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Prevalence and risk factors of helminth infections in cattle of Bangladesh

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Abstract

A cross-sectional survey was undertaken to identify risk factors and clinical signs associated with parasitic helminth infections of cattle in Mymensignh district of Bangladesh. A nonrandom convenience sampling method was used to select 138 animals from 40 farmers/herds. The eggs per gram of faeces (epg) for nematodes and trematodes were determined by McMaster and Stoll's methods respectively. Animal-level and herd-level data were recorded by means of a questionnaire. Multi-collinearity amongst explanatory variables were assessed using 2×2 X^2 test and one variable in a pair was dropped if $P \leq 0.05$ for multiple logistic regression models. Association study between outcome and explanatory variables was conducted using classification tree, random forests and multiple logistic regression. A positive epg was considered as infected. Analyses were performed using STATA®, version 8.0/Intercooled and R®, Version 2.3.0. Seventy eight percent of the cattle were found to be infected with at least one type of helminth. Twenty four pairs of combinations of explanatory variables showed significant associations. Male animals (OR=3.3, P=.006, 95% CI=1.4, 7.7) were associated with significantly increased prevalence of nematode infection. Female cattle of the study area are mostly cross-breed, kept indoor, fed relatively good diet and not used for draught purpose. Males are used for draught purpose thereby more exposed to nematode infective stage and provided with relatively poor diet. So stressed male cattle may become more susceptible to nematode infection. All of the three statistical techniques selected gender and rumen motility as most important variables in association with nematode infection in cattle. The result of this survey can only be extrapolated to the periurban cattle population of traditional management system.

Key words : Cattle, Helminth, Prevalence, Risk factors

INTRODUCTION

Inadequate feed, widespread diseases and inefficient extension services are considered among the most binding constraints of livestock development in Bangladesh (FAO STAT, 2003). The most important disease of cattle in Bangladesh is considered to be parasitic helminths infection (Rahman and Mondal, 1983). At least two third of the total livestock population (23 million) are infected subclinically with parasitic hel-minth infections (Garrels, 1975; Rahman and Mondal, 1983). Subclinical diseases are often ignored resulting in heavy economic loss. The economic losses due to sub-clinical problems in population are significantly higher than clinical problems in individual animals (Martin et al, 1994). A prerequisite for disease control/prevention is to identify factors responsible for that disease.

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Knowledge of the risk factors facilitates identification of categories of animals that are at particular risk of developing an infection (Thrusfield, 1995). Only age and seasons are two factors studied by some authors in relation to fasciola infection in cattle of Bangladesh.

Cattle more than one year of age, females and post-monsoon season were described significantly associated with liver fluke infection (Howlader et al, 1990; Chowdhury et al, 1994). Begum et al (2003) identified risk period of gastrointestinal nematodes in ruminants and suggested two strategic treatments. Risk factors for animal health and production problems include specific etiologic agents, host factors and environmental factors (Dohoo et al, 1996). Knowledge of the associated factors of a disease is an aid in differential diagnosis also. Pathognomonic clinical signs of a disease help in non-laboratory diagnosis which is rapid and cheaper than laboratory diagnosis. For a rational and sustainable helminth control programme, a comprehl anve know-ledge of the epidemiology of the disease in a specific clim cheand manageologysystem is a prerequisite (Keyyu et al, 2005 climre are very few reanags regarom-prerisk factors and clinical signs associated with helminth infections in cattle of Bangladesh. A preliminary cross-sectional survey including animal-level and herd-level factors of parasitic helminth infections in cattle of Mymensingh district of Bangladesh was undertaken for the following objectives: 1) to report apparent prevalence of parasitic helminth infections, 2) to identify risk factors of parasitic helminth infections, and 3) to identify clinical signs associated with parasitic helminth infections in cattle.

MATERIALS AND METHODS

Survey design and sampling

The survey was a cross-sectional observational type and conducted in three villages of Mymensingh district of Bangladesh. A non probability convenience sampling method was used to select animals from these villages. A total of 138 animals from 40 farmers/herds were selected and included in the study.

Sample collection and processing

Faecal samples from 138 animals were collected from the rectum and also from recently voided faeces using gloved fingers. The samples were labelled and few drops of 10% formalin were added to fix the eggs of schistosomes. The samples were then transported to the laboratory of Parasitology department of Bangladesh Agricultural University and refrigerated at 4°C The eggs per gram of faeces (epg) for nematodes and trematodes were determined by McMaster and Stoll's methods respectively (Soulsby, 1982). Count data should be analysed with poisson regression. But in individual helminth infection there were few positive observations. Also the treatment of helminth infections is usually performed group-wise. Foor that reason poisson regression was not tried. The individual animal was categ-poized infected by a positive epg. Considering therapeutic management of gastrointestinal helminths they were categorized into three groups amphifasc (amphistome and fasciola), schistosome and nematodes (strongyles, strongyloides, toxocara, trichuris, capillaria and bunos-tomum). Infected in the group was indicated by the pre-sence of at least one type of helminth. Apparent preva-lence of each group of parasite and their exact binomial 95% confidence intervals were calculated in STATA®.

Questionnaire based data collection and processing

Data from each animal and herds were recorded by means of a questionnaire. The animal-level covariates were age, breed, gender, physical condition, appetite, soiling, rumen motility, dehydration, conjunctiva and consistency of faeces. Age was determined by asking owner and also by dentition. The other covariates were recorded either by distance inspection or by physical examination. The herd-level covariates were production type, grazing, feeding of cut-grass, sanitation, diet and deworming. These covariates were recorded by interviewing owners. Farmers who sold surplus milk were considered commercial producers. The data were entered in Microsoft Office Excel 2003 and transferred by STATA®, version 8.0/Intercooled (Stata Corporation, Texas, USA, 2003) and to R®, Version 2.3.0 (The R Foundation for Statistical Computing, Vienna, Austria) for statistical analyses.

Statistical analysis

Screening for multicollinearity

Simple bivariable associations among explanatory variables were investigated by $\times 2$ test in STATA®. One of the pairs of significantly associated variables was selected for inclusion in the final multiple logistic regression analysis and the other was ignored (Dohoo et al, 1996).

Associations with helminth infections

Classification tree: Classification trees were produced for each of the three groups of parasitic helminth infections (binomial target variables) in R® following the procedure described by Speybroeck et al (2004). Random forests was also constructed in R® following the procedure described by Breiman (2001).

Multiple logistic regression

Multiple logistic regressions for three group of parasitic infections were performed in STATA® with all of the independent variables remained after checking for multicolinearity. Backwards elimination method was

Table 1. Description of variables, and distribution of trematode[a] and nematode infection in cattle

Variables	Category level	Number of observations	Amphistome and Fasciola		Schistosoma		Nematodes	
			Prevalence	95% CI	Prevalence	95% CI	Prevalence	95% CI
Age (years)	0 (.01, 1.5)[a]	52	.40	.27, .54	.06	.01, .15	.56	.41, .69
	1 (2, 5)	44	.45	.30, .61	.11	.04, .24	.45	.30, .61
	2 (6, 13)	42	.50	.34, .65	.07	.01, .19	.57	.40, .72
Breed	Indigenous	83	.45	.35, .57	.12	.06, .21	.55	.44, .66
	Cross	55	.44	.30, .57	.02	.00, .09	.49	.35, .62
Gender	Male	37	.41	.24, .58	.08	.02, .23	.73	.56, .86
	Female	101	.46	.36, .56	.08	.03, .15	.45	.35, .55
Physical condition	Slight thin	70	.46	.33, .58	.07	.02, .15	.52	.40, .64
	Moderate to very thin	68	.44	.32, .56	.09	.03, .18.	53	.40, .65
Appetite	Normal	123	.44	.35, .53	.09	.05, .15	.52	.42, .61
	Anorexia	15	.53	.26, .78	.00	–	.60	.32, .83
Soiling of perineum	Yes	61	.44	.32, .57	.08	.03, .18	.56	.44, .67
	No	77	.45	.34, .57	.07	.03, .16	.49	.36, .62
Rumen motility	Normal	117	.47	.37, .56	.07	.03, .14	.57	.47, .66
	Hyper motile	21	.33	.14, .56	.09	.01, .30	.28	.11, .52
Dehydration	Normal	14	.36	.13, .64	.00	–	.57	.28, .82
	Dehydrated	124	.46	.37, .55.	09	.04, .15	.52.	43, .61
Conjunctiva	Normal	30	.33	.17, .52	.10	.02, .26	.43	.25, .62
	Anemic	108	.48	.38, .57	.07	.03, .14	.55	.45, .65
Consistency of faeces	Normal	89	.41	.31, .52	.09	.04, .16	.49	.38, .60
	Liquid	49	.51	.36, .65	.06	.01, .17	.59	.44, .73
Diet	Good	75	.41	.30, .53	.05	.01, .13	.52	.40, .64
	Poor	63	.49	.36, .62	.11	.04, .21	.54	.41, .66
Deworming	Yes	20	.60	.36, .80	.15	.03, .37	.60	.36, .80
	No	118	.42	.33, .51.	06	.03, .13	.52	.42, .60
Production type	Subsistence	52	.54	.39, .67	.09	.03, .21	.52	.37, .65
	Commercial	86	.39	.29, .50	.07	.03, .14	.53	.42, .64
Grazing	No	104	.46	.36, .56	.07	.03, .14	.51	.41, .60
	Yes	34	.41	.25, .59	.09	.02, .23	.59	.41, .75
Feeding cutgrass	No	44	.45	.30, .61	.11	.04, .24	.57	.41, .71
	Yes	94	.45	.34, .55	.06	.02, .13	.51	.40, .61
Sanitation	Poor	68	.41	.29, .53	.03	.00, .10	.48	.36, .61
	Good	70	.48	.36, .60	.12	.06, .23	.57	.45, .68

[a]Here trematode means amphistome, fasciola and schistosoma

used for model building. The maximum model was fit and then variables were removed sequentially until all of them remaining in the model had a P-value ≤ 0.05. The better model was selected using likelihood ratio test (Dohoo et al, 2003). Considering the importance of the variables and based on descriptive statistics the following predictor variables were kept for maximum model: 1) Rumen and liver fluke infection: age, deworming, production type, appetite, dehydration and conjunctiva, 2) Nematode infection: age, breed, gender, deworming, grazing, appetite, conjuctiva, rumen motility and consistency faeces, and 3) Schistosome infection: age, breed, deworming, soiling and rumen motility.

RESULTS

Data exploration

A general overview of the sample is shown in Table 1. Female, indigenous, not dewormed and zero-grazed animals predominate in the sample. Mean age of the animals is 3.62 ± 1.7 years.

The cattle herd size is provided in Table 2. Largest herd size is 11. Most of the herds contain 2 or 3 animals.

Apparent prevalence

Apparent prevalence of individual helminth infections with their mean epg are shown in Table 3. Prevalence of helminth infections (groups) are in Table 4. Both the prevalence (37%) and mean epg (107) are highest in Strongyles infections. Whereas the lowest prevalence (2%) and mean epg (1) are observed in capillaria infection. Seventy eight percent cattle are infected with at least one type of helminth. The frequency of multiple helminth infections is given in Table 5. Thirty four per-

Table 2. Cattle herd size in helminth data of Mymensingh district

Herd size	Frequency
1	5
2	9
3	9
4	7
5	6
6	2
7	1
11	1
Total	40

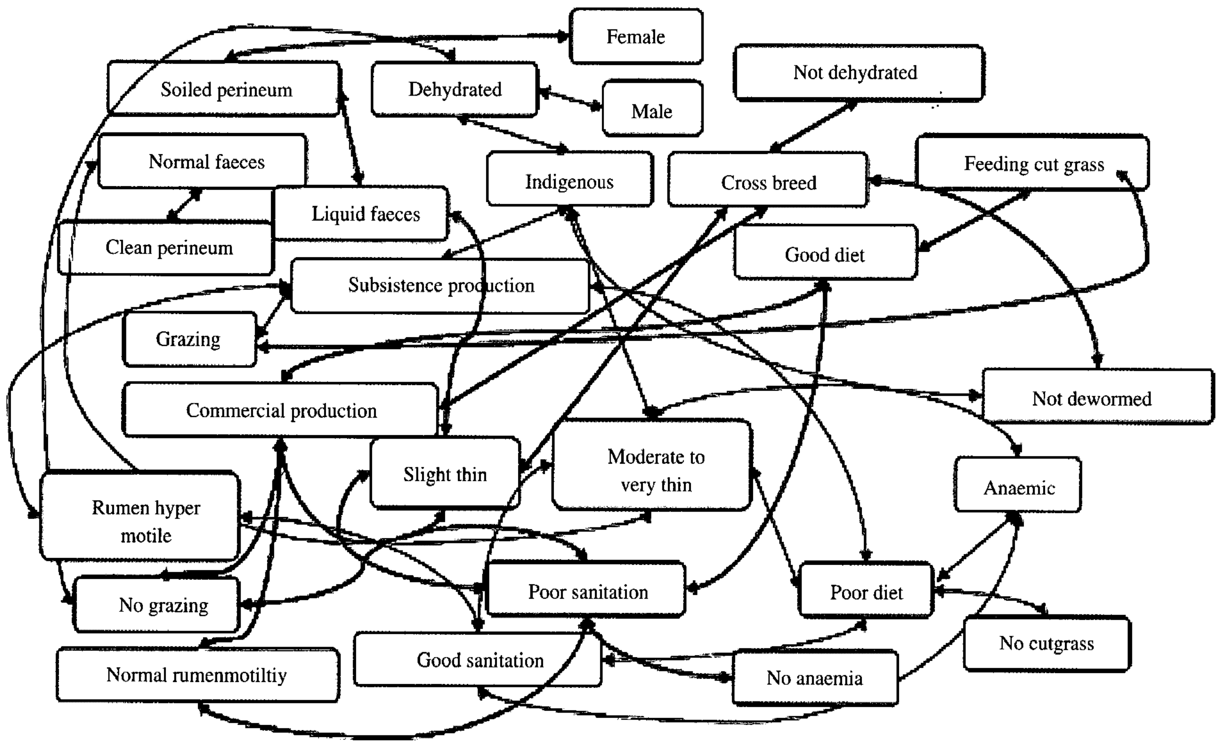

Fig. 1. Flow chart of association among explanatory variables (Green thick lines indicate cross breed, commercial production and good diet group. Red lines indicate indigenous, subsistence production and poor diet group).

Table 3. Apparent prevalence and epg of different helminth infections of cattle

Helminth	Mean	SE[a]	95% CI		Mean epg[b]	SE epg
Overall	.78	.03	.70	.84	–	–
Amphistome	.35	04	.27	.44	70	10
Fasciola	.15	.03	.10	.23	30	9
Schistosoma	.08	.02	.04	.13	9	6
Strongyles	.37	.04	.29	.46	107	18
Strongyloides	.09	.02	.05	.15	56	19
Toxocara	.03	.01	.00	.07	4	5
Trichuris	.08	.02	.04	.14	13	7
Capillaria	.02	.01	.00	.06	1	3
Bunostomum	.08	.02	.04	.14	20	8

[a]SE=standard error, [b]epg=eggs per gram of faeces

Table 4. Apparent prevalence of helminth infections (groups) in cattle

Helminth	Mean	SE	95% CI	
Overall[a]	.78	.03	.70	.84
Amphifasc[b]	.44	.04	.36	.53
Schistosoma	.08	.02	.04	.14
Nematode[c]	.52	.04	.44	.61

[a]Overall idicates infection by at least one type of parasite
[b]Amphifasc means liver fluke and/or rumen fluke infection
[c]Indicates infection by at least one type of nematode

Table 5. Multiple infections of helminths in cattle

Number of helminths	Frequency (%)
0	37 (26.81)
1	41 (29.71)
2	47 (34.05)
3	10 (7.25)
4	2 (1.44)
5	1 (0.72)
Total	138 (100.00)

cent animals are infected with two types of helminths.

The rumen and liver fluke infection is higher with the increase of age, in subsistence production system and in dewormed cattle. Indigenous and dewormed cattle have high level of schistosoma infection (Table 1). Higher prevalences of nematode infections are observed in male animals and in those animals having normal rumen motility (Table 1).

Association among explanatory variables

Significant 2×2 X^2 test results of explanatory variables are presented in Table 6. Twenty four pairs of the combinations showed significant associations. Breed-production type, gender-soiling, production typediet and grazing-cut-grass are the highly significant variable pairs.

The association among explanatory variables are illustrated in flow chart (Fig. 1). The flow chart shows two distinct grouping among variables. The indigenous animals are moderate to very thin in body condition and maintained on poor diet. These animals are anemic and not fed cut-grass. Indigenous animals are more related to subsistence type of production system. They are dehydrated, usually grazed and thereby sanitary condition of their houses is good.

Table 6. Significant association among explanatory variables

	Pair variables	X^2 p-value
Breed	Physical cond	0.005
	Dehydration	0.002
	Diet	0.01
	Production type	0.000
Gender	Dehydration	0.02
	Soiling	0.000
Physical cond	Conjunctiva	0.02
	Consistency feces	0.001
	Production type	0.01
	Sanitation	0.01
	Diet	0.007
Soiling	Consistency feces	0.009
Rumenmotility	Sanitation	0.001
	Production type	0.01
Dehydration	Grazing	0.04
Conjunctiva	Sanitation	0.03
	Diet	0.02
Production type	Diet	0.000
	Grazing	0.003
Grazing	Cut-grass	0.000
	Sanitation	0.02
Cut-grass	Diet	0.002
	Deworming	0.03
Sanitation	Diet	0.007

Table 7. Significantly associated variables in nematode infection of cattle

Nematode	OR	SE	p-value	95% Confidence Interval
Gender (Male)	3.3	1.4	0.006	1.4, 7.7
Rumenmotility (Normal)	3.5	1.8	0.02	1.2, 9.9

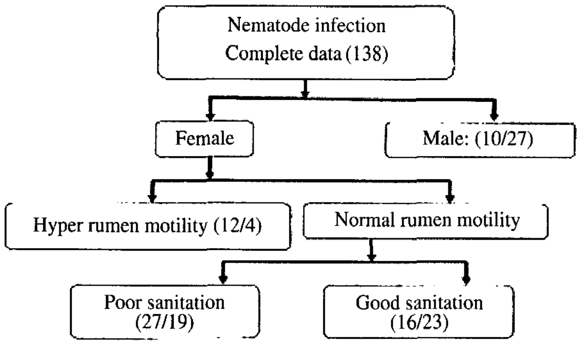

Fig. 2. Pruned tree of nematode infection in cattle (healthy/ infected).

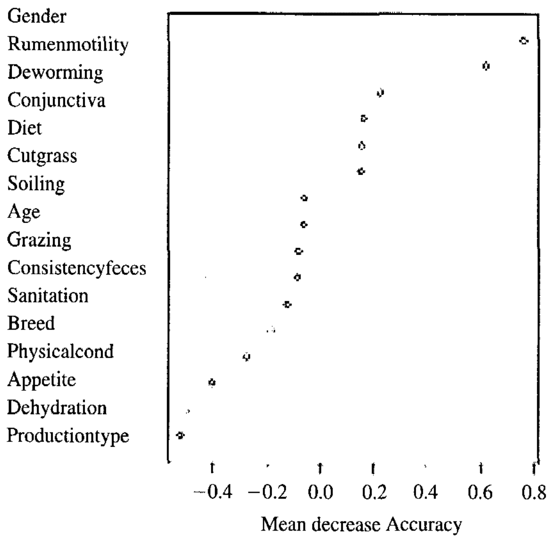

Fig. 3. Important variables for nematode infection in cattle obtained from random forests analysis. [Values in the x-axis indicate accuracy, gender and rumen motility has been classified as most important vari-ables associated with nematode infection].

On the contrary, cross-breed animals are slight thin and fed good diet. They are provided with cut-grass and not anemic. There are more cross-breed animals in commercial type of production system. Cross-breed animals are usually not dehydrated, kept indoor and thereby sanitary condition of their houses is relatively poor.

Associations with helminth infections, Classification tree, Risk factor and clinical signs

Data of mixed amphistome-fasciola and schistosome infections does not allow the construction of any tree (increase of relative cost at the first branch). The only tree remaining after pruning is that of nematode infec-Otion. Three variables are kept after pruning. In order of decreasing priority they are age, rumen motility and sanitation (Fig 2). Random forests provides important variables in order of decreasing priority for every group of helminths infection. The important variables for nematode infection only in order of decreasing priority are shown in Fig 3.

Multiple logistic regression, Risk factors and clinical signs

None of the variables are significantly associated with amphistome-fasciola mixed infection and schistosome infection in multiple logistic regression. The only animal level factor significantly associated with nematode infection is sex. Male animal (OR=3.3, P=.006, 95% CI=1.4, 7.7) has significantly increased prevalence of nematode infection (Table 7).

In multiple logistic regression, the only clinical sign significantly associated with nematode infection is rumen motility. Animals with normal rumen motility (OR=3.5, P=0.02, 95% CI=1.2, 9.9) has significantly increased prevalence of nematode infection (Table 7).

DISCUSSION

Apparent prevalence

To make inference about the frequency of outcome or prevalence of exposure in a target population, the study subjects should be obtained by a formal random sampling procedure (Dohoo et al, 2003). It was not in the case of this survey of helminth infections in cattle of

Mymensingh district of Bangladesh. The farmers having at least one cattle were approached without any prior thinking. Those who agreed to provide their animals for sampling were included. This study area is close to Bangladesh Agricultural University, Mymensingh, Bangladesh. The farmers get easy access of treatment, extension facilities, artificial insemination facility and free consultancy on livestock health and production problems. For that reason they are relatively more aware about the health of their animals. Relatively more cross-breed animals are found in periurban areas due to availability of artificial insemination centres. Similar type of above mentioned facilities are only available in periurban areas of Bangladesh. Periurban areas may include villages surrounding districts and even upa-zillas. This sample represents cattle managed traditionally in periurban condition. So the prevalence from this survey is only applicable for the cattle population of traditional management system in periurban areas of Bangladesh.

The tropical humid climate is very favourable for the endemicity of helminth infections in all parts of Bangladesh. Animals without any parasitic infection are rare (Rahman and Mondal, 1983). Mixed infections in host is the rule rather than the exception in most grazing systems. The occurrence of mixed infections may lead to decreased production due to the synergistic effect of each parasite, although this depends on the proportion of the pathogenic species (Garrles, 1975; Rahman and Mondal, 1983; Bari et al, 1991; Maichomo et al, 2004). Prevalence of fasciola infection is in agreement with others (Howlader, 1990; Garrels, 1975) and that of strongyloides infection with Rahman and Mondal (1983). Bari et al. (1991) observed similar prevalence of amphistomes.

The fecal egg counts can help to measure the severity of infection. According to the classification of Phiri et al. (2005), all fasciola cases have severe infection because the range of epg for fasciola in positive cases is 100~200.

The herd size represents the real picture of traditional cattle management system. Two or three animals are most prevalent in the farms. They are mainly used for draught purpose.

Higher prevalence of amphistome in dewormed cattle can be explained by the fact that available drug (Oxyclozanide) need to be repeated three days after the initial treatment. This is rarely done even if we consider that the drug is effective and administered at appropriate doses and time. In subsistence production system there are more indigenous animals and mainly used for ploughing land. They are also provided with poor diet. They may also be less dewormed than those for commercial production. These may be the reasons for relatively higher prevalence of amphistome and fasciola infections in subsistence production type. Indigenous cattle are used for ploughing land and more exposed to snaiessabitat. The only drug available against schistosoma infection inighegladesh is antimony compound (Anthiomaline®), in injectable form, expensive and not readily available. This imported drug is usually used for severe form of nasal schistosomiasis (snoring disease). These may be the reasons for higher prevalence of schistosoma in indigenous and dewormed cattle.

Association among explanatory variables

Solving the problem of multicollinearity by 2×2 X^2 test or pairwise correlation has some limitations. The choice of which independent variable is to be removed is arbitrary. If pairwise correlation is used then selecting the level of correlation coefficient that represent a problem is also arbitrary. Knowledge of the production system may help to make an appropriate decision (Dohoo et al, 1996).

Associations with helminth infections, Classification tree, Risk factor and clinical signs

Classification tree retained sanitation as third important variable for nematode infection. But sanitation is collinear with rumen motility (X^2 P-value=0.001). Classification tree can deal with multicollinearity in an intuitively correct way (Speybroeck et al, 2004). One explanation may be the high misclassification error rate (36%). Classification tree deals more effectively with large data sets and the issues of higher dimensionality (Speybroeck et al, 2004). The helminth data has 16 cova-riates but only 138 observations which is very little for a classification tree. Classification and regression trees (CART) are universally applicable to classification and regression problems without assumptions on the structure of the data. The picture of the tree structure gives valuable insights into which variables are important and where, terminal nodes give a natural clustering of the data into homogenous groups. Classification tree has some limitations. A small change in data can produce highly divergent trees (unstable out put). Misclassification error rate is higher than other techniques like bagging and random forests (Breiman, 2003).

Random forests can handle hundreds and thousands of input variables with no degeneration in accuracy (Breiman, 2001). It is not possible to examine individual trees separately (Prasad et al, 2006). Both of the techniques have kept gender and rumen motility as first two important variables for nematode infection. In the helminth data unusually misclassification error rate in random forests was higher than that of classification tree. The possible causes are small sample size and increased error rate in constructing individual tree.

Multiple logistic regression, Risk factors and clinical signs

In the multiple logistic regression model, the significantly higher prevalence of nematode infections in males should be interpreted with caution as it could be due to the large numbers of explanatory variables. However, the small sample size and thereby low power does not increase the risk of rejecting erroneously the null hypothesis. There are reports that entire male cattle (bullsThhave increased susceptibility to nematode infections (Armour and Urquhart, 1974). This phenomenon is related to androgen. In this survey all males are not bull. The male animals in fectioudy areahere ased for draught purpose and thereby more exposed to the pasture and also more stressed. Male animals are also fed relatively poor diet. Poor diet increase the susceptibility to nematode infection (Houdijk and Athanasiadou, 2003). So all these factors may increase the risk of being infected by nematode.

The prevalence of amphistome and fasciola infection has an increased trend with the age in cattle of Mymensingh district of Bangladesh (not significant). There are many reports of higher fluke prevalence in adults than calves (Cringoli et al, 2002, Molina et al, 2005; Pfukenyi et al, 2005, 2006). It is expected because age is a surrogate measure of amount of exposure time.

Rumen motiltiy is a clinical sign statistically associated with nematode infection. Animals with normal rumen motility have higher nematode infection. It can not be explained biologically. Clinically hyper rumen motility is observed with parasitic gastroenteritis. This finding should not be reported as significant because of lack of biological plausibility and small sample size.

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