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Full Length Research Paper

Prevalence and species of major gastrointestinal parasites of donkeys in Tenta Woreda, Amhara Regional State, Ethiopia

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A cross sectional study was carried out from January 2014 to May 2015 in Tenta Woreda, Amhara regional state, Ethiopia to identify the species and determine the overall prevalence of gastrointestinal (GI) parasites of donkeys. A total of 300 fecal samples were collected from randomly selected donkeys and examined with flotation, sedimentation, McMaster and Baerman fecal examination techniques. The overall prevalence was found to be 72.33. The major GI parasites identified based on qualitative faecal examination techniques were Strongyles spp. (57.2%), *Parascaris equorum* (11.2%), *Strongyloides* spp. (10.4%), *Gastrodiscus aegypticus* (5.1%), *Oxyrus equi* (2.7%), *Fasciola* spp. (2.0%) and *Anoplocephal* spp. (2.6%). The prevalence in female and male animals was 58.5 and 41.5%, respectively. There was a statistically significant difference ($p<0.05$) for the prevalence and mean epg count of strongyles and *P. equorum* among different age groups. Accordingly, the prevalence as well as mean epg count was higher in young than adults and old age groups. Further analysis of positive samples using coproculture revealed the occurrence of *Strongylus vulgaris* (27.7%), *Strongylus edentates* (16.6%), *Strongylus equinus* (4.8%), *P. equorum* (11.2%), *Trichostrongylus axei* (8.3%) *Strongyloides westeri* (9.5%), *Dictyocaulus arnfieldi* (7.5%), *Oxyuris equi* (6.5%) and *Triodonthophorus tencollis* (1.3%). There was concurrent infection of donkeys with a maximum of two different GI parasites with prevalence of 33.48%. The findings of the present study clearly suggest that GI parasites of donkeys in the study area are still widespread and economically important constraints for the productivity and use of donkeys in rural towns. Hence, further and strengthened intervention is highly recommended taking into account the importance of these animals to the economy.

Key words: Coproculture, donkeys, egg per gram of faeces (epg), faeces, GI parasites, prevalence, Tenata, Ethiopia.

INTRODUCTION

Donkeys (*Equus asinus*) are among the early-domesticated equines that have been existing, dating back to the time of early men (Saul et al., 1997). Today, there are more than 40 million donkeys distributed

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throughout the world (FAO, 2013). In Africa, the donkey population is estimated to be 13 million (Starkey and Starkey, 2013) and Ethiopia has about four million donkeys or 32% of all the donkeys in Africa and 10% of the world population (Alemu et al., 2004). Donkey in Ethiopia is most commonly found in the dry and mountainous areas. There is one equine for every four people in the agricultural sector and for every five persons of the total population (Yilma et al., 1991).

Despite the increase in mechanization throughout the world, donkeys are still well deserving of the name beasts of burden. Their prominent position in the agricultural systems of many developing countries is shown by the wide spread use of donkeys in rural and urban areas. It is suggested that donkeys can play a great role in the frame works of food security and social equity of high food insecure countries (Pearson and Krecek, 2006).

The low level of development of road transport and rough terrain of Ethiopia make donkeys the most valuable, appropriate and affordable pack animals under the small holder farming system (Gebrewold et al., 2004). Donkeys appear to be an effective entry point for assisting women in domestic responsibilities (Marshall and Ali, 2004) and also in areas where draft power is a constraint to crop cultivation; a pair of well-conditioned donkeys could be used as an alternative draft power sources for secondary and tertiary land preparation (Abayneh et al., 2002).

Certain impediments hinder the maximum utilization of these animals to their potential. The most important ones are parasitic disease (Chhabra et al., 2011; Sumbria et al., 2014; Sumbria et al., 2016), especially gastrointestinal parasites, harness sores; infectious disease likes strangles and poor management system of these animals (Ministry of Agriculture and Rural development, 2012). Donkeys harbor a large quantity of parasite that prevail in the GIT including round worms (families: Stronglidae, oxyuridae, Trichostronglidae and Ascaridae), flat worms (Fasciolidae) and tapeworm (family: Anoplocephalidae) which damage the intestine depend on the species and number present, nutrition, age and natural defense of the individual equine (Pereia and Vianna, 2006). In donkeys, infection with endoparasites is responsible for problems including poor body condition, reduced power output, diarrhea, colic, emaciation, impaired growth, poor reproductive performance, short lifespan and predisposition to other infectious diseases (Elisabeth and Sevendesen, 1997; Fikru et al., 2005).

Studies on endoparasites including haemoprotozoa in working donkeys across several countries of the world have disclosed the involvement of several species (Sotiraki et al., 1997; Wells et al., 1998; Matthee et al., 2002; Mushi et al., 2003; Uslu and Guclu, 2007; Sumbria et al., 2015). These investigations have revealed that in developing countries where nutrition and hygiene are generally poor, GI parasites are highly prevalent and are the major problems of donkeys. In Ethiopia where the health care is minimal, especially for equines, the prevalence, species composition and epidemiology of GI parasites affecting donkeys have not been investigated in detail (Getachew et al., 2009, 2010).

However, the available information suggests that gastrointestinal GI parasites are the main reasons for early demises of donkeys in the country (Yoseph et al., 2001; Fikru et al., 2005; Ayeleet al., 2006). Despite their invaluable contributions, donkeys in Ethiopia are the most neglected animals, accorded low social status. Apart from few studies in other parts of Ethiopia, there is no previous information on GI parasites of donkeys in Tenta woreda, Amhara Regional State, Ethiopia

Hence, the objectives of this study were to determine the overall prevalence of GI parasites of donkeys in Tenta woreda Amhara Regional State, Ethiopia. Besides, the level of infection based on the mean epg count and the relationship between measurable parameters and GI parasites were assessed.

MATERIALS AND METHODS

Study area

The present study was conducted in Tenta Woreda, Amhara Regional State, Ethiopia from January 2014 to May 2015. The livestock resources of the region consist of cattle, goats, sheep, camel, poultry and equines. Tenta is a town located in South Wollo Zone of the Amhara Region. It has a latitude and longitude of 11° 19′N 39°15′E / 11.317° N 39.250°E, with an elevation of 2972 m above sea level.

Study animals

Animals used in this study were systematically selected 300 donkeys from Tenta woreda Amhara Regional State, Ethiopia maintained under traditional small holder extensive management production system. The animals were owned by the individual farmers for the purpose of packing to generate income and for household use. Though selected animals represented different age and sex groups, but donkeys were randomly selected irrespective of age, sex and body condition scores and color.

Sample size determination

Sample size was calculated with an expected prevalence of 77.3% from the previous research work on prevalence of GIT parasite in similar highland area, South Wollo (Alemayehu and Etaferahu, 2013). The desired sample size for the study was calculated using the formula given by Thrusfield (2007) with 95% confidence interval and 5% absolute precision.

$$n = \frac{1.96^2 Pexp\ (1-Pexp)}{d^2}$$

Where, Pexp = expected prevalence; d= absolute precision; n=sample size. Accordingly, a total of 300 donkeys were used in this study.

Table 1. Overall prevalence.

Parasite	Number positive	Prevalence (%)	Standard error	[95% Confidence interval]
Strongyles	138	57.2	0.0291536	[0.49-0.60]
Oxyrus equi	8	6.5	0.0169264	[0.06-0.13]
Parascaris equorum	27	11.2	0.0095369	[0.01-0.05]
Strongyloides spp.	25	10.4	0.0163485	[0.05-0.12]
Faciola spp.	6	4.0	0.0082881	[0.01-0.04]
Gastrodiscus aegypticus	15	8.1	0.0128976	[0.03-0.08]
Anoplocephala spp.	2	2.6	0.0048184	[-0.01-0.01]

Study design and sampling strategy

A cross sectional study was conducted from January 2014 to May 2015 in Tenta Woreda, Amhara Regional State, Ethiopia to identify, assess and determine the prevalence of the GI parasites. Sampling method followed was a household based systematic random sampling in which the study animals were randomly selected from respective village of the district and from the selected village, the house were also randomized systematically in every second household.

Age and body condition estimation

During sample collection, various potential risk factors including sex, age, and body condition score of donkey was recorded. The age of the selected donkeys was determined from birth records of owners and by dentition (Crane and Svendsen, 1997). Body condition score (BCS) was subjectively estimated based on the guides published (Svendsen, 1997). Accordingly, donkeys were grouped into three age categories: donkeys from 1-3 years of age were classified as young; 3-10 years were considered as adult; and those beyond 10 years were classified as old. These age classes were based on age of first work, productive age and the life span of Ethiopian donkeys (Svendsen, 1997; Yosef et al., 2001). Regarding BCS, the studied animals were grouped poor, moderate, ideal, fat and obese (NEWC, 2005).

Sample collection and examination

Faecal sample collection

Fecal samples were collected from donkey per-rectum from fresh deposits using plastic rectal gloves. Each sample was labeled with the animal number, date of collection, age, sex, BCS, number of animal owned and place of collection in edible pen. The collected samples were kept in icebox, having adequate ice and able to close tightly and transported to Woreda laboratory. The samples were kept in plus four refrigerators if immediate processing was not possible, but it had been processed within 48 h.

Faecal sample examination

Microscopic faecal examination was done by different qualitative and quantitative faecal examination technique for the presence of parasitic eggs and identification of larvae.

Qualitative faecal examination was carried out by sedimentation and floatation technique. For identification of parasite to species level, fecal samples were cultured and the larvae were recovered using Baerman apparatus technique and then identified under lower power microscope (10x objectives) based on the shape, relative size and shape of larvae's tail and under oil emersion (100x objectives) based on number of gut cells (Kaufmann, 1997). The floatation fluid used in the study was supersaturated solution of sodium chloride (Nacl) salt prepared in the laboratory. The procedure given by Gutpa and Singla (2012) was followed for the above parasitological methods.

Egg counts were also conducted using McMaster age counting technique. Severity of infection as obtained from the number of eggs per gram of faeces was determined less than or equal to 500 eggs/g of faeces regarded as mild infection; 500-1000 eggs/g of faeces as moderate infection; and above 1000 eggs/g of faeces as severe infection (Urquhart et al., 1996).

Data analysis

The entire collected row data were entered into Microsoft Excel spread sheet and coded. Statistical analyses were performed using STATA, version 11 software packages. Percentage was used to calculate prevalence. Additionally Chi-square was used to calculate degree of association between risk factors and prevalence of gastrointestinal parasites. In the analysis, a difference was taken as significant at a p-value less than 0.05 and the confidence level was held at 95%

RESULTS

Coproscopical examination

The present study revealed an overall prevalence of 72.33%. The parasites encountered in the study period includes: *Strongyles*, *Oxyrus equi, Parascaris equorum, Fasciola* spp., *Gastrodiscus aegypticus*, *Strongyliodes* spp. and *Anoplocephala* spp. (Table 1).

Quantitative faecal examination

The McMaster technique applied to determine the number of GI parasites egg per gram of feces revealed minimum and maximum epg value of 0 to 9000. Likewise, the study showed that the mean epg and prevalence of

Table 2. Mean epg and prevalence in different age groups.

Parasites	Mean faecal egg count(epg)			Prevalence (%)		
	Young	Adult	Old	Young	Adult	Old
Overall egg count	442	530.754	410	10.5	79.8	9.7
Strongyles	359.09	213.24	355.26	91	74	7.9
Fasciola spp.	75	300	0	0.9	1.9	0
Parascaris equorum	750	314.58	225	13.8	11.5	10
Oxyuris eaui	50	175	0	2.6	0	0
Strongyloides spp.	100	290	383.3	11.2	11.5	1.6
Gastrdiscus aegypticus	100	233.33	160	4.3	2.9	0
Anoplocephala spp.	0	0	150	0.9	0	0

Table 3. Eggs per gram of faeces in different age groups.

Level of infection	Percent		
	Mild infection	Moderate infection	Sever infection
Young	45.7	20	2.9
Adult	43.3	16.9	9.5
Old	45.6	22.8	8.8

Table 4. Intensity of infection of GI parasites on the basis of epg.

Degree of severity	Number of donkey	Percentage (%)	Standard error	[95% Confidence interval]
Mildly infected	130	44.4	0.0290741	[0.39-0.50]
moderately infected	54	18.4	0.0226901	[0.14-0.23]
Heavily infected	25	8.5	0.0163485	[0.05-0.12]

strongyle type nematode is significantly greater than the other GI parasites (Table 2).

The study showed that young donkeys were with greater proportion of mild, moderate and heavy level of epg than both young and old age donkeys (Table 3)

Intensity of infection

Based on the result of epg counts in the study area, 130 (44.4%) were mildly infected, 54 (18.4%) were moderately infected and 25 (8.5%) were severely infected (Table 3).

Larvoscopic examinations

Identification of L3 of GI parasites isolated by Baerman technique from coprocultured faeces showed the predominance of *Strongylus vulgaris, Strongylus edentatus* and *Parascaris equorum* than the other GI parasites (Table 4 and 5). Infections with one species of helminthes were more common, 139 (66.52%) than infections with two, 70 (33.49%) species of helminthes (Table 6).

Analysis of risk factors

Analysis of different risk factors showed that age and body condition score was significantly associated with the risk of infection with GI parasites ($p<0.05$), whereas sex of the animal was not significantly associated with GI parasite infection(Table 7).

The prevalence of GI parasites in both body condition groups (good and poor) was determined in larvoscopy. The larvoscopy results revealed that, the prevalence of GI parasites in moderate body condition donkeys were *Dictyocaulus arnfieldi* (50%) which was the top species of parasites and the least were *Triodontophorus tenuicollis* and *Anoplocephala* spp. (0%). In ideal body condition, *T. tenuicollis* (100%) was the highest. Similarly, in poor body condition animals, the highest prevalent parasites was *Anoplocephala* spp. *(*100%) and least was *T. tenuicollis*

Table 5. Species of GI parasites identified from coprocultured faeces of donkey in Sendafa district, Oromia Regional State, Ethiopia.

Species of parasite	Number positive	Prevalence (%)	Standard error	[95%Confidence Interval]
Strongylus vulgaris	84	27.7	0264639	[0.23-0.34]
Strongylus edentatus	43	16.6	0.0207083	[0.11-0.19]
Strongylus equinus	11	4.8	0.011124	[0.02-0.06]
Dictyocaulus arnfieldi	16	7.5	0.0132966	[0.03-0.08]
Tricostrongylus axie	21	8.3	0.0150951	[0.04-0.10]
Strongyloides westeri	25	9.5	0.0136811	[0.03-0.08]
Triodonthophorus tencollis	1	1.4	0.003413	[0.003-0.01]
Cythostomum spp	17	7.9	0.0095369	[0.01-0.04]
Parascaris equorum	27	11.2	0.0095369	[0.01-0.05]
Oxyrus equi	8	6.5	0.0169264	[0.06-0.13]

Table 6. Concurrent infections.

Concurrent infection	Number of positive donkeys	Percentage (%)
One species	139	66.52
Two species	70	33.48

Table 7. The relative prevalence among the sex category.

Parasites	Total	Sex	
		Female	Male
Strongyles	159	95(59.9%)	64(40.3%)
Parascaris equorum	27	14(51.9%)	13(48.1%)
Oxyuris equi	8	4(50%)	4(50%)
Fasciola spp.	6	4(66.7%)	2(33.3%)
Anopleocephalus spp.	2	2(100%)	0
Gastrodiscus aegyrticus	15	9(60%)	6(40%)

Table 8. Prevalence between body conditions.

Species of parasite	Poor	Moderate	Ideal	X^2value	p-value
Strongylus vulgaris	59.5	36.9	3.6	33.511	0.000
Strongylus edentateus	55.8	41.9	2.3	14.264	0.003
Strongylus equinus	45.5	44.5	0	4.115	0.249
Strongyloides westeri	52.0	48.8	0	9.077	0.028
Parascaris equorum	51.9	44.4	0	8.23	0.042
Triodontophorus tenuicollis	0	0	100	28.397	0.000
Dictyocaulus arnfieldi	43.8	50.0	6.2	3.486	0.323
Gastrodiscus aegypticus	66.7	33.3	0	6.887	0.076
Anoplocephala spp.	100	0	0	3.073	0.381
Trichonema spp.	70.6	29.4	0	9.065	0.028
Trichostrongylus axei	57.1	33.3	9.5	4.72	0.254

(0%). There was statistically significant difference ($p<0.05$) in prevalence of *S. vulgaris, S. edentates Strongyloides westeri, P. equorum, T. tenuicollis* and *Trichonema* spp. between the body conditions as examined by larvoscopy (Table 8).

The prevalence of GI parasites in different age groups

Table 9. Age wise prevalence.

Species of parasite	Age group			X^{2-} value	p- value
	Young (%)	Adult (%)	Old (%)		
Strongylus vulgaris	14.3	61.9	23.8	2.458	0.293
Strongylus edentatus	7.0	79.0	14.0	2.630	0.268
Stronglus equinus	9.1	63.6	27.3	0.478	0.787
Stronyloides westeri	8.0	64.0	28.0	1.467	0.480
Oxyuris equi	12.5	75.0	12.5	0.256	0.880
Parascaris equorum	92.6	7.4	0	95.013	0.000
Triodontophorus tencollis	0	0	100	7.644	0.022
Dictyocaulus arnifieldi	18.8	43.8	37.5	5.013	0.082
Fasciola spp,	0	83.3	16.7	0.950	0.622
Anoplocephala spp,	0	50.0	50.0	1.308	0.520
Cythostomum spp,	11.8	58.8	29.4	1.172	0.557
Gastrodiscus aegypicus	0	86.7	13.3	2.945	0.229
Trichostrongylus axei	9.5	61.9	28.6	1.467	0.480

revealed that *P. equorum* (92.6%) was the highest and *G. aegypticus, T. tenuicollis, Anoplocephala* spp. and *Fasciola* spp. (0%) the lowest in the young. In old age, the highest was *T. tenuicollis* (100%) and the lowest was *P. equorum* (*0*%). In adults, *G. aegypticus* (86.7%) and *T. tenuicollis* (0%) were the highest and lowest, respectively. There was statistically significant difference ($p<0.05$) in prevalence of *P. equorum* between different age groups (Table 9).

DISCUSSION

The coprological examination done in this study using floatation and sedimentation method revealed an overall GI parasite prevalence of 72.33% in the study area, which was relatively lower than some of the earlier reports of 98.2% by Ayele et al. (2006), 84.4% by Gulima (2006), 96.9% by Ibrahim et al. (2011) and 92.71% by Mezgebu et al. (2013) at and around Gonder, Hawassa Town, Dugda Bora district and Awi Zone, respectively. This difference could be attributed to the variation in sampling time as seasonality. Additionally, accessibility of donkeys to grazing land, deworming habit of the donkeys and giving supplementary feed to these animals affect its occurrence.

The prevalence of strongyles was 57.2%. This figure was much lesser than the earlier finding of Zerihun et al. (2009), Yoseph et al. (2001), Muleta (2005), Fikru et al. (2005) who reported prevalence of 100, 100, 100 and 98.2% in Sululta and Gefersa, Wonchi, highlands of Wollo province and western highlands of Oromia, respectively. This is most probably attributed to the difference in the study area or due to nutritional status of the animal in the respective study area which can influence the level of immunity to be infected by the parasite. Additionally, it could be affected by deworming strategy and accessibility to veterinary clinic.

The prevalence of 11.2% *P. equorum* recorded in the current study is lower than the previous reports of Fikru et al. (2005), Ayele et al. (2006) and Zerihun (2008) who reported 43, 17.3 and 42.8% in Western highlands of Oromia, Dugda Bora district, and highlands of Wollo provinces, respectively. These differences in prevalence might be due to the variation in the length of the study period, the season of the study period, ecology of the study area, intervention with anthelminthic (deworming) and the ecological and climatic differences among localities. The prevalence and mean epg of *P. equorum* was significantly ($p<0.05$) higher in young donkeys than the other age groups. This is most probably due to the fact that young donkeys have less immunity against *P. equorum* infection than both adult and old donkeys. This agrees with the earlier report by Zerihun (2008) in central Showa, Ethiopia. However, this finding contrast the research of Ayele et al. (2006) and Getachew et al. (2009) who reported absence of statistically significant differences in the prevalence of *P. equorum* among donkeys of different age groups that may reflect differences in the study design and geographic locations.

The prevalence of 4% for *Fasciola* spp. recorded from intervention area in the current study is higher than the previous report by Ayele *e*t al. (2006) who reported 1.5% in Dugda Bora district. This higher prevalence suggests that *Fasciola* spp. is common in highlands where donkeys share the same grazing area with ruminants that are considered as primary hosts of liver fluke and favorable ecological conditions which allow multiplication and spread of intermediate snail host in both study districts as has been reported by Getachew et al. (2010). As compared to other reports in the central highlands of Ethiopia (Yoseph et al., 2001) in Wonchi (Muleta, 2005),

South and North Wollo zones) lower prevalence of *Fasciola* was recorded. This lower prevalence might be due to the differences in season of sample collection and effect of deworming.

Lower prevalence of *Anoplocephala* spp, 2.6% recorded in this study as compared to reports by, Yoseph et al. (2005), Fikru et al. (2005) and Getachew et al. (2010) might reflect the seasonality of orbited mite intermediate hosts and differences in study period and locations. The negative value of confidence interval for *Anoplocephala* spp. indicates that they are rare parasites in the study area that indicate cross sectional study which is not a better study method. The prevalence of 8.1% for *G. aegypticus* recorded in the current study is in agreement with previous research done by Zerihun et al. (2009) reporting 5.7% in Sululta and Gefersa districts of central Oromia.

O. equi with prevalence rate of 6.5% was lower when compared with the work of Yoseph et al. (2001) in Wonchi who reported 32.4% and relatively similar to Alemayehu and Etaferahun (2013) who have reported 4% in south wollo zone. The low prevalence in this study might be the effect of deworming by the woreda veterinary clinic, variation in management system and relatively dry season during sample collection time in the present study area which desiccates the highly susceptible *O. equi* eggs. The parasite is ubiquitous but greater prevalence in areas of high rainfall (Radostitis et al., 2007).

Lower prevalence (7.5%) of *D. arnfieldi* was recorded in the present study as compared to Ayele et al. (2006) who have reported 32% in Dugda Bora District and Bewketu and Endalkachew (2013) who have reported 22.17% in and around Bahir Dar town. This difference in prevalence might be due to the ecological and climatically differences among localities.

Analysis of the degree of infection by GI parasites as determined by epg of donkey showed that the greatest proportion of young donkeys were with mild degree (45.7%) followed by moderate degree (20.0%) whereas the majority of adult and old donkeys were with mild degree of infection 43.3 and 45.6%, respectively. This observation is lower than the previous research of Matthee et al. (2002) and Getachew et al. (2009). This might be due to the effect of deworming in the study area. The difference in parasite prevalence between sexes might be due to the fact that females are found to have higher infection rates as they might have lower immunity due to gestation and lactation as stated by Ram (2009). The co-infection pattern observed in this study showed that donkey has the high chance of concomitant exposure to different GI parasites. Similar findings were reported by Yoseph et al. (2001).

This study confirmed that there is significant difference in the prevalence of the parasite among the different body condition scores and it is shown that GI parasites are more prevalent in animals with poor body condition than well-conditioned animals which are in agreement with the results of Ayele et al. (2006), which implies that the body condition score is a good indicator of parasitic burden, which can be used by farmers to identify donkeys with immediate requirement of anthelmintic remedies.

This study result is in agreement with Ayele et al. (2006) that there was no statistically significant difference ($p>0.05$) between age groups for GI parasite infections except *P. equorum.* This might be due to increased land of cultivation which restricts donkeys on small communal grazing land which allows the animals for continuous larvae exposure.

Identification of infective larvae of GI parasites showed that *S. vulgaris* (29.7%) and *S. edentatus* (16.7%) were the major larvae encountered. The prevalence of *P. equorum* and *D. arnfieldi* were 11.2 and 7.5%, respectively. This finding disagrees with observations of Ayele et al. (2010) and Yoseph et al. (2001) who reported 100%. These differences in prevalence might be due to intervention with anthelminthic and the ecological and climatic differences among localities.

Conclusion

The current study showed a decrease in prevalence of helminthes of donkeys in both coproscopic and larvoscopic methods of examination in the study area. This suggests that the mass deworming given by Woreda Veterinary Clinic reduce the parasitic infection. This study revealed that the major GI parasites that occurred were *S. vulgaris, P. equorum, S. edentatus, O. equi, D. arnfiedi* and *Cythostomum* spp. In the light of the results, it is considered that the infections caused by GI parasites, especially the *S. vulgaris, S. edentatus* and *P. equorum* are common in the region of the study, so greater importance should be given to this situation. This research indicated that infection with one parasite was found to be very common in this study than concurrent infection with two and three parasites. This study confirmed that there is significant difference in the prevalence of the parasite among the different body condition scores and it is shown that GI parasites are more prevalent in animals with poor body condition than well-conditioned animals.

RECOMMENDATIONS

1. Owners should be trained to improve the management system, especially in terms of the level of nutrition so that the animal can have good body condition that confers some level of resistance against GI parasite infection.
2. The decrease in the prevalence of parasite due to mass deworming is not necessarily satisfactory, so strategic parasitic control program should be designed with broad spectrum anthelmintic drugs with regular

evaluation of efficiency of anthelmintic.
3. Donkey owners should be educated on the economic importance and methods of control of helminthes of donkey.
4. Government and non-governmental organization should work together to improve the health and welfare of donkeys in the study area.
5. Further research on the economic importance, epidemiology and time of treatment of GI parasites of donkey in the study area is recommended.

Conflict of interest

The authors declare that they have no conflict of interest

Abbreviations: BCS, Body condition score; **CI,** confidence interval; **epg,** egg per gram of faeces; **GI,** gastrointestinal; **MOARD,** Ministry of Agriculture Rural Development; **rpm,** revolution per minute.

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Full Length Research Paper

Bursa body index as a visual indicator for the assessment of bursa of Fabricius

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The objective of the present study was to identify the significance of the Bursal-Body index (BB index) as a tool in assessing the health status of a chicken flock especially those under experimental condition. With the unending debate on the bursa of Fabricius as a diagnostic tool, a total of 135 day-of-hatch Dominant black Cockerel hybrid were housed in six separate houses with positive pressure and filtered airflow. Houses were assigned to the three vaccines (two intermediate (A, and C), and one intermediate plus (B) vaccine strain), a challenge group (D) and control group (E). The birds were vaccinated according to manufacturer's directives on day 17th post hatch; challenge group was inoculated with 0.05 ml of very-virulent isolate of the infectious bursal disease virus (vvIBDV) on the same day. On day(s) 1, 2, 3, 4, 5, 7, 10, 14 and 21 post vaccination/challenge (Dpv/c) three birds were humanely sacrificed from each group with Bursal-Body ratio (BB ratio) and Bursal-Body index (BB index) recorded. The values (>0.7, that is, no atrophy (mild vaccine); 0.3-0.7 (relative or transient atrophy (for intermediate or intermediate plus vaccine) and <0.3 for a strong atrophy (hot vaccine or infection with vvIBDV) were recorded conforming to the first described standard, and there was not a statistical difference (p>0.05) observed between the groups. This indicating that BB index could be used as a tool in assessing the health status of a flock.

Key words: Avian, Bursa of Fabricius, bursal-body index (BB index), infectious bursal disease (IBD).

INTRODUCTION

The bursa of Fabricius (BF) is an organ unique to birds. It BF undergoes striking changes in size during development, growing rapidly during late embryogenesis and for several weeks (8 to 10 weeks) after hatching before regressing in sexually matured adult chicken at age of 6 to 7 months (Fang and Peng, 2014; Olah and Vervelde, 2012; Olah et al., 2014; Schat and Skinner, 2014). As a primary lymphoid organ, it plays a key role in the differentiation of B-lymphocytes (Cazaban et al., 2015:11). Stress related situations, that is, too low or too high temperature, too much or too little ventilation, other diseases (Marek's, Chicken infectious anaemia (CIA),

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infectious bursal disease (IBD), Newcastle disease and Avian influenza) (Jungbaeck and Nutolo, 2001), mycotoxins, management system (deep litter or battery cage) can directly impact BF size (van Herdeen et al., 2011).

With the absence of an ideal standard for the bursal size, it is difficult to evaluate and interpret bursa weight under field condition (Cazaban and Gardin, 2012). Although, Glick (1956) and Wolfe et al. (1962) addressed (BF) size and development in meat type and egg type chickens genetic lines kept in good ‘’normal’’ conditions and considering bursa free form any infection, sex, age and husbandry influences in the bursa weight and Bursa Body weight ratio (B:B ratio), they fail to issue standards (van Herdeen et al., 2011). With a minimum bursa-to-body weight ratio standard of 0.11 proposed for broilers from 7 to 42 days of age by Cazaban et al. (2015), there is the need to update and device means of standardizing this published standard considering the genetic selection in the poultry today (Cazaban and Gardin, 2012).

The objective of the study was to evaluate the changes in the bursa using BB index, a method developed to overcome the shortfalls of the earlier protocols used in assessing the bursa in some available vaccines in Nigeria and a Nigerian field isolate of the very virulent infectious bursal disease virus (vvIBDV) in cockerels.

MATERIALS AND METHODS

Birds

Two hundred (200) Dominant-Black hybrid commercial day-old cockerels were sourced from reputable hatchery (Terudee Hatchery, Oyo State, Nigeria). The chicks were hatched on the same day and came from the same breeder flock. The breeder flock was vaccinated against infectious bursal disease (IBD) with an intermediate live IBD vaccine at 4 and 10 weeks and inactivated IBD vaccine at 16 weeks. After quality sorting on arrival at the poultry experiment pens of the Avian medicine unit of Ahmadu Bello University, Zaria, and a total of 135 one-day-old chicks were used in the study.

Housing

The birds were housed in the standard avian research facility that mimics field conditions. The birds were placed in a 30 m^2 naturally ventilated. They were raised in the 30 m^2 houses for 17 days (rearing period), and then, from 17^{th} day of age onward they distributed into 5 houses at 3 birds/m^2. They were fed starter from 1 to 21 days of age and grower-finisher feed from 22 days to the end of the study (38 days). Standard lighting period was observed throughout the period of the study.

Vaccination and medication

As a tradition of the hatchery, the chicks were vaccinated against Marek’s disease at the hatchery via subcutaneous injection and were vaccinated against infectious bronchitis by aerosol spray. Coccidiostat was incorporated in the grower feed. No other medications were given for the remainder days of the study.

Experimental design and sampling

Guideline protocols of the Avian Medicine Unit standard research facility of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria was strictly adhered to. Observatory procedure of clinical signs and mortality was carried out daily throughout the period of the study. One hundred and thirty five (135) cockerels were randomly divided into five groups, with each group containing 27 birds each. Each of the group received different treatment; three were vaccinated with 2 intermediate vaccines, and 1 intermediate plus vaccine of the IBDV. The two remaining groups received 0.05 ml of vvIBDV isolate and 0.05 ml of distilled water respectively. The groups were housed in different houses and assigned A, B and C for the vaccinated (A, and C for intermediate and B for intermediate plus); D for the challenge and E for control groups. A total of 27 cockerels, 3 from each group were randomly sampled on day(s) 1, 2, 3, 4, 5, 7, 10, 14 and 21 post vaccination or challenged as follows: Necropsies were conducted at the avian pathology necropsy room of the veterinary pathology department postmortem room of Ahmadu Bello University, Zaria where all the measurements were taken.

Body weight and bursa weight were used to calculate the bursa-body (BB) ratio according to the following formula:

BB ratio = [bursa weight (g)/body weight (g)] × 1000

Bursa-body (BB) index was calculated according to the following formula:

BB index = BB ratio of infected (or vaccinated) birds/ BB ratio of the controls

Each of the BF was cut longitudinally into 2 parts and placed in a 20 ml bottle containing 10% neutral buffered formalin for histopathology and histopathological lesion score using Williams and Davison (2010) criteria with a scoring range of 0 to 5 (0, normal bursal follicle architecture and 5, complete loss of bursal).

Statistical analysis

BB indexes were subjected to statistical analysis between the sampling groups over the period of study using the ANOVA test at a confidence level of 5% (P = 0.05). Tukeys post hoc test was employed to determine differences between groups.

RESULTS

Clinical signs and mortality

Clinical signs were recorded in the challenge (infected) group at day one post challenge (1Dpc). Severe depression, ruffled feathers, anorexia and diarrhoea, characterised by whitish colour were observed in birds in the challenge group, with an increase in birds exhibiting clinical signs. Mortality began on 3Dpc, peaked on 4Dpc, and declined on 5Dpc. At 6Dpc, recovery was observed.

Lesions of the BF

Gross lesions were observed as early as 1Dpc in the challenge group. Slight oedema, hyperaemia and gelatinous yellowish transudate covering the serosal

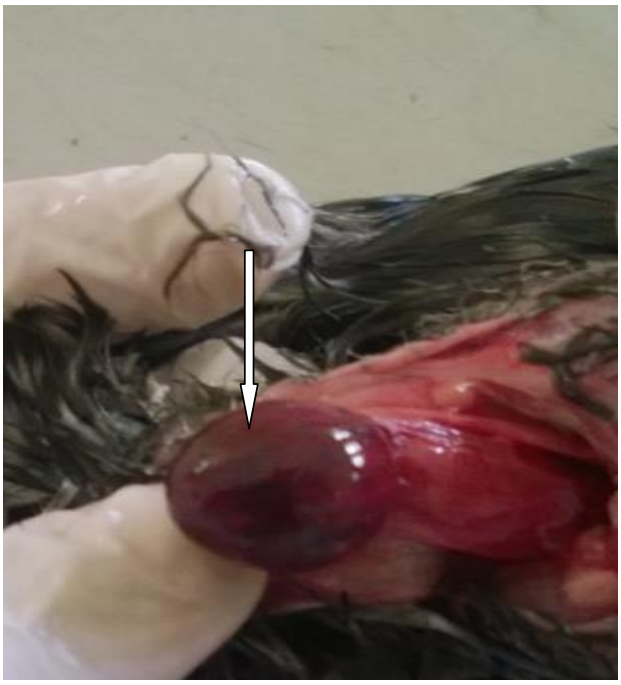

Figure 1. Severe bursal haemorrhage (white arrow) 3 Dpv/c in the challenge group.

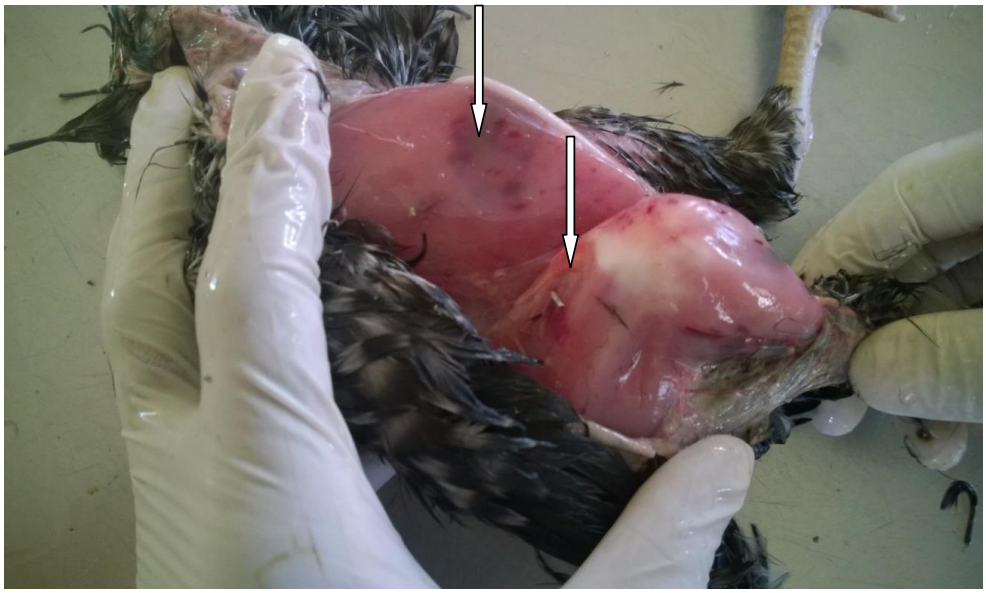

Figure 2. Severe petechial and ecchymotic haemorrhages on the breast and thigh muscle (white arrows) 3 Dpv/c in the challenge group.

surface; on mucosal surfaces, the BF as well as the thigh and breast muscle showed mild petechial haemorrhages. The thymuses were also haemorrhagic on 1Dpc. Bursal haemorrhages; ecchymotic haemorrhages and severe BF congestion (Figure 1) were observed at 3Dpc in the sacrificed and dead birds. At 5 Dpc, mild haemorrhages were observed in the bursae and thigh (Figure 2) of both sacrificed and dead birds, the spleen was also enlarged. The bursae have atrophied to about 1/3 of its size when compared with the control. No relevant gross pathology

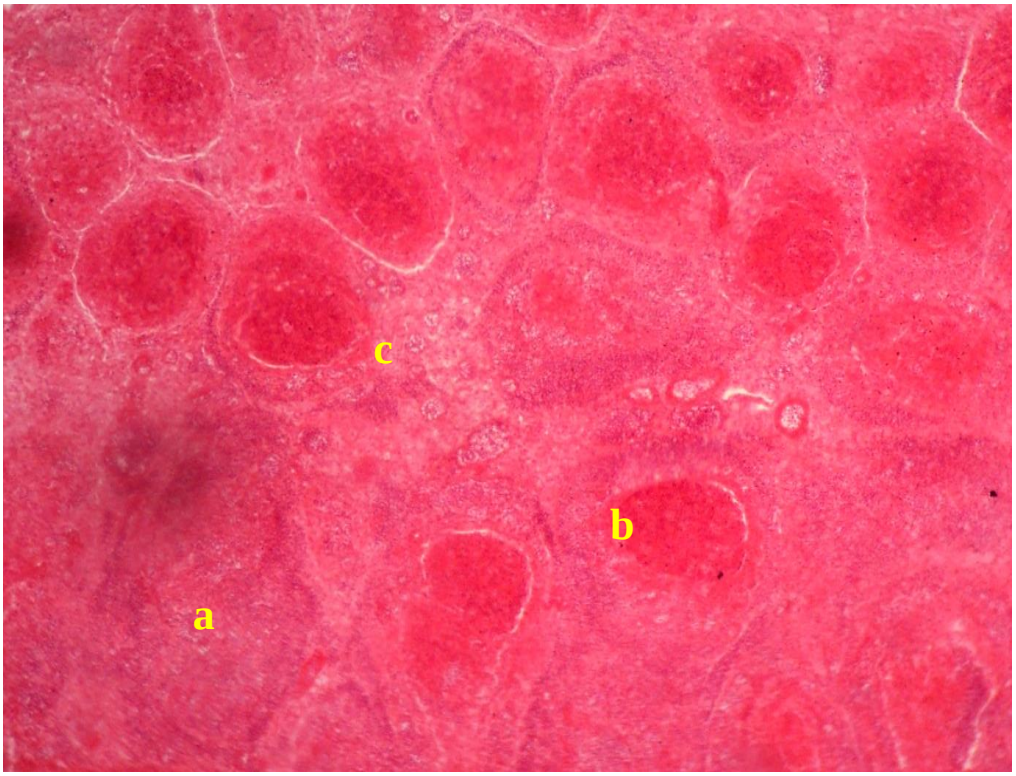

Figure 3. Bursa of Fabricius from Challenge group 5 Dpv/c; lymphoid follicular necrosis (a); Follicular focal haemorrhages (b); marked oedema in the interstitial connective tissue (c). H&E stain × 200.

was observed in birds in the control group.

Microscopically, lymphocytic depletion and haemorrhagic interfollicular interstitium in the medullary areas of the bursal follicles at 1Dpc were observed in the challenge group. Groups B and C presented haemorrhagic follicles at day-one post vaccination (1Dpv) (Figure 3). On days-three, post vaccination and challenge (3Dpv/c), challenge (D) group and group A presented necrosis and depletion of lymphocytes in the follicular medullar and cortex. At 5 Dpv/c, lymphoid necrosis was also observed in the challenge group, progressing to areas of coagulative necrosis within the follicles forming cystic areas (Figure 4) on 21Dpc. Moderate to marked atrophy of BF was observed in groups C and A respectively. Histopathologic lesions scores observed in the challenge group ranged from 4 to 5.

Morphometric of the bursa of Fabricius

Although the mean BF weight increased as birds grew older, however, the vaccinated groups were not able to reduce the bursal weight significantly when compared to the challenge and control groups. However, the challenge group when compared to the control group presented a significant variation in the BF weight on days 10, 14, and 21pv/c.

The BB ration showed a non-significant difference ($p>0.05$) between the groups (Table 1). On 21Dpv/c, all groups revealed a lower BB ratio, with the challenge group exhibiting an early reduction in the BB ratio on day 3 through days 21 post challenge.

The BB index values recorded on 7Dpv/c gave values of <0.3 in the challenge group, indicating strong atrophy; 0.3 to 0.7 in the vaccinated groups, indicating relative transient atrophy (Table 2). The standard is presented in Table 3.

DISCUSSION

For over 50 years bursa of Fabricius has become a major organ (lymphoid organ) of debate since its discovery by Hieronymus Fabricius *ab aquapendente* in the late 16th to early 17th century (Madej et. al., 2012). There exists agreed physiologic pattern of development; colonisation and migration of bursal lymphocytes (B-lymphocytes) in the bursa of bursa of Fabricius, its usage as a tool in diagnosis of infectious bursal disease (IBD) especially bursal size remains unending. Bursal weight, bursal body ratio and bursal diameter were some of the parameters used by Glick in 1956 to study normal bursal regression.

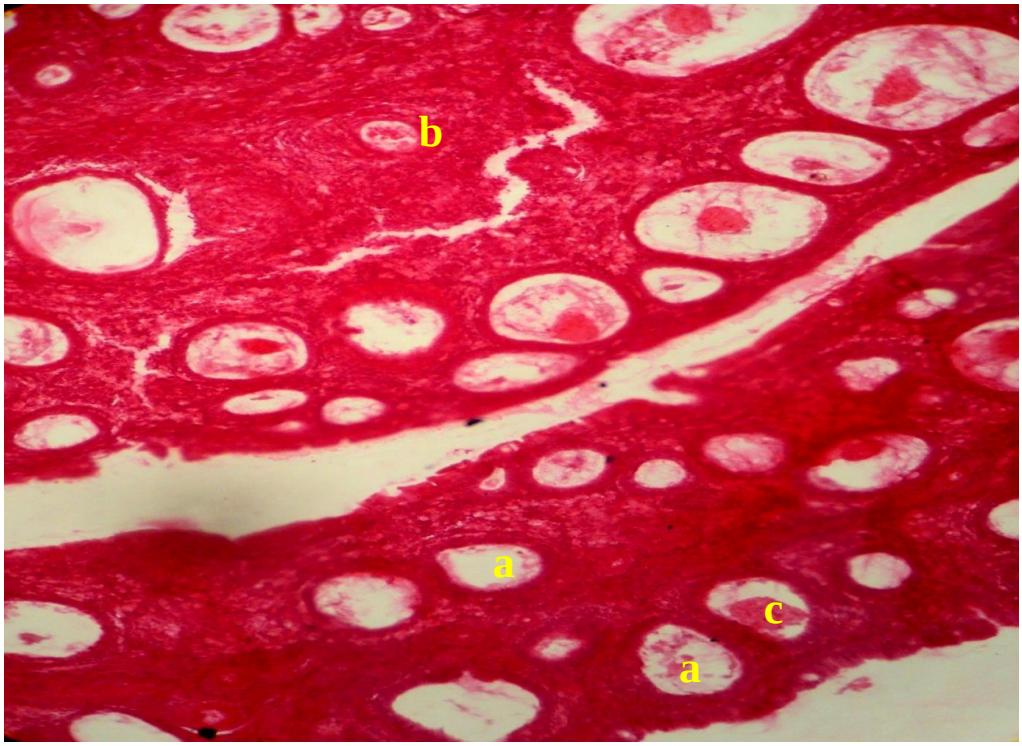

Figure 4. Bursa of Fabricius from MB® group 21Dpv/c: Vacuolar degeneration of the epithelium (a); Follicular atrophy and vacuolar degeneration of the medullary area (b); Follicular necrosis (c). H&E stain × 400.

Table 1. Mean bursa body weight ratio of different treatment and control groups.

Time	A	B	C	D	E
1Dpv/c	4.89±0.53	5.62±1.48	4.35±0.83	5.98±0.31	4.71±1.38
2Dpv/c	6.85±0.48	5.22±1.36	5.80±0.22	6.23±0.91	6.06±1.68
3Dpv/c	6.65±0.93	5.31±1.48	4.31±0.57	2.49±0.28	5.98±1.55
4Dpv/c	4.69±0.80	6.08±1.56	4.37±0.46	1.27±0.13	1.27±0.18
5Dpv/c	6.70±0.22	5.60±1.47	6.45±1.06	1.82±0.37	6.28±1.53
7Dpv/c	2.34±0.08	4.37±1.09	6.19±0.79	1.18±0.09	5.90±1.47
10Dpv/c	1.93±0.38	2.49±0.96	5.68±1.86	0.86±0.24	4.48±1.06
14Dpv/c	1.44±0.22	1.25±0.34	5.73±0.53	1.36±0.15	6.20±1.51
21Dpv/c	1.36±0.16	1.25±0.67	1.13±0.29	1.39±0.24	1.42±0.28

Dpv/c, Day(s) post vaccination or challenge.

But this extensive study of his and that of Jolly in 1914 faced with varying challenging factors ranging from breed type, sex, housing, stress and disease.

Although specific pathogen free (SPF) pullets would have been better for the purpose of this study; however, the cockerels (chicks) used were acquired from an independent, reliable and reputable hatchery where vaccination programme against Marek's disease and infectious bronchitis are routinely administered to day-old-chicks (DOCs). These vaccines have no effect on the integrity of the BF, and the use of the chicks reflected or mimics field conditions.

Vaccine selection was based on commonly available vaccine and as used on poultry farm with little modification. Of the two types of vaccines used in this study, one of the intermediate vaccine produced a moderate to marked reaction on the BF, whereas, the intermediate plus vaccine produced a non-to-mild reactions on the BF. The lesions observed were expected when live IBD vaccine is administered leading to impact

Table 2. Mean (± SEM) value of Bursal Body Index (BB index) of different treatment.

Groups	Bursa Index								
	1Dpv/c	2Dpv/c	3 Dpv/c	4 Dpv/c	5 Dpv/c	7Dpv/c*	10Dpv/c	14Dpv/c	21Dpv/c
A	1.04±0.60	1.13±0.65	1.11±0.64	3.69±2.13	1.07±0.62	0.40±0.23	0.43±0.25	0.23±0.13	0.96±0.55
B	1.19±0.69	0.86±0.50	0.89±0.51	4.79±2.76	0.89±0.52	0.74±0.43	0.55±0.32	0.20±0.12	1.61±0.93
C	0.92±0.53	0.96±0.55	0.72±0.42	3.44±1.99	1.03±0.59	1.05±0.61	1.27±0.73	0.92±0.53	0.79±0.46
D	1.27±0.73	1.03±0.59	0.42±0.24	1.00±0.58	0.29±0.17	0.20±0.12	0.19±0.11	0.22±0.13	0.98±0.56

SEM = Standard error mean; N=3; Dpv/c= Day(s) post vaccination or challenge. A and C = intermediate vaccines; B = intermediate plus vaccine; D = challenge (Infected) group; 7Dpv/c*= BB index calculation day.

Table 3. Standards of the BB index used in classifying IBD viruses, or conventional live IBD vaccines.

BB index	Conclusion
>0.7	Physiological variability = no atrophy Mild vaccines
0.3-0.7	Relative and transient atrophy Intermediate vaccines
<0.3	Intermediate plus vaccines Strong atrophy; Hot vaccines

BB index = BB ratio of infected (or vaccinated) birds / BB ratio of the controls.

on the BF size (Jungbaeck and Nutolo, 2001). Mazeriegos et al. (1990) showed that intermediate vaccine varied in their pathogenicity. They divided intermediate vaccines into 3 pathogenic categories based on bursal damage, bursal B/W and histopathological findings; low or mild pathogenic, moderate pathogenic and highly pathogenic. In line with this result, intermediate vaccine used in group ‘‘A’’ showed to be highly pathogenic.

Furthermore, no clinical signs and mortality were observed in the vaccinated and non-vaccinated (control group (group E)); however the challenge group (D) presented clinical signs exhibited by birds infected with vvIBD virus.

The presence of histopathological lesions on the BF confirmed the pathogenicity of the field IBD virus field isolate used. Although the scoring pattern followed that of Williams and Davison, (2010); it was compared with the European pharmacopoeia lesion scoring because of the live attenuated IBD vaccines used in this study, with lesion of 4 and 5 recorded in the challenge group.

With values of BF weight and size steadily increasing prior to vaccination and challenge as a result of variables due to continues genetic selection through output in egg and carcass. This variability as described by Cazaban and Gardin (2012) was observed during the course of this study.

Following infection or vaccination, the BF goes through several stages; (1) Acute inflammation stage, where bursa is getting larger, and lasts for about 4 days post infection (dpi); (2) Sub-acute stage where BF quickly regresses and gets back to its original size at around 5 dpi and the final stage; (3) Which is the relevant stage to record bursal atrophy, and calculate BB ratio and BB index (which is the main target of this study). As in the previous and only study on BB index by Cazaban and Gardin (2012), the relevant schedule to start recording BB index is from 7 dpi onwards (and say up to 14dpi). The sizes recorded in this study presented the picture of the vaccine virus strain used and likewise the strain of the field virus. The challenge virus isolate gave value of <0.3 BB index indicative of a hot vaccine or vvIBDV infection, whereas the intermediate and intermediate plus vaccines gave a value of between 0.3 to 0.7 which is indicative of the administration of either of the vaccines used.

Conclusively, this study has confirmed that BB index can be used in assessing the BF status (in vaccination or infection) as the BF histopathologic lesion scoring scale is used in designing the safety of live IBD vaccines.

Conflict of Interests

The authors have not declared any conflict of interests.

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