Openventio
PUBLISHERS

ISSN 2475-1286

VETERINARY MEDICINE

Open Journal

June 2022 | Volume 7 | Issue 1 |

www.openventio.org

ISSN 2475-1286

VETERINARY MEDICINE
Open Journal

Openventio PUBLISHERS

CONTENTS

ISSN 2475-1286

VETERINARY MEDICINE
OpenJournal

Openventio PUBLISHERS

Case Report

Colotomy to Resolve Constipation Secondary to Spinal Cord Injury: A Case Report

Cheru Telila, DVM, MVSc[1*]; Jiregna Dugassa, DVM, MVSc[2]; Zerihun Mulatu, DVM, MVSc[3]

[1]*Department of Veterinary Teaching Hospital, College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Oromia, Ethiopia*
[2]*Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Oromia, Ethiopia*
[3]*College of Agriculture and Veterinary Medicine, Jima University, P. O. Box 307, Jima, Oromia, Ethiopia*

[*]***Corresponding author***
Cheru Telila, DVM, MVSc
Department of Veterinary Teaching Hospital, College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Oromia, Ethiopia; Tel. +251913765416; E-mail: cheru.telila@aau.edu.et

Article information
Received: December 9th, 2021; **Revised:** January 4th, 2022; **Accepted:** January 17th, 2022; **Published:** January 21st, 2022

Cite this article
Telila C, Dugassa J, Mulatu Z. Colotomy to resolve constipation secondary to spinal cord injury: A case report. *Vet Med Open J*. 2022; 7(1): 1-4. doi: 10.17140/VMOJ-7-160

ABSTRACT

Urinary and bowel dysfunction is a condition that encompasses loss of bladder and bowel control which is characterized by partial or complete loss of defecation and urination. Urinary and bowel dysfunction is multifactorial and mainly caused by spinal cord injury at the lumbosacral level or more cranial. The present case report was aimed to show techniques and outcomes of colotomy to resolve constipation secondary to bowel dysfunction in a cat. A 1-year-old female cat weighing 1.3 kg was presented to Veterinary Teaching Hospital with a history of anorexia dullness, abdominal distension, and absence of defecation for the last 8-days. Clinical examination revealed stunted growth, poor body condition, very weak anal and pedal reflex, distended abdomen, dehydration, and lateral recumbency. Based on the history and clinical findings the case was diagnosed as constipation and exploratory laparotomy was indicated after sufficient stabilization. The caudal ventral midline was used to perform colotomy to remove accumulated feces. Post-operatively fluid therapy, antibiotics, laxative, and vitamins were administered accordingly. Upon 52-days follow-up; the patient was able to pass her feces completely, while urine retention and incontinence remains unresolved. Therefore, the owner was advised how to apply gentle external compression of the bladder to assist urination.

Keywords
Cat; Colotomy; Constipation; Incontinence.

INTRODUCTION

Urinary and bowel dysfunction is a condition that encompasses loss of bladder and bowel control which is characterized by partial or complete loss of urination and defecation or urine incontinence. The most common cause of constipation requiring surgical intervention is a prostatic disease and its sequela, and perineal hernia.[1] Urinary and bowel dysfunction is multifactorial that is mainly caused by spinal cord injury cranial or at the lumbosacral level. Spinal cord damage is mainly caused by traumatic injury due to different accidents. Traumatic injury of the sacrococcygeal area occurs frequently in young cats of either sex. This injury may lead to temporary or permanent urinary incontinence.[2] Lumbo-sacral epidural injections in cats have a rare but serious potential risk of causing damage to the spinal cord. Development of chronic urinary retention and constipation due to decreased perineal reflex was reported in a cat after single epidural administration of morphine.[3] Nerve injury at this point could eliminate voluntary and supraspinal control of voiding which initially leads to an are flexic bladder and complete urinary retention, followed by the slow development of automatic micturition and bladder overactivity mediated by spinal reflex pathways.[3,4] Urinary incontinence in cats was also associated with a variety of congenital and acquired disorders. Incontinent cats with spinal cord disorders warrant a more guarded prognosis than do cats with other bladder or urethral disorders.[5] Constipation resulting from pelvic nerve damage causes increased resting tone of the colon and rectum and decreased ability to evacuate feces. Fecal incontinence is rarely a management problem for the owners.[2]

In this condition, voiding is commonly inefficient because of simultaneous contractions of the bladder and urethral sphincter. Therefore, spinal cord injury can affect both urine storage and voiding.[4,6] Following traumatic injuries to the spinal cord,

Openventio PUBLISHERS

affection of other structures like the hind leg and tail were also recorded in 84.3% of the cats.[2] Occasionally there is a development of megacolon due to prolonged obstruction from intractable impaction to the rectum and colon.[7] Patients that had an anal tone and perineal sensation at the time of initial examination following injury have a higher chance of returning to normal urinary function after treatment intervention.[2] In companion animals, the characteristic clinical signs of severe and lower motor neuron spinal cord injury include detrusor hyporeflexia or areflexia and sphincter hypotonia or atonia, which can lead to increased bladder compliance and constant urine leakage.[6] The diagnosis of the present case was based on history, clinical and physical examination of the abdominal area. The present report was aimed to show techniques and outcomes of colotomy to resolve constipation secondary to bowel dysfunction in the cat.

CASE DESCRIPTION

History and Clinical Findings

A one-year-old female cat weighing 1.3 kg was presented to the Veterinary Teaching Hospital of Addis Ababa University with a history of anorexia, dullness, and absence of defecation for the last 8-days. As the owner reported, when they first noted the abdominal distention it was recognized as she was getting pregnant. However, since her abdomen distention was increasing with the unexpected speed they started to follow other conditions and found that she could not defecate, lost appetite, and lost her hind leg control. As a result, she started suddenly falling when she was in an attempt of walking as usual. Additionally, the owner informed us that the cat has not been walking normally since her birth and she was using her metatarsal bone as her footpad with flexed hock joint. Clinical examination revealed stunted growth, poor body condition, a very weak anal and pedal reflex, and she was in lateral recumbency due to highly distended abdomen that could not allow her to stand. Abdominal palpation revealed an elongated hard mass in the abdominal cavity. Other findings include a slightly pale and dry mucous membrane, weak and rapid pulse (140 beats/minute), moderate dehydration (8%), RT 36.8 °C. Based on history and clinical findings the case was diagnosed as constipation and exploratory laparotomy was indicated to resolve the problem.

Patient Stabilization and Pre-Operative Preparation

Pre-operatively the animal has received IV 10 ml of 40% glucose and lactated ringer solution that was fixed at 5 ml/kg/hour for 1 hour before surgical intervention to stabilize the patient. At the same time procaine penicillin G at dose rate of 20,000 IU/kg, IM was administered. Since the cat was weak and easily manageable, the caudal ventral midline was shaved and scrubbed before anesthetizing the animal to reduce prolonged exposure to the anesthetic agent.

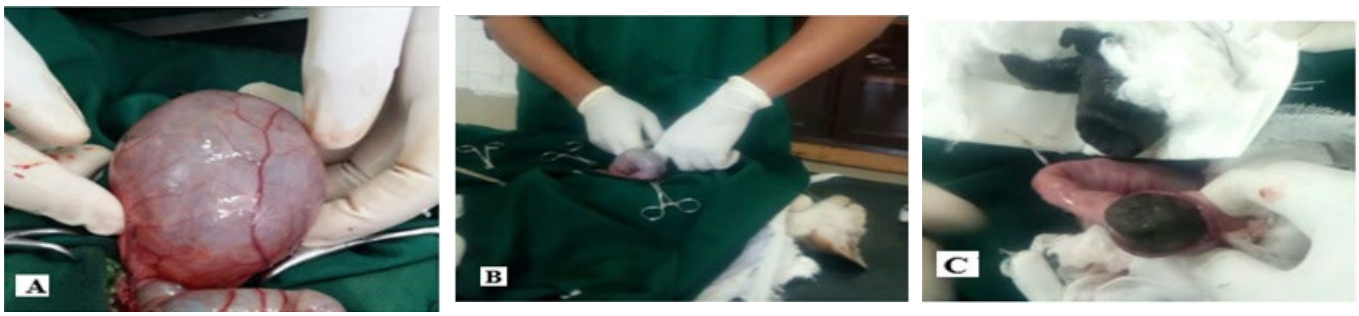
Anesthesia and Animal Control

The cat was premedicated with intramuscular tramadol, atropine sulfate, and xylazine hydrochloride @ 2 mg/kg, 0.02 mg/kg, and 1.1 mg/kg, respectively. Then she was controlled in dorsal recumbency with her legs tied to the surgical table. For induction anesthesia, ketamine hydrochloride was given with slow IV administration at 5 mg/kg. Finally, the surgical site was disinfected aseptically and draped with a fenestrated sterile drape.

Surgical Procedure and Techniques

About 6 cm long ventral midline incision was made onto the caudal abdomen. The abdominal cavity was entered after incising a very thin linea alba. The abdominal cavity was investigated and distended urinary bladder and impacted colon were identified. Then accumulated urine was voided by direct gentle compression of the bladder before undergoing colotomy (Figure 1A). Then the impacted intestine was exteriorized from the abdominal cavity and the laparotomy incision was packed off with sterile gauze to prevent accidental spillage of intestinal contents (Figure 1B). About a 5 cm long enterotomy incision was made on the anti-mesenteric border of the colon and fecal mass at the incision site was first removed by holding with gauze while gentle outward pushing is maintained from the mesenteric side of the colon (Figure 1C). The mass located proximal to the incision and in the rectum was removed by gentle milking to the incision site. After removing all hard fecal mass, the edge of the colotomy incision was cleaned with different gauze soaked in normal saline. The intestine was closed with a lambert suture pattern using 3-0 chromic catgut taking care of stricture formation. Pair of surgical gloves worn during

Figure 1. *Colotomy in Progress Following Urinary and Bowel Dysfunction*



(A) Distended urinary bladder

(B) Exteriorized impacted colon

(C) Removal of fecal mass after anti- mesenteric incision of the colon

colotomy was changed and laparotomy packs were removed to replace the intestine into its normal position and the abdominal cavity was flushed with 10 ml normal saline. The abdominal incision was closed with a single layer of simple interlocking suture pattern using the same suture material. The skin was closed with a simple interrupted suture using 2-0 suture silk (Figure 2A). The surgical procedure was completed without adding a maintenance dose.

Post-Operative Care and Outcome

Post-operatively the topical wound spray was applied on the surgical site and tramadol injection was prescribed 2 mg/kg, IM for the next 2-days. Intravenous fluid therapy was continued until she has received a total calculated fluid deficit and discontinued on the 3rd day since she was started drinking boiled milk. Antibiotic given pre-operatively was continued for 7-days. The owner was advised to provide liquid/soft feed for 10-days and to gradually return to a regular diet. Additionally, oral laxative bisacodyl 1 mg/day was administered after 2-days of operation and was given occasionally until the defecation difficulty was resolved. The patient was so alert and started feeding normally on 3rd day of surgery. However, she was unable to use her hind legs due to previous damage to the spinal nerve at the lumbosacral level. Therefore, neurobion forte tablet was administered for consecutive 20-days as supportive treatment. The skin suture was removed on 18-days of operation, after complete healing of the surgical wound (Figure 2B). The cat was able to pass her feces completely though she can not decide where she has to defecate. She faced urination incontinence and was unable to empty her bladder. Therefore the owner was advised how to manually compress the bladder to assist urination. Her hind leg was showed excellent improvement and she was able to run, play, and hide on 52-days follow-up. Though, the problem of passing feces was resolved after surgical intervention, fecal and urine incontinence were remain as the main problem during the follow-up period.

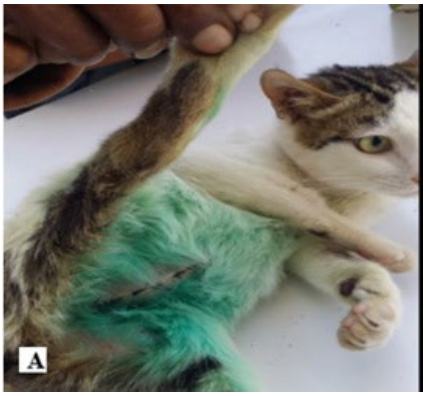
DISCUSSION

Urinary and bowel dysfunction is a common condition characterized by partial or complete loss of urination and defecation or urine incontinence.[3] This condition is multi-etiological that could be congenital and acquired disorders.[5] The acquired disorders are mainly caused by spinal cord injury at the lumbosacral level.[4,8] A radiographic examination can be conducted to identify the presence of impacted fecal material and other abdominal abnormalities.[7] Unfortunately in the current case, physical diagnosis and exploratory laparotomy were used to identify intra-abdominal abnormalities. The present surgical procedure was performed to remove fecal mass from the colon following constipation secondary to the unknown cause of spinal cord injury. In recent procedures directly treating the primary cause of intestinal obstruction-like[1] was found difficult due to equipment unavailability. To resolve this primary cause (spinal cord injury) sacral anterior root stimulation in combination with sacral deafferentation was developed. By using this method human patients with refractory voiding and bowel dysfunction were revealed good improvement.[9] As reported by Saha et al,[7] the cats with intestinal obstruction and found refractory to medical therapy were finally selected to undergo surgical intervention. In the present case, medical treatment was not tried before surgical intervention. Because the patient cannot wait or receive medical treatment as her abdomen was highly distended and in a recumbent position.

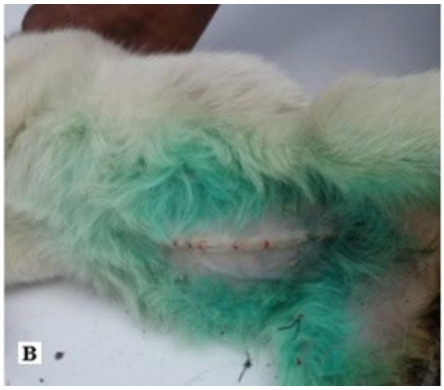
In the current case report, the animal was pre-medicated using xylazine, tramadol, and atropine sulfate while ketamine was used as induction anesthesia and the surgical procedure was completed without any problem. This is almost in agreement with Saha et al[7] work that used xylazine along with ketamine and diazepam. In contrary to this Song et al[3] used dexmedetomidine for sedation while ultra-short acting non-barbiturate propofol and midazolam were used for induction anesthesia and lumbosacral morphine epidural was used to provide peri-operative analgesia. In the present case, spinal cord injury was confirmed following hind limb paresis manifested by incoordination and urinary and fecal incontinence that was observed after the surgical procedure.

In the present case, the abdominal incision was closed with a single layer of simple interlocking suture pattern since the cat has poor body condition and a skinny abdominal wall. Skin closure was made with simple interrupted suture without burying knot since the skin and subcutaneous tissue was very thin and does not allow subcuticular suture. The management of persistent

Figure 2. *Skin Suture and its Removal*



(A) Simple interrupted skin closure



(B) On 18-days of operation and skin suture removal

lower urinary tract dysfunction resulted from severe thoracolumbar spinal cord injury can be challenging. The management of this condition consists of regular manual bladder compression that can result in better outcomes if the primary cause is corrected.[6] Currently, the site of nerve injury was not identified and management of urine incontinence was found difficult. Nonetheless, the owner was advised to manage it manually by applying gentle bladder compression.

Sacral nerve stimulation has proven to be an effective treatment modality for voiding dysfunctions that are refractory to conservative treatment, particularly for patients with urinary and fecal incontinence.[9,11] However, if clinical signs of constipation persisted for more than 6-months it is unlikely to function normally because of irreversible neuromuscular damage to the colon due to persistent pathologic dilation.[7] In the present case, the problem of constipation was completely resolved after surgical correction while fecal and urinary incontinence were continued as the main problem. Though skin sutures removal was possible on the 8th day operation as conducted by Osuna,[1] currently skin suture was removed after complete healing on the 18th day with no surgical complication. Suture removal was delayed to prevent wound dehiscence associated with increased intra-abdominal pressure during manual compression of the bladder.

CONCLUSION

Surgical removal of impacted fecal mass has enabled the cat to void feces without any support and played a major role in the survival of the patient. She also gradually returned to her previous gait after 20-days of operation following supportive treatment. Urine and fecal incontinence were continued as the main challenging condition despite the survival of the animal.

ACKNOWLEDGMENTS

The authors are thankful to Dr. Mahari Teklu for his help through provision of all necessary materials to perform surgical procedure and Haimanot Girma for his assistance with the post-operative management of this case.

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript preparation and final submission. The main surgical procedure and original manuscript writing were performed by Cheru Telila Feyisa, the manuscript structure modification and editing by Jiregna Dugassa Kitessa, while Zerihun Mulatu Tuji was participated as assistant surgeon and reviewing process. Finally, all authors read and approved the final manuscript submission.

ETHICAL CONSIDERATION

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.

REFERENCES

1. Osuna D. Surgical treatment of constipation. In: *Proc Combined Seminar, Society of Sheep & Beef Cattle Veterinarians and Companion Animal Society*. New Zealand Veterinary Association, New Zealand: FCE Publication; 1997: 176. 69-79.

2. Smeak D, Olmstead M. Fracture/luxations of the sacrococcygeal area in the cat a retrospective study of 51 cases. *Vet Surg.* 1985; 14(4): 319-324. doi: 10.1111/j.1532-950X.1985.tb00895.x

3. Song R, Cross J, Golder F, Callan M. Suspected epidural morphine analgesia induced chronic urinary and bowel dysfunction in a cat. *J Feline Med Surg.* 2011; 13(8): 602-605. doi: 10.1016/j.jfms.2011.03.014

4. Christopher M, Melnick A, Sheng L. Impact of bioelectronic medicine on the neural regulation of pelvic visceral function. *Physiol Behav.* 2016; 176(1): 100-106.

5. Lonc K, Kaneene J, Carneiro P, Kruger J. Retrospective analysis of diagnoses and outcomes of 45 cats with micturition disorders presenting as urinary incontinence. *J Vet Intern Med.* 2020; 34(1): 216-226. doi: 10.1111/jvim.15683

6. Hu H, Granger N, Jeffery N. Pathophysiology, clinical importance, and management of neurogenic lower urinary tract dysfunction caused by suprasacral spinal cord injury. *J Vet Intern Med.* 2016; 30(5): 1575-1588. doi: 10.1111/jvim.14557

7. Saha C, Mukherjee P, Ghosh D, Nandi S. Surgical management of pyometra in a cat. *Indian J Vet Surg.* 2016; 55(1): 89-90.

8. Santos-Pereira M, Charrua A. Understanding underactive bladder: A review of the contemporary literature. *Porto Biomed J.* 2020; 5(4): e070. doi: 10.1097/j.pbj.0000000000000070

9. Noblett K, Buono K. Sacral nerve stimulation as a therapy for patients with refractory voiding and bowel dysfunction. *Obs Gynecol.* 2018; 132(6): 1337-1345. doi: 10.1097/AOG.0000000000002968

10. Negrin A, Schatzberg S, Platt S. Neuroanatomic diagnosis and specific spinal cord diseases. *J Feline Med Surg.* 2009; 11(5): 361-372. doi: 10.1016/j.jfms.2009.03.004

11. Lycklama A, Siegel S, Jonas U, et al. Results of sacral neuromodulation therapy for urinary voiding dysfunction: Outcomes of a prospective, worldwide clinical study. *J Urol.* 2007; 178: 2029-2034. doi: 10.1016/j.juro.2007.07.032

ISSN 2475-1286

VETERINARY MEDICINE
OpenJournal

Openventio PUBLISHERS

Original Research

Prevalence and Public Health importance of Bovine Cysticercosis in Haramaya Municipal Abattoir, East Hararghe Zone of Oromia Regional State, Eastern Ethiopia

Mohammed Abdella, DVM[1*]; Mohammedkemal M. Ame, BVSc[2]

[1]*Department of Veterinary Medicine, Haramaya Msunicipal Abattoir, East Hararghe, Ethiopia*
[2]*Department of Veterinary Public Health, College of Veterinary Medicine, Haramaya University, P. O. Box. 138, Dire Dawa, Ethiopia*

[*]***Corresponding author***
Mohammed Abdella, DVM
Department of Veterinary Medicine, Haramaya Municipal Abattoir, East Hararghe, Ethiopia; Tel +251921171393; E-mail: mameabdella53@gmail.com

Article information

Received: January 13th, 2022; **Revised:** February 15th, 2022; **Accepted:** March 10th, 2022; **Published:** March 19th, 2022

Cite this article

Abdella M, Ame MM. Prevalence and public health importance of bovine cysticercosis in Haramaya Municipal Abattoir, East Hararghe Zone of Oromia Regional State, Eastern Ethiopia. *Vet Med Open J*. 2022; 7(1): 5-11. doi: 10.17140/VMOJ-7-161

ABSTRACT

Aim

This study was conducted by using the protocols of post-mortem examinations of meat (visual inspection) to determine the prevalence of bovine cysticercosis in the cattle slaughtered at Haramaya Municipal Abattoir, Eastern Hararghe, Ethiopia and to determine zoonotic significance of taeniasis.

Method

A cross-section study design was conducted from November 2020 to March 2021, based on routine meat inspection on simple randomly selected cattle slaughtered at the abattoir. Visual inspection of all exposed surface was made in all active organs. They are shoulder muscles, hearts, masseters (cheek muscle), diaphragms, tongues and livers. This is followed by incision of all those organs to be examined for *Cysticercus bovis* cysts.

Results

Twenty-one (21) of the 384 cattle examined utilizing the post-mortem examinations meat inspection methodology were positive for *C. bovis*, resulting in a prevalence of 5.5 %. The masseter muscle (11.5 %) had the highest prevalence of cysts, followed by the triceps (8.3%), heart (5.8%), liver (4.8 %), and tongue (2.9 %). The sex-based prevalence rates were 10 (3.4%) and 11 (12.1%), respectively. The predominance of bodily condition was found to be good (1.3%), medium (11.1%), and bad (50 %). The prevalence male and female e differed substantially by organ, sex, and bodily condition (p>0.05), but not statistically significant by age of the animals (young 2.8% vs. adult 6.1%) (p>0.05). Eight (20%) of the total 40 interviewees had contracted *Taenia saginata* infection at least once in their lives. Religion showed a significant difference (p>0.05) (Christian 66.7% and Muslim 6.5%). However, there was no statistically significant difference in meat consumption habits (raw 31.6% vs. cooked 9.5%), sex (male 26.5% vs. female 11.8%), age (young 33.3% vs. adult 17.7%), educational status (illiterate 22.2% vs. elementary 14.3% vs. high school 27.3% vs. college 16.7%) or latrine use (proper users 19.4% and non-proper users 25%).

Conclusion

This study to increasing public awareness of the disease, as well as strict routine meat inspections, should be prioritized in order to decrease the parasite's impact.

Keywords

Bovine, Cysticercus bovis; Haramaya Municipal Abattoir; Prevalence; Public health.

INTRODUCTION

In all parts of the world, animal illnesses are one of the most significant limitations of increasing the productivity of food animals. Among the various parasitic diseases that impair cattle output around the world, parasitism is one of the most serious. Tapeworms are commercially important intestinal parasites that have infected humans for thousands of years all throughout the world.[1] Besides *Taenia saginata* causes one type of taeniasis, while *Taenia solium* causes the other. Both infections are acquired indirectly, with humans swallowing parasite-infected beef or pork. Humans are the definitive hosts, while cows and pigs serve as intermediate hosts.[2]

Openventio PUBLISHERS

Tapeworm infection has been recorded in 1500-years ago and the occurrence of metacestode stage larvae (*C. bovis*) in cattle musculature causes bovine cysticercosis while the adult worm in human small intestine is the cause of taeniasis. Both adult and larval forms have hazardously affect health of their respective hosts, either directly or indirectly accompanied with several secondary infections, particularly in human.[3]

T. saginata is a worldwide zoonotic cestode whose epidemiology is ethnically and culturally determined by estimation of 50-77 million annually.[4] The parasite is very common in Africa and endemic in central and East African countries like Ethiopia, Kenya and Zaire. The custom of eating raw or undercooked beef dishes such as kourt, lebleb, kiteffo and the habit of defecating in open fields coupled with cattle to graze in such fields which are cause of cysticercosis for cattle and taeniasis for human.[5] A high-prevalence of human infection in different agro-climatic zones of Ethiopia has been reported and estimates made by different investigators on the prevalence is vary widely from 2-16% to over 70%.[6]

Transmission to animals occurs by the ingestion of food or water contaminated with the feces of infected humans. Bovine cysticercosis is asymptomatic after development in infected animals. However, it produces irreversibly effects to the beef cattle industry, when the disease is diagnosed at slaughterhouses by visual inspection of specific sectioned tissues.[7,8]

Humans become infected by eating raw or undercooked meat containing the viable cysticerci. The tapeworm develops within the small intestine and becomes sexually mature in about three months, producing gravid proglottids, which are mobile and either migrate from the anus of the infected host spontaneously or shed in feces.[9] Cysts of *C. bovis* can be found anywhere in the carcass and viscera, but its illustrated sites are predilection like masseter, tongue, heart, triceps, intercostals muscle and the diaphragm which organs are consumed at raw level and causes of public health hazardous.[10]

Haramaya district had, large head of cattle but production is low which may be due to parasitic diseases; among which bovine cysticecosis is the most important disease, causing direct and indirect economic loss on livestock production, particularly of cattle and the parasite has public health importance.[1] However, there was no study indicating the disease in Haramaya district which may vary epidemiologically from one area to another.

Therefore, the objectives of this study were:

- To determine the prevalence of bovine cysticercosis at the Haramaya municipal abattoir.
- To determine zoonotic significance of Cysticercosis in the study area.

MATERIALS AND METHODS

Study Area

The research was carried out in the Haramaya Municipal Abattoir, which is located in the Oromiya region's Eastern Hararghe Zone. Harar is 14 kilometers away, and Finfinne is 508 kilometers away. According to agricultural statistics from the Haramaya district, the district contains 63,723 cattle, 13,612 sheep, 20,350 goats, 15,978 donkeys, 530 camels, and 42,035 chickens. The district's production system is a hybrid. It is located at a height of 1600-2100 meters above sea-level, with an average yearly temperature of 18 °C and a relative humidity of 65%. The area receives roughly 900 mm of rain per year on average, with a bimodal distribution pattern peaking in mid-April and mid-August. It is situated at 41° 59' 58" north latitude and 9° 24' 10" south longitude.[11]

Study Population

The study was conducted on the local breed of cattle coming to the Haramaya municipal abattoir which were reared under semi-intensive and extensive farming system. Different risk factors were considered like sex, age, body condition and organ affected. Examination and evaluation of body condition were accomplished during ante-mortem examination. They were classified as poor, medium and good by observing the body condition of the animals in the field according to the method described by Nicholson et al.[12] The ages of animals were also estimated by the dentition method.[13]

Study Design and Sampling Strategy

Cross-sectional study was used to know the prevalence of bovine cysticercosis. The study was based on routine meat inspection on simple randomly selected cattle slaughtered at the abattoir. Visual inspection of all exposed surface was made in all active organs. They are shoulder muscles, hearts, masseters (cheek muscle), diaphragms, tongues and livers. This is followed by incision of all those organs to be examined for *C. bovis* cysts.

Moreover, a cross-sectional study was conducted by a structured questionnaire survey to assess the prevalence of *T. saginata* and associated risk factors.

Sample Size Determination

The number of animals required for the study was determined using the formula given by Thrusfield[14] for simple random sampling, by using 95% level of confidence, 50% expected prevalence and 0.05 desired absolute precision.

$$n = \frac{1.962\ Pexp\ (1\text{-}Pexp)}{d^2}$$

Were as: n=numbers of individuals to be sampled.
Pexp=expected prevalence
d^2=desired absolute precision
1.96=95% confidence level

There was no previously obtained data on the prevalence of *C. bovis* in extensive cattle production system in the study area. Therefore, an overall mean expected prevalence of 50% (0.5) was used with desired absolute precision of ±5%. Accordingly, 384

heads of cattle were sampled in the study.

Study Methodology

Active abattoir survey: The explanatory variables considered are age, sex, body condition and species of animal. The animals were examined before slaughter (ante-mortem inspection) and after slaughter (post-mortem inspection). During ante–mortem examination each animal was marked for identification by writing a code on its head by using un-washable ink and the tagged number of each animal was recorded. Ante-mortem examination on individual animal was done for the assessment of body condition, age, sex and species. During post-mortem inspection, examination of muscles and different organs for the presence of cysts was undertaken by incisions. Each individual was examined based on routine meat inspection on randomly selected cattle slaughtered at the abattoir for the presence of *C. bovis* in organs like Masseter, tongue, heart, triceps, intercostals muscle and the diaphragm. Post-mortem inspection is the most common method in use to detect bovine cysticercosis.

Questionnaire surveys on taeniasis: Questionary survey was used to collect data regarding human taeniasis by visiting hospital 40 patient in Haramaya town by using questionary servey. The potential risk factors of taeniasis such as habit of raw meat consumption, age, sex, religion, occupation, educational levels, presence and usage of sanitary facilities especially toilet and knowledge of *T. saginata* was assessed.

Data Analysis

Abattoir data and questionnaire collected were entered to Microsoft Excel spreadsheet and analyzed by using statistical package for the social sciences (SPSS) version 20 software. Associations between each factor were determined by using Chi square test (χ^2). A statically significant association between variables exists when $p<0.05$ and at 95% confidence level (CI).

RESULTS

Out of the total 384 cattle examined, 21 were found positive for the presence of *C. bovis* with overall prevalence of 5.5%. There is no statistically significant difference ($p>0.05$) in the prevalence of cysticercosis between the age groups. Analysis of the active abattoir survey proved that there is statistically significant difference between sex groups ($p<0.05$) as well as between body condition (Table 1).

An association with regard to the anatomical distribution of Cysticercus cysts in the inspected organs showed statistical significance. Out of single organ, the maximum intensity of infection was observed in masseter muscle followed by tongue and liver, 11.3%, 1.6% and 1.3% respectively. The cyst found in more than one organ, the maximum intensity were found in liver and masseter muscle (37.5%) followed by triceps muscle and tongue (8.3%), head and heart (5.8%) (Table 2).

Table 1. *Prevalence of Bovine Cysticercosis on the Basis of Sex,Age and Body Condition*

Varaible	Animals Examined	Positive	Prevalence (%)	χ^2	*p*-value
Sex					
Male	293	10	3.4		
Female	91	11	12.1	10.1	0.002
Total	384	21	5.5		
Age					
Young	71	2	2.8		
Adult	313	19	6.1	1.2	0.15
Total	384	21	5.5		
Body Condition					
Good	229	3	1.3		
Medium	153	17	11.1	24.8	0.00
Poor	2	1	50		
Total	384	21	5.5		

Table 2. *Frequency Distribution of C. bovis in Different Organs and Tissues of Affected Animals*

Organ infected	No. of Infection	Percent (%)	χ^2	*p*-value
Liver	1	1.3		
Tongue	2	1.6		
Masseter muscle	12	11.3		
Liver and masseter	3	37.5	30.6	0.00
Triceps and tongue	1	8.3		
Head and heart	2	5.8		
Total	21	5.5		

Questionnaire Survey on Taeniasis

Of the total 40 interviewed respondents who participated in this study, 20% (8/40) had contracted *T. saginata* infection at least once in their life time, of which, 17.5% and 2.5% reported using modern drugs and traditional drugs, respectively. The majority of the respondent had an experience of raw meat consumption as a result of traditional and cultural practice.

The analysis of the risk factors showed a significant difference ($p<0.05$) in the prevalence of taeniasis with religion, but there was no significance difference between age, sex, educational status, address, habit of eating meat and latrine using behavior of the respondents ($p>0.05$) (Table 3).

DISCUSSION

In the current study, the overall prevalence of bovine cysticercosis was 5.5%. This is comparable with finding of Gomol et al,[15] (3.6%) and Megersa et al,[16] (4.4%) in Jimma Municipal Abattoir as well as result by Dawit[17] (4.9%) at Gondar. However, this was lower than findings reported by Abunna et al,[18] (26.3%) in Awassa, Hailu[19] (17.5%) in East Shoa, Getachew[20] (13.8%) in Debre Zeit, Regassa et al,[21] (13.3%) in Ahmed[22] (21%) in Nekemte abattoirs. This difference might be because most of the animals slaughtered

Table 3. *Association between Prevalence of Human Taeniasis with Risk Factors in Haramaya Town*

Varaible	Interviewed People	No. of Infected	Prevalence (%)	χ^2	*p*-value
Sex					
Male	23	6	26.1		
Female	17	2	11.8	1.25	0.18
Total	40	8	20		
Age					
Young(1-20)	6	2	33.3		
Adult(>20)	34	6	17.7	0.78	0.26
Total	40	8	20		
Education Status					
Illiterate	9	2	22.2		
Elementary	14	2	14.3		
High school	11	3	27.3	0.72	0.87
College	6	1	16.7		
Total	40	8	20		
Address					
Rural	12	1	8.3		
Urban	28	7	25	1.46	0.19
Total	40	8	20		
Religion					
Christian	9	6	66.7		
Muslim	31	2	6.5	15.8	0.001
Total	40	8	20		
Meat Eating Habit					
Raw	19	6	31.6		
Cooked	21	2	9.5	3.03	0.07
Total	40	8	20		
Use of Latrine					
Proper User	36	7	19.4		
Non proper user	4	1	25	0.69	0.43
Total	40	8	20		

in the abattoir were brought from fattening systems flourishing in different district of east Hararghe zone in which animals from such are less exposed for eggs of *C. bovis* as they graze on relatively clean pasture and restricted indoor husbandary system used by Hararghe farmers.[23]

The prevalence of this study is also lower than to some reports from African countries, such as 20% in Senegal, 27% in Tanzania and 38-62% in Kenya Opera et al.[24] Similarly, Opara et al,[24] had reported prevalence of 26.2% from slaughter animals in Nigeria. Conversely, lower prevalence was reported from developing countries, such as 0.26% in Croatia (Zivkovic et al,[25] 0.48-1.08% in Germany Abuseir et al,[26] and 0.9% in Suarez et al).[27]

The different prevalence reported in these studies might be due to diagnosis of bovine cysticercosis by meat inspection underestimates the true prevalence, especially when infection is light Dorny et al.[9] The higher prevalence of cysticercosis in developing countries might indicate poor sanitary infrastructure, low awareness and improper disposal of sewage, which also pertains to Ethiopia, where the widespread habit of eating raw meat is an additional important risk factor.

In Ethiopia, available literature reveals different percentages of cattle positive for *C. bovis*. Tembo[28] found 70(3.11%) out of 2250 randomly selected adult of bovine carcasses in the central highlands (Akaki, Debrezeit, Nazereth) positive for cysticercosis. Similarly, in Gondar area, the prevalence of *C. bovis* was reported to be 9.7% and 4.9% by Demissie[29] and Hailu[19] respectively. In contrast to low prevalence reported above, such studies in export abattoirs like Mojo, ELFORA, Dukem and Luna revealed a comparatively higher prevalence was reported as 17.9%, 13.6%, 19.2% and 27.6% respectively.[30] In Amhara regional state, Kebede[31] reported (18.49%). These reports indicate variation in the prevalence within Ethiopia. Difference in the prevalence of cysticercosis within a country probably reflects the difference in the expertise/or diligence of meat inspectors. The high prevalence reported in modern and export abattoirs may be due to a thorough meat inspection by incising all organs and muscles and also inspectors in such abattoirs are more experienced and

Openventio PUBLISHERS

also have awareness to the economic importance of *C. bovis* in the exportation Gracey et al.[32]

In the present study there is statistically significant difference ($p<0.05$) between both sexes (male 3.4%, female 12.1%) and this is in contrary with report of Gomol et al,[15] Kebede,[31] Jemal et al[33] and of Garedaghi et al.[34] The possible reason is that, the sample size of female cattle is not comparable to that of male cattle slaughtered at Haramaya municipal abattoir. Prevalence based on body condition were (1.3%), (11.1%) and (50%) in good, medium and poor, respectively which has significant difference ($p<0.05$). This is contrary with report by Abunna et al.[18] The reason might be due to sample size of medium body condition slaughtered is greater and/or immune system of poor body condition animal is weak and easily exposed to infection.

In current study there is no statistically significant difference ($p>0.05$) between age group (young 2.8% and adult 6.1%). It agrees with observation by Hailu[19] and Tembo[28] but not in agreement with report of Gomol et al,[15] and Jemal et al[33] The possible explanation for this might be that young animals have close susceptibility due to poor immunity. Animals brought to the abattoir are in the same age group that means nearly adult and also the sample size is a factor for its insignificancy.

The study also revealed that the highest prevalence of cysts was found in the masseter muscle (11.5) followed by triceps (8.3), heart (5.8), liver (4.8) and tongue (2.9) as observed on Table 2. This finding is in agreement with the reports of Amsalu,[35] Dawit[17] and Opara et al.[24] Report by Wanzala et al,[8] indicates *C. bovis* are commonly found in muscles of mastication, particularly masseter muscles, shoulder muscles, heart, tongue, and occasionally in liver, lungs and lymph nodes. The variations in anatomical distribution depend on a number of factors, such as blood kinetics and animals' daily activities.

Human taeniasis was a health problem in the study area with prevalence of 20%. The occurrence of the disease had significant association ($p<0.05$) taeniasis, prevalence was higher among the Christian community than Muslims. This might be because of raw meat consumption is not common in Muslims as in Christians and Christians also celebrate several annual festivals with the tradition of raw meat consumption, which was in agree with reports by Abunna et al,[18] and Hailu.[19]

Non-statistical variation between people and different risk factors were as follows; Prevalence with educational backgrounds was 22.2%, 14.3%, 27.3% and 16.7 in illiterate, elementary, high school and college, respectively. This might be because of the deeply rooted tradition of raw and undercooked meat consumption regardless of the educational level status. Association between human taeniasis and sex was 26.5% in male and 11.8% in female. The higher prevalence of taeniasis among male could be due to economic reasons and cultural practices in that male do not prepare their dish at home, rather consume at restaurants and butcheries. This study also indicate higher prevalence of Taeniasis among individuals who often consume raw meat than those with less frequent raw meat consumers (31.6% and 9.5% raw and cooked meat consumers, respectively) and use of latrine (proper users 19.4% and non-proper users 25%) indicate being higher in non-proper latrine users similar with Magarsa et al.[16] But disagree with Magarsa et al,[16] Age wise prevalence was 33.3% in young and 17.7% in adult, the reason associated with children play outdoor with in contaminated soil and pasture and feed most of the time without washing their hand unless their families control them. Bovine cysticercosis/teniasis is a serious illness in both bovine and human populations, according to the current study. The disease causes financial losses due to the rejection of infected organs and the degradation of carcasses, as well as the high costs of human treatment. As a result, regular meat inspections should be done to ensure that contaminated carcasses and organs are rejected appropriately.

CONCLUSION AND RECOMMENDATION

Both the active abattoir and the questionnaire survey concluded that bovine cysticercosis caused by *C. bovis*/*T. saginata* is a major livestock disease that causes human health problems in Haramaya and the surrounding district areas. This is linked to risk factors such as raw meat consumption, incorrect bathroom usage, and a lack of other hygienic measures. According to the findings, the condition mostly affects the mastication muscle, resulting in significant production losses.

The following recommendations were made based on the aforesaid conclusion.

- Strengthening training should raise public understanding about the disease's importance and impact on health.
- Lessen the disease's impact on humans and animals, there should be strong and tight collaboration between medical and veterinary professionals.
- Sewage disposal and latrines should be well-designed.
- Strict routine meat inspection is required, as well as a comprehensive examination of all organs for cyst.

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my advisor, Dr. Geremew Haile, for his invaluable support, dedication of time in editing this manuscript, unwavering and consistent advice, encouragement, painstaking correction to give the paper its form, and brotherly approach in all aspects.

My special gratitude go to my co-advisor, Dr. Natenael Tamiret, who assisted me in all facets of my work during my externship program.

Finally, I'd want to express my gratitude to Haramaya University, the College of Veterinary Medicine, and the Haramaya Municipal Abattoir inspector for their assistance during this project, as well as my family for their financial and moral support.

REASON FOR NOT HAVING THE APPROVAL FROM ETHICAL COMMITTEE AND THE INSTITUTIONAL REVIEW BOARD

Keeping in view the public health significance of bovine

cysticercosis, the present study was proposed to find the occurrence of bovine cysticercosis in some parts of Eastern Ethiopia and its implication on public health. The current study was proposed in light of the public health significance of bovine cysticercosis. Its goal was to determine the prevalence of bovine cysticercosis in specific sections of eastern Ethiopia, as well as its implications for public health.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Radostits MO, Gay CC, Hinchcliff KW, Constable PD. *Veterinary Medicine. Text Book of the Disease of Cattle, Sheep, Goats, Pigs and Horses*. 10th ed. Philadelphia, Pennsylvania: Saunders; 2007: 1581-1583.

2. Marquardt WC, Demaree RS, Grieve RB. *Parasitologyand Vector Biology*. 2nd ed. San Diengo, nd London, UK: Accadamic Press; 2000: 327-334.

3. Minozzo JC, Gusso RLF, DeCastro EA, Lago O, Soccoi VT. Experimental bovine infection with *T. saginata* eggs: Recovery rates and cysticerci location. *Braz Arch Biol.* 2002; 45. doi: 10.1590/S1516-89132002000600008

4. Carlos E, Armando N, William A. Taenia solium cysticercosis/taeniosis: Potential linkage with FAO activities; FAO support possibilities. *Acta Trop*. 2003; 87(1): 145-148. doi: 10.1016/s0001-706x(03)00037-8

5. Kumar A, Tadesse G. Bovine cysticercosis in Ethiopia: A review. *Ethiop. Vet J*. 2011; 15: 15-35. doi: 10.4314/evj.v15i1.67681

6. Mohammed AA, Waqtola C. Medical Parasitology for Medical laboratory Technology students. Ethiopia Public Health Training Initiative. https://www.cartercenter.org/resources/pdfs/health/ephti/library/lecture_notes/med_lab_tech_students/ln_parasitology_final.pdf. 2006: 339-341. Accessed January 11, 2022.

7. Geysen D, Kanobana K, Victor B, et al. Validation of meat inspection results for Taenia saginata cysticercosis by PCR restriction fragment length polymorphism. *J Food Prot*. 2007; 70(1): 236-240. doi: 10.4315/0362-028x-70.1.236

8. WanzalaW, Onyango AG, Kang KE, Zessin HK, Kyule MN. Control of Taenia saginata by post-mortem examination of carcasses. *Aft Health Sci*. 2003; 3: 68-76.

9. Dorny P, Praet N. Taenia saginata in Europe. Vet Parasitol. 2007; 149: 22-24. doi: 10.1016/j.vetpar.2007.07.004

10. Gyles CL, Prescott JF, Songer JG, Thoen CO. *Pathogenesis of Bacterial Infection in Animals and its Product Hygiene*. 3rd ed. NY, USA: Blckwell Science; 2004: 122-119.

11. Haramaya woreda agricultural development bureau (HBD). 2009: Haramaya, Ethiopia.

12. Nicholson M, Butterworth T. *A Guide to Condition Scoring of Zebu Cattle*. Addis Ababa, Ethiopia.1986:

13. Gatenby R. *The Tropical Agriculture, London and Beging Stock Mc Millan Education Ltd*. Washington, D.C., USA: ACCT; 1991: 6-10.

14. Thrusfield M. *Veterinary Epidemiology*. 2nd ed. Oxford, USA: Black Well Science tropics; 2005:

15. Gomol T, Achnef M, Basazenuw B, Mersha C. Cyst viability, body site distribution and public health significance of bovine cysticercosis at Jimma, South West. Ethiopia. *Global Veterinaria*. 2011; 7(2): 164-168.

16. Megersa B, Tesfaye E, Regassa A, Abebe R, Abunna F. Bovine cysticercosis in cattle slaughtered at Jimma municipal abattoir, South Western Ethiopia: Prevalence, cyst viability and its socio-economic importance. *Vet World*. 2011; 3: 257-262. doi: 10.5455/vetworld.2010.257-262

17. Dawit S. *Epidemiology of Taenia Saginata Taeniasis and Cysticercoids in North Gondar Zone, North Western Ethiopia*. [dissertation]. Debre zeit, Ethiopia; Addis Ababa University; 2004.

18. Abunna F, Tilahun G, Megersa B, Regassa A, Kumsa B. Bovine cysticercosis in cattle slaughtered at Hawassa Municipal Abattoir, Ethiopia: Prevalence, Cyst Viability, Distribution and its public health implication. *Zoonoses Public Health*. 2008; 55(2): 82-88. doi: 10.1111/j.1863-2378.2007.01091.x

19. Hailu D. *Prevalence and Risk Factor for T. saginata Cysticercosis in Three Selected Areas of Eastern Shoa*. [master's thesis]. Debre zeit, Ethiopia; Addis Ababa University; 2005.

20. Getachew B. *Prevalence and Significance of Cysticercus Bovis among Cattle Slaughtered at Debre Zeit Abattoir*. Debre zeit, Ethiopia; Addis Ababa University; 1990.

21. Regassa A, Abunna F, Mulugeta A, Megersa B. Major metacestodes in Wolaita Soddo municipal abattoir, Southern Ethiopia: Prevalence, cyst viability, organ distribution and socioeconomic implication. *Trop Anim Health Prod*. 2009; 41: 1495-1502. doi: 10.1007/s11250-009-9338-3

22. Ahmed I. *Bovine Cysticercosis in Animals Slaughtered at Nekemte Abattoir*. [dissertation]. Addis Ababa University; Debre Zeit, Ethiopia;1990.

23. Over HJ, Jansen J, van Olm PW. *Distribution and Impact of Helminth Diseases of Livestock in Developing Countries (No 96) (FAO Animal Production and Health Paper S.)*. Rome, Lazio, Italy: Food & Agriculture Organization of the United Nations; 1992.

24. Opara M, Ukpong I, Okoli C, Anosike J. Cysticercosis of slaughter cattle in southeastern Nigeria. *Ann N Y Acad Sci*. 2006;

1081: 339-346. doi: 10.1196/annals.1373.048

25. Zivkovic J, Velimirovic D, Dzaja P, Grabarevic Z. Prevalence of *C. bovis* in measles with particular reference to histopathological changes in meat. *Arch Lebensmittelhyg.* 1996; 47: 66-68.

26. Abuseir SC, Epe T, Schnieder G, Klein M. Visual diagnosis of Taenia saginata cysticercosis during meat inspection: Is it unequivocal? *Prasitol Res.* 2006; 99: 405-409. doi: 10.1007/s00436-006-0158-3

27. Suarez HM, Santizo RM. Epidemiology of the Taenia saginata complex and *C. bovis* in Ciego de Avila, province of Cuba. *Rev Patol Trop.* 2005; 34: 43-52.

28. Tembo A. *Epidemiology of Taenia saginata, Taeniasis/Cysticercosis in Three Selected Agro Climatic Zones.* [master's thesis]. Debre zeit, Ethiopia; Addis Ababa University; 2001.

29. Demissie A. *Prevalence and Significance of Cysticercus Bovis Among Slaughtered Cattle at Gondar Meat Factory.* [dissertation]. Debre zeit, Ethiopia; Addis Ababa University; 1989.

30. Degefu H. *Prevalence and Risk Factors for Taenia Saginata Taeniasis/Cysticercosis in Three Selected Areas of Eastern Shoa.* [master's thesis]. Debrezeit, Ethiopia; Addis Ababa University; 2005.

31. Kebede N. Cysticercosis of slaughtered cattle in north western Ethiopia. *Res Vet Sci.* 2008; 85: 523-526. doi: 10.1016/j.rvsc.2008.01.009

32. Gracey J, Collins DS, Huey R. Diseases caused by helminthes and arthropod parasites. In: *Meat Hygiene.* 10th ed. London, UK: W. B. Saunders Company Ltd.; 1999: 673.

33. Jemal E, Haileleul N. Bovine cysticercosis: Prevalence, Cyst viability and distribution in cattle slaughtered at Kombolcha Elfora meat factory, Ethiopia. *American Eurasian Journal of Agriculture and Environmental Science.* 2011; 11: 173-176.

34. Garedaghi Y, Saber P, Khosroshahi MS, Prevalence of bovine cysticercosis of slaughtered cattle in meshkinshahr abattoir. *American Journal of Animal and Veterinary Sciences.* 2011; 6(3): 121-124. doi: 10.3923/javaa.2012.785.788

35. Amsalu D. *Prevalence and Significance of Cysticercus bovis Among Slaughtered Cattle at Debrezeit Abattoir.* [dissertation]. Debrezeit, Ethiopia; Addis Ababa University; 1989.

ISSN 2475-1286

VETERINARY MEDICINE
OpenJournal

Openventio PUBLISHERS

Case Report

A Case Report on Surgical Management of Dystocia in Heifers Due to Narrow Pelvis and Immaturity

Endale Mekoria, DVM[1*]; Jabessa Dhaba, DVM[1]; Mohammedkemal M. Ame, BVSc[2]

[1]*Department of Veterinary Medicine, Bedeno Woreda Veterinary Clinic, East Hararghe, Ethiopia*
[2]*Department of Veterinary Public Health, College of Veterinary Medicine, Haramaya University, P. O. Box. 138, Dire Dawa, Ethiopia*

[*]***Corresponding author***
Endale Mekoria, DVM
Department of Veterinary Medicine, Bedeno Woreda Veterinary Clinic, East Hararghe, Ethiopia; Tel. +251915129775;
E-mail: endalemekuriyabebe@gmail.com

Article information
Received: February 27th, 2022; **Revised:** March 23rd, 2022; **Accepted:** April 7th, 2022; **Published:** April 13th, 2022

Cite this article
Mekoria E, Dhaba J, Ame MM. A case report on surgical management of dystocia in heifers due to narrow pelvis and immaturity. *Vet Med Open J.* 2022; 7(1): 12-15. doi: 10.17140/VMOJ-7-162

ABSTRACT

Narrow pelvis is characterized as an insufficient maternal bony pelvis that does not allow for fetal transit due to a variety of factors. Animal immaturity is one of these reasons. This communication was written with the goal of documenting surgical management of dystocia and its impact on a two-year-old heifer who was with a full-term pregnancy. A vaginal delivery was tried but failed due to the narrow maternal pelvis. To avoid any post-operative problems, the surgical management was carried out under strict aseptically. A volume of 30 ml 2% Lidocaine Hydrochloride was infiltrated on left flank with inverted L-block techniques. While the cow was on her right lateral recumbency. The uterus was emptied of a deceased fetus but fetal membrane with the intact umbilical cord was returned back to the womb. Suturing was done using standard surgical procedures, and post-surgical care was followed-up for ten-days. The heifer was successfully rehabilitated.

Keywords
Dystocia; Heifer; Immaturity; Narrow pelvis; Surgical management.

INTRODUCTION

Dystocia is a condition in which parturition is delayed or difficult. It's the dam's inability to give birth to her young on her own.[1] Dystocia is caused by congenital or acquired abnormalities of the pelvis, cervix, vagina, or vulva. Pelvic abnormalities, vulvar or vaginal stenosis, neoplasms of the vagina and vulva, vaginal cystocele, inadequate cervical dilatation, uterine torsion, and ventral displacement of the uterus can all cause constriction/obstruction of the birth canal.[2] Small pelvis, exostoses or pelvic deformities, pelvic fracture, osteomalacia, and hypoplasia of the vagina and vulva are some of common pelvic abnormalities that can cause dystocia.[2-4] Hereditary reasons of pelvic defects, such as tiny size and insufficient pelvic ligament development, or small breeds with outsized fetuses, are other causes of dystocia due to a narrow or defective pelvis. The presence of a small bony pelvis was identified as a symptom of sacral luxation or displacement.[3,5] added to the genetic cause of a small-size pelvis.[5] The rate of occurrence of dystocia is closely related to the maturity of the dam. It is widely accepted that heifers calving at roughly two years of age have difficulty delivering the calf, even with good feeding and treatment. Often, intervention is required to save the life of the calf and/or cow. When a heifer can give birth to a calf without assistance, however, it is common to notice that labor lasts longer than in older cows.[6] Early heifer breeding, breeding of underdeveloped heifers, and breeding of heifers and calves with pelvic fractures can result in a smaller mother's pelvis, resulting in dystocia at parturition.[3] The dimensions of bony pelvis are too small to allow passage of the fetus.[7] Feto-maternal dystocia is caused by circumstances such as feto-pelvic disproportion, in which the foetus is either too large to travel through the birth canal or the dam's pelvis is too narrow to allow easy foetal ejection after parturition.[8] In such dystocia, parturition may not proceed over first stage, as the chances of calf being struck in the pelvic inlet are more. Surgical intervention is the only way to opt for delivering the calf, as forced traction may endanger life of both dam and calf.[4,9] There are various reasons for performing a caesarean section (C-Section), including maternal and fetal reasons. Immature heifers, pelvic abnormalities, failure of cervical dilation, uncorrectable uterine torsion, uterine tear, and prepartum paralysis are among the maternal indicators. When compared to multiparous cows, the risk factors in cattle are raised when the heifer is less than two years old.[10] A case of dystocia due to narrow pelvis associated with immaturity in heifer and its successful surgical management is presented in this report.

CASE HISTORY AND FIELD EXAMINATION

On Friday, September 17th, 2021 at midday, the farmer was called to Bedeno Veterinary Clinic and complained that his two-year-old (2-years-old) heifer, with full term of pregnancy, had been unable to deliver a fetus since yesterday mid-night. The owner said that he and other locals attempted unsuccessfully to birth the fetus. The vets traveled by a motorcycle and arrived at the scene. The vets continued to examine the cow and upon field examination, the rectal temperature was 38.80 °C, and tachycardia (130 beats per minute) and dyspnea (16 breaths per minute) were present. There was a rupture of water bag, swelling of vulva, absence of straining, abdominal distension. The heifer was recumbent and unable to stand. After the external genitalia was washed with water and soap solution, then cleaned with savlon (Cetrimide 3%+chlorohexidine gluconate 0.3%), solution (by disolving 250 ml of Savlon with 1000 ml of warm water) and sugar solution (500 grams of sugar disolved with 250 ml water) was made and added to the vulva to remove water content from swollen vulva to ease manual manipulation. Here, sugar is not a standard treament for dystocia but the vets were used in reduction of water from swollen vulva. Accordingly, the water content was drained and the swollen vulvar size was reduced. After the vulvar edema was reduced and lubricated with 100 ml of paraffin oil, per-vaginal delivery was attempted but failed because the maternal bony pelvis was very narrow. Locals damaged the calf's lower mussle while attempting to remove the fetus before the veterinarian team arrived. Even fetotomical birth was impossible because the recognized or palpable or reachable part of the fetus was only the fetal mussle. Only C-Section was left as a final resort to save the heifer. As there was no operation room built in the field, the place (floor) where the cow had been recumbent (just nearby the cow) was covered by polyvenyl plastic material (4 m×5 m) about the area of 20 m^2 and with the help of many assistances, the heifer was moved on the plastic. Additinally, tent was made over the recumbent cow from the same type of material with the same area coverage. So, by doing this the surgical procedure was conducted under aseptic nature.

SURGICAL MANAGEMENT

The surgical site was prepared aseptically on the left flank region after the cow was secured by tying legs front and back and placed on its right lateral recumbency. Then, using an inverted L-block approach, 30 ml of 2% lidocaine hydrochloride (2% Lignocaine 20 mg/ml, Scott-Edil pharmaceutical Cot. Ltd, India) was injected subcutaneously to generate a wall of analgesia encircling the operative field. Here, the vets do not want to give full dose of lignocaine beacause of the increased heart rate and decreased respiration rate. This may cause unwanted negative effect on compromised animal. A surgical blade was used to make a 45 cm long vertical incision on the skin. To reach the uterus, the dissection continued through subcutaneous tissues, all abdominal muscle layers, and the peritoneum. Subsequent to incision of uterus, the dead fetus was disconnected from the intact umbilical cord and taken from the womb (Figure 1A). The fetal membrane with the intact umbilical cord were left behind in the uterus. the The uterus was dusted with procaine penicillin G powder (Fortified Procaine Pencilline 400,000 I.U., CSPC Zhangnuo Pharmceutical (Shijiazhuang Co., Ltd, Hebei Province, China) and sutured in two layers using absorbable catgut size 2 (6 m) (Shandong Sinorgmed International Co., Ltd, China) in an inversion (Lambert) pattern. The peritoneum was sutured with absorbable catgut sutures in a basic continuous suture pattern (2-0). Interlock pattern with chromic catgut size (3-0) was used to seal the abdominal wall (Assault suture, Switzerland). Finally, using nylon size (2-0) the skin was closed using a simple interrupted suture procedure (Figure 1B). Antibiotics injection (Penstrip 400, a combination of Procaine penicillin G 200,000 IU and dihydrostreptomycin sulphate 200 mg at a dose of 1 ml/20 kgs (15 ml/300 kgs body weight, IM), Interchemie Werken, Holland product, Western Australia, Australia) for 5 consecutive days, oxytetracycline aerosol wound spray (oxy voicid 3.58%, provet, Ankara, Turkey) was sprayed on surgical wound of operated heifer once a day until complete healing, along with multivitamin (Introvit, dose; 10-15 ml for cattle, Interchemie Werken, Holland product, Western Australia, Australia) injection were all used as post-operative managements. After 5-hours of operation, the fetal membrane was expelled through birth canal spontaneously. The skin sutures were removed on the 20th day after the operation, the wound had healed and was quite active (Figure 1C). The heifer recovered completely without any post-operative difficulties and now looks like the one in the picture (Figure 1D). The owner was advised to closely monitor the cow and giving good nutrition and bedding (cleaning of cow pen and making comfortable) to facilitate wound healing. The day of operation was too cold. So, the owner was also advised to provide charcoal heat/wood fire in the cow pen so as to resist the cold weather.

Figure 1A. *Dead Fetus Taken Out from Womb*

Figure 1B. *Simple Interrupted Skin Suture*

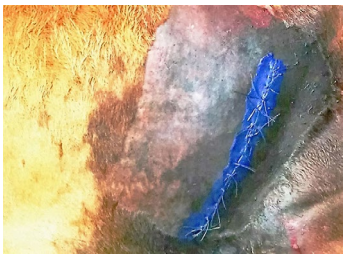
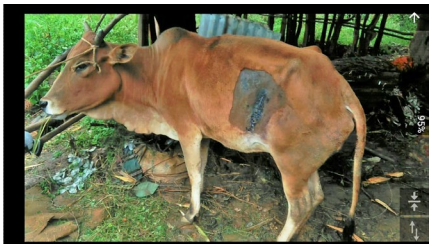
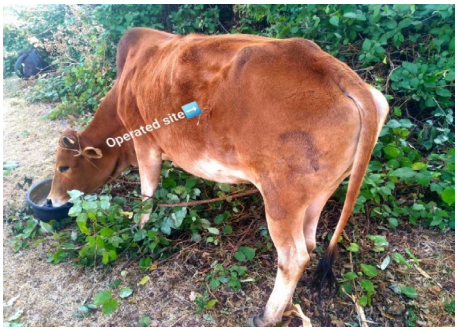


Figure 1C. *The Healed Wound on 20th Day*



Openventio PUBLISHERS

Figure 1D. *Completely Healed Heifer (2-Years-Old)*



DISCUSSION

Dystocia was caused in this case by a constricted pelvis as a result of the heifer's immaturity, which may have been avoided with adequate reproductive care. Heifers are more susceptible to dystocia than cows because they are smaller. The heifer was about two-years-old (its pelvic bone had not fully formed) and was housed with the rest of the cattle herd. This resulted in early mating, which made calving difficult. The current case report adhered to the following guidelines[11]: in that the size of heifers at breeding should attained the average size of mature cow weight, with a minimum of 60%. Otherwise, it is pre-disposed to calving difficulties. This case report was communicated with[12] Primiparous cows had a smaller pelvic area, lower live weight, and more difficulties calving, according to the study. Manual delivery was impossible due to the narrowness of the maternal bony pelvis (pelvic cavity). This case had a lot in common with[3] who reported that, if the birth canal is too narrow, it is advised to optics for C-Section. Thus, surgical management of the case was the last option. After aseptic preparation of the animal, local analgesic agent is deposited in the form of an inverted L to create a wall of analgesia enclosing the surgical field. This presentation was agreed with Hendrickson[13] in the usage of local analgesic. Accordingly, the case was managed surgically while the heifer was on right lateral recumbency. This case study was related with a report of Kolkman[14] and Masterson[15] who described that recumbent left paralumbar celiotomy approach in a recumbent animal is most suitable in case of cow that is not able to stand for the C-Section throughout the procedure. Following subcutaneous administration of analgesia, surgical intervention was made *via* left oblique paralumbar fossa. After removal of the fetus, fetal membranes were not loosed, so returned back into the uterus. The uterus was covered with procaine penicillin G and sutured in two layers using absorbable catgut size 2 in an inversion (Lambert) pattern. Abdominal wall was closed using interlock pattern with chromic catgut size (3-0) followed by subcutaneous suturing by chromic catgut (1-0) and Oxytetracycline wound spray smeared on. Finally, the skin was closed routinely. As described by Fesseha et al[16] for early recovery and positive outcomes of the surgical procedure, the wound should always be managed and regularly monitored for the fast healing process. Hence, post-operative care was followed-up. As result the animal was recovered completely on 20th days of operation. Here, the main goal of C-Section was to save the heifer only because the fetus was already died. In conclusion, mating of immature animals is one of a cause of dystocia. Surgical management (C-Section) is a live saving obstetric method if it is done early. So, it recommended that mating of immature animals should be avoided and every dystocia case should be presented to the veterinarian for early interventions.

CONCLUSION

A 2-year-old heifer with a full-term pregnancy was presented with dystocia in the current case report. The altered vital organs indicators were tachycardia and dyspnea, according to a field examination. However, the temperature of rectum was within the biological range. There was also a ruptured water bag, no straining, vulva edema, and abdominal distension. The case was identified as dystocia due to a constriction of birth canal linked to the dam's immaturity based on the history and vaginal inspection. After the failure of vaginal birth, C-Section was performed using normal techniques, with aseptic procedures and post-operative care being thoroughly monitored. As a result, the cow was fully recovered. The owner was advised not to mate or inseminate juvenile animals or those who have a pelvis that is either tiny or too narrow. Local attempts should be ignored and it is preferable to consult a veterinarian as soon as possible once it occurs.

ACKNOWLEDGEMENTS

The authors are grateful to Bedeno Woreda Agricultural and Livestock Resource Development Office, Directorate of Animal Health for providing us with computer access and overseeing the work, Bedeno Woreda Veterinary Clinic for providing us with surgical materials and a motorcycle for the success of the work, and our colleague Abdela Abrahim Jibro for arranging surgical materials and capturing all photographs used in this communication belong to the authors while they were performing the surgery, as well as the owner and his entire neighborhood for their help in handling the animal during the treatment.

ETHICAL APPROVAL

The study was carried out under the tight supervision of the Directorate of Animal Health of the Bedeno Agricultural and Livestock Development Office, which concluded that the case report was ethically done and met all animal ethics and welfare criteria. The ethical authorization letter was also delivered to the office for your confirmation.

CONSENT STATEMENT

The authors confirmed that the family member of the animal's owner was photographed in the case report. The involvement of the owner's family was limited to handling their animal, and their written and signed agreement was obtained. As a result, we declared that their participation has no bearing on the publication.

CONFLICTS OF INTEREST

It was confirmed that the authors have no potential competing interests in terms of authorship and/or publication of this case report.

REFERENCES

1. Regmi B, Gautam G. Management of dystocia in Bovine: A review. *The Blue Cross*. 2020; 16: 31-35.

2. Rathore AK, Kumar A, Agrawal JK, Satena A. Dystocia in a non-descript cow due to pelvic fracture and left hock flexion. *Res. J. Vet. Pract*. 2018; 6(1): 7-9. doi: 1017582/journal.rjvp/2018/6.1.7.9

3. Pruhit NG, Barolia Y, Shekhar C, Kumar P. Maternal dystocia in cows and buffaloes: A review. *Open Journal of Animal Sciences*. 2011; 1(2): 41-53. doi: 10.4236/ojas.2011.12006

4. Dutt R, Singh G, Gahalot SC, Sharma K, Yadav V, Patil SS. Successful management of a rare case of dystocia due to narrow pelvis associated with pelvic fracture in water buffalo. *Res. J. Vet. Pract*. 2017; 5(3): 34-36. doi: 10.7582/journal.rjvp/2017/5.3.34.36

5. Rahawy MA. Clinical dystocia in Iraqi buffaloes in Mosul City. *Adv Anim Vet Sci*. 2019; 7(8): 715-719. doi: 10.17582/journal.aavs/2019/7.8.715.719

6. Jensen D. *Causative Factors and Some Consequences of Dystocia in Two Year Old Heifers*. [master's thesis]. Logan, Utah: Utah State University; 1979; 2-4.

7. Weldeyohanes G, Fesseha H. Dystocia in domestic animals and its management. *Int. J. Phar. Biomed. Res*. 2020; 7(3): 1-11. doi: 10.18782/2394-3726.1089

8. Aro SO, Faluyi OB, Adebayo IA. Dystocia in a primigravida Heifer: A case report. *ARI*. 2016; 13(3): 2518-2522.

9. Kamalakar G, Devarathnam J, Sumiran N, Mahesh R, Prasad VD. Surgical management of dystocia due to narrow pelvis in a non-descript buffalo. *International Journal of Science, Environment and Technology*. 2016; 5(6): 3889-3892.

10. Ali AA, Fadhil AM, Aayed JK, Nuhaia AA. Caesarean section in ruminants referred to the aL-muthanna veterinary hospital. *Mirror of Research in Veterinary Sciences and Animals*. 2019; 8(2): 30-41. doi: 10.22428/mrvsa-2019-0082-04

11. Kalita MK, Acharya C, Chhetri B, Ahmed K, Bhuyan M. Successful manegment of a case of dystocia due to fetopelvicincompatabilityim heifer cow. *International Journal of Chemical Studies*. 2019; 7(2): 30-32.

12. Bureš D, Bartoň L, Zahrádková R, Teslík V, Fiedlerová M. Calving difficulty as related to body weights and measurements of cows and calves in a herd of Gascon breed. *Czech J. Anim. Sci*. 2008; 53(5): 187-194.

13. Hendrickson DA. *Techniques in Large Animal Surgery*. 3rd ed. Iowa, USA: Blackwell Publishing; 2007: 7-8.

14. Kolkman I. *Calving Problems and Calving ability in the Phenotypically Double Muscled Belgian Blue Breed*. [dissertation]. Ghent, Belgium; Ghent University; 2010; 41.

15. Masterson MA. Calving detours: Cesarean section. *AABP-PROCEEDINGS*. 2020; 53(1): 36-38.

16. Fesseha H, Negash G, Gebrekidan B. Caesarean operation in cow due to prolonged pregnancy. *Vet Med Open J*. 2020; 5(1): 9-13. doi: 10.17140/VMOJ-5-141

ISSN 2475-1286

VETERINARY MEDICINE
OpenJournal

Openventio PUBLISHERS

Retrospecitive Study

Effect of Breed and Risk Factors Affecting Conception Rate to Artificial Insemination in Dairy Cows of Tullo District Western Haraghe, Ethiopia

Abas M. Abdula, DVM[1*]; Ziyad M. Bilal, DVM[2]

[1]*College of Veterinary Medicine, Midega Woreda Veterinary Clinic, East Hararghe, Ethiopia*
[2]*Department of Veterinary Medicine, Bedano Woreda,Furda Veterinary Clinic, East Hararghe, Ethiopia*

[*]***Corresponding author***
Abas M. Abdula, DVM
College of Veterinary Medicine, Midega Woreda Veterinary Clinic, East Hararghe, Ethiopia; Tel. +251984116210; E-mail: abbasmohamed012@gmail.com

Article information
Received: March 29th, 2022; **Revised:** May 16th, 2022; **Accepted:** May 24th, 2022; **Published:** May 26th, 2022

Cite this article
Abdula AM, Bilal ZM. Effect of breed and risk factors affecting conception rate to artificial insemination in dairy cows of Tullo District Western Haraghe, Ethiopia. *Vet Med Open J.* 2022; 7(1): 16-21. doi: 10.17140/VMOJ-7-163

ABSTRACT

Aim
This study was conducted using the questionnaire method to assess the effect of breed and factors affecting conception rate on artificial insemination in dairy cows in Tullo district, Western Haraghe, Ethiopia.

Methods
A follow-up study design was conducted from December 2018 to June 2019 to determine the effect of breed and factors affecting conception rate to artificial insemination in dairy cows, taking breed, age, parity, body condition and timing of insemination as risk factors. The demographic factors were recorded by interviewing the owners. Most of the cows were examined for pregnancy diagnosis by rectal palpation of the genital tract at 60-80-days post-artificial insemination.

Results
Out of 114 artificially inseminated cows/heifers, 59 became pregnant, giving an overall first service conception rate of 51.8%. Although the breed, age, parity and body condition score did not affect the conception rate significantly, the pregnancy rate was better in the cross-breed (62.5%), in cows of 5-7-years of age (54.6%), in cows of parity 2-3 (59.2%) and in cows of good body condition score (57.7%). The conception rate in cows inseminated at 12-18-hours after the onset of estrus was significantly higher (62.3%) than those inseminated after 18-hours (31.2%) and before 12-hours (52.4%) after the onset of estrus. Therefore, cows with good body condition score (BCS) and artificial insemination (AI) service at 12-18-hours after the onset of estrus are the best choice of selection for obtaining the best result in the first service conception rate to AI in dairy cows/heifers.

Conclusion
This study reveals that the conception rate was influenced by the time of AI, so awareness should be given to cattle owners, as they should give AI for their cattle at optimum time within 12-18-hours after onset of heat sign.

Keywords
Artificial insemination, breed, conception rate, Tullo district.

INTRODUCTION

Animal production has an important role to play as food of animal origin representing approximately one-sixth of food energy and one-third of the human food protein on a global basis. In this ratio, the formidable role that milk and its products play in human nutrition has made scientists to play a pivotal role in developing technologies to improve the reproductive efficiency in dairy cattle, in turn to increasing the efficiency and profitability of milk production.[1] Among these technologies, artificial insemination has become one of the most important techniques ever devised for the genetic improvement of farm animals.[2] It is the manual placement of semen in the reproductive tract of the female by a method other than natural mating, and it is among the group of technologies commonly known as assisted reproduction technologies (ARTs), where by offspring are generated by facilitating the meeting of male and female gametes.[3] Semen collected from the bull is deep frozen and stored in a container with liquid nitrogen at a temperature of -196 °C made for use.[3]

Openventio PUBLISHERS

In Ethiopia, artificial insemination (AI) was introduced in 1938 in Asmara, then part of Ethiopia, which was interrupted due to the Second World War and restarted in 1952.[4] The National Artificial Insemination Centre (NAIC) was established in 1984 to coordinate the overall AI operation at the national level.[5]

The efficiency of AI service in the country, however, has remained at a very low-level due to the low accessibility of infrastructure, management (such as methods of husbandry, feeding, estrus detection, semen handling and transition cow management), and financial constraints, as well as poor heat detection, improper timing of insemination and death of embryos[6,7] Cattle breeding is mostly uncontrolled in Ethiopia, making genetic improvement difficult, and an appropriate bull selection criteria have not yet been established.[8] In the case of technical, financial and managerial problems.[9,10]

The total cattle population for the rural sedentary areas of Ethiopia is estimated at 55.03 million, of which 55.38% are females, of which 6,675,466 and 10,731,656 were dairy and milking cows, respectively.[11] With an average lactation length of 6-months and an average daily milk production of 1.67 litres per cow, the total milk produced during the year 2015/16 was recorded to be 3.06 billion liters.[12] This shows unsuccessful cross-breeding work. Therefore, Ethiopia needs to work hard on improving the work of productive and reproductive performance improvements of cattle through appropriate breeding and related activities.[12]

The conception rate was influenced by cattle rearing systems (intensive *vs.* extensive), the purpose of rearing cows, and poor heat detection rate to AI.[13] Furthermore, the parity, breed, body condition, time of insemination, and age of the cows inseminated were also found to affect the conception rate to AI.[14]

The effects of age, parity and breed of the cows on conception rate and number of services required per conception are poorly documented with respect to Ethiopia despite having a many dairy and draught farms. In the study area, the success rate of AI and factors affecting the conception rate have not yet been determined and documented. Therefore, the current study was undertaken with the following objectives: To estimate the conception rate to AI with frozen semen, and determine the effect of breed, age, parity, body condition scoring and time of insemination on conception rate.

MATERIALS AND METHODS

Study Area

The study was conducted in Tullo district, Oromia Regional State, Ethiopia, from December 2018 to June 2019. Tullo district is located in the West Hararghe zone of the Oromia regional state. The district has a daily mean temperature ranging from 18 °C to 26 °C and a mean annual rainfall ranging from 550 mm to 800 mm. The agro-ecological zones of the district are highland (dega) 40%, medium high land (Weyna dega) 57% and kola 3%. The district has an altitude ranging from 1500 meters to 2500 meters above sea level and the relative humidity ranges between 22 and 65%. The livestock populations of the district are 125,915 cattle, 37,973 goats, 13,177 sheep, 171,499 poultry, 5,905 donkeys, 338 horses and 274 mules.[15]

Study Population

The study population was represented by smallholder dairy owners who were beneficiaries the of artificial insemination service, and all animals were artificially served during the study period in Tullo district. In the rural areas, mainly local Zebu breeds are found grazing on communal land under a traditional extensive animal husbandry system, while cross breed cows (HF×Local Zebu, Jersey×Local Zebu) were housed and fed with a cut and carry system.

Study Design

A follow-up study design, was carried out to determine the rate of conception and the effect of breed and other major factors affecting the conception rate to artificial insemination in dairy cows from December 2018 to June 2019 in Tullo district, West Hararghe zone. A retrospective study was also used to collect data from AI service records from 2015 to 2018. In the follow-up study, 114 animals were followed at the Tullo District veterinary clinic and house hold level by telephone calls to AI technicians.

Study Methodology

Semi-structured questionnaires were prepared in a format predesigned to collect relevant data on the status of the animal, AI services and interviews with to owners of each cow/heifer receiving AI services during the study period and with AI technicians directly involved in the inseminations were made. Individual animal data, such as age, body condition, parity, breed, and time of AI from onset heat, were recorded, and the conception rate of inseminated animals was determined. The body condition score (BCS) of inseminated cows was determined by estimation according to the methods described by Nichonlson et al[16] and the ages of cows were determined by the statement of Cringoli et al.[17] The cows were inseminated between 6-24 h of onset of heat; history obtained from the owners. A standard semen handling and insemination procedure recommended by International Atomic Energy Agency (IAEA)[18] was used to inseminate the studded cows/heifers. All inseminated cows were checked for the presence or absence of oestrus signs in the next cycle post-AI by telephone call-up to the owners. Only first-service pregnancies were was included in this study. Most of the animals under this study were subjected to pregnancy diagnosis per rectum after 60-90-days post-AI, some by visiting the owner's house and some at the Hirna Veterinary Clinic, where animals are brought by the owners.

Data Analysis

The data collected using the questionnaire were checked for errors, incomplete and inconsistencies were corrected when possible and removed otherwise. After complete check-up, the data were coded and entered into Microsoft Excel and transported to STATA Version 12. Descriptive statistics such as frequency or percentage were used to summarize the data as needed. The chi-square (χ^2) test was computed to determine the association of risk factors with target

Openventio PUBLISHERS

Table 1. *Conception Rate to AI and Effect of Breed and other Factors on Success Rate*

	No. Inseminated	No. Conceived	Conception Rate (%)	χ^2	*p* value
Breed					
Local	90	44	48.9		
Cross breed	24	15	62.5	1.4058	0.236
Total	114	59	51.8		
Parity					
<2	46	21	45.6		
2-3	49	29	59.2	1.9155	0.384
>4	19	9	47.4		
Total	114	59	51.8		
Body Condition					
Poor	18	9	50		
Medium	70	35	50	0.4756	0.788
Good	26	15	57.7		
Total	114	59	51.8		
Age					
<4-years	41	21	51.2		
5-7-years	55	30	54.6	0.5615	0.755
>8-years	18	8	44.4		
Total	114	59	51.8		
Time of Insemination					
Before 12-hours	21	11	52.4		
12-18-hours	61	38	62.3	8.1058	0.017
After 18-hours	32	10	31.2		
Total	114	59	51.8		

variables of interest. The variation between variables was considered significant when the *p* value was less than 0.05.

RESULTS

Out of 114 artificially inseminated cows/heifers, 59 became pregnant, giving an overall first service conception rate of 51.8%, of which 48.9% were local cows and 62.5% were crossbred cows respectively with a non-significant difference. Out of a total of 21 cows/heifers, 11 (52.4%) were found to be pregnant when AI was performed during the period of less than 12-hours after the onset of estrus. A total of 61 animals, 23 (37.7%) were found to be non-pregnant when AI was performed during the period of 12-18-hours after the onset of estrus, and 38 (62.3%) were pregnant. Out of 32 cows/heifers, what showed signs of and inseminated at >18-hours from the onset of estrus, 10 (31.2%) were pregnant. Statistical association showed that there was a significant difference ($p<0.05$) between the time of insemination and the conception rate to AI. However, parity and BCS did not show a significant association with the conception rate to first service (Table 1).

Retrospective data obtained from an artificial insemination service recording book from year 2015-2018 showed an increasing rate of conception to AI. High numbers of conceived animals 82(47.1%) were recorded by the year 2018, and at the least 32 (27.4.4%) were recorded by 2015. There was statistically significant difference with good improvement from year-to-year (Tables 2 and 3).

Table 2. *Retrospective Data on Conception Rate to AI from 2015-2018 in Dairy Cows*

Year	No. Inseminated	No. Conceived	Conception Rate (%)	χ^2	*p* value
2015	117	32	27.4		
2016	132	40	30.3		
2017	144	63	43.8	17.0592	0.001
2018	174	82	47.1		
Total	567	217	38.3		

Table 3. *Retrospective Data on Differences in Conception Rate to AI from 2015-2018 Dairy Cows*

	No. Inseminated	No. Conceived	Conception Rate (%)	χ^2	*p* value
Breed					
Local	449	168	37.4		
Cross breed	118	49	41.5	17.0592	0.001
Total	567	217	38.3		

DISCUSSION

The present study was carried out to determine the conception rate to AI and the effect of factors such as animal breed, parity, age, body condition and time of insemination on the first service conception rate of cows/heifers in Tullo District.

Openventio PUBLISHERS

The overall first service conception rate in cows/heifers that received AI using frozen semen was 51.8%. This is in agreement with a previous study performed by Haque et al,[19] Shamsuddin et al,[13] Khan et al[14] and Mollah[20] who found pregnancy rates of 52.6%, 54.9%, 57.3% and 55.1% respectively, in cows which were lower than the findings reported by Shiferaw et al[21] and Jemal et al,[22] 86.4% and 63-71%, respectively. Factors that may cause differences in the conception rate may be the sexual health status of the female reproductive organs, proper maintenance of the liquid nitrogen level in the container and faulty technique of using frozen semen in AI practice.

The present finding showed that (48.9%) local breed cows/heifers were conceived, whereas (62.5%) cross breed cows/heifers were conceived. Although, the results of this study showed that the conception rates of crossbred cows were higher than those of locally bred cows, the breed and conception rates were not statistically significant (p>0.05). Some of the possible reasons for lower proportions of indigenous cows conceiving at first insemination are related to the difficulty of detecting estrus signs in Zebu cattle.

The present study demonstrates parity as a non-significant (p>0.05) influencing factor in the conception rate of cows/heifers. This is in agreement with a previous study performed by Haque et al.[19] In contrast to the present study, Belete et al[23] showed that there was a statistically significant (p<0.05) association between the conception rate and parity of cows. In the present study, more of the cows belonged to 2-3 parities, which may be considered the parity of grown cows. Although no significant variation in the conception rate of cows was obtained among different parity groups, obtaining a lower conception rate (45.6%) in 0-1 parity cows supports the earlier finding reported by Khan et al.[14]

The results of the present study revealed that the body condition of the animals did not significantly affect the conception rate. The finding of this study disagreed with Shamsuddin et al,[13] who reported a higher pregnancy rate in cows with good BCS than in cows with poor BCS. Nevertheless, the findings of this study agreed with those of Kaziboni et al,[24] who also noted no appreciable difference in conception rate among different body conditions of cows kept by smallholder farmers in Zimbabwe. Generally, cows with good BCS are more responsive to hormonal stimulation than their poor BCS counterparts resulting in a good pregnancy rate. However, the reason for obtaining no variation in conception rate between BCS in the present finding may be due to the small difference in body condition of cows/heifers included in this study.

The cows/heifers aged <4, 5-7 and >8-years had conception rates of 51.2%, 54.6% and 44.4% after the first service to AI, respectively.

The present study indicated that no significant variation in conception rate among different age group of cows. Agreement to the current finding, there is a report that fertility is the highest in cows between four and nine years of age and declines after 10-years of age.[25] Contradict to our results, Fonseca et al[26] reports, age of animals significantly affect conception rate, that the conception rate decrease as age of animals increase, the older cows might have more chance to get subclinical uterine infection resulting in lower conception rate.

The present study showed that the first service conception rate in cows/heifers is influenced by the time interval between oestrus and insemination, as indicated by a significantly higher conception rate (62.3%) in cows that received insemination at 12-18- hour intervals than in those that received insemination at <12-hours and >18-hours, which were 52.4 and 31.3% conception rates, respectively. This is in agreement with a the previous study performed by Gonzalez[27] which reported the highest conception rate when insemination was performed 12-18-hours after the onset of oestrus, which means that the afternoon inseminated in the following morning. As Dessalegn,[28] Tessema et al[29] reported, fertilization is highly dependent on the interval from insemination to ovulation. When cows are inseminated early, the aged sperm may not be capable of fertilizing the ovum. When insemination is late, fertilization and the formation of viable embryos may not be possible because of an aging egg. It tended to be higher in animals that were inseminated at the optimum time within 12-18-hours after the onset of the heat sign, which supports earlier findings reported by Gonzalez.[27]

Retrospective data obtained from the artificial insemination service recording book from 2015-2018 indicated that there is consistency in the number of animals inseminated and the conception rate. High numbers of inseminated animals (174) were recorded by 2018, and the lowest numbers (117) were recorded by 2015, with conception rates of 47.1 and 27.4%, respectively. In line with this finding, Sisay et al[30] reported that there was a consistent increase in the number of animals inseminated and calves born. This indicates that there is a consistent increment in farmers' awareness of the advantage of AI over natural service and that there might be an improvement in AI technicians, experience from year-to-year.

CONCLUSION AND RECOMMENDATIONS

The conception rate of cows/heifers to first service AI may not be affected by factors such as breed, parity, age, and body condition and may be affected by the time of insemination. According to the results of this study, the first service conception rate was not significantly influenced by breed, BCS, parity or age. However, the time of insemination had a profound impact on the conception rate of cows. The conception rate (CR) was found to be lower in animals that inseminated very early after onset of estrous and very late more than 18-hours of estrous. The highest CR was recorded within 12 to 18-hours. Therefore, to improve the CR, one should consider all factors related to the cow, and AI should be performed at the proper time of insemination after the onset of oestrus (12-18-hours) to increase the first service conception rate.

ACKNOWLEDGEMENTS

First of all, we praise Almighty Allah who helped us with uncountable blessing and favours in our life and gave us the opportunity

to complete this work. Finally, we would like to give our deepest gratitude to our families, our relatives and all of our friends.

REFERENCES

1. Kailasam M. Reproductive performance of dairy cows following different estrus-synchronization protocols. Universitat Autònoma de Barcelona; Bellaterra, Spain; 2003; 1-157.

2. Bearden HJ, Fuquary JW, Willard ST. Applied animal reproduction. 6th ed. NJ, USA: Pearson; 2004: 155-233.

3. Morrell JM. Artificial isemination, current and future trends. In: *Artificial Insemination in Farm Animals*. 1st ed. London, UK: Intechopen; 2011: 1-14.

4. Mengistu Z. A review on artificial insemination of cattle in Ethiopia. *Glob J Reprod Med.* 2019; 6(5): 118-125. doi: 10.19080/GJORM.2019.06.555700

5. GebreMedhin D. All in one: A Practical Guide To Dairy Farming. Agri-Service Ethiopia Printing Unit; Addis Ababa, Ethiopia; 2005; 15-21.

6. Shiferaw Y, Tenhagen BA, Bekana M, Kasa T. Reproductive performance of cross bred dairy cows in different production systems in the central highlands of Ethiopia. *Trop Anim Health Prod.* 2003; 25: 551-561. doi: 10.1023/a:1027377722576

7. Tesfaye A, Alemayehu L, Tefera Y, Endris A. Factors affecting the reproductive performance of smallholder dairy cows in two regions of Ethiopia. *Livestock Research for Rural Development*. 2015; 27: 32-41.

8. Tegegn A, Kassa T, Mukassa-Mugerwa E. Aspects of bull production with emphasis on cattle in Ethiopia. II. Sperm production capacity and semen characteristics. Paper presented at: The 3rd National Conference of Ethiopian Society of Animal Production. 1995; 83-99.

9. AzageT, Lahlou-Kassi A, Mukassa-Mugrwa E. Biotechnology in animal production. Development Opportunities in Livestock Agriculture. Paper presented at: The 2nd Annual Conference of the Ethiopian Society of Animal Production; Addis Ababa, Ethiopia; 1995; 49-80.

10. Woldu T, Giorgis YT, Haile A. Factors affecting conception rate in artificially inseminated cattle under farmer's condition in Ethiopia. *Journal of Cell and Animal Biology*. 2011; 5: 334-338. doi: 10.5897/JCAB11.067

11. Central Statistical Agency (CSA). Agricultural sample survey report on livestock and livestock characteristics (Private peasant holdings). Statistical Bulletin 570. Addis Ababa, Ethiopia; Federal Democratic Republic of Ethiopia; Volume II. 2013.

12. Central Statistical Agency (CSA). Agricultural sample survey report on livestock and livestock characteristics (Private Peasant Holdings). Statistical bulletin 583. Addis Ababa, Ethiopia; Federal Democratic Republic of Ethiopia; Volume II. 2016.

13. Shamsuddin M, Bhuiyan MMU, Sikder TK, Sugulle AH, Alam MGS, Galloway D. Constraints limiting the efficiency of artificial insemination of cattle in Bangladesh. Paper presented at: Final Research Co-ordination Meeting on Radioimmunoassay and Related Techniques to Improve Artificial Insemination Programs for Cattle Reared under Tropical and Sub-tropical Conditions; Uppsala, Sweden; 2001; 9-27.

14. Khan MRK, Uddin J, Gofur MR. Effects of age, parity and breed of the cows on conception rate and number of services per conception in artificially inseminated cows in Bangladesh. *Bangladesh Livestock Journal*. 2015; 1: 1-4.

15. Agricultural and Rural Development Office (ARDO). Agricultural and Rural Development Office of Tullo district. 2016.

16. Nichonlson MJ, Butterworth MH. A guide to condition scoring of Zebu Cattle. International Livestock Center for Africa; Addis Ababa, Ethiopia; 1989; 34-45.

17. Cringoli G, Rinaldi V, Venezino G, Capelli J, Malona B. Across sectional coprological survey from an area of Southern Italian Appennines. *Vet Parasitol.* 2002; 108: 137-143. doi: 10.1016/s03044017(02)00183-8

18. International Atomic Energy Agency (IAEA). Improving Artificial breeding of cattle in Africa. Guidelines and recommendations. A manual prepared under the framework of an IAEA Technical Cooperation Regional AFRA Project on Increasing and Improving Milk and Meat Production. technical support from the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. 2005.

19. Haque M, Gofur K, Saduzzaman A, Bhuiyan M. Factors limiting the pregnancy rates in artificially inseminated cows in Bangladesh. *International Journal of Dairy Science*. 2015; 10: 278-287. doi: 10.3923/ijds.2015.278.287

20. Mollah MFK. *Post AI Conception Rate of Zebu Cows at Shaghata, Gaibandha*. [master's thesis]. Mymensingh, Bangladesh: Bangladesh Agricultural University; 2011.

21. Shiferaw T, Shibiru T, Cherinet M. Experience on field AI management in Ethiopia. *Ethiopian Society of Animal Production*. 2002; 5: 323-335.

22. Jemal H, Lemma T, Bekana M. Assessment of the reproductive performance of dairy cows in smallholder dairy farms using artificial insemination. *Livestock Research for Rural Development*. 2016; 28: 123-126.

23. Belete Y, Abraham J, Abebe A, Atilaw W, Alemayehu H. Factors affecting the efficiency of artificial insemination in dairy cows in

and around bishoftu (debre zeite), Oromia regional state, Ethiopia. *Journal of Reproduction and Infertility*. 2018; 9(2): 28-35. doi: 10.5829/idosi.jri.2018.28.35

24. Kaziboni S, Kusina NT, Sibanda S, Makuza S, Nyoni O, Bhebhe E. Performance of artificial insemination in smallholder dairies of Nharira-Lancashire in Zimbabwe. *Livestock Research for Rural Development*. 2004; 16(4): 51-59.

25. Schilling PE, England NC. Some factors affecting reproduction in beef cattle. *J Anim Sci*. 1968; 27: 1363-1367. doi: 10.2527/jas1968.2751363x

26. Fonseca FA, Britt JH, McDaniel BT, Wilk JC, Rakes AH. Reproductive traits of holsteins and jerseys. Effects of age, milk yield and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate and days open. *J Dairy Sci*. 1983; 66: 1128-1147. doi: 10.3168/jds.S0022-0302(83)81910-9

27. Gonzalez SC. Factors affecting fertility at first service in crossbred cows. *World Rev. Animal Production*. 1981; 16: 123-124. doi: 10.1371/journal.pone.0264029

28. Dessalegn G. Assessment of problems associated with artificial insemination service in Ethiopia. [master's thesis]. Addis Ababa, Ethiopia; Addis Ababa University; 2008.

29. Tessema R, Atnaf A. Assessment of problems associated with artificial insemination services in and around Gondar Town, North West Ethiopia. *Journal of Reproduction and Infertility*. 2015; 6: 22-26.

30. Sisay W, Tamene D, Worku G, Kidanu D, Getahun B, Nuraddis I. Evaluation of artificial insemination efficiency in and around Ejere District, Western Shoa Zone, Ethiopia. *Journal of Reproduction and Infertility*. 2017; 8: 66-71. doi: 10.5829/idosi.jri.2017.66.71

ISSN 2475-1286

VETERINARY MEDICINE

OpenJournal

Openventio PUBLISHERS

Review

Review of Diagnostic and Vaccination Approaches of Infectious Bursal Disease of Poultry

Guta W. Adino, MSc[1*]; Morka D. Bayu, BSc, MSc[2]

[1]*Department of Inactivated Bacterial Vaccine Quality Control, National Veterinary Institute, Bishoftu, Ethiopia*
[2]*Department of Veterinary Laboratory Technology, Ambo University, Ambo, Ethiopia*

[*]***Corresponding author***
Guta W. Adino, MSc
Senior Veterinary Vaccine Quality Control Officer, Department of Inactivated Bacterial Vaccine Quality Control, National Veterinary Institute, Bishoftu, Ethiopia; E-mail: gutawakjira@gmail.com; waksifguta@gmail.com

Article information
Received: April 12th, 2022; **Revised:** April 26th, 2022; **Accepted:** April 29th, 2022; **Published:** June 4th, 2022

Cite this article
Adino GW, Baye MD. Review of diagnostic and vaccination approaches of infectious bursal disease of poultry. *Vet Med Open J*. 2022; 7(1): 22-28.
doi: 10.17140/VMOJ-7-164

ABSTRACT

Infectious bursal disease, also known as Gumboro disease, is a highly contagious and acute viral disease of poultry characterised by the destruction of lymphoid cells. Diagnosis of infectious bursal disease involves consideration of the flocks' history, clinical signs, and lesions. The objectives of this paper are to highlight various commonly used diagnostic methods for infectious bursal disease and to review advances made in diagnostic methods and vaccination strategies for infectious bursal disease, with special emphasis on the strengths and weaknesses of each of those techniques. Isolation of infectious bursal disease virus followed by its serological assay and histopathological examination of the bursa is regarded as the gold standard method of infectious bursal disease diagnosis. Serological tests such as agar gel, immune diffusion, enzyme-linked immuno sorbent assay, and viral neutralisation tests are commonly used laboratory assays in diagnosing infectious bursal disease viruses. Recently, the most accurate and relatively fast diagnostic method, the molecular technique, is widely used. The molecular diagnostic technique is the simplest and most sensitive of the diagnostic techniques reviewed. The virus causes immunosuppression, so the infected chicken recovers from the acute disease but becomes more susceptible to infections by other pathogens. Therefore, prevention is important and vaccination has become the principal control measure of infectious bursal disease virus infection in chickens. Conventional attenuated live and killed vaccines are the most commonly used vaccines. With the advancement of knowledge and technology, new generation or genetically-engineered vaccines like deoxyribonucleic acid and subunit vaccines have been used. Various vaccination strategies, such as *in ovo*, at hatch, and post hatch vaccination, are used. Hatchery vaccination is becoming a common practice. Based on this review paper, more affordable and effective infectious bursal disease vaccines that are affordable and readily available must be identified with further cost-benefit analysis.

Keywords
Infectious bursal disease; Infectious bursal disease virus; Diagnosis; Vaccine; Vaccination.

INTRODUCTION

Infectious bursal disease (IBD), also known as Gumboro disease, has been a great concern for the poultry industry worldwide. It was first reported in broiler flocks in the area of Gumboro, Delaware in 1957.[1]

Infectious bursal disease is caused by the infectious bursal disease virus (IBDV), which is an acute and very contagious disease that affects growing chickens between the ages of 3 to 6 weeks.[2] It is caused by a virus that is a member of the genus Avibirnavirus of the family Birnaviridae[3] which is characterised by the destruction of lymphocytes in the bursa of fabricius.[4] It is a non-enveloped, double-stranded ribonucleic acid (RNA) and bi-segmented virus, that is, segment A and B.[5] There are two serotypes of IBDV, namely serotypes 1 and 2. In chickens, serotype 1 is pathogenic and consists of three viral strains: classical (ca), very virulent (vv), and variant (va) IBDV. In chickens, serotype 2 is non-pathogenic). There are two serotypes of IBDV, namely serotypes 1 and 2. In chickens, serotype 1 is pathogenic and consists of three viral strains: classical (ca), very virulent (vv), and variant (va) IBDV. In chickens, serotype 2 is not pathogenic.[1]

Infectious bursal disease is a commonly encountered lymphocytolytic disease that adversely affects the defence mechanism of birds and results in immunosuppression and a failure to develop satisfactory immunity.[6]

Infectious bursal disease virus infections, clinical signs, organ lesions, and immuno-suppression correlate with the status of immunity, age, and genetic background of affected chickens and with the virulence of the infecting virus strain. After an incubation period of 2-3-days, young chickens show symptoms of ruffled feathers, watery diarrhea, trembling, severe prostration, severe depression, vent picking, the presence of urate stains on the vent, dehydration, loss of appetite, and elevated water consumption, and death may follow 1-3-days later. Mortality will peak and recede, usually in a period of 5-7-days.[7]

In most cases, a preliminary diagnosis can be made based on flock history, clinical signs, and post-mortem (necropsy) examinations. A necropsy will typically reveal changes in the Fabricius bursa, such as swelling, oedema, haemorrhage, the presence of a jelly serosa transudate, and eventually bursal atrophy.[8]

Various diagnostic methods, such as the virus neutralisation test (VNT), enzyme-linked immunosorbent assay (ELISA), and agar gel immunodiffusion test (AGIDT), are used infrequently to detect IBDV, whereas molecular techniques, such as reverse transcriptase-polymerase chain reaction (RT-PCR), are frequently used to detect viruses from field samples.[9] Laboratory confirmation was achieved by virus isolation followed by its serological assay and histopathological examination of the affected bursa.[10] Isolation of the viruses is laborious, nonspecific, and time-consuming. The more frequently used molecular method is the reverse transcription polymerase chain reaction (RT-PCR).[11]

The main effective way to control IBD is vaccination, and different vaccination programmes are regularly implemented globally, including in Africa.[12] Vaccination has become the principal control measure of IBDV infection in chickens since the virus is resistant to different physical and chemical methods of decontamination.[13] Vaccines and vaccination programmes vary widely depending on several local factors (e.g. type of production, level of biosecurity, the local pattern of disease, the status of maternally derived antibodies, vaccines available, costs and potential losses).[6]

The objectives of this paper are:

• To highlight various commonly used diagnostic methods for infectious bursal disease.
• To review advances made in diagnostic methods and vaccination strategies for IBDV, with special emphasis on the strengths and weaknesses of each of those techniques.

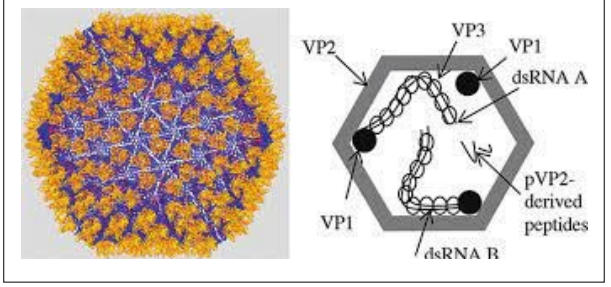
INFECTIOUS BURSAL DISEASE

Infectious bursal disease is an acute and highly contagious viral infection of immature chickens. IBD is characterised by the destruction of lymphocytes in the bursa of Fabricius and, to a lesser extent, in other lymphoid organs. Infectious bursal disease virus is the cause of infectious bursal disease, also known as "Gumboro-disease".[14,15]

Etiology

Infectious bursal disease virus is a double strand RNA virus (dsRNA) and a non-enveloped, icosahedral capsid with a bi-segmented genome.[16] The larger segment, A, is 3261 nucleotides long and contains two open reading frames (ORFs) that encode four viral proteins known as VP2, VP3, VP4, and VP5. The smaller segment B encodes only VP1, which has polymerase activity. The two viral proteins, VP2 and VP3, are structural proteins that make up the viral capsid. The epitopes responsible for the induction of neutralising and protective antibodies are found in the VP2 protein (Figure 1).[17]

Figure 1. *Structure of Infectious Bursal Disease Virus Particles*



Mutations in the IBDV genome have impacted antibody recognition and led to variations in the antigenicity, immunogenicity, virulence, and tropism of circulating infectious bursal disease virus strains.[18]

General Characteristics of the Infectious Bursal Disease Virus

Two serotypes of infectious bursal disease, serotypes one and two, have been recognised as having considerable antigenic variation within each serotype.[19] It is a naked virus, devoid of an envelope, known for its resistance to physical and chemical agents and to potential of hydrogen (pH) conditions of 2-11, but it is inactivated at pH 12. Due to this ability of stability and hardiness, it persists in poultry premises even after thorough cleaning and disinfection for up to 4-weeks in the bone marrow of infected chickens. The virus has been shown to remain infectious for 122-days in a chicken house and for 52 days in feed, water, and faeces.[20]

Pathogenesis of Infectious Bursal Disease

Following host entry *via* oral ingestion or inhalation, IBDV may bind to host cell proteins such as N-glycosylated polypeptide (s) expressed on the cell membrane of immature IgM+ B-cells during the viral entry process. It is transported by infected macrophages to the bursa of Fabriciaus, where the virus undergoes intracytoplasmic replication in IgM+ B lymphocytes.[21] Due to its short incubation periods, which range from 2 to 3-days, a pore-forming peptide of the virus (pep46), which is associated with the outer capsid of the IBDV particle, may facilitate viral entry into the cytoplasm of infected cells.[22]

Mature and competent lymphocytes will expand as a result of stimulation by the virus, whereas immature lymphocytes will be destroyed. The bursa is infiltrated by heterophils and undergoes hyperplasia of the reticulo-endothelial cells and of the interfollicular tissue.[23]

Openventio PUBLISHERS

Diagnosis

Diagnostics of infectious bursal disease involves consideration of the flocks' history and of the clinical signs and lesions. Clinical manifestations and post-mortem findings of affected birds may aid in the diagnosis of IBD disease, but laboratory diagnosis is necessary for its confirmation.[24] Chickens less than 3-weeks of age present no clinical signs of disease, but chickens greater than 3-weeks of age present clinical signs.[25]

Gross and histopathological examinations of the bursa are used to diagnose IBD in young chickens or in those having maternal antibodies.[20] However, other methods used in diagnosis include isolation and detection of IBDV using embryonated chicken eggs, cell culture, RT-PCR and serology, such as virus neutralisation, indirect ELISA, and agar gel immune diffusion test.[26]

Virus isolation: IBDV can be isolated (grown) in chicken embryos and primary cell cultures, especially chicken fibroblast cells. Isolation and identification of the agent provide the most certain diagnosis of IBD, but they are not usually attempted for routine diagnostic purposes as the virus may prove difficult to isolate.[10]

(a) Isolation of virus in embryos: The most sensitive diagnostic method for virus isolation is the inoculation of bursal homogenates from IBDV-infected chickens into the chorioallantoic membrane of 9-10-days old embryonated specific-pathogen-free (SPF) chicken eggs. The most sensitive route of inoculation is the chorio allantoic membrane; the yolk sac route is also practicable.[27] It is especially important for wild-type IBDV, which usually does not replicate in conventional cell culture, can also be regenerated by the reverse genetics approach, but can grow in embryonated chicken eggs.[26] Some strains grow well in embryos but are not readily adapted to grow in chicken embryo fibroblasts or chicken embryo kidney. Variant viruses, however, do not kill the embryos but cause embryo stunting, discoloration, splenomegaly, and hepatic necrosis.[20]

(b) Isolation of virus in cell culture: In about 3-5-days, IBDV grows in chicken embryo fibroblasts and produces cytopathogenic effect (CPE) characterised by the appearance of round retractile cells.[21] Because the virus is difficult to culture, IBDV isolation in cell culture is not commonly used as a diagnostic test. In cell cultures, some field strains did not grow at all.[28] In tissue culture, wild-type infectious bursal disease virus strains, particularly the most virulent strain, do not grow. A comparison of the genome sequences of wild-type and tissue culture adapted IBDV strains revealed several mutations that may be responsible for IBDV invitro growth in tissue culture.[29]

Serological characterization: Typically, blood can be collected from the wing vein, allowed to clot, and serum separated by centrifugation and stored at -20 °C until tested. Agar gel immuno diffusion (AGID) test, ELISA, and viral neutralisation (VN) test are the most commonly used serological tests for detecting IBDV.[30]

(a) Agar gel immunodiffusion test: The AGID test is the most effective serological test for detecting specific antibodies in serum or viral antigens in bursal tissue.[23] The test is specific because it cannot produce false positives but can produce false negatives. AGID can detect the presence of IBDV antigen in bursal tissue for 5-6-days after infection.[31]

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, 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.[3]

(b) Virus neutralization tests: Viral neutralisation tests are carried out in cell culture. The test is more time-consuming and costly than the AGID test, but it is more sensitive for detecting antibody.[23] This sensitivity is not required for routine diagnostic purposes, but may be useful for evaluating vaccine responses or differentiating between IBDV 1 and 2 serotypes. To reduce test-to-test and operator-to-operator variation, a standard reference antiserum may be included with each batch of tests, and the titer of the virus suspension must be reassessed in each new experiment using a sufficient number of repeats (wells) per virus dilution.[3]

(c) Enzyme-linked immunosorbent assay: The ELISA is the most commonly used test for the detection and quantification of IBDV antibodies to check for response to vaccination, natural field exposure, and decay of maternal antibody titer. It is economical, simple, and quick to test a large number of samples at the same time and is adaptive to automation by computer software.[20]

The test sera are diluted according to the established protocol or kit instructions, and each is dispensed into the requisite number of wells. After incubation under the appropriate conditions, the serum is discarded from the plates, and the wells are washed thoroughly. Wells are dispensed with anti-chicken immunoglobulins conjugated to an enzyme and the plates are again incubated as appropriate. The plates are emptied and rewashed before a substrate containing a chromogen that gives a colour change in the presence of the enzyme used is added to the plate. After a final incubation step, the substrate/chromogen reaction is stopped by the addition of a suitable stopping solution, and the colour reactions are quantified by measuring the optical density of each well. The sample to positive (S/P) ratio for each test sample is calculated.[3]

Identification by molecular method: Molecular detection and characterisation, involving sequencing and phenotypic and genotypic analyses, have been utilised in the diagnosis of IBD. This method can detect the genome of IBDV, which is unable to grow in cell culture or embryonated eggs because it is unnecessary to grow the virus before amplification, even when the virus is present in a very minute quantity and has lost its infectivity.[32] The classical methods for molecular characterization and differentiation of IBDV field isolates include RT-PCR, restriction fragment length

polymorphism (RFLP), nucleotide sequence analysis, and quantitative real-time RT-PCR (qRT-PCR).[3]

Reverse transcriptase polymerase chain reaction offers a rapid, highly sensitive and specific test for the confirmative diagnosis of the disease which would help in controlling the disease, thereby reducing the economic losses significantly. RT-PCR in combination with restriction enzyme analysis allows the rapid identification of IBDV. Nucleotide sequencing of RT-PCR products is widely used for further characterization of IBDV strains.[18]

The *VP2* gene of IBDV contains a variable region, which suggests the potential of this region for differentiation of IBDV strains. RT-PCR followed by digestion with multiple restriction enzymes or RFLP and nucleotide sequencing of the *VP2* gene have been used for the differentiation of IBDV strains. The molecular differentiation of IBDV strains using VP2 has been improved by the use of labelled probes in real-time RT-PCR.[33]

Post-mortem findings: Pathological change observed at the bursa of Fabricius is characteristic and histopathological investigations combined with the demonstration of viral antigen by immunohistochemistry confirm an IBDV infection.[34] Diagnostic lesions include muscle haemorrhages and bursal enlargement. Pathognomonic gross lesions are observed in the bursa of Fabricius, which show doubling in size with a yellowish gelatinous film that may surround it and sometimes haemorrhages may be seen on the surface of it (Figure 2).[1]

Figure 2. *Gross Lesions Observed in the IBDv Affected Bursa of Broiler Chicken*

Histopathology examination: The lymphoid structures primarily affected by IBDV are: BF, spleen, thymus, Harderian glands, caecal tonsils, gut-associated lymphoid tissue (GALT) and head-associated lymphoid tissues (HALT). Lymphocytic degeneration and necrosis in the medullary region of the BF at 1-day post-infection are the first signs.[6]

Microscopic examination of tissues shows moderate haemorrhages in the muscles and kidneys, and the spleen shows moderate lymphoid depletion in the lymphoid nodules. There is marked interfollicular oedema and depletion of 13 lymphocytes from the lymphoid nodules in the BFs. Other lymphoid nodules of the BF show degeneration and necrosis of lymphocytes and cystic cavitations with heterophil infiltrates.[5]

Vaccine and Vaccination against IBD

Vaccination of chickens with high quality vaccines is the primary method of control of many poultry infectious diseases, including IBD (Gumboro) disease.[35]

With a proper vaccination schedule, it is possible to protect chickens. Many studies have identified rational vaccination schedules and strict biosecurity measures as critical tools for IBD management.[36] Despite the fact that different types of IBD vaccines are being developed, two of them are commonly used for IBD control. These are either live attenuated or inactivated oil-emulsion adjuvanted vaccines.[8] Currently, plant-based vaccines are available, and a live recombinant vaccine expressing IBDV antigens has also been approved.[17]

Live-attenuated vaccines: Live viral vaccines can activate the target host's immune system. They have the ability to replicate and induce both cellular and humoral immunity. They do not require an adjuvant to be effective and can be given to chickens in large quantities, but they may have unfavourable side effects. Horizontal and vertical transmission (though not in the case of IBD vaccines), reversion to virulence, and vaccine reactions that may result in disease or production loss are examples of these. In general, the live IBDV vaccines used in the poultry industry have been attenuated through serial passage in tissue culture, eggs, or embryo-derived tissues, with the goal of maintaining the immune response induced by the parent virus while attenuating the vaccine virus's ability to cause disease.[37]

Inactivated vaccines: Inactivated IBD vaccines are mostly formulated as water-in-oil emulsions, usually combining several antigens, and have to be injected into each bird. It has been observed that inactivated IBD vaccines are able to induce IBDV-specific T-cell and inflammatory responses in chickens. It has been reported that inactivated IBD vaccines must have either a high or an optimised antigenic content in order to induce in breeders an immunity that helps protect the progeny from infection by variant IBDV strains.[15]

Killed-virus vaccines with an oil adjuvant are frequently used to increase maternal antibody levels and confer longer-lasting immunity in breeder hens. The concentration and antigenic specificity of the vaccine strain may influence the duration and uniformity of this immunity. Because these vaccines are not ideal for inducing a primary antibody response, they are most effective in chicks that have been "primed" with a live virus vaccine or have been naturally infected through field exposure to IBDV.[5] Many oil adjuvant vaccines now include both classic and variant IBDV strains. Killed-virus vaccines are administered by subcutaneous or intramuscular injection between the ages of sixteen and twenty-weeks.[12]

New generation or genetically-engineered IBD vaccines: Genetically engineered IBD vaccines have also been developed as a result of improved understanding of the molecular structure and immunology of IBDV. The viral capsid protein VP2, encoded by genomic segment A and derived from a large precursor protein VP0 by a series of proteolytic processes, carries immune determinants that control antibody-dependent neutralisation and protection. In general, these could be divided into two categories based on their replicative nature upon delivery into the chicken.[38]

(a) Non-replicative IBD vaccines: Immunisation with deoxyribonucleic acid (DNA) or subunit vaccines involves the use of non-replicating IBDV for the induction of an immune response in birds. DNA vaccination is based on direct inoculation of plasmid DNA encoding a target immunogen gene into subjects of study.[39] Under the influence of a mammalian promoter, the target genes were expressed to produce proteins *in vivo* that are able to induce immune responses in the injected host. Repeated injections of DNA vaccines carrying the IBDV genes, either the polyprotein genes or the gene of *VP2* alone, were shown to protect the chickens from challenge virus.[40]

However, the presence of maternally derived antibody (MDA) could affect the efficacy of DNA vaccines, and a high dose of DNA vaccines was required to overcome the interference of MDA and induce an immune response in chickens. It was shown that a booster vaccination with inactivated IBD vaccine after priming with DNA vaccine provided better and higher protection to the chickens compared to injection with DNA vaccines alone.[41]

(b) Replication-competent IBD vaccines: Replication-competent viral vectors have been utilised to express and deliver immunogens of interest to chickens. In contrast to DNA and subunit vaccines, vaccination by live recombinant virus vectors employs the use of live and replicating viruses to produce IBDV antigen upon *in vivo* infection. They have been shown to elicit both humoral and cell-mediated immune responses in chickens. As they could persistently infect the chickens, the potential for having a long-term protective immunity is high.[42]

Besides, the recombinant viral vectors are less sensitive to MDA and could therefore evade neutralisation by the maternal anti-IBDV antibody.[12] Several viruses have been engineered to express the VP2 protein of IBDV. This includes fowlpox virus, fowl adenovirus, Marek's disease virus, Newcastle disease virus, and avian adeno-associated virus, among others.[42] The VP2 protein expressed *in vivo* from these various studies has been shown to confer from partial to full protection to vaccinated chickens from mortality, although they do not prevent the damage to the bursa.[43]

Plant-produced IBD vaccines: The plant-based expression system is becoming a more popular alternative platform for animal vaccine production and development.[44] Because VP2 capsid protein is one of the most important pathogenic agents in poultry, a plant-based expression system using the stable,[45] transient,[46] or chimeric viral particles[47] approach was used to create an IBD vaccine containing it. Transgenic rice expressing the VP2 protein was shown to protect the chickens from challenge following oral immunisation.[16]

Recently, the VP2 protein of IBDV has been transiently expressed in Nicotiana benthamiana leaves and extracted for subunit vaccination in chicken.[46] The recombinant VP2 protein emulsified in oil adjuvant, injected intramuscularly to chicks at 18-days of age and followed by booster doses after 22 and 35-days, was shown to induce the production of anti-IBDV antibodies with neutralising ability.[47]

***In ovo* vaccination and post-hatch vaccination:** *In ovo* vaccination and post-hatching vaccination technology have recently been developed to deliver live vaccine into eggs during the incubation period. After 18-days of incubation, the complex of live vaccine viruses and IBD antibodies is injected *in ovo*. When the chicks are about 7-days old, the vaccine virus is released and the eggs hatch. The problem of maternally derived IBD antibodies is thus solved, and the chicks are effectively immunized.[27] Compared to post-hatch vaccination, *in ovo* injection of a live intermediate vaccine allowed faster recovery from bursa lesions, although both methods exhibited similar protection against challenge.[48] Although *in ovo* vaccine delivery is an appealing alternative to post-hatch vaccination, several factors, including dosage, virulence, and efficacy, must be properly optimised before pursuing large-scale vaccinations.[49]

CONCLUSION AND RECOMMENDATIONS

Infectious bursal disease is caused by the IBD virus that affects the immune cells of chickens. It is mainly a disease of young chickens between 3-6-weeks old and causes secondary problems due to the effect of the virus on the bursa of Fabricius. Diagnosis of IBD depends on clinical signs, differential diagnosis, gross lesions, histopathological lesions, virus isolation, serological and molecular diagnosis. Isolation and identification of the agent can deliver the most confident diagnosis of infectious bursal disease.

From the recommended serological tests for IBD virus, AGID is the simplest but least sensitive, whereas ELISA is a rapid and sensitive method, but it cannot differentiate serotypes. The virus neutralisation test is the gold standard and the only serologic test that differentiates antibodies of two serotypes and is sensitive, but it is more laborious and expensive than the AGID. Molecular Identification Reverse Transcription-Polymerase Chain Reaction is used to detect IBDV without considering the viability of the virus by working on VP2 found in segment A of the viral capsid.

Vaccination is the principal control measure of IBDV infection in chickens. Of the available vaccines, the live vaccine is the most protective and widely used IBD vaccine. Vaccination strategies *in ovo*, at-hatch or on-farm vaccinations, determine the choice of vaccines used on the farm. Therefore, based on the above conclusion, the following recommendations are forwarded:
The virus neutralisation test is the most sensitive, but it is laborious and time-consuming.

- The reverse transcriptase polymerase chain reaction has been the most simple and sensitive molecular diagnostic technique.
- Further cost-benefit analysis must be conducted on more safe and effective IBD vaccines that are affordable and readily available.
- Ivo vaccination will be the best vaccination strategy against infectious bursal disease.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Liew PS, Mat Isa N, Omar AR, Hair-Bejo M. Vaccines and vac-

cination against infectious bursal disease of chickens: Prospects and challenges. *Pertanika Journal of Scholarly Research Reviews*. 2016; 2(2): 23-39.

2. Saidi R, Shirima G, Buza J, Kasanga C. Factors associated with infectious bursal disease vaccination failure in Dar es salaam, Tanzania. *Journal of Veterinary Medicine and Animal Health*. 2020; 12(3): 99-109. doi: 10.5897/JVMAH2020.0834

3. Office International des Epizootics (OIE). Infectious bursal disease (Gumboro disease). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 4th ed. Paris (in press): OIE; 2008.

4. Mutinda WU. *Isolation, Pathological and Immunological Characterization of Kenyan Infectious Bursal Disease Virus Strains for Vaccine Development*. [dissertation]. Nairobi, Kenya: University of Nairobi; 2016.

5. Eterradossi N, Saif YM. Infectious bursal disease. In: *Diseases of Poultry*. Ames, Iowa: Blackwell Publishers; 2008: 185-208.

6. Getachew Y, Fesseha H. Infectious bursal disease in poultry production. *Global Veterinaria*. 2020; 4: 185-196. doi: 10.5829/idosi.gv.2020.185.196

7. Zahid B, Aslam A, Tipu Y, Yaqub T, Butt T . Conventional and molecular detection of infectious bursal disease virus in broiler chicken. *Pakistan J. Zool.* 2016; 48(2): 601-603.

8. Aregitu M. Review on infectious Bursal disease: Threat for Ethiopian Poultry industry. *International Journal of Advanced Life Sciences*. 2018; 11: 52-55. doi: 10.26627/IJALS/2018/11.01.0047

9. Mathivanan K, Kumanan K, Nainar AM. Characterization of a newcastle disease virus isolated from apparently normal Guinea Fowl (Numida melagridis). *Vet Res Commun*. 2004; 28: 171-177. doi: 10.1023/B:VERC.0000012116.60726.9b

10. Yousif MGO. *Evaluation of Various Techniques Used for the Diagnosis of Infectious Bursal Disease and its Prevalence in Khartoum State*. [master's thesis]. Khartoum, Sudan: University of Khartoum; 2005; 99. doi: 10.1008/s11250-012-0280-6

11. Wu CC, Lin TL, Zhang HG, Davis VS, Boyle JA. Molecular detection of infectious bursal disease virus by polymerase chain reaction. *Avian Dis.* 1992; 36(2): 221-226.

12. Van den Berg TP. Acute infectious bursal disease in poultry: A review. *Avian Pathology*. 2000; 29(3): 175-194. doi: 10.1080/03079450050045431

13. Liew S, Kraemer C, Vanmarcke J, Montiel E. Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. *Avian Dis*. 2015; 49: 231-237. doi: 10.1637/7310-112204R

14. Mahgoub H, Bailey M, Kaiser P. An overview of infectious Bursal disease. *Arch Virol*. 2012; 157(11): 2047-2057. doi: 10.1007/s00705-012-1377-9

15. Muller H, Mundt E, Eterradossi N, Islam M. Current status of vaccines against infectious bursal disease. *Avian Pathol*. 2012; 41: 133-139. doi: 10.1080/03079457.2012.661403

16. Wu J, Yu L, Li L, Hu J, Zhou J, Zhou X. Oral immunization with transgenic rice seeds expressing VP2 protein of infectious bursal disease virus induces protective immune responses in chickens. *Plant Biotechnol J*. 2007; 5(5): 570-578. doi: 10.1111/j.1467-7652.2007.00270.x

17. Wagari A. A review on infectious bursal disease in poultry. *Health Econ Outcome Res Open Access*. 2021; 7(2): 167 (018- 023). doi: 10.35248/2471-268X.21.7.167

18. Zierenberg K, Raue R, Nieper H, et al. Generation of serotype 1/serotype 2 reassortant viruses of the infectious bursal disease virus and their investigation *in vitro* and *in vivo*. *Virus Research*. 2004; 105(1): 23-34. doi: 10.1016/j.virusres.2004.04.009

19. Jackwood D, Sommer-Wagner S. Genetic characteristics of infectious bursal disease viruses from four continents. *Virology*. 2018; 365: 369-375. doi: 10.1016/j.virol.2007.03.046

20. Lukert PD, Saif YM. Infectious bursal disease. In: *Diseases of Poultry*. 11th ed. Ames, Iowa: *Iowa State University Press*; 2003: 161-10.

21. Orakpoghenor O, Oladele SB, Abdu PA. Infectious bursal disease: Transmission, pathogenesis, pathology and control - an overview, *World's Poultry Science Journal*. 2020; 76: 292-303. doi: 10.1080/00439339.2020.1716652

22. Yip W, Hon C, Zeng UF, Leung C. Cell culture-adapted IBDV uses endocytosis for entry in DF-1 chicken embryonic fibroblasts. *Virus Res*. 2012; 165: 9-16. doi: 10.1016/j.virusres.2011.12.016

23. Office International des Epizootics (OIE). Infectious bursal disease (Gumboro disease): In: *Manual of Diagnostic Test and Vaccine for Terrestrial Animals*. Paris (in press): OIE; 2004

24. Banda A. Characterization of field strains of infectious bursal disease virus (IBDV) using molecular techniques. *Tropical Animal Health and Production*. 2002; 30: 70-80.

25. Kegne T, Chanie M. Review on the incidence and pathology of infectious bursal disease. *Br J Poultry Sci*. 2014; 3: 68-77. doi: 10.5829/idosi.bjps.2014.3.3.8556

26. Brandt M, Yao M, Liu R, Heckert A, Vakharia N. Molecular determinants virulence, cell tropism, and pathogenic phenotype of IBDV. *J Virol*. 2001; 75: 11974-11982. doi: 10.1128/JVI.75.24.11974-11982.2001

27. Office of International des Epizooties (OIE). Manual of diagnostic tests and vaccines for terrestrial animals. In: *Infectious Bursal Disease*. Paris (in press): OIE; 2012; 3: 549-565.

28. Bumstead N, Reece RL, Cook JKA. Genetic differences in susceptibility of chickens lines to infection with infectious bursal disease virus. *Poult Sci.* 1993; 72: 403-410. doi: 10.3382/ps.0720403

29. Islam MR. Developing vaccines. In: *Georg Forster.* 2002

30. Shaimaa I, Mohamed MH, Mohga FB. Effect of infectious bursal disease field vaccines on avian influenza vaccination immunity. *Alexandria J Vet Sci.* 2014; 43: 33-36. doi: 10.5455/ajvs.162984

31. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ. Infectious bursal disease. In: *Veterinary Virology.* 3rd ed. MA, USA: Academic press; 1999: 405-409.

32. Mittal D, Jindal N, Gupta SL, Kataria RS, Tiwari AK. Detection of infectious bursal disease virus in field outbreaks in broiler chickens by reverse transcription-polymerase chain reaction. *International Journal of Poultry Science.* 2005; 4(4): 239-243. doi: 10.3923/ijps.2005.239.243

33. Jackwood D, Sommer S. Molecular studies on suspect very virulent infectious bursal disease virus genomic RNA samples. *Avian Dis.* 2005; 49: 246-251. doi: 10.1637/7294-102604R

34. Zeleke A, Gelaye E, Sori T, Ayelet G, Sirak A, Zekarias B. Investigation on Infectious Bursal Disease outbreak in Debre Zeit, Ethiopia. *Int J Poult Sci.* 2005b; 4(7): 504-506. doi: 10.3923/ijps.2005.504.506

35. Dacic M, Zugic G, Petkovic J. The effect of a gumboro disease control program on the reduction of economic losses. *Acta. Vet. (Beogard).* 2008; 58: 53-62. doi: 10.2298/AVB0801053D

36. Farooq M, Durrani F, Imran N, Durrani Z, Chand N. Prevalence and economic losses due to infectious bursal disease in broilers in Mirpur and Kolti districts of Kashmir. *Int J Poultry Sci.* 2003; 2: 267-270. doi: 10.3923/ijps.2003.267.270

37. Schijns VEJC, Sharma J, Tarpy I. Practical aspects of poultry vaccination. In: *Avian Immunology.* 1st edn. London, UK: Academic Press; 2008; 373-393.

38. Delmas B. Birnaviruses. In: Mahy BWJ, van Regenmortel MHV, eds. *Encyclopedia of Virology.* 3rd edn. London, UK: Academic Press Inc; 2008: 321-328.

39. Oshop GL, Elankumaran S, Heckert RA. DNA vaccination in the avian. *Vet Immunol Immunopathol.* 2002; 89: 1-12. doi: 10.1016/s0165-2427(02)00189-7

40. Chen YY, Hsieh MK, Tung CY, Wu CC, Lin TL. Infectious bursal disease DNA vaccination conferring protection by delayed appearance and rapid clearance of invading viruses. *Arch Virol.* 2011; 156: 2241-2250. doi: 10.1007/s00705-011-1127-4

41. Hsieh MK, Wu CC, Lin TL. DNA-mediated vaccination conferring protection against infectious bursal disease in broiler chickens in the presence of maternal antibody. *Vaccine.* 2010; 28(23): 3936-3943. doi: 10.1016/j.vaccine.2010.03.066

42. Tsukamoto K, Saito S, Saeki S, et al. Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. *J Virol.* 2002; 76(11): 5637-5645. doi: 10.1128/jvi.76.11.5637-5645.2002

43. Zhou X, Wang D, Xiong J, Zhang P, Li Y, She R. Protection of chickens, with or without maternal antibodies, against IBDV infection by a recombinant IBDV-VP2 protein. *Vaccine.* 2010; 28(23): 3990-3996. doi: 10.1016/j.vaccine.2010.03.021

44. Liew PS, Hair-Bejo M. Farming of plant-based veterinary vaccines and their applications for disease prevention in animals. *Adv Virol.* 2015; 2015: 936940. doi: 10.1155/2015/936940

45. Wu H, Singh NK, Locy RD, Scissum-Gunn K, Giambrone JJ. Expression of immunogenic VP2 protein of infectious bursal disease virus in Arabidopsis thaliana. *Biotechnol Lett.* 2004; 26(10): 787-792. doi: 10.1023/b:bile.0000025878.30350.d5

46. Gómez E, Lucero MS, Zoth SC, Carballeda JM, Gravisaco MJ, Berinstein A. Transient expression of VP2 in Nicotiana benthamiana and its use as a plant-based vaccine against infectious bursal disease virus. *Vaccine.* 2013; 23: 2623-2627. doi: 10.1016/j.vaccine.2013.03.064

47. Chen TH, Chen TH, Hu CC, et al. Induction of protective immunity in chickens immunized with plant-made chimeric bamboo mosaic virus particles expressing very virulent infectious bursal disease virus antigen. *Virus Res.* 2012; 166: 109-115. doi: 10.1016/j.virusres.2012.02.021

48. Rautenschlein S, Haase C. Differences in the immunopathogenesis of infectious bursal disease virus (IBDV) following *in ovo* and post-hatch vaccination of chickens. *Vet Immunol Immunopathol.* 2005; 106: 139-150. doi: 10.1016/j.vetimm.2005.02.011

49. Corley MM, Giambrone JJ, Dormitorio TV. Evaluation of the immune response and detection of infectious bursal disease viruses by reverse transcriptase-polymerase chain reaction and enzyme-linked immunosorbent assay after *in ovo* vaccination of commercial broilers. *Avian Dis.* 2022; 4: 803-809. doi: 10.1637/0005-2086(2002)046[0803:EOTIRA]2.0.CO;2