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Retrospective Study

Survival Rate of Calves and Assessment Reproductive Performance of Heifers and Cows in Dida Tuyura Ranch, Borana Zone, Southern Ethiopia

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ABSTRACT

Background and Aim
The study was conducted at Dida Tuyura Cattle Breeding and Improvement Ranch in Borana zone, southern Ethiopia, with the aims of determining the mortality rate of Ethiopian Boran calves and assessment of the reproductive performance of heifers and cows.

Materials and Methods
The study was based on the retrospective record of calves, heifers, and cows. The data was taken from 1994-2010 on the calf survival rate and 1994-2005 on reproductive performance of the breed in the ranch were used for this study. The retrospective data collected over the years were used to identify factors associated with calf survival or death.

Results
The mortality rate of calves before weaning and after weaning was 13.3% and 11.2%, respectively. Age, sex and birth weight of calf had a significant effect on survival rate. The overall mean values age at first service, age at first calving, calving interval, days open and gestation length were 42.52, 51.67, 20.67, 11.30 and 9.30 months, respectively. Parity had a significant effect on both the calving interval and days open. Gestation length was not significantly affected by parity, sex, and birth weight of calf.

Conclusion
From the present study, it can be concluded that the obtained calf survival rate and reproductive performance are not sufficient to achieve the established objective of the ranch.

Keywords
Boran, Calf survival, Ethiopia, Reproductive performance.

INTRODUCTION

Ethiopia, with its 52 million heads of cattle has the largest cattle population in Africa.[1] Cattle production plays an important role in the economies of farmers and pastoralists and the country at large. The agricultural sector in Ethiopia, engaging 80% of the population, contributes 52% of the gross domestic product (GDP) and 90% of the foreign exchange. The livestock sub-sector contributes an estimated 12% total GDP and over 45% to agricultural GDP.[2] Cattle produce a total of 1.5 million tones of milk and 0.331 million tones of meat annually.[3] In addition, 14 million tones of manure are used annually primarily for fuel, and six million oxen provide the draught power required for the cultivation of cropland in the crop-livestock mixed production system.[4]

Boran, a popular cattle breed, is predominantly utilized and widely distributed across various countries of Africa. The Ethiopian Boran breed is one of the cattle breeds widely used in

Ethiopia.[5,6] Available archaeological records indicate that Zebu cattle are the most recent types of cattle to be introduced into Africa. Recent molecular genetics, as well as archaeological evidences[7,8] also showed that the introduction of Zebu cattle into Africa centered in East Africa rather than through the land connection between Egypt and the Near East. Their hardened hooves and lighter bones enable them to endure long migrations. These adaptive attributes have facilitated their importation and spread by Indian and Arabian merchants across the Red Sea to the drier agro-ecological regions of the Horn of Africa.[9]

The Ethiopian Boran breed originally descended from the first introduction of Zebu into Africa from West Asia. The breed established its presence first in semi-arid and arid pastoral Borana plateau of southern Ethiopia. The Borana pastoralist community maintains it. Pastoral movements and migrations led to spread of the Ethiopian Boran to the eastern rangelands in Ethiopia as well as into northern Kenya and southwestern Somalia. The Orma Boran, the Ethiopian Boran, and the Kenya Boran have evolved from these migrations, whereby only the Orma and the Ethiopian Boran are existing on the Borana plateau.[5,8]

The Ethiopian Boran subtype is considered to be the original pure one. The breed is well survived to semi-arid tropical conditions, has a high degree of heat tolerance, is tolerant to many of disease prevailing in the tropics and has the ability to survive long periods of feed and water shortage.[10] Besides, comparisons of the reproductive performance of Ethiopian Boran with other indigenous Ethiopian breeds indicated that Boran cattle calve at a younger age and have shorter calving interval.[5,11]

Now-a-days the existence of this breed is threatened due to various underlying causes, the most important include: bush encroachment and recurrent droughts, poor herd management and difficulties in access to markets.[12] With these facts in mind, Dida Tuyura cattle breeding and improvement ranch are established which is the only available ranch involved in the improvement of Ethiopian Boran cattle. It was established in 1987 on 5550 hectares of land with the objective of conserving and improving Ethiopian Boran breed through selection and controlled breeding. It also supplies pure Ethiopian Boran bulls and heifers for local pastoralist community and other concerned bodies. Calf survival rate and reproductive parameters are among the most important traits affecting progress in selection. So far, little is known about the calf survival rate and reproductive performance of this breed, especially in Dida Tuyura ranch. Therefore, the present study was designed to:

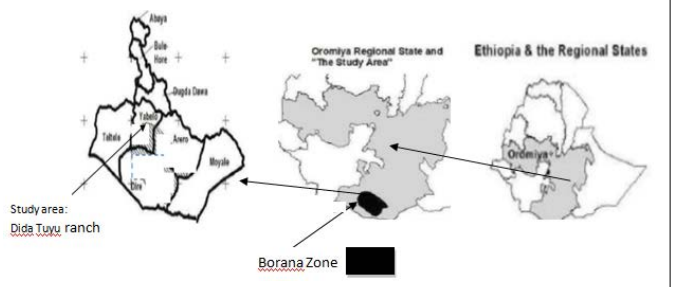
- To depict the long-term trend of calf mortality or survival rate in Ethiopia Boran calves
- To identify factors that are associated with a calf mortality rate
- To evaluate the reproductive performance of Ethiopia Boran cattle and
- To assess the non-genetic factors affecting the reproductive performance under ranch condition.

MATERIALS AND METHODS

Study Area

The Dida Tuyura Ethiopian Boran cattle breeding and improvement ranch is found in the Borana plateau (southern rangelands) Borana Zone of Yabello district and is situated at about 550 km south of Addis Ababa and 20 km north of Yabello town. It is part of the Borana plateau which covers 95,000 km², or 8.5% of the total area of Ethiopia and 14.6% of the lowland areas. Yabello district is characterized by a rather semi-arid climate. Annual mean daily temperature varies from 19 to 24 ºC. The average annual rainfall, as registered by the National Meteorological Service Agency of Yabello station is 600 mm. The rainfall distribution is bimodal, but erratic and unreliable in distribution. About 59% of annual precipitation occurs from March to May and 27% from September to November.[12]

Figure 1. *Map of Borana zone, Southern Ethiopia*



Study Animals and Management

In Dida Tuyura Cattle Breeding and Improvement Ranch, the Ethiopian Boran breed is maintained. They are reasonably large and have a good general body conformation. Their color is mainly white, light gray, fawn or light brown with gray, black or dark brown shading on head, neck, shoulders, and hindquarters. The horns are thick at the base, very short, erect and pointing forward. The hump is well developed in the male, is of pyramidal shape and overhanging to the rear or to one side. The dewlap is well developed. In the male, the preputial sheath is pendulous while in the female, the udder is well developed. Average wither height is 118 to 124 cm in males and 116 to 120 cm in females. Body weight ranges from 318 to 680 kg in males and 225 to 454 kg in females. The breed is known by its tolerance to heat stress and seasonal feed shortage.[13]

The management of the ranch is an extensive system. Animals are herded in a group based on age and sex. They constantly graze natural pasture, year-round. Dry (hay) grass was collected from the field, stored and used as a source of feed in the dry

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period. Supplementary feed during the long dry season was given in limited amount for all groups of cattle. Ponds in ranch are the water source for cattle (once per day) except calves. There is ad libitum tape water source for calves and weak animals.

Calves suckle their dams once a day from 4 or 5-days of birth up to 2 months of age. After 2-months; calves are allowed to run with their dams until weaning at six months of age. Based on weight, body conformation, and health status, both males and females are selected for the further breeding program. Animals were grouped into 1 (male) ratio to 30-50 (females) during the mating period (June, July, and August). Heat detection was practiced during grazing time by observation of unskilled herd's men and experience in natural mating. Pregnancy was confirmed by rectal palpation. Different categories of cattle like heifers, cows, bulls and weaned calves were kept separately outside but calves less than six months age were kept in common pens.

Veterinary medicaments and requirements budget were supplied by Oromia Pastoral Development Commission. Cattle greater than six month age in the ranch were routinely vaccinated against Anthrax, Blackleg, Pasteurellosis, Contagious Bovine Pleura Pneumonia and Foot and Mouth disease. Preventive measures for both external and internal parasites were also carried out regularly.

Study Design

A retrospective type of study was carried out to evaluate the calf survival rate and reproductive performances of Ethiopia Boran cattle in the ranch. Recorded data from 1994-2010 on the calf survival rate and 1994-2005 on reproductive performance of the breed in the ranch were used for this study. Recent data can't be obtained due to poor recording system on the ranch for both calves and cows. Only the data of cows and calves with complete information were included in the study.

The retrospective data collected over the years were used to identify factors associated with calf survival or death. For reproductive performance traits, Ethiopian Boran heifers born from 1994 to 2005 were used to determine the age at first service and age first at calving. Additional cows that gave birth from 1994 to 2005 at varying parity levels were included in the study to determine calving interval (CI), days open (DO), gestation length (GL) and non-genetic factors associated with them.

Data Collection and Analysis

Data of Ethiopian Boran cattle breed calves in Dida Tuyura ranch, collected from 1994 to 2010, were used. Information on calf identification number, birth date, sex of calf, birth weight, weaning date, a terminal event like death and their dates were collected. A total of 929 survival records were available for analyzing the survival rate of Ethiopian Boran calves. At the same time, reproductive trait records (1994-2005) were used to estimate age at first service (AFS), age at first calving (AFC), calving interval (CI), days open and gestation length (GL), respectively.

Collected data were entered into Microsoft spread excel sheet and analyzed using SPSS (version-20). The percentage of calves died was calculated with descriptive statistics. The effect of age, sex and birth weight calf on calf survival rate was analyzed by X^2. In addition, descriptive statistics were used to summarize mean and standard error of reproductive performance parameters. The effect of different factors on the reproductive performance parameters was analyzed by General linear model.

RESULTS

Calf Mortality and Survival Rate

The mortality and survival rates of 929 calves were presented in Table 1. The cumulative mortality rate of calves was 24.5 % with 13.3% before weaning and 11.2% after weaning. The mortality rate of calves was compared for different age, sex, and three birth weight categories. The comparison indicated a statistical significance difference ($p<0.05$) in the probability of calf survival between different age, sexes and varying body weight categories (Tables 1 and 2).

Table 1. *Calf Mortality and Survival Rate in Dida Tuyura Cattle Breeding and Improvment Ranch from 1994 to 2010*

Out Come	Number of Animals	Percentage	*p* value
Dead before weaning age	124	13.3	0.023
Dead after weaning age	104	11.2	
Survived	701	75.5	
Total	929	100.0	

Table 2. *Associations of Sex and Birth Weight of Calf with Calf Survival Rate*

Factors	Number at birth	Dead (%)	Survived (%)	*p* value
Over all	929	24.5	75.5	
Calf sex				
Males	701	75.5	929	0.026
Females	929	100.0	929	
BW of calf				
≤20Kg	286	11.2	19.6	0.041
21-25Kg	443	9.1	38.5	
>25 Kg	200	4.2	17.4	

Reproductive Performance

The overall mean values and standard errors of age at first service, age at calving, calving interval, days open and gestation length of Ethiopian Boran heifers and cows at Dida Tuyura cattle breeding and improvement ranch were 42.52±1.02, 51.67±1.00, 20.67±0.57, 11.30±0.57 and 9.30±0.05 months, respectively (Table 3).

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Table 3. *Mean Reproductive Parameters of Heifers and Cows in the Study Site*

Parameters	No. of observations	Mean±SE
Age at first service	70	42.52±1.02
Age at first calving	70	51.67±1.00
Calving interval	155	20.67±0.57
Days open	155	11.30±0.57
Gestation length	155	9.30±0.05

Calving interval and days open were significantly affected by parity ($p<0.05$) at Dida Tuyura ranch. Gestation length was not significantly influenced ($p>0.05$) by parity level, sex and birth weight of calf (Tables 4 and 5).

Table 4. *Squares Means and Standard Error (LSM±SE) of CI and DO in Association with Parity*

	CI (Months)	Days Open (Months)	*p* value
Parity level	**Mean±SE**	**Mean±SE**	
Overall	20.67±0.57 (n=258)	11.30±0.57 (n=258)	
1	-	12.64±0.77 (n=153)	
2	22.00±0.77 (n=153)	9.89±1.01 (n=72)	<0.05
3	19.31±1.00 (n=72)	8.99±1.68 (n=21)	
4	8.36±1.69 (n=21)	6.73±1.52 (n=12)	
5	16.12±1.54 (n=12)	-	

Table 5. *Least Squares Means and Standard Error (LSM±SE) of Gestation Length and Its Association with Parity, Sex and Birth Weight of Calf*

Factors	GL(Months)	*p* value
	Mean±SE	
Overall	9.30±0.05(n=427)	
Parity		
1	9.23±0.05(n=169)	>0.05
2	9.30±0.05(n=153)	
3	9.41±0.08 (n=72)	
4	9.33±0.12 (n=21)	
5	9.25±0.19 (n=12)	
Calf sex		
Male	9.33±0.72 (n=219)	>0.05
Female	9.26±0.60 (n=208)	
BW of calf		
≤20Kg	9.33±0.09 (n=118)	
21-25Kg	9.22±0.08 (n=190)	>0.05
>25Kg	9.38±0.58(n=119)	

DISCUSSION

The cumulative mortality rate of calves in the present study was 24.5%. Globally, mortality rate over 5% is considered to be too high[14,15] and a mortality rate of 20% can reduce net profit by 38%.[16] The mortality rate was higher in pre-weaned than weaned calves and this was found statistically significant. In early age immune system of young calf is under development. Colostrums can provide passive immunity only against those diseases for which dam possesses antibodies.[17] The pre-weaning mortality rate of the study was higher than findings of Amuamuta et al[18], Kivaria et al[19] who reported 9.4 % and 10%, respectively. This difference in pre-weaning mortality rate might be attributed to the absence of individual pens and poor housing system in the current study site. However, Lobago et al[20,21] were reported higher pre-weaning mortality than present finding but within ranges of 15%-25% for dairy farms, which might be associated lack of colostrums and poor management system in the farms studied. Post eaning mortality rate was lower than previous reports.[22,23,18] The difference in post-weaning mortality could be due to the difference in management which includes timely vaccination, deworming and proper feeding of the animals.

The present study also showed that calves with lower birth weight had a significantly higher mortality rate compared to moderate and higher body weight calves ($p<0.05$). This finding was in harmonious with previous literatures[24-26] who illustrated that calves with lower birth weight have poor vitality and survival ability. The sex-dependent study showed lower mortality rate for females compared to males. This finding conforms well to report of Amuamuta et al,[18] Debnath et al,[25] Mekonnen et al.[27] This difference between sexes in mortality rate might be due to preferential care and management for females for the purpose of early growth and breeding.

The mean age at first service observed in the present study at Dida Tuyura cattle breeding and improvement ranch was 42.52 months. The current result was in line with the previous finding of Ali et al[28] who reported 42.45 months for non-descript Deshi/Indigenous cows in Bangladesh. The present finding was much greater than the published findings by Mureda et al[29] with 26.5 months in Dire Dawa town,[30] with 23.2 months in Gondar town and Dinka[31] with 24.9 months in Asella town for cross breeds. However, the mean AFS obtained in the current study was lower than local Horro heifers who indicated 48.9 months.[32] The variation between Ethiopia Boran breed and other breeds of age at first service might be due to the difference in management, environment, and difference in genotype. Association of management, environment, and genotype with AFS was evidenced by Gifawosen et al.[33]

The AFC of Ethiopian Boran heifer in Dida Tuyura ranch was found to be 51.67-months. This finding is within the range expected for Bos indicus cattle in tropics and reported values the range 35.1 to 53-months.[34,35] In addition, the present finding was comparatively supported by Melaku et al[36] who reported 50.83 months for Fogera cattle. However, other authors indicated lower age at first calving were Mekuriaw et al[37] for Ogaden cattle,[38,39] for cross breeds. Obtained age at first calving in the current study was shorter than 58.3 months for cattle under smaller holder condition in Zimbabwe, 59.73-months for Horro heifers and 54.1-months for Kereyu Sanga cattle reported by Demissu et al,[32] Masama et al,[40] Garoma,[41] respectively. The longer average age at first calv-

ing reported for Ethiopian Boran cattle might be associated with scarcity of feed and shortage of water for the long dry season of the year in the study area. Regardless of the breed, the association of feed availability with attaining age at first calving for heifers was reported.[42]

The calving interval of this study was within estimates of 12.2 to 26.6-months for Zebu cattle reported by Abrha[43] and also closely agreed with the finding of Yifat et al[44] for Boran cows at Tatesa cattle breeding center who reported 20.75-months. This finding was higher than 17.81 months found by[45] for Ethiopian Boran herd maintained at Abernossa ranch and 15.00 months reported by Habtamu et al[46] for Jersey breed in Wolaita Sodo dairy farm. In addition,[47] for Boran cows at Mkwaja ranch of Tanzania,[38] for crossbreed and Habib et al[48] for Red Chittagong cattle at Nucleus herd in Bangladesh were reported shorter calving interval than present finding. But the finding was much lower than 26-months of traditionally managed Ethiopian high land Zebu.[35] The variation of calving interval among the observation of different researches might have resulted due to different sample size, genotype, number of parity, forage availability in any particular year, disease condition and days open.

The current study showed that calving interval becomes shorter as the parity increased. Generally, longer calving interval was seen in second parity might be due to the stress of sucking calf in young growing animals in early parities thus delays the onset of postpartum heat. In later parities, there is physical maturity with advancing of the age of cows. The report was consistent with finding of Ibramhim et al,[49] Negussie et al[50] on indigenous and cross breeds. However, other scholars Agyemang et al,[51] Haile-Mariam et al[52] reported a non-significant effect of parity on calving interval (CI).

The mean days open was 11.30-months which was in accordance with finding of Yifat et al[44] who indicated for 11.34-months. However, this finding was higher than previous reports.[53,54] Relatively longer do in the present study might be due to sucking of calves up to weaning age which may interfere with ovarian function. The interference of sucking of calves until weaning age on ovarian function was indicated.[55] Significant association of parity on DO was obtained in this study. The finding was in harmonious with other authors[56,57] who reported a significant effect of parity on DO. However, Gifawosen et al,[33] Yohannes et al[58] indicated non-significances of calving parities on DO.

The overall mean gestation length in the current study was 279-days (9.30-months) which was in comparison with a report of Tegegne et al.[59] Although gestation length is more or less constant within a given species[60] but relatively shorter GL has prevailed from studies of Swensson et al[61] for Arsi cattle. Slightly longer GL (291-days) was found by the studies of Alberro[34] for Ethiopian high land Zebu cattle.

In the current study, GL was not significantly affected by the parity level (p>0.05). The result was in agreement with the report of Yifat et al,[39] Habib et al[48] who found an absence of significant effect of parity on GL[36,62] indicated a significant effect of parity on GL. In addition, the present study also noted the non-significant effect of sex and birth weight of calf on the GL. Likewise, the non-significant influence of sex of calf on GL was reported by Melaku et al[36], Haile-Mariam et al,[52] Addisu.[63] However, Mukasa-Mugerwa et al,[64] Getinet et al[65] were found a significant influence of sex of calf on the GL. The significant effect of the birth weight of calf on the GL was reported by[37] which were not supported by the present study.

CONCLUSIONS AND RECOMMENDATIONS

From the result of this study, it could be concluded that calf survival rate and reproductive performance of Ethiopian Boran cattle are within the range of values reported for other tropical and particularly Ethiopian cattle breeds. Given the fact that the study ranch raise their own replacement stock and distribute heifers and bulls to the community and other concerned bodies, obtained calf survival rate and reproductive performance have great hindrance to fulfill the demand of community and concerned bodies and to improve productivity through the distribution of heifers and bulls. Considered factors associated with calf survival and reproductive performance (parity) have a significant effect on traits indicating great effort should be made towards mitigating negative impacts of those factors associated with calf survival and reproductive performance. Thus, the present study suggests that factors associated with calf survival rate and reproductive performances are the serious problems in achieving established the objective of the ranch. Therefore, it is recommended that a detailed study on the factors affecting calf survival rate and reproductive traits as well as the determination of the impacts of each factor on the calf survival rate and reproductive traits should be studied especially through follow-up to improve calf survival rate and reproductive performance.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

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Review

Overview of Methods Used in the Diagnosis of Infectious Bursal Disease

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ABSTRACT

Infectious bursal disease (IBD) is an infectious viral disease of poultry. It is caused by infectious bursal disease virus (IBDV) that is a member of the genus *Avibirnavirus* of the family *Birnaviridae*. The virion is non-enveloped and consists of a bi-segmented RNA molecule. The disease occurs in a clinical and subclinical form depending on age at infection. Only young chickens are clinically affected. Severe acute disease of 3-6 week old birds is associated with high mortality but a less acute or subclinical disease is common in 0-3-week-old birds. This can cause secondary problems due to the effect of the virus on the bursa of Fabricius. There are two serotypes of IBDV; These are serotype 1 which is pathogenic to chickens and commonly leads to the development of the clinical form of the disease and serotype 2 is avirulent to chickens. Clinical IBD can be diagnosed by the combinations of a characteristic sign and post-mortem lesions. Gross lesions are characterized by marked haemorrhages in the pectoral and thigh muscles. At post-mortem examination bursa of *Fabricius*, thymus, spleen and kidneys are initially enlarged, however, bursa of Fabricius and thymus are later become atrophic. Histologic lesions showed marked edema, infiltration of heterophiles, hyperaemia and lymphoid depletion and hyper plastic corticomedullary layer in the bursa of Fabricius. Serological diagnosis of IBD by agar gel immunodiffusion (AGID), enzyme linked immunosorbent assay (ELISA), virus neutralization test (VNT) and agar gel precipitin test (AGPT) are also possible. Subclinical IBD can be confirmed in the laboratory by detecting viral antigens in tissues. In the absence of such tests, histological examination of the bursa may be helpful.

Keywords
Gross lesions; IBDV; Serology; Molecular techniques; Bursal disease; Gumboro disease.

Abbreviations
AGID: Agar gel immunodiffusion; AGPT: Agar Gel Precipitin Test; BF: Bursa Fabricius; CAM: Chorio Allantoic Membrane; CDNA: Complementary Deoxy Ribonucleic Acid; CEF: Chicken Embryo Fibroblast; CPE: Cyto-pathic effect; DsRNA: Double Stranded Ribonuclic Acid; ELISA: Enzyme linked immunosorbent assay; IBD: Infectious Bursal Disease; IBDV: Infectious Bursal Disease Virus; MDA: Maternally Derived Antibodies; ORF: Open Reading Frame; PMOLE: Picomole; SAN: Specific Antibody Negative; SPF: Specific Pathogen Free; VNT: Virus Neutralization Test.

INTRODUCTION

Infectious bursal disease is an acute and highly contagious disease which is mostly affects young chickens of 3-6 weeks old. The disease, also named "Gumboro disease" according to the location of the first outbreaks in Gumboro, Delaware, USA, was initially described as avian nephrosis due to damage seen in the kidneys,[1] but was later designated infectious bursal disease (IBD) according to varying morphologic and histological changes observed in the bursa of Fabricius.[2]

The causal agent of IBD is infectious bursal disease virus (IBDV), which is a non-enveloped double stranded RNA (dsRNA) virus belonging to the genus Avibirnavirus, family Birnaviridae.[3-5] Infectious bursal disease virus genome has two double-stranded RNA segments; these are segment A and segment B.[6] Segment A

contains two partially overlapping open reading frames (ORFs), these are; ORF1 and ORF2. The small ORF1 encodes a non-structural protein VP5, whereas the large ORF2 encodes a precursor polyprotein (NH2-VP2- VP4-VP3-COOH), which is processed to produce mature protein like VP2, VP3 and VP4. The largest ORF contains a polyprotein that is proteolytically cleaved to form three polypeptides: namely VP2 and VP3 are the structural proteins, whereas VP4 is a protease.[3,7] Segment B contains one ORF encoding VP1,[7,8] with the multifunctional protein having the polymerase (an RNA-dependent RNA polymerase) that is responsible for viral genome replication, mRNA synthesis and capping enzymatic activity.[9]

The IBDV is one of the most economically important diseases that affect the growth of young chickens which results in significant economic losses in the poultry industry.[10] The virus has a selective tropism for actively dividing bursal B-lymphocytes which leads to massive destruction of B-lymphocytes in the bursa and to a lesser degree, in other lymphoid organs thereby causing prolonged immunosuppression of chickens infected before 3 weeks of age.[11,12]

Commonly young chickens at 0-2 weeks old have a high level of maternally derived anti bodies (MDA) hence, resistance to IBD. Nevertheless, the MDA level declines with age and so also bursa of Fabricius. Once the target organ reaches its maximum development between 3 to 6 weeks, thereafter the chickens will be highly susceptible to IBD.[13]

Strains of IBDV can be grouped within two distinct serotypes by virus neutralization test.[14] Serotype 1 is pathogenic in chickens and serotype 2, isolated from turkeys, is not pathogenic in chickens or turkeys.[6] Serotype 1 strains are further classified into very virulent (VV), classical virulent, and sub-clinical on the basis of pathogenicity.[15] Very virulent strains of serotype 1 IBDV are common worldwide and cause serious disease. Clinical IBD can be diagnosed by a combination of characteristic signs and post-mortem lesions. Subclinical IBD can be confirmed in the laboratory by demonstrating a humoral immune response in unvaccinated chickens, or by detecting viral antigens or viral genome in tissues. In the absence of such tests, histological examination of bursae may be helpful.[16]

Clinical IBD is responsible for heavy economic losses due to impaired growth, death and also from the excessive condemnation of carcasses because of skeletal muscle haemorrhages[17,18] and losses due to immunosuppression.[19] The clinical features of the IBD included whitish or watery diarrhoea, followed by anorexia, depression, trembling, severe prostration, and death. At necropsy, the birds exhibited dehydration, haemorrhages in the leg and thigh muscles, urate deposits in kidneys and enlargement of the bursa of Fabricius.[20] Infectious bursal disease has not been reported to have any zoonotic potential.[21]

The infection, when not fatal, cause immunosuppression, in most cases temporary, depending on the virulence of the strain, the age, breed and the presence or absence of passive immunity. In chicken flocks, clinical picture and the course of the disease usually are indicative of an IBDV infection, and the pathological changes observed at the Bursa of fabricius (BF) are characteristic.[13] Presence of this disease causes alterations in different haematological and serum biochemical parameters in poultry.[18] The immunosuppressive effects of IBDV infections enhance the chicken's susceptibility to secondary bacterial infections such as gangrenous dermatitis, Escherichia coli infections, with a viral infection like chicken anemia agent, inclusion body hepatitis, respiratory diseases.[11]

Therefore, the objective of this paper is to highlight various commonly used diagnostic techniques of IBD.

DIAGNOSIS OF INFECTIOUS BURSAL DISEASE

Infectious Bursal Disease is diagnosed by considering the flock's history and of the clinical signs and post mortem lesions. Obviously, chickens less than 3 weeks of age present no clinical signs of disease but chickens greater than 3 weeks of age present clinical signs.[22] Clinical manifestations and postmortem findings of affected birds may aid to diagnose a disease but laboratory diagnosis is necessary for confirmation of the diseases.[23]

Isolation and identification of the agent provide the most certain diagnosis of IBD but are not usually attempted for routine diagnostic purposes because the virus is difficult to isolate. In practice, laboratory diagnosis of IBD depends on detection of specific antibodies to the virus, or on detection of the virus antigen and nucleic acid in tissues, using immunological or molecular methods.[16] Confirmatory diagnosis of IBDV is most commonly performed by serology using Enzyme linked immunosorbent assay (ELISA), Agar gel precipitin test (AGPT) and Virus neutralization test (VNT) of bursal sections.[24]

Clinical Signs

Infectious bursal disease virus has a short incubation period of 2-3 days and the infection generally last 5-7 days. One of the earliest sign of IBDV infection is the tendency for the bird to engage in vent picking.[25]

In a typical outbreak in 2- to 15-week-old chickens, some 10-20% of the flock may show sudden signs. Observing any signs is difficult during the very early stages. One of the earliest signs is whitish or watery diarrhoea, with vent feathers soiled by urinary material. This is followed by anorexia, depression, trembling, severe prostration, and death.[26] The disease is manifested by debilitation, dehydration and development of depression with the swollen and bloodstained vent.[27]

Differential Diagnosis

The lesions and symptoms of coccidiosis are very similar to IBD. Similar to IBD in coccidiosis there are sudden onset, ruffled feathers bloody droppings but no bursal lesion.[28] However, muscular

haemorrhages and edema of bursa differentiate IBD from coccodiosis. Other diseases that resemble IBD are infectious bronchitis virus, haemorrhagic syndrome, Marek's disease.[29]

Quinn reported that if there are enlarged muscular haemorrhages and enlarged edematous or haemorrhagic cloacal bursas, it would suggest IBD, however, the involvement of cloacal bursa usually will distinguish IBD from other nephrosis causing condition. Marek's (causes bursal atrophy, but nerve lesion is very distinct and also marek's forms tumors). Haemorrhages syndrome (cause bursal muscular mucosal haemorrhage, but with no bursal lesion) is the usual manifestation of the diseases.[28]

Non-infectious disease like mycotoxins are causes of bursal atrophy.[30] In aflatoxins there is a degeneration of both thymus and the bursa and trichothecenes generally depress lymphocytopoesis. High doses of zearalenon also cause decreased bursal weight. As in other species, steroidal anti inflammatory drug like, corticosteroids cause apoptosis and decrease the production of lymphocytes and smaller bursas can be occurred in animals that are in poor nutritional states or stressed from other reasons.[31,32]

Gross Lesions

Changes in lymphoid organs are typical of the disease. The BF, which is the main target of the virus, undergoes major changes beginning at 3 d post-infection (PI) when it increases in size reaching twice the normal size by 4 d PI followed by atrophy, reaching one third of its original weight by 8 d PI. The increase in size is accompanied by a red coloration.[33] Autopsies performed on birds that died during the acute phase (three to four days following infection) indicate hypertrophic, hyperaemic and oedematous bursas. The most severe cases are characterized by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish colour. This appearance is often accompanied by petechial and haemorrhages. The affected birds are severely dehydrated, and many birds have hypertrophic and whitish kidneys containing deposits of urate crystals and cell debris.[34]

Haemorrhages in the pectoral muscles and thighs are frequently observed, probably due to a coagulation disorder.[35] Office International des Epizootics[16] stated that the bursae of chickens infected with virulent serotype 1 IBDV appeared yellowish (sometimes haemorrhagic) with black cherry appearance and turgid, with prominent striations (Figures 1 and 2).

Figure 1. *Haemorrhages on Thigh Muscles and Breast Muscles*[36]

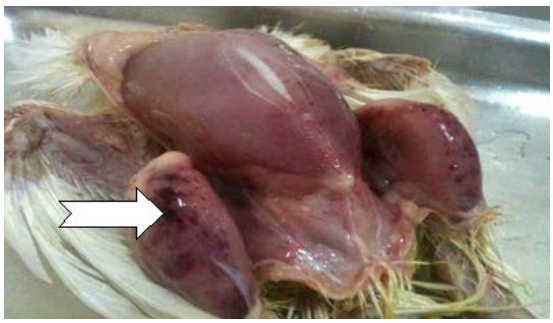
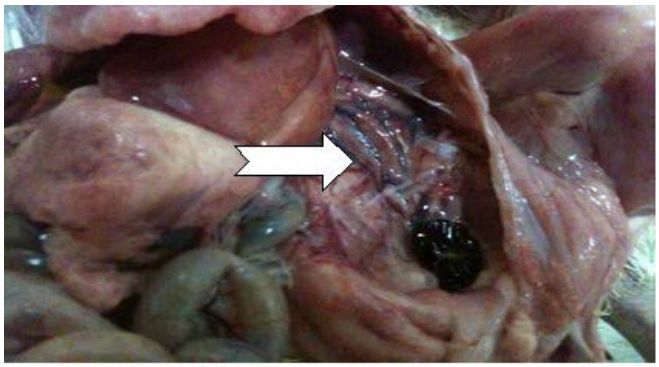


Figure 2. *The Presence of Haemorrhages on the Serosal Surface of Bursa*[36]



Histopathological Lesions

According to OIE,[16] tissue of bursa of fabricius was removed aseptically from affected chickens in the early stages of the disease. Bursae is Chopped by using two scalpels, add a small amount of peptone broth containing penicillin and streptomycin (1000 μg/ml each), and homogenise in a tissue blender. The homogenate is centrifuged at 3000 g for 10 minutes. Harvest the supernatant fluid for use in the investigations. The bursal tissue are fixed by immersion in 10% buffered formalin and processed for histologic examination by standard methods of paraffin embedding, sectioning and haematoxylin and eosin staining.[37] The tissue sections were examined by light microscope and the severity of lesions was graded on the basis of the extent of lymphocyte necrosis, follicular depletion and atrophy.[38]

Histological diagnosis is based on the detection of modifications occurring in the bursa. The ability to cause histological lesions in the non-bursal lymphoid organs, such as the thymus,[10] the spleen or bone marrow[39] has been reported as a potential characteristic of hypervirulent IBDV strains.

Necrosis and infiltration of heterophils and plasma cells occur within the follicle, as well as, the interfollicular connective tissue. In addition, a fibroplasia, the inter follicular connective tissue, may appear and the surface epithelium of the bursa becomes involutes and abnormal.[40] Histological lesions in the kidney are nonspecific and probably occur because of severe dehydration of affected chickens. Lesions observed consisted of large casts of homogeneous material infiltrated with heterophils, and also glomerular hypercellularity.[11] Proliferation of the bursal epithelial layer generates a glandular structure of columnar epithelial cells that contains globules of mucin. During this stage of the infection, scattered foci of repopulating lymphocytes were observed; however, these did not develop into healthy follicles.[41]

Virological Diagnosis

In the acute phase of infection, Infectious bursal disease virus may be detected in the bursa of Fabricius of chicks ideally within the first three days following the appearance of clinical signs.[34]

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Virus Isolation in Cell Culture

In order to observe cytopathic effect, 0.5 ml of sample is inoculated into each of four freshly confluent chicken embryo fibroblast (CEF) cultures (from a specific pathogen free (SPF) source) in 25 cm^2 flasks. Adsorb at 37 °C for 30-60 minutes, wash twice with Earle's balanced salt solution and add maintenance medium to each flask. Then it is Incubated at 37 °C, observing daily for evidence of cytopathic effect (CPE). This is characterized by small round refractive cells. If no CPE is observed after 6 days, discard the medium, then freeze-thaw the cultures and inoculate the resulting lysate into fresh cultures. The more pathogenic IBDV strains usually cannot be adapted to grow in CEF unless the virus has first been submitted to extensive serial passage in embryos.[16]

Cell cultures containing 50% bursal lymphocytes and 50% CEF have been used to isolate and serotype IBD virus successfully.[29] The traditional isolation method for IBDV using the chorioallantoic membrane of 9 to 11-day-old chicken embryos is no longer reliable, as some variant strains of the virus cause no embryo mortality.[42] In addition, most field isolates cannot be readily adapted to grow in primary chicken cell cultures.[43,44] However, embryo- or cell-culture adapted strains of IBDV replicate and produce cytopathic CPE in the avian continuous cell line[45] and in mammalian continuous cell lines such as rabbit RK-13 cells[46] and monkey Vero cells.[47] Such cell lines provide sensitive media for assay of the virus.[48,49]

Virus Isolation in Embryos

To identify serotype 1 and serotype 2 inoculate 0.2 ml of sample into the yolk sac of five 6- to 8-day-old specific antibody negative (SAN) chicken embryos and on to the chorioallantoic membrane of five 9 to 11 day-old SAN chicken embryos. Specific antibody negative embryos are derived from flocks shown to be serologically negative to IBDV. Candle daily and discard dead embryos up to 48 hours post-inoculation. Embryos that die after this time are examined for lesions. Serotype 1 IBD produces dwarfing of the embryo, subcutaneous oedema, congestion and subcutaneous or intracranial haemorrhages but Serotype 2 IBDV does not induce subcutaneous oedema or haemorrhages in the infected embryos, but embryos are of a smaller size with a pale yellowish discolouration.[16]

Viral isolation was performed on bursas from infected chickens. Bursal tissue homogenate was inoculated onto the chorioallantoic membrane (CAM) of 10-day-old SPF embryonating chicken eggs.[30]

Serological Diagnosis

For serological investigations, usually blood can collected collect from the wing vein, allowed to clot and serum separated by centrifugation and stored at -20 °C until tested.[50] Serological tests generally used for the detection of IBDV are ELISA, VN and Agar Gel Immunodiffusion (AGID). The ELISA is the most commonly used test for the detection of antibodies to IBDV.[29] The infection usually spreads rapidly within a flock of birds. Because of this, only a small percentage of the flock needs to be tested to detect the presence of antibodies. If positive reactions are found in unvaccinated birds then the whole flock must be regarded as infected.[51]

Agar Gel Immunodiffusion (AGID)

Agar gel immunodiffusion test is one of the alternative tests recommended for IBD diagnosis by Organisation for Animal Health (OIE) in its list of tests for international trade.[52] The AGID test is the most useful of the serological tests for the detection of specific antibodies in sera, or for detecting viral antigen or antibodies in bursal tissue. Blood samples should be taken early in the course of the disease, and repeat samples should be taken 3 weeks later. Because the virus spreads rapidly, only a small proportion of the flock needs to be sampled. Usually 20 blood samples are enough. For detection of antigen in the bursa of Fabricius, the bursae should be removed aseptically from about ten chickens at the acute stage of infection. The bursae are minced using two scalpels in a scissor movement, and then small pieces are placed in the wells of the AGID plate against known positive serum. Freeze-thaw cycles of the minced tissue may improve the release of IBDV antigens from the infected bursal tissue.[51]

The result of tested serum is interpreted as when a clear precipitin line is formed a "line of identity" with that of the positive control antiserum and with the antigen in the central well but, when no line is formed the tested sera is considered as negative result. The test is repeated when no clear precipitin line is formed at the positive control well or when a suspicious reaction at the tested serum well observed.[53]

AGID is the simplest, but least sensitive technique. Results are obtained after an incubation period of 48 h. Variability in results may be due to the investigator, as well as the nature of the viral strain used as an antigen.[54-58]

The AGID test can also be used to measure antibody levels by using dilutions of serum in the test wells and taking the titre as the highest dilution to produce a precipitin line.[59] This can be useful for measuring maternal or vaccinal antibodies and for deciding on the best time for vaccination; however, this AGID quantitative determination has now been largely replaced by the ELISA.[60]

Enzyme Linked Immunosorbent Assay (ELISA)

The principle of ELISA is that antibodies are attached to their specific antigen by linking an enzyme to an antibody following the addition of the substrate. A serum sample is added and if there are specific antibodies they will bind to the antigen. If there is a positive sample, the antibody will attach and react with the substrate.[61] Thus the positive samples will develop colour.[62,63]

The ELISA allows the quantification of antibodies to IBDV and is therefore used for monitoring the immune status of

the chicken flocks to check response to vaccination, natural field exposure and decay of maternal antibody titer.[64] The ELISA is the most rapid and sensitive method and presents the fewest variations due to the viral strain used as an antigen. It is economical, simple, and quick and tests a large number of samples at the same time and is adaptive to automation to computer software.[64] However, ELISA cannot differentiate between the antibodies specific to the two serotypes.[64,65]

Agar Gel Precipitation Test (AGPT)

Another method used to detect antibodies to IBDV is the AGP test. This test has been adapted to the quantitative format.[59] Antigen was prepared from a saline suspension of bursae from chickens infected with IBD virus. Briefly, a 50% suspension was homogenized and then clarified by centrifugation. The antigen was checked for sensitivity and specificity against known positive and negative sera but was not standardized otherwise. Test sera were placed in wells adjacent to positive control sera to enhance sensitivity and to establish specificity of lines.[54]

It is rapid but insensitive. It does not detect serotypic differences and measures primarily group-specific soluble antigens.[64]

Virus Neutralization Test (VNT)

In virus neutralization neutralization test (VNT) is the gold test and the only serologic test that discriminates between antibodies elicited by the two serotypes and various subtypes of the serotype 1 strains.[66]

VN tests are carried out in cell culture. The test is more laborious and expensive than the AGID test, but is more sensitive for detecting antibody. This sensitivity is not required for routine diagnostic purposes, but may be useful for evaluating vaccine responses or for differentiating between IBDV 1 and 2 serotypes.[51]

When a virus is mixed with homologous antiserum it will be neutralized and not infectious. This is the principle of a VNT and can be visualized in cell cultures. If the virus usually produces a CPE in cell culture, the neutralized virus will not be able to produce it and thereby the effect of the serum can be observed.[61]

Table 1. *Bursal Lesion Scoring System*

Level of severity description	
0	No lesion
1	Mild scattered cell depletion in a few follicles
2	Moderate, 1/3 to 1/2 of the follicles have atrophy or depletion of cells
3	Diffuse, atrophy of all follicles
4	Acute, inflammation and acute necrosis typical of IBD

Source[67]

Molecular Diagnosis of IBD

Another method that is used to detect IBDV is molecular technique. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) is one of the most important frequently used molecular method that is used to detect the genome of IBDV.[68] Reverse transcription-polymerase chain reaction (RT-PCR) enable us to detect viral RNA in homogenates of infected organs or embryos, as well as in cell cultures, without considering the viability of the virus present.[34] It is also used to detect the genome of viruses that don't replicate in cell culture because it doesn't require the growth of the virus before amplification. There are three steps in which RT-PCR is performed .These are; extraction of nucleic acids from studied sample, change of IBDV RNA into cDNA by Reverse Transcription (RT) and amplification of cDNA by PCR. The IBDV double stranded RNA stranded RNA (dsRNA) can't be degraded by RNAases, unlike single stranded RNA.[69]

Extraction of IBDV RNA

Infectious bursal disease RNA can be extracted from infected tissues by using some kits which is available from commercial suppliers of molecular biology reagent. In another way IBDV RNA can be extracted by adding 1% sodium dodecyl sulphate and 1 gm/ml proteinase K to 700 µl of bursal homogenate and Incubated for 60 minutes at 37 °C. Nucleic acids are harvested from the final aqueous phase by ethanol precipitation and are resuspended in RNase-free distilled water or a suitable buffer. Water-diluted RNA should be kept frozen at a temperature below -20 °C until use.[69]

First Strand cDNA Synthesis

The extracted RNA is used for the synthesis of cDNA. The following reagents are mixed in PCR tubes to a final volume of 25 µL. These are; Template RNA 1 µg, OligodT primer 1 µL and Nuclease free water to 12 µL. Then the above mixture was kept at 65 °C for 5 min in a thermal cycler, followed by the addition of the following components in the indicated order: 5 X reaction buffer 4 µL, Rnase inhibitor 1 µL, 10 mM dNTP 2 µL and Reverse transcriptase 1µL. The above mixture was kept at 42 °C for one hour and 5 minutes at 70 °C in a thermal cycler.[70] Synthesized cDNA was used as template for polymerase chain reaction (PCR).[71]

Reverse Transcriptase -Polymerase Chain Reaction (RT-PCR)

The cDNA was amplified in a 25 ml reaction mixture as given as below; Mastermix 12.5 µL, Forward primer (20pmol) 5'-GGTAACYGTCCTCAGCTTA-3' 1 µL, Reverse primer (20 pmol) 5'-GTTCAGGATTTGGGATCAGC-3' 1 µL, Template DNA 3 µL and Nuclease-free water 7.5 µL. After amplified the reaction mixture was subjected to initial denaturation of 95 °C for 5 minutes followed by 35 amplification cycles at 95 °C for 45 seconds, 51°C for 45 seconds and 72 °C for 1.30 minutes with final extension at 72 °C for 10 minutes.[70]

The extracted RNA is changed into cDNA then RT-PCR. Reverse Transcription-Polymerase chain reaction is work on VP2. The VP2 is the major structural protein that contains the antigenic regions responsible for the production of neutralizing antibodies in the chicken. VP2 contains a hypervariable region that

displays the greatest amount of amino acid sequence variation between strains.[72,73] This region is responsible for antigenic variation, tissue-culture adaptation and it is partially responsible for viral virulence.[74]

A commercial cDNA synthesis kit (Fermantas, USA) is used to make cDNA. Hair-Bejo M[4] also reported that for amplification of a 743 bp fragment of VP2 hypervariable region, two region, two primers are used these are; forward primer 5'-GCCCAGAGTCTACACCAT-3 and Reverse primer s5'-CCCGGATTATGTCTTTGA-3'. Then the amplification products were detected by gel electrophoresis in 1.5% agarose gel in buffer. Gels were run for 1.5 h at 80 V, stained with ethidium bromide (0.5 µg/ml), exposed to ultraviolet light and photographed (Visi-Doc-It system, UVP, UK).

If four amino acids (alanine 222, isoleucine 256, isoleucine 294 and serine 299) are present simultaneously present, it simultaneously, it is considered as indicative of vvIBDV.[75,76]

CONCLUSION AND RECCOMMENDATIONS

Infectious bursal disease is one of the viral diseases that affect poultry all over the world. It mainly affects young chickens between 3-6 weeks old. This disease is highly affects bursa of Fabricius. Economic losses due to IBD is directly associated with mortality and in directly with immunosuppression. Infectious bursal disease is also called "gumboro disease" according to the location of first outbreaks in Gumboro Delaware, USA, was initially described avian nephrosis due to damage seen in kidney but later the name of avian nephrosis is changed to infectious bursal disease depending on morphologic and histologic change occurred in bursa of Fabricius. Infectious bursal disease virus is the highly contagious disease that causes IBD. Two serotypes are recognized. These are; serotype 1 which is pathogenic to chickens and serotype 2 which are not pathogenic to chickens. Diagnosis of IBD is depending on clinical signs, differential diagnosis, gross lesions, histopathological lesions, virus isolation, serological and molecular diagnosis. Agar gel immunodiffusion is the simplest but least sensitive where as ELISA is a rapid and sensitive method a rapid and sensitive method is but cannot differentiate serotypes. Virus neutralization test is the golden standard and the only serologic test that differentiate test that differentiates antibodies of two serotypes and sensitive but sensitive but it is more laborites laborious and expensive than AGID. Another method that is used to detect IBDV is molecular methods. Reverse Transcription-Polymerase Chain Reaction is used to detect IBDV without considering the viability of the virus by working on VP2 which is a major structural protein that is responsible for the production of neutralizing antibodies in chickens and it is a place where a greatest amount of amino acid variation occurred between strains. Depending on the above conclusion, the following recommendation is forwarded.

Early diagnosis of IBDV must be conducted because of it is highly contagious disease. Infectious bursal disease should be differentiated from other disease that has the same lesions. More sensitive and rapid test should be selected.

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Original Research

Canine Urolithiasis and Concurrent Urinary Bladder Abnormalities: Symptoms, Haematology, Urinalysis and Comparative Radiographic and Ultrasonographic Diagnosis

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ABSTRACT

Background
Urocystoliths are difficult to palpate and physical examination findings, complete blood cell count (CBC) and serum biochemical analysis are usually normal and the clinical signs are not definitive. Thus diagnostic imaging is a crucial tool required to confirm the diagnosis of urolithiasis in dogs presented with non-specific clinical signs of urogenital affection.

Aim
The aim of this study was to compare the capability of radiography and ultrasonography in detecting uroliths and concurrent urinary system abnormalities and to evaluate clinical, haematological and urinalysis findings of dogs affected with urolithiasis during the presentation.

Methods
Findings of signalment, history, physical and laboratory examination of blood and urine were performed and recorded. All dogs presented with complete or partial urinary obstruction, haematuria and renal failure were subjected to both radiographic and ultrasonographic evaluation. Uroliths were retrieved by a cystotomy, urethrotomy, and at necropsy from kidney failure cases confirming urolithiasis.

Results
The result revealed occult clinical haematuria in 56.5%, microscopic haematuria in 78.3% and dysuria/anuria in 34.8% of the affected dogs. Crystalluria is detected in seven (30.4%) of urolithiasis affected dogs. The total leukocyte count was significantly elevated ($p \leq 0.05$) in partially and completely obstructed dogs. Radiography diagnosed 19 of 23 urolithiasis cases in the urinary bladder (UB), 2 of 2 in the kidney and 12 of 13 in the urethra while ultrasonography diagnosed 17 of 23 urolithiasis cases in the UB and one in the urethra. From a total of 15 dogs presented with either neoplastic growth and/or cystitis concurrent with urolithiasis, ultrasound detected six while pneumocystogrpahy detected only one.

Conclusion
The study showed haematuria as the leading clinical sign of urolithiasis. Detection of urolithiasis and concurrent cystitis and/or urinary bladder growth increases when ultrasonography and radiography were employed together.

Keywords
Dog; Urolithiasis; Urinary bladder growth; Diagnosis; Radiography; Ultrasonography.

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INTRODUCTION

Canine urolithiasis is a common cause of emergency urinary tract disease requiring a rapid definitive diagnosis for immediate surgical and/or medical therapy.[1-5] Generally, dogs with urolithiasis are presented with serious clinical conditions such as occult haematuria and partial or complete obstruction. Haematuria, pollakiuria, stranguria, and dysuria are common clinical signs of lower urinary tract disease that are non-specific to cystic calculi.[6] In dogs affected with urocystoliths, urocystoliths are difficult to palpate and physical examination findings are often normal unless the urethral obstruction is present, complete blood cell count (CBC) and serum biochemical analysis are usually normal and the clinical signs are not definitive.[7] Reports indicate that haematuria can occurs in more than 50% of dogs with urinary bladder and renal neoplasia or other disorders are known to damage the mucosal surfaces of the urogenital tract such as infection, inflammation, trauma, vascular disease, and coagulopathies.[8] Although haematuria is one of the most common clinical signs exhibited by dogs with urolithiasis, it can lead to wrong etiological diagnosis due to its multiple causes.

Thus, definitive diagnosis of urolithiasis cannot be made on history, clinical signs, haematology, urinalysis and other findings except with diagnostic imaging. Therefore, for dogs with lower urinary tract signs, imaging is crucial when clinical signs persist or recur and some of the breeds are susceptible to urolithiasis.[6] Survey and contrasts abdominal radiography, as well as ultrasonography, are often used to definitively diagnose and localize uroliths. Survey radiography and/or ultrasonography are the initial step in the sequential evaluation of the urinary system problems.[9,10] Positive and/or negative contrast radiography is necessary to overcome inherent limitations of survey radiography to identify non-radiopaque uroliths and all free or attached soft tissue filling defects.[9] Ultrasonographic evaluation of canine urinary system was found valuable in the diagnosis of, renal, bladder and urethral uroliths and bladder filling defects caused by neoplasia and granulomas except in the distal urethra.[9,10] An ultrasound scan shows a clear hyperechoic area with acoustic shadows in all urinary bladder stones.[8,9] Survey radiographs is an important tool to image the entire urinary tract and the complete length of the urethra to detect radiopaque urinary stones[9,10] that should be used as a diagnostic complement to ultrasonography if the extent of urinary bladder disease cannot be adequately evaluated by ultrasonography.[10]

However, except a few retrospective studies reported on comparative canine urolithiasis detection capability of radiography and ultrasonography.[11] Most of the studies conducted on canine urolithiasis were focused on the mineral composition and associated risk factors.[2-6] Thus, this study was conducted considering the limited availability of recent information on the clinical haematological and microscopic urinalysis pictures at presentation and comparative diagnostic detection capability of ultrasonography and radiography in canine urolithiasis and/or concurrent urinary bladder growths.

MATERIALS AND METHODS

Study Animals

This study was conducted on 23 dogs affected with urolithiasis and presented to the Veterinary Teaching Hospital of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. About half of the animals were presented from Ludhiana and the remaining from various cities of Punjab State.

Clinical and Haematobiochemical Examination

Signalment: Age, breed, sex, neuter status and body weight of all the animals were recorded at the time of presentation.

History: The features recorded for each case were the duration of the illness, the severity of the problem, history of the previous ailment, along with the medication, clinical symptoms such like anorexia, lethargy, depression, weight loss, vomiting, enlargement of the abdomen, polydipsia, polyuria, dysuria, haematuria, vaginal discharge.

Physical examination: Respiratory rate (RR), pulse rate (PR), rectal temperature, colour of mucous membrane and hydration status were recorded. Abdominal palpation was also done.

Haematology: Venous blood from cephalic/saphenous vein (2-3 ml) were collected in vials containing ethylenediamine-tetraacetic acid (EDTA), at the time of presentation and the relevant parameters were recorded including haemoglobin (Hb, g/dL) determined by acid haematin method using Sahli's haemocytometer method and the value expressed in g%, total leukocyte count (TLC $x10^3$ per μL) evaluated using Neubar's counting chamber method, packed cell volume (PCV, %) determined by Wintrobe method as described by Bellwood et al[12] Differential leucocyte count (DLC, %) was determined as per method given by Harvey.[13]

Microscopic urinalysis: Urine samples were collected aseptically in sterile syringes by cystocentesis and analyzed for microscopic examination. The urine sample was centrifuged for 5 minutes at 2000 rpm after mixed well in a graduated conical tube. The supernatant was discarded leaving behind the sediment. A drop of the sediment was then transferred onto a microscopic slide and covered with a coverslip and examined first under a light microscope at low power (10X) to assess the quantity and type of casts, cells, and sediments. Then samples were examined under high power (40X) to identify any abnormal structures.

Radiography: All animals were subjected to radiography using the 160 mA X-Ray machine. Right lateral survey abdominal radiographs were taken for visualization of uroliths in the urinary bladder, urethra, and kidney while additional ventrodorsal views were taken for imaging kidneys. Radiographic factors given were 60-80 mAs and 70-95 kvp at a focal film distance of 32 inches. Potter Bucky grid and high speed intensifying screen were used. Plain or negative contrast cystography was done as per standard procedure

is given by O'Brien.[14] When plain radiography, failed to reveal a detectable abnormality in the urinary bladder in dogs presented with clinical signs of urinary tract affection, pneumocystography was employed using atmospheric air after aseptically passing catheter into the urinary bladder.

Ultrasonography: In the present study, ultrasonography was carried out using a Concept/MVC veterinary ultrasound Scanners (Dynamic Imaging Limited, 9 Cochrane Square, Brucefield Industrial Park, Livingstone, Scotland, UK), with 3.5 MHz micro convex or 7.5 MHz linear array transducers. The images were recorded on thermographic printing paper of UPP-110 S series (Sony Corporation, Tokyo, Japan) with UP-595 CE (Sony Corporation, 6-7-35 Kitashinagawa, Shinagawa-Ku, Tokyo, Japan) video graphic printer for later reference.

Animal preparation and ultrasound scanning procedure: The body area from the costal arch to the pelvic inlet was prepared by clipping and shaving hair and by clearing any grease or dirt for ultrasonographic examination. A coupling medium (Gel) was applied liberally over the area to increase the skin transducer contact. The animals were restrained in dorsal, right or left lateral recumbency as per the requirement on a padded table. The transducer of the appropriate frequency was selected. The machine gains were set appropriately and reset while scanning with a different probe. Scanning was carried out in a low light room with the scanner placed in such position that the screen could be viewed without altering its position in relation to the animals.

Ultrasonographic scanning was done following a systematic approach with the animal in the dorsal or lateral recumbency as desired. The examination was started at the cranial aspect of the abdomen by evaluating the liver and gall bladder and then preceded in a circular fashion around the left abdomen, next imaging the spleen, left kidney, urinary bladder and the prostate caudally. Similarly, the right kidney was scanned after locating the liver using liver and gall bladder as landmarks.

Organs of interest were scanned in transverse, sagittal and/or frontal planes to evaluate the internal architecture, boundaries/silhoutte, organ size, shape and position. The xiphoid cartilage, linea alba and pubis were used as the basic reference points. The landmarks were labeled and measurements, wherever required, were made with the help of in-built electronic calipers.

The amplitude of returning echoes (echogenicity) as visualized on two-dimensional, gray-scale images, were classified as increased (hyperechoic), normal (isoechoic), decreased (hypoechoic) or absent (anechoic) when compared with the normal echo amplitudes for that organ. Acoustic shadowing was used as a definitive or confirmatory diagnosis of uroliths.

Surgical procedure: Cystotomy was performed in all dogs with urolithiasis in the urinary bladder and urethra except in four cases where retrograde hydropropulsion was failed and both cystotomy and urethrotomy was done in dorsal recumbency. Ventral abdominal area extending from xiphoid to the pelvic inlet was prepared for aseptic surgery. An intravenous cannula (Kit Kath, Hindustan Syringes and Medical devices Limited, Ballabgarh, India) was fixed in the cephalic vein and premedication was done with atropine sulphate (Atropine sulphate I.P., Jackson Laboratories Limited, Amristar, India) at 0.04 mg/Kg body weight and diazepam (Calmpose, Ranbaxy Laboratories Limited, Indore, India) at 0.5 mg/kg body weight administered slow IV. Five minutes later anaesthesia was induced with thiopentone sodium (Interval Sodium, Rhone-Poulenc (India) Limited, Bombay-25, India) (5% solution) "till effect." The IV line was maintained with 0.9% normal saline solution (Sodium chloride injection I.P., Punjab Formulations Limited, Jalandhar, India) at 10-12 mL/kg body weight per hour. Endotracheal intubation was done and animal was secured in dorsal recumbency. Maintenance of surgical plane of anaesthesia was done with thiopentone sodium (5%) IV or halothane. Surgical site was thoroughly scrubbed with chlorhexidine gluconate, cetrimide and isopropyl alcohol mixture (Aceptic. lCI India Limited, Chandigarh, India) (1:30 solution). Spirit was finally sprayed over the surgical site. Surgeons followed a routine scrubbing schedule.

For urethrotomy, after aseptic preparation, draping of surgical site was done and a urinary catheter was inserted through the penile urethra to the site of obstruction. Two to three centimeters ventral midline skin incision was made over the site of obstruction. Subcutaneous tissue was dissected to expose the retractor penis muscle, which was retracted laterally. With the penis stabilized in one hand, the corpus spongiosum urethra was incised on its exact midline over the site of obstruction to expose urethra. Then a longitudinal incision was made on the urethra. Once the urethra was entered the uroliths were removed and the catheter was gently extended through the urethral lumen into the bladder. Urethral incision was not closed. The skin incision was closed by simple interrupted or cross mattress suture pattern using nylon.

For cystotomy, after aseptic preparation, draping of surgical site was done. Following a ventral midline celiotomy, the bladder was exteriorized and abdomen was packed with sterile drape. The bladder was then drained through retrograde catheterization. About one inch cystotomy incision was made on the dorsal aspect of bladder wall in the least vascular area. The urocystoliths were retrieved with help of forceps/index finger. The lumen of bladder and the bladder neck was explored with index finger to detect any remaining uroliths. The catheter was then pulled back up to neck of bladder and retrograde flushing was done three to four times with sterile normal saline through the catheter to force any remaining urolith from bladder neck and urethra back into the bladder. The cystotomy incision was closed in two layers of continuous inverting suture pattern (Lambert followed by cushing) using No. 2/0 polyglactin 910 (Johnson and Johnson limited, Aurangabad, India). The abdomen was flushed with sterile normal saline. The abdominal wall was sutured in a single layer of interrupted suture pattern using No. 1 Vicryl. The subcutaneous tissue was sutured in a simple continuous suture pattern using No. 1 Vicryl. The skin incision was closed by interrupted horizontal mattress pattern using nylon.

Statistical Analysis

Quantitative data collected on signalment, vital signs, haematological parameters and microscopic urine analysis were summarized and simple arithmetic mean, standard error and 95% confidence interval were calculated. Mean values were compared by using student *t*-test. All the required statistical calculations were done using SPSS 16.0 statistical software.

RESULTS

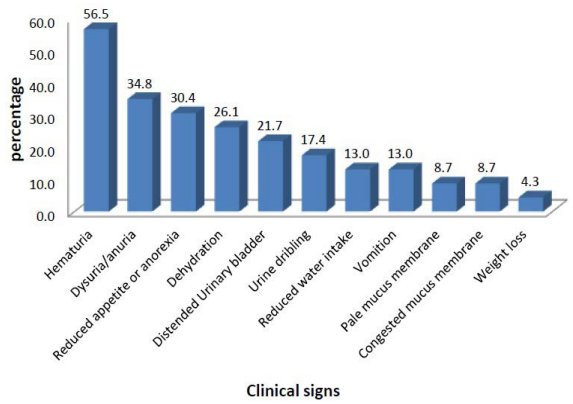
Signalment: Age, Breed and Sex

Recorded signalment and vital signs of dogs affected with urolithiasis in the study are presented in Table 1. The mean and SE of the age of dogs suffering from urolithiasis was 5.4±0.5 years with 95% CI of 4.3-6.4 years. Amongst eight different breeds affected with urolithiasis in this study, the highest occurrence was recorded in German Shepherd, Spitz and Mongrel dogs (17% each) followed by Doberman and Labrador (13% each), Dalmatian and Pomeranian (9% each) and Boxer (5%). In this study gender wise occurrence of urolithiasis was found to be more in males with a ratio of 22:1.

Table 1. *Recorded Signalment and Vital Signs of Dogs Affected with Urolithiasis in the Study*

Signalment and vital parameters	Recorded Mean ± SE (95% CI) of the evaluated parameter
Mean age in years	5.4±0.5(4.3-6.4)
Mean weight in kg	23.7±1.9(19.6-27.7)
Male to female sex ratio	22:1
Breeds	German Shepherd, Spitz and Mongrel (n=4, 17% each)
	Doberman and Labrador (n=3, 13% each)
	Dalmatian and Pomeranian (n=2, 9% each) and
	Boxer (n=1, 5%)
Mean body temperature in °F	102.2±0.2 (101.6-102.6)
Mean pulse rate in beats/min	113±5(103-122)
Mean respiratory rate in breaths/min	43±3(36-50)

Figure 1. *Clinical Signs Exhibited by Dogs Affected with Urolithiasis*



History and Clinical Signs

In this study, the most common clinical sign exhibited by dogs affected with urolithiasis were macroscopic haematuria (56.5%) followed by dysuria/anuria (34.8%), reduced appetite or anorexia (30.4%), dehydration (26.1%), distended urinary bladder (21.7%), urine dribbling (17.4%), reduced water intake (13.0%), vomiting (13.0%), pale mucus membrane (8.7%), congested mucus membrane (8.7%) and weight loss (4.3%) in decreasing order (Figure 1).

Physical Examination

In urolithiasis affected dogs, mean and standard error (SE) of rectal temperature (°F), heart rate (beats/minute) and respiratory rate (breaths/minute) recorded were 102.2±0.2, 113±5 and 43±3 with 95% confidence interval of (101.6-102.6), (103-122) and (36-50), respectively, which were within the normal reference range. However, increased rectal temperature (>103°F) and heart rate (>120 beats/minute) were recorded in three and five dogs, respectively. In this study, distended urinary bladder was easily palpable in four dogs presented with complete obstruction.

Haematological Evaluation

Haematological evaluation of dogs affected with urolithiasis without obstruction *versus* partially or completely obstructed is presented in Table 2 below. The mean values of haemoglobin and packed cell volume (PCV) recorded in this study were within the normal range for both groups of dogs. The mean total leukocyte count (TLC) value was normal for unobstructed group while it was significantly elevated ($p \leq 0.05$) in partially plus completely obstructed group. The differential leukocyte count (DLC) (%) depicted relative neutrophilia in the case of unobstructed group while it showed absolute neutrophilic leukocytosis in partially plus completely obstructed dogs.

Table 2. *Recorded Haematological Findings in Urolithiasis Affected Dogs with and without Urethral Obstruction*

Type of Haematological examination	Urolithiasis without obstruction (n=7)	Urolithiasis with partial plus complete obstruction (n=15)
	Mean ± SE (95% CI)	**Mean ± SE (95% CI)**
Hb (g/dL)	13.7±1.3[a](10.3-17.0)	14.5±0.4[a](13.7-15.2)
PCV (%)	42.3±2.7[a](35.6-49.0)	41.1±1.1[a](38.7-43.4)
TLC (x103 µL)	12.5±2.1[a](7.4-17.6)	21.1±2.8[b](15.1-27.0)
DLC (%):		
Neutrophil	77±3[a](69-85)	86±2[b] (82-90)
Lymphocyte	21±3(12-29)	13±2(9-16)
Eosinophil	3±2(1-7)	13±2(9-16)
Monocyte	0.3±0.3(0-1)	0.4±0.3(0-1)

Microscopic Urinalysis

The result of microscopic urinalysis reveled haematuria (>10 RBC/HPF) in 18 (78.3%) of the dogs and pyuria (>5 WBC/HPF)

Table 3. *Abnormal Condition Observed During Microscopic Urine Examination in Dogs Affected with Urolithiasis (n=23)*

Abnormality	Number observed
Haematuria (>10 RBC/HPF)	18 (78.3%)
Pyuria (>5 WBC/HPF)	11 (47.8%)
Epithelial cells or casts (positive)	6 (26.1%)
Crystaluria (positive)	7 (30.4%)
Bacteria (positive)	4 (17.4%)

in 11 (47.4%) (Table 3).

Radiographic and Ultrasonographic Evaluation

In this study, 19(82.6%) of the dogs had multiple uroliths and radiography diagnosed 19 of 21 urolithiasis cases in urinary bladder 2 of 2 in the kidney and 12 of 13 cases in the urethra while ultrasonography diagnosed 17 of 21 uroliths in the urinary bladder, one in the urethra, and none in the kidney. In this study, both ultrasonography and radiography failed to detect numerous small sized (1-5 mm) radiolucent uric acid stones in the urinary bladder of one dog but the largest of the stones that blocked the urethra in this same case was slightly radiopaque and detected by both radiography and ultrasonography. One radiolucent stone was detected by ultrasonography alone, but US failed to detect uroliths less than 5 mm in three other dogs. Among the 23 dogs diagnosed with urolithiasis, 13(56.5%) were in the urinary bladder and urethra, eight (34.8%) were in the urinary bladder alone, one (4.3%) was in the kidney alone and one (4.3%) was both in the kidney and urinary bladder. In the present study, from a total of 13 urethral stones seven (53.8%) were at the ischial arch, five (38.5%) were behind the os-penis and in one (7.7%) case uroliths were along the whole length of the urethra forming a chain. In the present study, the majority, (n=20, 87.0%) of the stones were radiopaque (++ to ++++) relative to the soft tissue density. In one case with numerous urate uroliths in UB were radiolucent except two found blocking the urethra while in remaining two (8.7%) cases they were slightly radiopaque (+).

From a total of 15 dogs that had either neoplastic growth and/or mild to severe cystitis concurrent with urolithiasis, ultrasound detected thickening of urinary bladder wall in six including dogs that had cystitis concurrent with urolithiasis (n=4), urolithiasis concurrent with transitional cell papilloma (n=1) and carcinoma *in situ* alone (n=1). In this study, the recorded mean and SE of urinary bladder wall thickness recorded by ultrasound in dogs with cystitis was 6.4±1.0 mm with 95% CI of (4.0-8.8 mm). The carcinoma *in situ* diagnosed by ultrasonography in this study was a misdiagnosis as ultrasound showed acoustic shadowing, which is a diagnostic characteristic of urolithiasis, but both plain and pneumocystography didn't detect this case of carcinoma *in situ*.

In this study, both pneumocystography and ultrasonography diagnosed transitional cell papilloma that was concurrently presented with cystolithiasis while plain radiography was not diagnostic. In the case of transitional cell papilloma, diagnosed in this study, pneumocystography has revealed a mass grown from cranioventral part of the urinary bladder into the lumen whereas ultrasonography shown hyperechoic mass with thickening of urinary bladder wall (Table 4).

Table 4. *Number of Urolithiasis Cases and Concurrent Abnormalities Detected by Radiographic and Ultrasonography in the Affected Dogs*

Affection	Number of abnormalities detected		
	Anatomical location	**Radiography**	**Ultrasonography**
Urolithiasis	Renal (n=2)	2	0
	UB (n=22) [8 in UB alone, 13 in UB+urethra and 1 in UB+kidney]	19	17
	Urethral (n=13)	12	1
	Total (n=23)	21(91.3%)	17(73.9%)
Mild to chronic cystitis and urinary bladder (UB) growth concurrent with urolithiasis	UB (n=15)	1(6.7%)	6(40.0%)

DISCUSSION

Dogs affected with urolithiasis are often presented with nonspecific clinical signs of urinary tract affection that requires running multiple diagnostic tests. Uroliths can cause partial or complete urinary obstruction of the urethra leading to emergency situation that requires rapid detection and removal to avoid life threatening conditions such as postrenal azotemia, urinary bladder rupture and uroabdomen. In this respect, radiography and ultrasonography are excellent diagnostic tools for detection of urinary calculi each with its own complementary superior aspects and limitations. In addition to the diagnostic imaging recommended for definitive diagnosis and confirmation of uroliths, routine clinical, physical, haematological and urine examinations are also highly valuable to detect underlying concurrent disease processes occurring with urolithiasis and/or consequent to it.

Signalment: Age, Breed and Sex

The mean age of dogs suffering from urolithiasis in this study was 5.4 years. This is in agreement with previous reports that indicated dogs suffering from cystolithiasis and/or urethral calculi were above 4.8-year-old depending on the chemical composition of uroliths.[2,4,15,16] Eight different dog breeds were affected with urolithiasis; the highest occurrence recorded in German shepherd, Spitz and Mongrel dogs in this study. The association of cystic and urethral urolithiasis with a variety of breeds was reported by several previous studies.[2-5,15-17] Variation in the pathogenesis of urinary stones between species, breeds, genetics, metabolism and nutrition was also documented[3] where nutrition is implicated as the major factor responsible for formation of uroliths in dogs and cats.[6] Toy and small breed dogs were significantly associated with calcium oxalate urolithasis while struvite uroliths tended to be over-represented in medium and large breed dogs.[4] The variation in the frequency of occurrence of urolithiasis in different breeds in the present study as well as previous reports may also be attributed to the changing trend in the preference of dog owners to different breeds of dogs. In this study gender wise occurrence of urolithiasis was more in males with a ratio of 22:1. Similar predominant occurrence of urolithiasis in male than female dogs was reported by previous studies.[1,2,16,19] This is also in agreement with higher occurrence of urolithiasis reported in male equines and bovines.[20,21] This high occurrence in male than female animals is likely due to the anatomy of the urethra, which is short and wide in females that may allow voiding most uroliths before obstruction and subsequent recognition of clinical signs as opposed to the male urethra, which is long with curved path and distally surrounded by ospenis predisposing dogs to frequent obstruction.[1,2,21] However, previous reports showed female dogs predominating males in the occurrence of struvite urolith.[2,4,5] The reported higher occurrences of struvite containing urolithiasis in female dogs was ascribed to infection-induced nature of struvite stones to which female dogs are at greatest risk due to their short and wide urethra.[2]

History and Clinical Signs

The most common clinical sign exhibited by dogs affected with urolithiasis in this study were haematuria, dysuria, reduced appetite or anorexia, dehydration, distended urinary bladder, urine dribbling, reduced water intake, vomiting, pale mucus membrane, congested mucus membrane and weight loss in decreasing order of occurrence. The clinical signs exhibited by the dogs in this study were in accordance with earlier studies of canine urolithiasis.[6,22-24] The observation of haematuria and dysuria as the most frequent clinical signs in canines affected with urolithiasis are most probably associated with irritation of bladder mucosa and blockage of the urethra caused by uroliths, respectively.

In this study, the mean recorded values of vital signs were within the normal reference range except an increased rectal temperature (>103 °F) recorded in three dogs and increased heart rate (>120 beats/minute) in five dogs. The average respiratory rate recorded in affected dogs in this study was above the normal reference resting respiratory rate documented by Aiello et al.[25] This is in agreement with the reported near normal rectal temperature and pulse rate but markedly elevated respiratory rate in dogs presented with urolithiasis.[17]

Haematological Evaluation

In this study, the recorded mean values of haemoglobin and packed cell volume (PCV) were within the normal range for all dogs, but the mean TLC was significantly elevated ($p \leq 0.05$) in partially and completely obstructed dogs. The DLC (%) depicted relative neutrophilia in the case of unobstructed group while it showed absolute neutrophilic leukocytosis in partially and completely obstructed dogs. This is in concordance with presurgical normal values of haemoglobin and PCV reported by previous studies in dogs with urolithiasis.[16,19] The observed neutrophilic leukocytosis in obstructed dogs evaluated in this study may be due to uremia, stress and inflammation associated with the obstruction or mucosal damage caused by uroliths. Both acute and chronic inflammations are recognized common-causes of leukocytosis with neutrophilia in dogs.[26] Pre-surgical elevation of TLC recorded in this study is in agreement with previous reports.[16,18,23,24] In the DCL, the relative neutrophil and lymphocyte count were significantly different ($p \leq 0.05$) in dogs with partial and complete obstruction compared to unobstructed dogs. This is in agreement with presurgical neutrophilia and lymphopenia reported by previous studies in canine urolithiasis at presentation.[16,23,24] Lymphopenia in cases of urethral obstruction might be due to stress and/or infection associated with the urolithiasis. Concurrent lymphopenia and eosinopenia are typical owing to release of endogenous corticosteroids in response to stress superimposed on the inflammatory neutrophilic leukogram.[26]

Microscopic Urinalysis

In this study, clinically occult haematuria was seen in 56.5% of dogs affected with urolithiasis while microscopic urine examination revealed subclinical microscopic hematuria (>10 RBC/HPF) in 18(78.3%) of the same dogs. This shows the importance of microscopic urine examination in order to detect subclinical microscopic hematuria that could be missed in gross urine examina-

tion to undermine the problem. A recent retrospective study also reported microscopic haematuria in 80.7% of urolithiasis affected dogs.[11] Pyuria (>5 WBC/HPF) is recorded in 47.4% of dogs in this study. The pyuria may be caused by urinary tract infection (UTIs) s that might have caused some of the uroliths or the uroliths may have also predisposed the patient to a bleeding and/or UTI leading to pyuria. Association of infection with the most commonly analyzed struvite uroliths (43.8%,7,287/16,647) was documented.[2] The severe injurious effect of uroliths is evident from the blood bathed rough cystoliths recovered from urinary bladder of affected dogs in this study that may lead to pyuria as a result of blood leak into urine in the bladder. Although, all the dogs evaluated in this study, had urolithiasis, urine crystals were detected only in seven (30.4%) dogs. This is agreement with the low detection of crystalluria (28.0 to 38.1%) reported by previous studies in urolithiasis affected dogs.[11,16,23] This indicates that crystalluria is not a consistent finding in microscopic urinalysis of urolithiasis affected animals that could not be relied on as a diagnostic method for urolithiasis.

Radiographic and Ultrasonographic Evaluation

In this study radiography diagnosed 19 of 21 urolithiasis cases in urinary bladder, 2 of 2 in the kidney and 12 of 13 in the urethra while ultrasonography diagnosed 17 of 21 uroliths in the urinary bladder, one in the urethra, and none in the kidney. Thus radiography showed better capability in urolithiasis detection in the kidney, urinary bladder and urethra while ultrasonography was weaker in detecting uroliths in the kidney and urethra, but equally capable in detection of urolith in the urinary bladder. The ultrasonographic appearance of radiopaque or radiolucent renal calculi is described as showing bright echoes and acoustic shadowing.[27] But ultrasonography didn't show the characteristic acoustic shadowing in both renolith cases in this study, except revealing renal calcification characterized by increased focal parenchymal echogenicity in one case and hyperechoic medulla with loss of corticomedullary junction, both of which were in conclusive and non-diagnostic of renolith. In this study, ultrasonography was also better than plain and negative contrast cystography in the detection of cystitis and urinary bladder growths that were present concurrent with urolithiasis or alone. However, we cannot aver that ultrasonography less capable than radiography in the detection of either renal or urethral urolith since the number of renal urolith cases were very few in this study and ultrasonography has known inherent limitation for imaging the urethra due to the presence of pelvic bones in that anatomic area limiting the passage of ultrasound beam. It was opined that urethral calculi are difficult to visualize with ultrasound unless they are lodged near the neck of the bladder.[6]

In this study, both ultrasonography and radiography failed to detect numerous small sized (1-5 mm) radiolucent uric acid stones in the urinary bladder of one dog. According to Gatoria et al[28] majority of urate uroliths were not radiolucent due to minor proportion of calcium phosphate content and[29] also concluded that radiodense urocystoliths less than 3 mm in size and radiolucent uroliths were difficult to detect by survey radiography. In this study, however, in one male dog both plain radiography and ultrasonography diagnosed two moderately radiopaque ammonium acid urate urethrolith, in a case from which numerous small ammonium acid urate urocystoliths were retrieved during cystotomy. This shows that urate uroliths can be detected by radiography when the sized is large enough. It is opined that except for single, very small stones, cystic calculi are easy to detect sonographically regardless of their radiopacity.[30]

In this study, 82.6% of the dogs had multiple uroliths. Similarly occurrence of multiple uroliths was reported in 66.7% of studied dogs.[23] In this study, 60.9% of dogs had stones in multiple anatomical locations with more common occurrence in the urinary bladder and urethra. This finding is in agreement with previous studies that reported more common occurrence of uroliths in the urinary bladder followed by the urethra in dogs, cattle and horses by Osborne et al.[1,3,4,20,21,23,28,31] Reports showed the ventral groove of the ospenis is the most common site of urethral obstruction (66.7%) followed by post-ospenis (25%) and ischial arch region (8.33%).[16,32] The long curved path of the urethral and the presence of ospen is surrounding the distal part makes urethra less distensible and more prone to obstruction creating acute emergency condition in dogs.

In the present study, the majority, (n=20, 87.0%) of the stones were radiopaque (++ to ++++) relative to the soft tissue density. In one case with numerous urate uroliths in UB were radiolucent except two found blocking the urethra while in remaining two (8.7%) cases they were slightly radiopaque (+). One report indicated that majority of uroliths were strongly radiodense (52.38%) followed by moderately radiodense (28.57%) and weakly radiodense (19.05%).[28] This variation in this reported proportion of graded radiodensity of uroliths could be due to the subjective nature of grading the degree of stone density or variations of the radiation exposure factors used. The two most prevalent mineral types in cats and dogs are calcium oxalate and struvite uroliths comprised 80.8% in 75,674 uroliths analyzed in a recent study Houston et al[4] and that both types of stone are generally radiopaque 6 explaining the reason for strong radiodense characteristics of most of the uroliths.

From 15 dogs presented with either neoplastic growth and/or cystitis concurrent with urolithiasis in this study, ultrasound diagnosed six cases, pneumocystogrpahy detected in only one case with transitional cell papilloma and plain radiography detected none. In the transitional cell papilloma, peumocystography revealed a mass grown from cranioventral part of the urinary bladder into the lumen whereas ultrasonography shown hyperechoic mass with thickening of the urinary bladder wall. In this study ultrasound also detected thickening of urinary bladder in five dogs and mean and SE of 6.4±1.0 mm (95% CI of 4.0-8.8 mm) bladder wall thickness was recorded. The thickness of the normal bladder increases with body weight and depends on the degree of distension where in the near-empty normal bladder, the mean wall thickness was 2.3±0.43 mm while in moderately distended urinary bladder (4 ml/kg) it was 1.4±0.28 mm.[30] The mucosa of urinary bladder responds to inflammation or urinary tract infection by hyperplasia

and hypertrophy, which causes thickening of the wall,[33] that is easily detected in acute cystitis for the echogenicity of the bladder wall is hypoechoic due to edema whereas focal or diffuse thickening of the cranioventral bladder wall in response to chronic inflammation may be difficult to detect unless changes are dramatic.[34]

It was opined that diseases identifiable with survey radiographic findings are uncommon except cystic calculi and the inability of survey radiography compared to the detection capability of contrast cystography to diagnose intramural and intraluminal bladder tumors and other changes such as bladder wall outline, thickness, mural masses, and intraluminal defects was previously documented.[35,36] The ultrasonographic appearance of the transitional cell papilloma seen in this study is analogous with stated observable focal hypoechoic mass in ultrasonographic imaging of urinary bladder is most likely a tumor.[10] In this study, one carcinoma *in situ* showed acoustic shadowing, which is a diagnostic characteristic of urolithiasis.[10] But no urolith was recovered during cystotomy except greatly swollen urinary bladder wall was observed associated with neoplastic growth. This case of carcinoma *in situ* is discussed here for its relevance as a false positive diagnosis of urolithiasis by ultrasonography otherwise it is not counted with the 23 urolithiasis cases. The case was later confirmed carcinoma in situ by histopathology using a tissue sample taken from the resected tumor during surgical treatment of the case. This showed that urinary bladder carcinoma may show acoustic shadowing in ultrasonographic evaluation leading to misdiagnosis of urolithiasis. Although, the possibility of false positive diagnosis was mentioned in the case of bladder tumors and polyps, they are not supposed to show acoustic shadowing, except hyper-echogenicity.[10]

In the present study, both radiography and ultrasonography failed to detect fibropapilloma, which was noticed only during surgery. This may be due to the small size of the fibropapilloma that was noticed during surgery. The size of urinary bladder tumor lesion was reported as an important determining factor in the rate of detection by ultrasonography such that the rate of detection of tumors less than 0.5 cm in diameter is less than 33.0% compared to 83.3% detection rate for tumors larger than 1.0 cm and 95.0% for tumors larger than 2.0 cm.[10] It is opined that accuracy of sonographic diagnosis of urinary bladder tumor depends on the size of the mass where small masses of less than 0.3 cm, and those located in the trigone area are difficult to detect.[34]

CONCLUSION

The study showed that clinical signs as well as haematological and microscopic urinalysis findings can insinuate to urolithiasis, but they won't conclusively enable to identify the etiology and localize the problem. The most common clinical signs exhibited by dogs affected with urolithiasis are haematuria and dysuria that should be taken as the leading clinical symptoms to consider urolithiasis in order to select an appropriate tool for further definitive diagnosis. Both ultrasonography and radiography are valuable and complementary diagnostic tools to confirm urolithiasis and concurrent cystitis and neoplastic growth in the urinary bladder. Radiography is relatively better in detecting uroliths in the kidney, urinary bladder and urethra with additional benefit of enumerating the number of uroliths enabling removal of all uroliths for best surgical management of the patient. Ultrasonography is superior in detecting urinary bladder inflammation and neoplastic growths with equal uroliths detection capability in the urinary bladder. However, ultrasonography is incapable to enumerate the number of uroliths and it is unsuitable for imaging the urethra due to pelvic bones. Therefore, both radiographic and ultrasonographic imaging is recommended for dogs presented with nonspecific signs of haematuria and dysuria for early confirmation and effective intervention.

DISCLOSURE

The approval of ethical committee was taken for conducting this study and followed all the animal ethics and welfare guidelines.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Review

Factors Affecting Rumen Microbial Protein Synthesis: A Review

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ABSTRACT

There is a diversified microbial ecosystem in the rumen for efficient utilization of diet by providing essential nutrient to their host. But there are different factors affecting rumen microbial protein synthesis which are physical factors, chemical factors, dietary factors, biological factors and endogenous factors. Among the details of factors, dietary factors and ruminal pH are the dominant factors influencing rumen microbial protein production. The effects of some dietary factors, on the amount and efficiency of microbial protein synthesis, are discussed in this review. Specifically, these factors include forage quality diets, level of feed and types of feed. It seemed that diets containing a mixture of forages and concentrates increase the efficiency of microbial protein synthesis because of an improved rumen environment for the growth of more diverse bacterial species. This review describes physical and chemical factors which include: pH and buffer system, oxygen concentration, rumen outflow rate and synchronized release of nitrogen and energy from the diet, a nitrogen compound, energy spilling, vitamins and minerals and antimicrobials chemicals, respectively. Age, species, physiological status, sex, and stress are among endogenous factors that mostly affect microbial protein synthesis of a ruminant. Bacteriophages, protozoa predation and bacterial lysis are biological factors affecting the efficiency of microbial protein synthesis. All these factors have a direct effect on the synthesis of microbial protein in the rumen. Therefore, the cumulative effects of the above factors are resulted in the depopulation of rumen microflora and finally reduction of animal product. So, improvement in quantitative aspect of microbial protein synthesis solves many problems from simple to complex so that, the quantitative aspect of rumen microbial biomass are invaluable for health and productivity of ruminants than qualitative aspect hence, maintain health rumen ecosystem means having healthy ruminant.

Keywords
Rumen; pouvoir hydrogène (pH); Microbial protein; Rumen ecosystem; Ruminant; Rumen microflora; Nutrient; Haematological parameters reference ranges; Healthy status.

Abbreviations
MBP: Microbial protein; MCP: Microbial crude protein; NDF: Neutral detergent fiber; NPN: Non-protein pitrogen; NSC: Non-structural carbohydrate; OM: Organic matter; OMTDR: Organic matter truly digested in rumen; RDP: Rumen degradable protein; RUP: Rumen undegradable protein; SCFA: Short chain fatty acid; TDN: Total digestible nutrient; VFA: Volatile fatty acid; ATP: Adenosine Tri-phosphate; CP: Crude protein; CS: Concentrate supplementation; DM: Dry matter; DOMI: Dry organic matter intake; EMPS: Efficiency of microbial protein synthesis; FOM: Fermented organic matter.

INTRODUCTION

Ruminants have diversified microbial ecosystem consisting of bacteria (1010-1011 cells/mL), ciliate protozoa (104-106/mL), anaerobic fungi (103-105 zoospores/mL) and bacteriophages (108-109/ mL). The synergism and antagonism among the different groups of microbes and even among different genera of the same group is so diverse and complicated that it is difficult to

quantify the role played by any particular group of microbes present in the rumen. The net result of these reactions in the rumen is responsible for the bioconversion of feed into a form that is utilizable by the animal as a source of energy (short-chain volatile fatty acids) and microbial protein (as single-cell protein).[1]

Ruminants are distinguished from the rest of the animals by the morpho-physiological adaptation of the upper part of their stomach. This peculiarity allows them to turn roughages and low quality protein, even non-protein nitrogen (NPN) into quality nutrients for themselves such as microbial protein and volatile fatty acid.[2] Microbial protein synthesis is important in ruminants because microbial protein synthesized in the rumen provides 50% of amino acids required for ruminants. Synthesis of microbial protein and growth of ruminal microbes largely depend on adequate energy (ATP), resulting from the fermentation of organic matter in the rumen, and N resulting from degradation of non-protein and protein nitrogen sources and this can be affected by either natural or diet-related factors.[3] Ruminants' foregut microbial community the structure could be expected to be constrained by, physical, chemical, physiological, and even biological characteristics that evolved along with the varied feeding strategies in the various ruminant lineages.[4]

Adaptation has resulted in a diversity of rumen sizes and passage rates of rumen contents, allowing ruminant species to exploit a range of feed types. In addition, feed composition effects, and the host adaptations might also play a role in regulating rumen microbial community structure. Host and diet effects on rumen microbial community structure could be separated. Microbial communities could clearly be discriminated by both host and diet, with bacteria being the main drivers behind the observed differences. This probably reflects their more diverse metabolic capabilities compared with the less versatile archaea and protozoa.[4]

Among the factors that affect the synthesis of microbial protein, the availability and synchronization between energy and nitrogen compounds (N) in the rumen have been recognized as the most important factor. Although the other most important factors such as dietary factors, animal factors, biological and chemical factors can influence the efficiency of microbial protein synthesis in the rumen.[5]

Therefore, this paper is to highlight major factors affecting the rumen microbial protein synthesis.

FACTORS AFFECTING MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN

Due to the complexity of microbial protein synthesis, there are many factors affecting the performance of the same.[2] The contributions of energy and nitrogen in the rations, as the most limiting factors for microbial protein synthesis in the rumen,[6] although other nutrients such as sulfur, volatile fatty acids, fatty acids of branched-chain, minerals and vitamins, are also very important for microbial growth, which is in a lesser extent.[7]

Physical Factors

pH and buffer system: One of the important factors affecting on the level of synthesis of microbial protein in the rumen is the acidity of the forage pouvoir hydrogène (pH).[8] Functional performance of rumen will be greater when rumen pH is above 6.0 and pH above 5.7 is necessary for protein synthesis. When rumen pH fell below 6, microbial enzymes in rumen do not function effectively and bacterial growth decline markedly.[9]

Cerrato-Sánches et al[10] reported that the negative effect on rumen fermentation started as soon as pH decreased to 5.50. However, fiber digestion rates decrease when ruminal pH declines below 6.00-6.20 which reduces access of bacteria and enzymes to the protein thus decreasing crude protein degradability.[11] A low pH value is also expected to reduce the digestibility of fibrous plant tissues and due to low pH value, the energy within the rumen is diverted to non-growth functions, i.e. maintaining neutral pH in bacterial cells.[12] Apart from affecting congenital prosopagnosia (CP) degradation, rumen pH could also affect membrane co-factor protein (MCP) synthesis, the efficiency of MCP synthesis and yield of MCP which are affected by rumen pH and outflow rate of solid particles and liquid from the rumen.[13] Different bacterial species grow in different pH range; for instance, cellulolytic bacteria are sensitive to acid pH; whereas, amylolytic species are more acid tolerant.[12]

Rumen pH is largely a function of the volatile fatty acid (VFA) concentration,[14,15] and pH will drop if there is a reduced rate of VFA absorption.[16] In a diet with high neural stem cell (NSC) and rumen degradable protein (RDP), VFA concentrations are high and ruminal pH is low.[17] Feed intake and salivary secretion affect pH in the rumen.[18] At a higher level of feed or dry matter (DM) intake, the pH of the rumen is lower.[17]

Rumen under normal conditions has Na^+, K^+, bicarbonate and short-chain fatty acids as the main buffering component. Forages encourage buffering through increased salivation and cation exchange of fiber.[9] The rumen is usually well buffered, due to the presence of bicarbonates and phosphates founded in the continuous flow of saliva.[19] Rumen, although well buffered by bicarbonate, phosphate, protein and VFA can vary in pH from approximately 7.0 to less than 5 under different dietary condition.[20] Rumen buffering could avert the reduction in pH and could enhance rumen microbial growth, diversity and activity, fermentation end product and microbial protein synthesis.[21] Ammonia from degraded protein or NPN would also act as a buffer in the regulation of the ruminal pH.[16] The rumen is well buffered by salivary secretion; however, if the amount of dietary non-deliverable forward (NDF) is restricted and the rate of carbohydrate fermentation is fast, the pH may decline.[12]

Oxygen concentration: The rumen is a suitable environment for the development of a large number of anaerobic microorganisms, having unique characteristics such as temperature around 38 to 42 °C.[22] But normally, the temperature was more commonly found to

be 39 °C.[23,24] Rumen environment is anaerobic, and hence most of the bacteria are obligate anaerobes. Some of them are so sensitive to oxygen that these are killed on exposure to oxygen.[1] Oxygen sequestration up to 16 L of O_2 can enter the rumen daily through water intake, rumination, and salivation, and inhibit the growth of obligate cellulolytic anaerobes like *Fibrobacter succinogenes*. So, yeasts can make the rumen environment more conducive for anaerobic, autochthonous microbes by scavenging O_2.[25] About 10 to 20 liters/day of O_2 could enter from capillaries through the mucosal lining. Strictly anaerobic species, e.g. methanogens, survive in the rumen under O_2 tensions previously found to be inhibitory to these organisms. Therefore, the ruminal microbial population must be able to rapidly utilize O_2 and remove it from the environment of highly O_2 sensitive organisms.[26]

Rumen outflow rate: It is one of the important factors which influencing the level of synthesis of microbial protein in the rumen is the rate of passage of food masses through the rumen. Passage of food masses through the rumen at high-speed increases the number of microorganisms without high energy consumption. Faster outflow rate is visualized to reduce the maintenance expenses of microorganisms (microbes) since they contribute less time inside the rumen.[27] According to the Agricultural and Food Research Council (AFRC)[28] data increasing the rate from 0.02 to 0.08-hours increases the level of synthesis of microbial protein in the rumen to 20%. The presence of dry matter in the forage increases the rate of passage of food passes through the rumen and the level of synthesis of microbial protein in the rumen. Rumen outflow rate is a function of dry matter intake and therefore it can be assumed that the efficiency of microbial protein synthesis in the rumen can be increased as dry matter intake increases (5 and 13). Level of DM intake, the residence time in the rumen and fractional outflow rate has an effect on degradability and extent of cerebral palsy (CP) degradation in the rumen.[29]

The rate of passage of ingested feed depends on the feed intake by the animal,[30] and the improvement of growth and microbial efficiency is due to a reduction in the maintenance requirements of the microorganisms.[31] Therefore, ensuring an adequate intake of dry matter is a way of increasing the production of MCP and reducing the need of rumen undegradable protein (RUP) in the diets.[32] The increased passage of microbial protein to the small intestine occurred as a result of the increased passage of both fluids and solids with increased intake.[33]

Chemical Factors

Synchronized release of nitrogen and energy from diet: Synchronization means both energies in the form of carbohydrates or organic matter (OM) and protein in the form of N or peptides are available in the rumen throughout the day, and neither OM nor N is exceeded or limited for maximal microbial synthesis at any point of time. Synchronization of rumen available protein and energy is one of the conceptual methods to increase the efficiency of utilization of nutrients by the ruminants. Formulation of diets that are synchronous for energy and nitrogen release in the rumen has been shown to increase the efficiency of maltose-binding protein (MBP) synthesis in the rumen.[34] Matching the release of ammonia-N from dietary protein with the release of usable energy may improve N utilization.[5]

Synchronizing energy and N availabilities in the rumen seems to have the potential to enhance the output of microbial protein from the rumen and efficiency of ruminal fermentation, thereby improving feed utilization and animal performance.[35] The optimal RDP balance of a diet is close to zero and corresponds to rumen degradable N to fermented OM ratio equal to 25 g of N/kg of fermented organic matter (FOM), which reflects a well-balanced availability of energy and N to rumen microbes. When the RDP balance is positive for a diet, N losses from the rumen occur. Negative RDP balance indicates a shortage of nitrogen and consequently, the microbial activity may be impaired. Matching degradation of carbohydrate and protein rates of degradation in rumen allows efficient MBP yield and overall dietary protein incorporation.[36]

Nitrogen compound: Rumen microorganisms act normally if the level of raw protein in the feed is more than 11%. To ensure the growth and progression of rumen microorganisms it is important to use feed with nitrogenous compounds in the feed. Nitrogenous compounds and degradability of feed proteins in the rumens are important for meeting the needs protein in ruminants in protein. And modern protein systems indicate that microorganisms' requirement for nitrogen is satisfied by a degrading protein in the rumen, yielding oxidized amino acids and nitrogen,[5,8] Showed that nitrogen compounds, which are released during the protein degradation, are crucial for microbial growth in the rumen. It seems that proteins which have lower rates of ruminal degradation tend to improve the efficiency of microbial protein synthesis, probably because of the better capture of released N by rumen microbes.

Microbial protein is largely dependent upon the availability of energy generated by the fermentation of carbohydrates. On average, 20 grams of bacterial protein is synthesized per 100 grams of organic matter fermented in the rumen. The percentage of protein in bacteria ranges from 38 to 55%. Non-protein nitrogen from the feed and urea recycled into the rumen through saliva or the rumen wall also contribute to the pool of ammonia in the rumen. In addition, ruminants possess a mechanism to spare nitrogen. When feeding a low nitrogen diet, large amounts of urea (typically excreted in the urine) recycles into the rumen, where it can be used again by the microbes. If ammonia levels in the rumen are too low, there will be a shortage of nitrogen available to bacteria and feed digestibility will be reduced.[5]

Energy spilling: Energy spilling is energy dissipated as heat when the amount of ATP available from the fermentation of feedstuff exceeds the amount used for growth and maintenance.[37] Energy spilling can be a major detraction from efficient growth in bacteria. Those bacteria that spill energy fermented glucose 10-fold faster than those that did not.[38] Energy spilling diverts energy away from growth, decreasing the efficiency of the microbial growth and thus the amount of microbial protein available for digestion. Energy spilling has been measured in rumen bacteria but could

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not be in rumen protozoa (which make-up 10-50% of the microbe biomass.[39]

Vitamins and minerals: In addition to N and carbohydrate supply, the microbial yield is affected by the concentrations of trace minerals and vitamins. Dietary sulfur concentration has been found to affect microbial growth.[7] The amount of sulfur required by rumen microorganisms for the synthesis of methionine and cysteine ranges from 11 to 20% of the total diet based on the status of the cattle.[29] Limited intake of sulfur may restrict microbial protein synthesis when large amounts of non-protein nitrogen are fed to ruminant animals, such as urea.[7] Sodium sulphate and methionine have been shown to stimulate riboflavin and B12 vitamin synthesis by rumen microorganisms to a greater extent than cysteine or elemental sulphur. It is essential in the synthesis of sulphur containing amino acids that are needed in the elaboration of the MBP.[40] Phosphorus (P) is another mineral required for the synthesis of ATP and protein by rumen microbes. Microbial protein synthesis can be limited by an insufficient supply of P for microbial growth.[5]

Magnesium activates many bacterial enzymes including phosphohydrolases, phosphor transferases and pathways involving ATP and thiamine pyrophosphate reactions. Its concentration in the ribosomes makes it essential for the protein synthesis process but it can be partly replaced by manganese.[9] Vitamin B2 is required only 0.38 mg/d but pantothenic acid (B5) is required about 360 mg/d to dairy cows for the optimum rumen fermentation. MBP production in control, water and fat-soluble vitamins were 163 and 140 g/d, respectively thus, indicating B-complex vitamin supplementation improves rumen MBP production.[41]

Antimicrobial chemicals: Effect of plant extracts like garlic and ginger extracts were found to have decreased the protozoa population resulting in a reduction of methane emission in the rumen and thus inhibiting methanogenesis and decrease rumen protein production. The other one is essential oils in the rumen which resulted in the reduction of protein and starch degradation, due to selective action on certain rumen microorganisms like Gram-positive bacteria due to the barrier of the cell wall structure not tolerating the inflow of the secondary metabolites.[42] Ionophores (such as monensin, lasalocid, laidlomycin, salinomycin and narasin) are antimicrobial compounds that are commonly fed to ruminant animals to improve feed efficiency. These antimicrobials specifically target the ruminal bacterial population. They are lipophilic compounds that exert their effects at the membrane level, and are most effective against gram-positive bacteria and alter the microbial ecology of the intestinal microbial consortium. Ionophores transport ions across cell membranes of susceptible bacteria, dissipating ion and uncoupling energy expenditures from growth, killing these bacteria.[43] The efficiency of microbial protein synthesis was greater in forages containing saponin and tannins, which reduce ruminal N degradability.[5] The readily degradable fraction of protein is higher in forages than in grains. Approximately 40% of the protein in fresh alfalfa is soluble in the rumen environment.[44]

Dietary Factors

Forage quality: The yield and efficiency of synthesis of microbial protein have frequently been recorded as high (30-45 g microbial-N per kg OM apparently digested in the rumen), when high-quality grass is grazed.[45,46] Much lower microbial efficiencies (<20%) have been noted with lower-quality autumn-grass, though in these experiments season was confounded with the physiological state of the animals.[46] MPS is often increased by supplementing silage-based diets with moderate levels of readily-fermented carbohydrates.[47,48]

Level of feed: Increasing the level of feeding in ruminants is expected to reduce maintenance costs of microbes because they spend less time within the rumen.[2] Experimental evidence is available which suggest that the frequency of feeding improve the efficiency of microbial protein synthesis and was certainly observed through stimulation models of rumen function. Also, frequent feeding increases the rate of passage of liquid and solids from rumen and influence in microbial protein synthesis so, increasing the feeding frequency of dried grass meal from 2 to 8 times increased MBP synthesis from 36 to 46 g/kg of dry organic matter intake (DOMI).[27] The level of feeding effect appears to hold true for maximum electronic music plotting system (EMPS) since there are no occurrences of high EMPS at low intakes.[28] However, no significant effect was found in the diets containing rolled barley which indicated that the frequency of feeding leads to increase in MBP production mainly due to the impact on the roughage diet. Feeds associated with lower outflow rates, for example, processed-grain rations, have a higher total energy production but lower efficiency of MBP production.[9] Therefore increased feeding frequency should lessen variation in ruminal ammonia N concentration and improve microbial protein yield.[49]

Types of feed: The efficiency of microbial protein synthesis greatly differs in animals fed different diets, even within similar diets. The average efficiency of microbial protein synthesis was 13.0 g membrane cofactor protein (MCP)/l00 g organic matter truly digested in the rumen (OMTDR), ranging from 7.5 to 24.3 for forage-based diets. For mixed forage-concentrate diets, the average efficiency of microbial protein synthesis was 17.6 g MCP/100 g OMTD in the rumen, ranging from 9.1 to 27.9 g.[3] Efficiency of microbial protein synthesis for high concentrate diets was 13.2 g MCP/100 g OMTD in the rumen, ranging from 7.0 to 23.7. Overall, the average efficiency of microbial protein synthesis is 14.8 g MCP/100 g OMTD in the rumen, ranging from 7.0 to 27.9 g MCP/100 g of OM truly digested in the rumen.[29]

The efficiency of microbial protein synthesis was predicted to be around 13 g MCP/100 g of total digestible nutrient (TDN) for beef cows. Sources of carbohydrates, such as different ratios of structural to nonstructural carbohydrates, would have little effects on the efficiency of microbial protein synthesis. It is well known that the rapid digestion of nonstructural carbohydrate results in reduced ruminal pH. The efficiency of microbial protein synthesis is reported to be low in animals fed high-concentrate diets because of reduced ruminal PH.[36] Also, the efficiency of MBP production varied widely between forages. MBP production in grass and maize silages was from 115 to 158 and 165 to 217, respectively while with green forage and hay was 145 to 199 and 126 g/kg of fermentable OM.[3]

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Biological Factors

Bacteriophages: Bacteriophages are the viruses of bacteria and are reported to be present in the rumen in large numbers.[1] The phage densities ranging from 109 to 1010 particles per milliliter of rumen fluid and considerable morphological diversity has been observed, with 26-40 morphologically distinct types from three viral families (Myoviridae, Siphoviridae, and Podoviridae) being reported.[50] Viruses of prokaryotes (phages) are ubiquitous to the gastrointestinal tracts of all animals, and particularly dense and diverse populations occur in the rumen of herbivores these viruses have characteristics that can be both detrimental (reduce feed efficiency, transfer toxin genes) and advantageous (bacterial population balance, lateral gene transfer, phage therapy, novel enzymes), very little is known about their biological properties or genetic make-up.[51] One cause of reduced efficiency is the non-specific lysis of bacteria within the rumen and subsequent fermentation of the bacterial protoplasm. This phenomenon has not been explained but at times a large proportion of the bacterial pool can be affected.[52] Bacteriophages (bacterial viruses) are implicated in this lysis. Bacteriophages are obligate pathogens of bacteria and occur in dense populations in the rumen.[53] Because, they lyse their bacterial hosts within the rumen and, the process is identified as reducing the efficiency of feed.[50]

Protozoa predation: Protozoa engulf bacteria and digest them to cover their nutritional needs. Bacterial proteins are degraded into peptides and amino acids inside the protozoa. Nearly half of the ingested amino acids are used by ciliates. The other half reappears in the medium where they are deaminated by bacteria as it was calculated that as much as 90 g of bacterial dry matter can be engulfed by protozoa each day in a sheep rumen, which corresponds to a loss of 27 g of bacterial protein. As a consequence of the predation, the turnover of bacterial protein is increased by the presence of protozoa.[40] Protozoa predate on bacteria as their main protein source and as a result, defaunation makes the rumen more efficient in terms of proteosynthesis increasing the duodenal flow of microbial protein (+30%, $p<0.001$) and total non-ammonia N flow (+31%, $p<0.001$). Defaunation also increased the efficiency of microbial protein synthesis (+27%, $p=0.008$) as a result of both better microbial proteosynthesis and a lower OM digestion. Protozoal generation time is far higher than that of bacteria, thus the energetic requirements for maintenance are higher when expressed as a ratio of protein leaving the rumen.[54] As a result, the presence of protozoa has a negative impact on the overall energetic efficiency of the rumen ecosystem. In addition, defaunation can also modify the composition of rumen bacteria.[55] The ability of protozoa to engulf exogenous fatty acids[56] may divert more carbon toward VFA production in preference to fatty acid synthesis and ultimately increase VFA production. On the basis of stoichiometry, such a shift in rumen VFA production should result in a decrease in methane production as less metabolic H_2 will be available as a substrate for methanogenesis.[57]

The effect of the presence of rumen protozoa on pathogen's survival in the rumen and pathogen shedding is another area of interest. As noted above rumen protozoa engulf and digest a wide range of bacteria[54] and can reduce the shedding of potential pathogens from the animal, although the effect is highly dependent on the composition of the protozoal population present.[58] However, it has also been shown that rumen protozoa enhance the pathogenicity of certain pathogens leaving the rumen[59] suggesting that more work is needed in this area.

Bacterial Lysis: The microbial turnover is estimated in defaunated sheep using N and autolysis of bacteria in the rumen environment. In gram-positive rods first deposited peptidoglycan at the inner surface while their outer layers were cut by autolytic enzymes and stress is gradually transferred to more recently synthesized portions of the peptidoglycan. The low pH, in turn, would inhibit autolysis. Conversely, starvation could dissipate the membrane potential, increase pH, and activate the autolysins. Compounds those decreases the membrane potential accelerates the lysis of rumen bacterium. However, bacterium e.g., *Fibrobacter succinogenes*, appeared to be regulating its autolysis *via* a mechanism involving the proteolytic degradation of autolysins.[9,54]

Endogenous Factors

Age: DM and CP degradability in calves differs from that of mature cows up to the age of 10-12-weeks after which the calves ability to degrade feed CP approaches that of mature cows This is to be expected due to the young calf's rumen still being in development.[60] MBP reaching the duodenum is increasing with age. However, studies based on excretion of purine derivatives (PD) in the urine showed that their excretion was lesser in adult sheep than in the lambs or yearlings because of reduced efficiency of MBP production and increase in proteolytic activity.[9]

Species: Microbial protein production rate estimated by Sodium sulfate in cattle fed straw alone or supplemented with CS or urea-molasses-mineral block (UMMB) licks was 80, 269 and 251 g/d, respectively.[9] MBP production in sheep varies from 15 to 35 g/kg of fermented OM (FOM). Such large variation within species was due to feeding regime, pattern and feed intake. Although the extent of MBP production varies between species, its efficiency is diet dependent.[9] If sheep and cattle were fed the same diet, microbial populations would be expected to be similar. However, there are differences because of different outflow rates.[61]

Physiological states: The rumen microorganism appears to provide sufficient protein for maintenance, slow growth and early pregnancy.[9] In the early lactation, the dry matter intake of high-producing dairy cows is increasing but the energy intake is not sufficient to support milking outputs, therefore the body weight is dropping dramatically. Following peak lactation, the consumption of high-quality diet peaks and milk production drops. During this stage, dairy cows tend to maintain body weight. In the mid and late lactation, energy required for milking is less demanding because of milk production is declining. Dairy cows still need more energy because of pregnancy and energy reserve for the next lactation. Maintaining body condition during the dry period is important for ensuring dairy cows have adequate body reserve for the next lactation. The transition period, defined as three weeks before to three

weeks after parturition, is characterized by dramatic changes in physiology and nutrient metabolism and imposes great challenges to the dairy cow.[62] Shifts in the rumen microbial composition of the cows in this period and in general, may alter the rumen fermentation characteristics, influencing parameters like short-chain fatty acid (SCFA) and methane production and thereby affecting feed efficiency of the cow.[63] Lesser MBP production during pregnancy may relate to poor energetic efficiency of just 20% for fetal growth.[64]

Multiparous cows tend to have a lower pH in the rumen than primiparous cows because higher feed intake leads to more fermentation acids produced in the rumen, which is not compensated by increased salivary secretions associated with increased chewing.[18]

Sex: Microbial protein synthesis is not affected by sex being male or female, bull *vs* heifer and ox *vs* cows. No suggested evidence that microbial protein synthesis since sex does not affect the intake of DM and digestibility of OM but the intake of digestible OM (DOM) tended to be greater for bulls than for heifers.[65]

Stress: Stress factors is a condition that affects the welfare of the animal. Animal welfare implies that the animals will develop physically and mentally in good conditions, and that nutritional, social, management, health and comfort factors do not adversely affect production.[66]

Health disorders which result from diet stress specially acidogenic diets, in acute acidosis are based on the degree of pH ruminal decrease which causes great challenge from decreasing the buffering capacity of the ruminal intake, change the population of microorganisms, rumen motility and the systemic fluid balance.[67]

Under heat stress conditions, lactating dairy cows exhibit several physiological responses including a voluntary reduction of feed intake, an increase in maintenance requirements, a decrease in milk yield, and a decline in the quality of milk for manufacturing. Milk protein composition is subject to significant detrimental changes under the effects of heat stress. It is clear that heat stress has an effect on milk protein and casein production and composition that is greater than the indirect effect of reduced intake.[68]

CONCLUSION AND RECCOMMENDATIONS

Conclusion

Ruminants have diversified microbial ecosystem consisting of bacteria, ciliate protozoa, anaerobic fungi and bacteriophages. Microbial organism in the rumen plays a key role for the production of single-cell protein and volatile fatty acid for ruminants and they solve issues with nutrition to methanogens. If one factor is not properly managed it causes other factors to worsen the problem on microflora and resulted in affection on the host. Microbial protein synthesis can be affected by many factors that reduce efficiency of MPS in rumen. Factors influencing them are microbial factor, chemical factors and dietary factors even physical factors together with endogenous factor and there is enormous information on how they affect rumen microbial protein synthesis.

If each factor is properly maintained and quantitatively improved microbial protein production they improve the nutrition, health condition, immunity, production, environmental stress as well as methanogens. Quantitative improvement of microbial protein synthesis has improved feed conversion efficiency also increased the synthesis of B-complex vitamins in the rumen by rumen microflora and available to host animal for stress tolerance and metabolism of energy in dairy cattle than qualitative improvement. Since ruminants rely on around 75% of microbial proteins so they obtain these proteins from them and build their body mass and improve milk yield. So, if we improve factors affecting them we will also get improved milk yield and meat. Therefore, understanding the effect of each factor on microbial protein production is a key for resolution of an animal problem.

Recommendation

Recommendation is based on:

- Understanding the effect of each factors on microbial protein synthesis and the way to solve those problems should be imposed upon them.
- Use accurate feed nutrient composition value in formulating ration
- Avoid access the ruminants to highly fermentable carbohydrate
- Diet containing a mixture of forages and concentrates should be provided to increases microbial protein synthesis.

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Original Research

Prevalence of Major Gastrointestinal Tract Parasite of Cattle at Municipal Abattoir of Jimma Town, Oromia, South Western Ethiopia

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ABSTRACT

Aim
The objectives of this study were to assess the prevalence of major gastrointestinal tract (GIT) parasite of cattle's and associated major risk factor at Jimma municipal abattoir.

Method
A cross-sectional study with a simple random sampling method was conducted from November, 2018 to April, 2019.

Result
Based on the carpological examination, from 400 animals that were presented for slaughter at Jimma municipal abattoir, 46.8% (187) of animals have at least one GIT parasite. The study detected five genera of GIT parasite which were Strongyle-type, *Trichuris* spp., *Monezia* spp., *Paramphistomum* spp. and *Eimeria* spp. with prevalence of 28.9% (54), 4.8% (9), 3.2% (6), 38.5% (72) and 13.4% (25), respectively while mixed parasites has 11.2% (21) of prevalence. The diversity of those mixed parasite were Strongyle type with *Paramphistomum* spp., *Paramphistomum* spp. with *Eimeria* spp., *Eimeria* spp. with *Trichuris* spp., Strongyle spp. with *Monezia* spp., *Paramphistomum* spp with *Monezia* spp,, Strongyle type with *Emeria* spp. and *Paramphistomum* spp. with *Trichuris* spp. with the prevalence of 42.9%, 14.3%, 14.3%, 9.5%, 14.3%, 23.8% and 10.0% respectively. The prevalence of gastrointestinal parasite based on origin of the study animals was recorded and showed statistically significant difference with p-value of 0.001 ($p \leq 0.05$) and the prevalence was highest in Seka Chekorsa and followed by Kersa, Mena, Oma Nada and Dedo in decreasing order. The risk factor related to the age showed statistically significant difference ($p<0.05$) in which GIT parasite has highest prevalence in young and least in old and moderate in adult cattle. This study showed that infection prevalence was highest in animal with poor body condition followed by medium and good body condition scores and difference was statistically significant ($p<0.05$). In addition, the difference between the season also recorded and found statistically significant ($p<0.05$).

Conclusion
The finding showed that good management and strategic anthelmintic treatment need to be applied in the area to reduce the prevalence of the GIT parasites of cattle and their risk factor to lessen economic loss caused by the parasite.

Keywords
Abattoir; Carpological examination; GIT parasite; Jimma.

Abbreviations
ELISA: Enzyme-linked immunosorbent assay; GDP: Gross domestic product; GIT: Gastrointestinal tract; Spp: Species; BW: Body weight.

INTRODUCTION

Livestock systems occupy about 30 per cent of the planet's ice-free terrestrial surface area and are a significant global asset with a value of at least $1.4 trillion.[1] They are important source of animal protein in many countries of the world, supplying a good percentage of the daily meat and dairy products in cities and villages, flexible income for family units, employment, farm energy and manure.[2,3] It is increasingly organized in long market chains that employ at least 1.3 billion people globally and directly support the livelihoods of 600 million poor smallholder farmers in the developing world.[1] According to Herrero et al,[4] the total demand for livestock products might almost double by 2050, mostly in the developing world owing to increases in population density, urbanization and increased incomes.

Ethiopia is one of the African country that possess about 59.5 million cattle, 30.7 million sheep, 30.2 million goats and 59.5 million chickens.[5] They contribute about 16.5% of the national gross domestic product (GDP) and 35.6% of the agricultural GDP.[6] In spite of the large population of cattle, productivity in Ethiopia is low due to poor nutrition, reproduction insufficiency, management constraints and prevailing animal disease. Gastrointestinal parasites are considered as the major diseases of cattle in the country.[7] It is one of the major causes of wastage and decreased productivity exerting their effect through mortality, morbidity, decreased growth rate, weight loss in young growing calves and late maturity of slaughter stock, reduced milk and meat production and working capacity of the animal mainly in developing countries.[8]

The numbers of gastrointestinal tract (GIT) parasite species are known to infect cattle worldwide. The most important ones include nematodes like *Strongyle* species (Haemonchus, Ostartagia, Trichostrongylus, Cooperia) and trematodes of economic importance *Fasciola* species (*Fasciola hepatica* and *Fasciola gigantica*) and *Paramphistomum* species (*Paramphistomum* cervei), while cestodes like *Monezia* species (*Monezia benideni* and *Monezia expanza*) could also be important constraints in animal production.[8] There are many associated risk factors influencing the prevalence and severity of GI helminths. These include age, sex, and weather condition and husbandry or management practices.[9]

Many *cross sectional* study on GIT parasite of cattle were carried out in many part of Ethiopia. According to Etsehiwot,[10] the study conducted in and around Holleta indicated that the overall prevalence parasitic infection of cattle was 82.8%. The predominant helminths egg identified were trematodes (*Fasciola* and *Paramphistomum* spp.) 80.6%, *Strongyles* 66.25%, mixed infection (*Trematodes* and *Strongyles*) 63.12%, while others such as *Trichuris* and *Monezia* 1.5%.[10] Other study conducted on gastrointestinal (GI) parasite of ruminants in Western Oromia also showed that the overall prevalence of GIT parasites was 69.6% in cattle with predominant prevalence of Strangles and Eimeria parasite.[11] In addition according to Tulu et al[7] the study on major gastro-intestinal helminths parasites of cattle in Tulo District, West Hararghe Zone shows that 50.08% was recorded with one or more species of GI helminthic parasites.

Furthermore, the abattoirs are instruments for the insurance of wholesome meat and meat products as well as providing abattoir by-products for livestock base industries.[12] More importantly, abattoirs are used for the purpose of surveillance against animal and zoonotic diseases.[12] The importance of abattoir records in analysis of prevalence rate and planning strategy for the control of livestock diseases cannot be undermined. However, there was no enough study that was carried out on GIT parasite of cattle that slaughtered at different municipals' of abattoirs of Ethiopia including Jimma municipal abattoir yet. Therefore, the objectives of this study were to assess the prevalence of GIT parasites of cattle come for slaughter and associated major risk factor at Jimma municipal abattoir. This is with a view of providing a baseline epidemiological data on this group of parasites and other livestock diseases of economic and zoonotic importance in an ongoing study in Ethiopia.

MATERIALS AND METHODS

Study Area Description

The study was carried out on the cattle that were come for slaughter at Jimma municipal abattoir from November 2018 to April 2019. Jimma is the largest city in south-western Ethiopia. It is a special zone of the Oromia Region about 352 km southwest of Addis Ababa. It has latitude of 7013' to 8056' N and longitude of 35052' to 37037' E, and an elevation ranging from 880 to 3360 m above sea level.[13] The area receives a mean annual rainfall of about 1,530 mm, which comes from long and short rainy seasons. The average minimum and maximum annual temperature ranges between 14.4 and 26.7 °C, respectively.[14] The predominant economic activities involve mixed farming, which broadly includes cultivation of cereal crops, cash crops including primarily coffee and production of livestock. The total livestock population of Jimma zone is estimated to constitute, 2.02 million cattle, 288,411 goats, 942,908 sheep, 152,434 equines, 1,139,735 poultry and 418,831 bee hives.[15]

Study Population

All cattle that were presented for slaughter at Jimma municipal abattoir during the study periods were considered as study animals for the presence of gastrointestinal parasite. Those animals were transported to the abattoir from different district of Jimma zone and all of them were zebu cattle. Sex of examined animals was male. Female animals were not slaughtered in abattoir during this study.

Study Design

A cross-sectional study was conducted to determine the prevalence of GIT parasite of cattle which were presented for slaughter at Jimma abattoir and to investigate the major risk factors influencing the prevalence of parasite infection in cattle.

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Sampling Methods and Sample Size

The sampling method that used in this study was random sampling method. Animals were selected in the lottery method of simple random sampling in which all the ID of the cattle that were transported to Jimma municipal abattoirs written on separate slips of paper of the same size, shape and colour and they were folded and mixed up in a container. The required numbers of slips were selected at random for the desire sample.

The sample size was determined by the formula stated in Thrustfiled[16] with 95% confidence interval and 5% of absolute precision and considering that expected prevalence is 50% used since there is no reported studies at Jimma municipal abattoir. Hence, the sample size was calculated to be 384.

$$N= (1.96)^2 P_{exp}(1-P_{exp})/d^2$$

Where, N=required sample size P_{exp}=Expected prevalence (50%), d=desired absolute precision (0.05). Accordingly, 384 samples were needed, however, 400 cattle was sampled and examined to increase precise of the mean. It was increased by 4%.

Data and Sample Collection

Fecal samples was collected directly from rectum of animals in clean universal bottle then labeled and kept in icebox and immediately transported to parasitology laboratory of Jimma University, College of Agriculture and Veterinary Medicine and was examined. Those that were not examined on that time were stored in refrigerator at 4 °C and examined in the following day. During sample collection various potential risk factors including sex, age, breed, and body condition score were recorded. The age of cattle was determined by dentition using the given standard. Cattle were grouped into three age categories; under 5-years of age, they were categorized as young, those in range of 5 to 10-years were grouped as adult and those above 10-years were classified as old. In addition to that body conditions of animals were recorded based on the scoring system described by Nicholson et al[17] in Zebu cattle. Accordingly the cattle were categorized in to poor, medium and good.

Carpological Examination

The collected fecal samples were examined by using floatation and sedimentation techniques simultaneously. The presence of at least one parasite egg in either of the tests revealed that the result was positive. The egg morphology, appearance, color and presence of blastomeres were used to identify the parasites.

Data Analysis

The information and data that were collected on GI parasite of cattle and its risk factors during the period were recorded in excel Sheet and analyzed using SPSS version 20. Descriptive Statistics was used determine the prevalence through percentage and frequency. The significance of association between and among the considered variables was determined using *p*-value, chi-square (χ^2) test statistics. Association between variables was said to exist if the calculated level of significance is less than 5% ($p<0.05$) at 95% confidence level.

RESULTS

Overall Prevalence of GIT Parasite

Based on the carpological examination, from 400 fecal samples of animals that were come to Jimma municipal abattoir for slaughter, 46.8% (187) of animals had GIT parasite. Variation had been observed on the prevalence of different types of gastrointestinal nematode parasites. 166 (88%) of the animals were positive with single parasite whereas 21 (11.2%) of the animals were positive for mixed types of GIT parasite. The study was detecting five genera of GIT parasite. Those were *Strongyle* type egg, *Trichuris* spp., *Monezia* spp, *Paramphistomum* spp and *Eimeria* spp. with the prevalence of 28.9% (54), 4.8% (9), 3.2% (6), 38.5% (72) and 13.4% (25), respectively. The diversity of those mixed parasite were Strongyle type with *Paramphistomum* spp., *Paramphistomum* spp. with *Eimeria* spp., *Eimeria* spp. with *Trichuris* spp., *Strongyle* spp. with *Monezia* spp., *Paramphistomum* spp. with *Monezia* spp., *Srongyle* type with *Eimeria* spp. and *Paramphistomum* spp. with *Trichuris* spp. and their prevalence were 42.9%, 14.3% , 14.3%, 9.5%, 14.3%, 23.8% and 10.0% , respectively (Tables 1 and 2).

Table 1. *Prevalence of Major Git Parasite of Cattle that Slaughtered at Jimma Municipal Abattoir*

Species of Parasite	No. of sample	Number of positive	Prevalence in %
Strongyle type	400	54	28.9
Trichuris spp.		9	4.8
Paramphistomum spp.		72	38.5
Monezia spp.		6	3.2
Eimeria spp.		25	13.4
Mixed parasite		21	11.2
Total	400	187	100

Table 2. *Prevalence of Mixed Git Parasite of Cattle Slaughtered at Jimma Municipal Abattoir*

Types of Parasite Genera	No of Animal Examined	No of Positive Animals	Prevalence in %
Strongyle type Paramphistomum spp.	400	9	42.9
Paramphistomum spp. with *Eimeria* spp.		3	14.3
Eimeria spp. with *Trichuris* spp.		3	14.3
Strongyle type with *Moneizia* spp.		2	9.5
Paramphistomum spp. with *Monezia* spp.		3	14.3
Srongyle type with *Eimeria* spp.		5	23.8
Paramphistomum spp. with *Trichuris* spp.		2	10.0

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Table 3. *Prevalence of Git Parasite Cattle Slaughtered at Jimma Municipal Abattoir in Related to Risk Factor*

Risk Factor	Number of Animal Examined	Number of Positive Sample	Prevalence in %	χ2	*p*-value
Origin of Animals					
Kersa	75	38	50.7		
Dedo	87	30	34.5		
Seka Chekorsa	95	62	65.3	22.971[a]	0.001
Oma Nada	89	32	36.0		
Mana	54	25	46.3		
Age Categories					
Young	142	107	75.4		
Adult	190	67	35.3	77.591[a]	0. 001
Old	68	13	19.1		
BCS					
Poor	114	72	63.2		
Medium	182	90	49.5	34.411[a]	0.001
Good	104	25	24.0		
Months					
November	80	52	65.0		
December	80	39	48.8		
January	80	30	37.5	15.927[a]	0.003
February	80	35	43.8		
March	80	31	38.8		

Prevalence of Gastrointestinal Parasite Based on the Risk Factor

The prevalence of gastrointestinal parasite based on origin of the study animals was identified and out of the total 75 animals from Kersa, 87 from Dedo, 95 from Seka Chekorsa, 89 from Oma Nada, and 54 from Mena, 50.7% (38), 34.5% (30), 65.3% (62), 36.0% (32), and 46.3% (25), respectively of them were positive at least for one GIT parasite. The difference was statistically significant with *p*-value of 0.001 ($p \leq 0.05$) and Chi-square value of 22.971. Young, adult and old animals were found to be infested with a prevalence of 75.4%, 75.4% and 19.1, respectively with statistically significant difference with *p*-value of 0.001 ($p<0.05$) and χ^2 of 77.591[a]. Infection prevalence was significantly highest in animal with poor body condition followed by medium and good body condition scores and difference was statistically significant with *p*-value was 0.001 ($p<0.05$) and χ2 of 34.411[a]. The overall infection prevalence according to body condition grades, 63.2%, 49.5% and 24.0% with poor, medium and good, respectively (Table 3).

In addition, the prevalence of GIT parasite of cattle in the different month was recorded and the associations found statistically significant ($p<0.05$) and its *p*-value was 0.001. The prevalence was 65.0%, 48.8%, 37.5%, 43.8% and 38.8% in November, December, January, February and March, respectively.

DISCUSSION

Gastrointestinal tract parasites cause severe infection to domestic animals worldwide. Those GIT parasite mostly caused by nematode, cestode, trematode and protozoa in domestic animals and affects fertility, work capacity, involuntary culling, reduction in food intake, weight & milk production and higher mortality rate.[18,19]

The findings of present study show that from 400 of the cattle screened, 46.8% (187) of animals had at least one GIT parasite infection which was similar with the result study of Adedipe et al[20] on the prevalence gastrointestinal helminths in slaughtered cattle in Ibadan, South-Western Nigeria which was 41.6% and of Lemy and Egwunyenga[21] on the prevalence of parasitic helminthes at various abattoirs in Abraka, Delta State, Nigeria which was 50.4%. However, it was less than that of other study result of Waruiru et al[22] in the central Highlands of Kenya, of Elele et al[23] at selected abattoirs in Port Harcourt, South-south, Nigeria, of Usman et al[24] in Katagum Abattoir Of Bauchi State, Nigeria, of Okike et al[25] at Aba, Nigeria, of Luka et al[26] at Gombe Abattoir, Gombe State, North-Eastern Nigeria and of Bisimwa et al[12] which were 86.8%, 62.1%, 61.8%, 87.41%, 80.72% and 74%, respectively. In addition to that the result of the study was greater than other study results which were conducted in Wukari Local Government abattoir, in Taraba State, North-Eastern Nigeria[20] and in Wudil Local Government Area abattoir in Kano State, Nigeria[27] with the same overall prevalence of 34.9%. These differences could be due to the periods or seasons in which the studies were conducted, the management system, topography climatic condition that favors the survival of infective stage of the parasite and intermediate hosts as well as the sources of cattle sampled in the various regions

In this study different genus of parasite was found. Five genus of parasite was observed. Those are *Strongyle* type, *Trichuris* spp., *Paramphistomum* spp., *Eimeria* spp. and *Monezia* spp. Those GIT were encountered in the study had been reported by other

researcher in different parts of the other country.[12,20-26]

Furthermore, the study showed that paramphistomum eggs were the most prevalent among the parasite which has prevalence of 38.5%. This prevalence was greater than the reported study that was conducted at Wukari Local Government abattoir, in Taraba State, North-Eastern Nigeria which was 23.70%[28]; furthermore, it was disagree with reported result of Ayalew et al[29] who reported paramphistomum prevalence which was greater than the study which was 51.82% in Gondar Elfora Abattoir.[30] This difference might be associated with the differences in geographical and/or climatic conditions and ecology of the region, health management of the animals and availability of the intermediate hosts.

In addition, the mixed infection such as *Strongyle* type with *Paramphistomum* spp., *Paramphistomum* spp. with *Eimeria* spp., *Eimeria* spp. with *Trichuris* spp., *Strongyle* type with *Monezia* spp., *Paramphistomum* spp. with *Monezia* spp. and *Srongyle* type with *Eimeria* spp. which also reported in different study at Various Abattoirs in Abraka, Delta State, Nigeria.[21] Mixed infection was characterized by the presence of two or more helminths. The phenomenon of mixed infection has been suggested to be an important cause of morbidity and reduced production in livestock.[28] Furthermore, the immune suppression of the host immune system by mixed infections increases host susceptibility to other diseases or parasites.

In the study the single infections were found more prevalent in comparison to mixed infections. Out of 400 samples examined 166 (88%) had GIT one type of GIT parasite and 21 (11.2%) had mixed parasite which was disagree with reported result of Yuguda et al[31] which was (55.67%) had single and 56 (18.67%) had mixed infection with different helminths species. The possible reason was the difference in management of animals and ecology of the area.

From the present result of study, cattle with poor body condition score had highest prevalence of gastrointestinal parasite when compared to those that were moderate and good body condition. The cause might be related to nutritional deficiencies which may have interfered with the development of acquired immunity in cattle.[31] Possible reason for this could be that those with moderate and good body condition for a number of reasons, including good nutrition, tolerated helminth infections better or that both host and parasites had reached a state of equilibrium and were asymptomatic at the point of faecal collection.[32]

The present study also shows that the presence of significant difference ($p \leq 0.05$) among the origin of cattle in related to GIT parasite. The prevalence of GIT parasite highest in Seka Chekorsa (65.3%) and followed by Kersa (50.7%), Mena (46.3%), Oma Nada (36%) and Dedo (34.5%) in decreasing order. The difference in the prevalence obtained could be attributed to the existence of favorable environmental factors necessary for the prolonged survival and development of infective larval stage of most helminthes.[33] District of Seka Chekorsa, Kersa, Dedo, Seka Chekorsa, Oma Nada, and Mena have different swampy area which were accounted as part of swampy, degraded unusable part of their land of 22.8%, 18.9%, 14%, 11.7% and 5.4%, respectively which is favorable environmental factors necessary for the prolonged survival and development of infective larval stage and other factor.

Statistically significant difference among the age of animals as risk factor for GIT parasite also found in this study. The young animals were most infected when compared with adult and old animals with prevalence of 75.4%, 35.3% and 19.1%, respectively. There was a decrease in infection rate (prevalence) as age increased. This may be due to the result of acquired immunity (natural) with age which is manifested by humoral immune response through frequent challenges and expel the ingested parasite before they establish infection. The natural immunity of the animals influenced by nutrition and general condition of the animal.[34] The result was similar with the study result of Shitta et al[35] in which young has prevalence of 45.30% than the adult examined which had 30.10%.

In addition the study shows that there was statistically significant difference of GIT parasite prevalence in different month in which sample was collected. The highest prevalence of helminths parasitic infections (65.0%) was recorded in November which was included in the rainy season and lowest prevalence (37.5%) was recorded in January which was categorized in dry season. This finding was in agreement with Wadhwa et al[36] and Kumar et al[37] who recorded higher incidence of parasitic infection during rainy season and lower prevalence during dry season. This may be due to high-moisture content and temperature which favours the growth and development of larvae on pasture resulting in increased contact between the host and parasites. In addition to that the reason of the lowest prevalence (37.5%) was recorded in January might be the January was come followed driest month of the December (en.climate-data.org). Thus, there were no available larvae of parasite whose cycle depends on optimum temperature and moisture that were infecting the cattle.

CONCLUSION AND RECCOMMENDATIONS

Generally, gastrointestinal parasites are considered as the major diseases of cattle which are one of the major causes of wastage and decreased productivity exerting their effect through mortality, morbidity, decreased growth rate, weight loss in young growing calves and late maturity of slaughter stock, reduced milk and meat production and working capacity of the animal mainly in developing countries. The study was performed to identify and find the prevalence of GIT parasite at abattoir. Five types of GIT parasites were identified including *Strongyle* type, *Trichuris* spp., *Paramphistomum* spp., *Eimeria* spp. and *Monezia* spp. In addition this study identified the potential risk factors such as age, body condition and the origin of the animals and season of the year which showed statistically significant difference associated with high-prevalence rate. Therefore based on the above conclusion the following recommendations are forwarded:

- Animals should be management in good manner to lessen their susceptibility

- periodical deworming need to be applied in the area to reduce the prevalence of the GIT parasites of cattle
- The habitat of the intermediate host should be the destructed if it is possible

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ANNEXES

Collection of Fecal Samples and Laboratory Procedures

Collection of fecal samples: Collection of faecal samples is performed according to the following procedure;

- Faecal samples for parasitological examination were collected from the rectum of the animal
- Then it was put in to universal bottles
- Each universal bottle was clearly labeled with animal identification, date and place of collection.
- Then Samples were packed and dispatched in a cool box to avoid the eggs developing and hatching.
- As soon after passage from the animal as possible examination was carried out.
- But when the processing of a fecal specimen delayed for some reason, it was preserved for the followed day be used; it was fixed with 10% formalin. Fixative added to feces at a ratio 3:1 (v:v) and mixed well.

Processing fecal samples

Floatation method

Principle: The simple test tube flotation method is a qualitative test for the detection of nematode and cestode eggs and coccidiaoocysts in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity.

Application: This is a good technique to use in initial surveys to establish which groups of parasites are present.

Equipment

- Beakers or plastic containers
- A tea strainer (preferably nylon) or double layer cheesecloth
- Measuring cylinder or other container graded by volume
- Fork, tongue blades or other type of stirring rod
- Test tube
- Test tube rack or a stand
- Microscope
- Microslides, coverslips
- Balance or teaspoon
- Flotation fluid

Procedure

a. Put approximately 3 g of faeces (weigh or measure with a precalibrated teaspoon) into Container 1.
b. Pour 40 ml flotation fluid into Container 1.
c. Mix (stir) faeces and flotation fluid thoroughly with a stirring device (tongue blade, fork).
d. Pour the resulting faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
e. Pour the faecal suspension into a test tube from Container 2.
f. Place the test tube in a test tube rack or stand.
g. Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a coverslip on top of the test tube.
h. Let the test tube stand for 16 minutes.
i. Carefully lift off the coverslip from the tube, together with the drop of fluid adhering to it, and immediately place the coverslip on a microscope slide (Table 1.1).

Table 1.1. *Specific Gravity of Some Helminth Eggs*

Species	Mean Specific Gravity	Range
Ancylostomacaninum	1.0559	1.0549-1.0573
Toxocaracanis	1.0900	1.0791-1.0910
Toxocaracati	1.1005	1.1004-1.1006
Taenia sp.	1.2251	1.2244-1.2257
Physalopterasp.	1.2376	1.2372-1.2380
$ZnSO_4$ Solution	1.18	
Saturated Salt or Sugar	1.20	

Source: David and Lindquist, 1982. J. Parasitology 68:916-919.

Sedimentation technique (for trematode eggs)

Principle: The sedimentation technique is a qualitative method for detecting trematode eggs (Paramphistomum) in the faeces. Most trematode eggs are relatively large and heavy compared to nematode eggs. This technique concentrates them in sediment.

Application: This is a procedure to assess the presence of trematode infections. It is generally run only when such infections are suspected (from previous postmortem findings on other animals in the herd/flock area), and is not run routinely. The procedure can be used to detect liver fluke (Fasciola) and Paramphistomum eggs.

Equipment

- Beakers or plastic containers
- A tea strainer or cheesecloth
- Measuring cylinder
- Stirring device (fork, tongue blade)
- Test tubes
- Test tube rack
- Methylene blue
- Microslide, coverslips
- Balance or teaspoon
- Microscope

Procedure

a. Weigh or measure approximately 3 g of faeces into Container 1.
b. Pour 40 ml of tap water into Container 1.
c. Mix (stir) thoroughly with a stirring device (fork, tongue blade).
d. Filter the faecal suspension through a tea strainer or double-

layer of cheesecloth into Container 2.
e. Pour the filtered material into a test tube.
f. Allow to sediment for 5 minutes.
g. Remove (pipette, decant) the supernatant very carefully.
h. Resuspend the sediment in 5 ml of water.
i. Allow to sediment for 5 minutes.
j. Discard (pipette, decant) the supernatant very carefully.
k. Stain the sediment by adding one drop of methylene blue.
l. Transfer the sediment to a microslide. Cover with a coverslip.

Microscopically examination of prepared samples: the prepared samples on microslides from the simple test tube flotation method, the simple flotation method and the sedimentation method are examined under a microscope at the magnifications listed in Table 1.2

Table 1.2. *Magnification Levels for Examining Prepared Samples*

Magnification	Parasites
10×10	Nematode and cestode eggs
10×40	Coccidia oocysts
10×4	Trematode eggs

Age Determination Based on Dentations

Table 2. *Age Determination Based on Dentations Principle*

Age (year)	Characteristics change
1.5-2	I_1 erupt
2-2.5	I_2 erupt
3	I_3 erupt
3.5-4	I_4 erupt
5	All incisors and canine are in wear
6	I_2 is level and the neck has emerged from gum
7	I_2 is level and neck is visible
8	I_3 is level and the neck is visible, I_4 may be level
9	I_4 is level and the neck is visible
10	The dental stars are squire in I_1 and in all teeth by 12-years
15	The teeth that are not fallen out are reduced (small round pags)

Accordingly cattle was categorized in to 3: 1. if≤5, young; 2. if 5-10, adult; 3. if≥10, old. Source: Delauta and Habel (1986). (De-Lahunta, A and Habel, R.E (1986): Teeth applied Veterinary Anatomy. Webester Saunder Company. 4-6).

Body Condition Score

Table 3. *Body Condition Score Principle*

Score	General Feature
1	Marked emaciation (animal would be condemned at ante mortem examination).
2	Transverse process project prominently.
3	Individual dorsal spines are pointed to touch, hip and pin. Tail, head and ribs are prominent transverse process visible, usually individual.
4	Ribs, hip and spines clearly visible muscle mass between hook and pines slightly concave, slightly more flesh above the transverse process.
5	Ribs usually visible, little fat cover, dorsal pins are barely visible.
6	Animal smooth and well cover, dorsal pins cannot be seen but are easily felt.
7	Animal smooth well covered, but fat deposits are no marked. Dorsal spines can be felt with firm pressure, but rounded rather than sharp.
8	Fat cover in critical areas can be easily seen and felt, transverse process cannot be seen.
9	Heavy deposit of fat clearly visible on head brisket, dorsal, spines, ribs, hooks and pins fully, covered and cannot be felt even with firm pressure.

According to above table cattle was categorized in to 1. Poor: 1, 2, 3; 2. Medium: 4, 5, 6 and; 3. Good: 7, 8 and 9. Source: Nicholson and Butterworth, 1986

Data Collection Form

Table 4. *Monthly Prevalence of Gastrointestinal Helminthosis and Parasite Types of Cattle at Jimma Municipal Abattoir in 2018-2019*

Season/ Month	Cattle	Sex	Origin	Age	BCS	Parasite that Identified During Fecal Examination (FE)				
						Strongyle type	*Trichuris* spp.	*Paramphisomum* spp.	*Eimeria* spp.	*Monieziaspp.*