18 (66.7) ^c	2.96±0.52	Summer vs rainy=1.52

n=310, values with the same superscript in the same column are statistically insignificant but values with different superscripts are statistically significant (p<0.05).

Table 3. Effect of age, sex and seasons on the prevalence of *O. armillata* infection in cattle

Parameters		Animals affected (%)	Parasitic burden	
			Mean ±SE	Odds Ratio
Age	Yearlings (1-2 yrs, n=14)	3 (21.4) ^a	0.71±0.44	Young vs yearlings=4.16
	Young (>2- 4 yrs, n=79)	42 (53.2) ^b	2.14±0.28	Adult vs young=1.08
	Adult (> 4yrs, n=58)	32 (55.2) ^b	1.90±0.27	Adult vs yearlings=4.51
Sex	Male (n=69)	31 (44.9) ^a	1.58±0.24	Female vs male=1.56
	Female (n=82)	46 (56.1) ^b	2.20±0.27	
Seasons	Rainy (n=68)	34 (50.0) ^a	1.81±0.27	Rainy vs winter=1.12
	Winter (n=66)	31 (46.9) ^a	1.76±0.28	Summer vs winter=2.7
	Summer (n=17)	12 (70.6) ^b	2.94±0.58	Summer vs rainy=2.5

n=310, values with the same superscript in the same column are statistically insignificant but values with different superscripts are statistically significant (p<0.05).

Breed and management systems did not significantly affect bovine onchocercosis: Our study revealed that the prevalence of onchocercosis in cattle was little bit higher in cross bred cattle (58.0%)

than in indigenous (50.4%) cattle (Figure 2). Prevalence of onchocercosis was almost similar in cattle reared in intensive system (59.1%) and in those reared in semi-intensive system (52.9%) (Figure 2),

indicating that breed and management system did not play vital roles in the prevalence of the disease.

Seasons of the year significantly influenced prevalence of onchocercosis: During this study, it was observed that seasons of the year had a profound effect on the prevalence of onchocercosis in cattle. In case of *O. gutturosa*, the prevalence was significantly ($p < 0.01$) higher in summer season (66.6%) followed by rainy (56.7%) and the lowest in winter (47.2%) seasons. Calculated odd ratio implied that in the summer season cattle were 2.26 and 1.52 times more susceptible to *O. gutturosa* infection than in winter and rainy seasons, respectively.

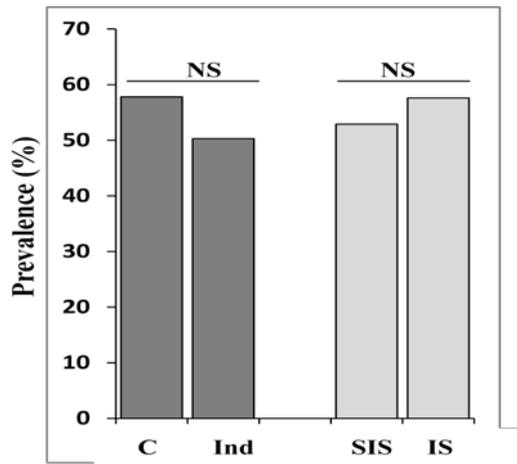


Figure 2. Effects of breed and management system on the prevalence of onchocercosis. We randomly selected and examined 310 cattle, and breed and information about rearing were recorded. Subcutaneous nodules were detected by clinical examinations. Parasites were collected and identified. Breed and management system did not play a vital role in the prevalence of the disease. C, cross-bred; Ind, indigenous; SIS, semi-intensive system; IS, intensive system, NS, non significant.

On the other hand, in rainy season, cattle were 1.46 times more vulnerable to *O. gutturosa* than in the winter (Table 2). Also, prevalence of *O. amillata* was significantly ($p < 0.01$) higher in summer season (70.6%) followed by rainy (50%) and winter (46.9%) seasons. And, in the summer season cattle were 2.7 and 2.5 times more susceptible to *O. amillata* than in winter and rainy seasons, respectively. In rainy season, cattle were 1.12 times more vulnerable to *O.*

armillata than winter season (Table 3). Our findings were in contrast to the findings of Pazhanivel et al (2006) who observed the highest prevalence of *O. armillata* during monsoon (6.73%). The variation among the present and previous studies might be due to the spatial variation and climatic changes. Onchocercosis is a fly-borne disease so temporal distributions of the infection observed in this study was more logical in our country contest.

Distribution of subcutaneous onchocercosis in cattle: Initially, we detected subcutaneous nodules through ante-mortem examinations then we confirmed subcutaneous onchocercosis by post-mortem examination and parasite collection from the suspected lesions, and subsequent identification of the parasites. Through detail ante-mortem and post-mortem examinations, we found nodular lesions of subcutaneous onchocercosis in neck 49 (20.85%), abdomen 5 (2.12%), flank 8 (3.40%), brisket 33 (14.04%) and thigh/legs 32 (13.61%) regions (Figure 3). It is difficult to explain exactly why nodular lesions were higher in the neck region, however, it may be due to the organ specificity.

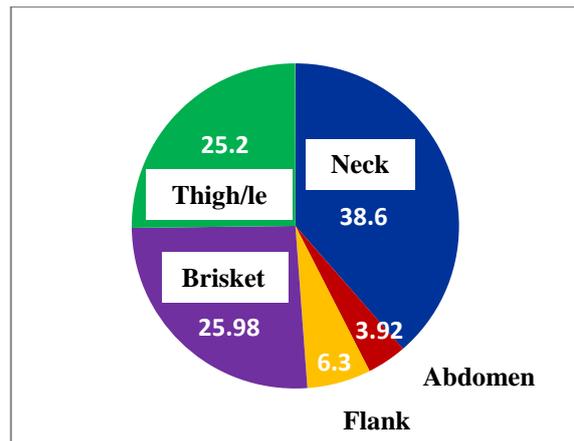


Figure 3. Distribution of nodules of subcutaneous onchocercosis on the body surface. Subcutaneous nodules from 310 randomly selected cattle were detected by clinical examinations. Parasites were collected and identified.

Gross and histopathological changes: Grossly, the affected aorta showed thickened, dilated and elevated tortuous tracts of atheromatous plaques on the intimal surface. The worms within a nodule were usually observed. These findings were similar with

the findings of Pazhanivel *et al.* (2006) who observed that the affected aorta became thickened, dilated, elevated and tortuous with the tracts of atheromatous plaques. Microscopically, numerous sections of the parasite (*O. armillata*) were found in the wall of aorta. Parasitic granulomas were infiltrated with eosinophils. Parasites were embedded or encysted in the inner most layer of blood vessel. Multifocal area of accumulation of macrophages and fibroblasts were found in the wall of aorta and *ligamentum nuchae*. This finding was supported by the findings of Solismaa *et al* (2008) and Kmmenou *et al* (2002) who reported mild to intense infiltration with eosinophilic granulocytes and multifocal nodular lymphoplasmacytic aggregations in aorta. There were granulomatous lesions with central necrotic tissue and cell detritus surrounded by eosinophilic granulocytes, lymphocytes, plasma cells and histiocytes and some multinucleated giant cells (Figure 4 A-D).

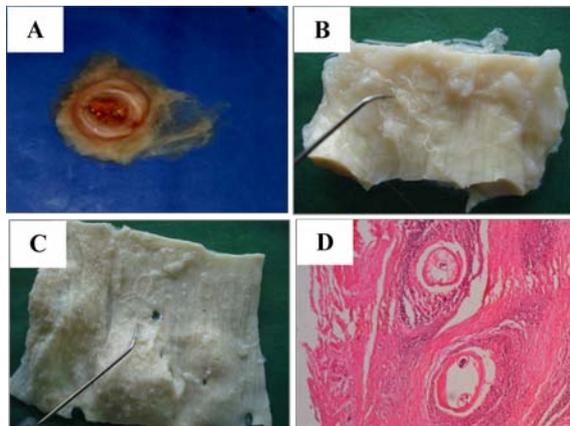


Figure 4. Gross and histopathological features of onchocercosis. (A-C) Gross features of onchocercosis. The slaughtered animals were skinned off and suspected tissues were collected. (A) Subcutaneous nodule, (B) *ligamentum nuchae* and (C) aortic lesion. (D) Histology of vascular onchocercosis. Heart and great vessels (especially, aorta) were collected and examined carefully to detect parasites and the pathological changes. Formalin fixed tissue were processed, and thin sections were prepared and stained with haematoxylin and eosin.

Culicoides spp. were detected of from the study areas: Plethora of literatures support that both *Culicoides* spp and *Simulium* spp act as a vector for bovine onchocercosis (Soulsby 1982 and Urquhart *et al* 1996). However, we could detect only *Culicoides* spp., but no *Simulium* spp., from the study areas (Figure 5). We also studied the temporal distribution of vector flies. Multiple visit to the same areas confirmed that *Culicoides* spp. were more abundant in the early morning followed by in the evening and least at noon. Flies mostly attacked to the neck and umbilical regions of the animals. Seasons also had significant effect on the abundance of vector flies. *Culicoides* spp. was most abundant in the winter followed by rainy season and the least in the summer.



Figure 5. Vector fly of onchocercosis. Vector flies were collected and identified. *Culicoides* spp. were detected from the study areas.

Our study revealed that the prevalence of onchocercosis was fairly common in cattle in Bangladesh. Age of the host and season of the year are the leading determinants which greatly influence the occurrence of the disease. Vector flies are available in our country and most abundant in winter season. Baseline data generated from the study will be helpful for the formulation of a fruitful control strategy.

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