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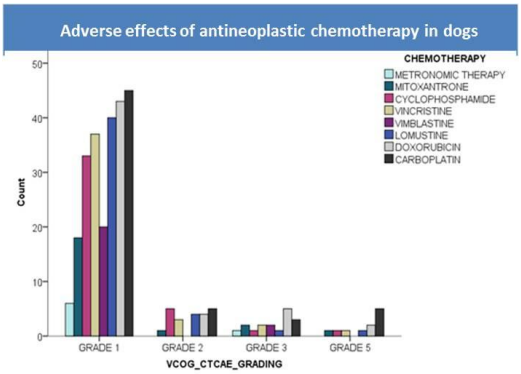
Research Paper

Adverse Effects of Chemotherapy in Dogs.

Cunha SCS, Silva FBF, Corgozinho KB, Silva KVG and Ferreira AMR.

World Vet. J. 7(3): 74-82, 2017; pii:S232245681700010-7
DOI: http://dx.doi.org/10.5455/wvj.20170896

ABSTRACT



Owners of dogs with cancer are often offered chemotherapeutic treatment. However, clients who seek veterinary care for pets with cancer are often concerned about the potential negative impact of chemotherapeutic treatments on their animals' quality of life. The purpose of this retrospective case series was to investigate the delayed acute effects of chemotherapy drugs in dogs receiving cancer treatment and their owners' opinions regarding chemotherapy acceptance by their pet. In this study, 292 dogs that were treated with chemotherapy as a definitive and/or adjuvant treatment for cancer. Medical records were reviewed to determine the chemotherapy agent used and if they had any delayed adverse effects or not. Side effects were classified according to VCOG-CTCAE grading of adverse effect severity veterinary co-operative oncology group. Lomustine, carboplatin, vincristine, doxorubicin, cyclophospha-mide, mitoxantrone, and vinblastine were administered in 16%, 20%, 15%, 18%, 16%, 8%, and 7% of the cases respectively. The most common adverse effects were neutropenia (22%), vomiting (21%), diarrhea (20%) and inappetence (20%). Cyclophosphamide and vincristine were the agents that had caused more adverse gastrointestinal effects, while lomustine was the drug that had caused more hematologic effects. In some dogs receiving lomustine and carboplatin, neutropenia (some of them severe) had occurred as early as in the sixth day. According to the current grading system of adverse effects induced by chemotherapy, general tolerance to chemotherapy is referred to as grade 1, which was observed in 83% of the cases. Owner opinion was positive in most cases, and 77% of the owners had evaluated that the treatment was well tolerated by their dogs. In contrast, 8% of the treatments were poorly tolerated and they had negatively impacted the affected dogs' quality of life. Based on the data examined, we would recommend that gastrointestinal adverse effects must be prevented with antiemetic medication, especially in dogs receiving cyclophosphamide, vincristine, carboplatin and doxorubicin. Hematologic profile must be performed as early as in the 6-7th day after lomustine and carboplatin, as severe neutropenia can occur. Adverse chemotherapy effects may occur in about 20-25% of canine patients.

Key words: Canine, Oncology, Chemotherapy, Side effect, Tolerability

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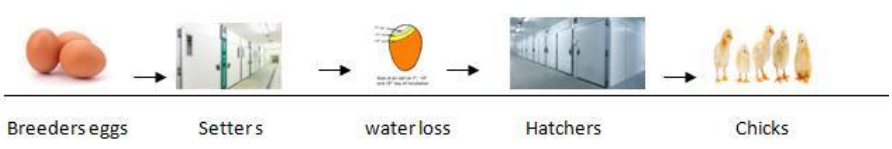
Research Paper

Incubation Duration of Broiler Breeder Egg and Post Hatch Performance.

Jabbar A and Ditta YA.

World Vet. J. 7(3): 83-88, 2017; pii:S232245681700011-7
DOI: http://dx.doi.org/10.5455/wvj.20170897

ABSTRACT



Incubation duration is a most essential factor to achieve standard hatchability, water loss and chick yield.Ross-308 different breeder flocks which were 42-46 weeks old and standard eggs that weighed up to 55-60g were selected. In the current study, two experimental groups each consisting of (n= 538560) eggs were selected to investigate the effect of eggs incubation duration on hatchability and post-hatch performance. Therefore, this experiment was conducted in order to evaluate the exact duration of incubation and its effects on broiler performance at the farm. Group A was incubated for 444hrs in setter and 62 hrs in hatcher. Hatch pulling for A was performed twice 1st after 494 hrs and remaining un-hatch eggs were again shifted to hatchers for next 12 hrs for 2nd pulling (conventional method of hatch pulling in Pakistan). For B hatch pulling was performed after 456hrs in setters and 50 hrs in hatcher, complete hatch pulling only once. Eggs weight at transfer was (53.9±0.8gm and 54.9 ±0.6gm), water loss at transfer was (11.67±0.7% and 10.6±0.7%) and chick weight was at (41.6±0.3gm and 42.7±0.3gm) on day one were significantly better for group B than A respectively. Similarly, hatchability (86.16±1.02% and 85.16±1.02) and dead in shell (5.10 ±0.8% and 6.61±1.5%) were also significantly better for group B then A respectively. Candling (8.23±0.9% and 8.23±1.33%) was

same for both groups. Mortality was estimated to be at (1.80±0.06and 2.47±0.23%), weight gain was determined to be at (2001.33±24.33gram and 1955.66±25.02gram), feed intake (g/bird) was at (3245.02±18.03 and 3260.51±13.47) and feed conservation ratio at day 35(1.44±0.02 and 1.716±0.03) were found to have been significantly better for B than A respectively. So, incubation of eggs for 456 hrs in setters and 50 hrs in hatchers, along with single pull is better in term of water loss, chick yield, hatchability and post hatch performance.
Key words: Broiler, Chicks quality, Incubation duration, Post hatch performance

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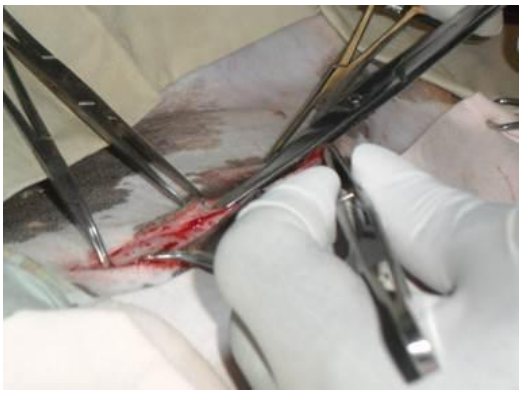
Research Paper

Haematological and Biochemical Changes in Nigerian Dogs with Short Bowel Syndrome.

Kisani AI, Adeyanju JB and Sonfada ML.

World Vet. J. 7(3): 89-100, 2017; pii:S232245681700012-7
DOI: http://dx.doi.org/10.5455/wvj.20170898

ABSTRACT



The purpose of this study was to evaluate the haematological and biochemical changes in Nigerian dogs with short bowel syndrome. Thirty adult dogs each weighing approximately 12.4kg (range 7-18kg) were used in this study. The dogs were randomized into five groups of six dogs each. Group 1 is the control group. The dogs here were not placed on any treatment. Group 2 dogs were supplemented with glutamine. Group 3 dogs were supplemented with honey. Group 4 dogs were supplemented with ascorbic acid and group 5 dogs were supplemented with glutamine, honey and ascorbic acid combination. Haematological parameters, serum electrolytes (Sodium, potassium, bicarbonate and, chloride) and enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) were also evaluated. There was no depletion in sodium, potassium, bicarbonate and chloride in all the animals as the value of these electrolytes had remained at normal range in all five groups. There was a significant decrease in the value of alkaline phosphatase in the five groups and non significant changes in the value of alanine aminotransferase in all the animals. It was therefore, concluded that patients with resection of proximal small intestinal tract have better chances of survival than patients with a resected distal small intestinal tract.
Keywords: Adaptation, Alanine aminotransferase, Electrolytes, Haematology, Alkaline phosphatase, Short bowel syndrome

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Research Paper

An Abattoir based Study on Bovine Tuberculosis in Debre Zeit, Ethiopia.

Pal M, Zenebe N, Amare T and Woldemariam T.

World Vet. J. 7(3): 101-107, 2017; pii:S232245681700013-7
DOI: http://dx.doi.org/10.5455/wvj.20170899

ABSTRACT

Members of the Mycobacterium complex group cause tuberculosis, it recognized as one of the most important threats to humans and animals causing mortality, morbidity and economic losses in many countries of the world, particularly in developing nations. Therefore, a cross-sectional study on bovine tuberculosis conducted in order to determine its prevalence in cattle at the ELFORA export abattoir in Debre-Zeit in the period from November 2014 to April 2015. Routine and detailed meat inspection methods used to detect lesions. Three hundred cattle inspected; their body condition scores and ages recorded before slaughtering. Of the total animals, 5.7% (17/300) had lesions of tuberculosis. Out of these, routine abattoir inspection had detected only 2.7% (8/300) with visible lesions and there was poor agreement (kappa=0.09) between routine and detailed inspection methods. The proportion of lesions found in the lung and associated lymph nodes, mesenteric lymph nodes and lymph node around head were determined to have been at 12.3%, 2% and 3.3%, respectively. The prevalence of the disease was significantly ($P < 0.05$) varying with body condition scores but it did not significantly ($P > 0.05$) vary with

age groups of the animals. This study demonstrated the prevalence of bovine tuberculosis in cattle slaughtered at ELFORA export abattoir and low sensitivity of routine abattoir inspection. Hence, the carcass must thoroughly examine well to reduce the chance of missing lesions of tuberculosis.
Key words: Bovine tuberculosis, Meat inspection, Prevalence, Public health, Zoonosis

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Research Paper

Influence of Enzymatic and Mechanical Liquefaction of Seminal Plasma on Freezability of Dromedary Camel Semen.

El-Bahrawy KA.

World Vet. J. 7(3): 108-116, 2017; pii:S232245681700014-7
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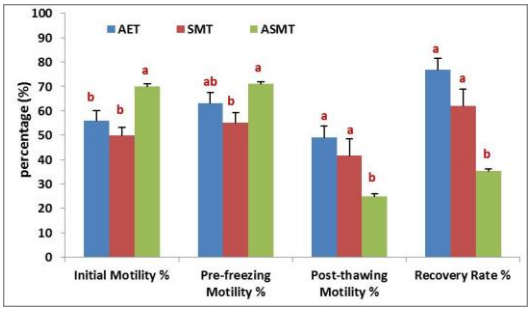
ABSTRACT



This study aimed to investigate the efficiency of mechanical and enzymatic elimination of semen viscosity in adult dromedary camel bulls' semen on cryopreservation potential of spermatozoa during the breeding season. Bulls showed reaction time 40.0±8.23 seconds and 251±24 seconds mating duration. Physical properties of raw semen showed volume mean value 5.28±0.66 ml, initial viability 2.5±0.6, initial raw motility 59.34±4.99%, livability 95.3±2.36%, first and second abnormalities 4.13±0.88% and 7.01±1.254%, respectively and acrosomal integrity 5.03±1.05%. The researcher examined three different treatments for viscosity elimination; namely; Amylase Enzymatic Treatment (AET), Syringe Mechanical Treatment (SMT) and Amylase Syringe Mixed Treatment (ASMT). The results revealed that, a significant deleterious effect of the ASMT on the post-thaw motility (M_{PT}) 25.00±3.69% was observed, with sperm Recovery Rate (RR) 35.02±5.02%, contrary to a clear superiority of AET treatment on (M_{PT}) 49.00±4.87%, followed by the SMT treatment (M_{PT}) 41.67±6.72%, with significantly higher RR% (76.86±4.63% and 62.10±6.65%) respectively. The AET recorded the highest acrosomal reaction (10.17±1.11%), followed by the mixed treatment (8.33±0.14%), with the least significant effect ($P < 0.05$) on the mechanically treated group (7.33±0.99%). The results also showed the same trend for first and second abnormalities. Computer assisted semen analysis showed a significant superiority for the AET on mostly all sperm kinetics (DCL, DAP, VAP, VSL), except for DSL, VCL that showed highest significant value for SMT treatment. Conversely, the study recorded the lowest significant values for LIN, STR and WOB in the SMT. These results clarified that both enzymatic and mechanical methods have a positive influence on dromedary camel semen cryopreservation.
Key words: Amylase, Cryopreservation, Dromedary, Semen syringing, Viscosity

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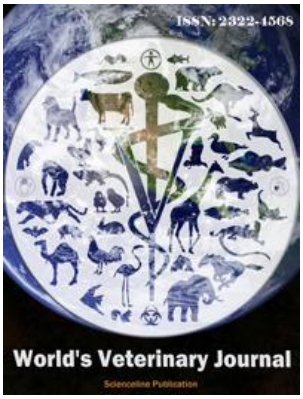

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Adverse Effects of Chemotherapy in Dogs

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ABSTRACT

Owners of dogs with cancer are often offered chemotherapeutic treatment. However, clients who seek veterinary care for pets with cancer are often concerned about the potential negative impact of chemotherapeutic treatments on their animals' quality of life. The purpose of this retrospective case series was to investigate the delayed acute effects of chemotherapy drugs in dogs receiving cancer treatment and their owners' opinions regarding chemotherapy acceptance by their pet. In this study, 292 dogs that were treated with chemotherapy as a definitive and/or adjuvant treatment for cancer. Medical records were reviewed to determine the chemotherapy agent used and if they had any delayed adverse effects or not. Side effects were classified according to VCOG-CTCAE grading of adverse effect severity veterinary co-operative oncology group. Lomustine, carboplatin, vincristine, doxorubicin, cyclophosphamide, mitoxantrone, and vinblastine were administered in 16%, 20%, 15%, 18%, 16%, 8%, and 7% of the cases respectively. The most common adverse effects were neutropenia (22%), vomiting (21%), diarrhea (20%) and inappetence (20%). Cyclophosphamide and vincristine were the agents that had caused more adverse gastrointestinal effects, while lomustine was the drug that had caused more hematologic effects. In some dogs receiving lomustine and carboplatin, neutropenia (some of them severe) had occurred as early as in the sixth day. According to the current grading system of adverse effects induced by chemotherapy, general tolerance to chemotherapy is referred to as grade 1, which was observed in 83% of the cases. Owner opinion was positive in most cases, and 77% of the owners had evaluated that the treatment was well tolerated by their dogs. In contrast, 8% of the treatments were poorly tolerated and they had negatively impacted the affected dogs' quality of life. Based on the data examined, we would recommend that gastrointestinal adverse effects must be prevented with antiemetic medication, especially in dogs receiving cyclophosphamide, vincristine, carboplatin and doxorubicin. Hematologic profile must be performed as early as in the 6-7th day after lomustine and carboplatin, as severe neutropenia can occur. Adverse chemotherapy effects may occur in about 20-25% of canine patients.

Key words: Canine, Oncology, Chemotherapy, Side effect, Tolerability

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INTRODUCTION

Owners of dogs with cancer are often offered chemotherapeutic treatment. However, clients who seek veterinary care for pets with cancer are often concerned about the potential negative impact of chemotherapeutic treatments on their animals' quality of life. This concern may arise from the owner's knowledge or experience with chemotherapy in human medicine, and often owners hesitate in proceeding with chemotherapy (Vail, 2009; Vols et al., 2016).

Anticancer drugs primarily target dividing cells to interfere with the processes involved in the mediating progression of the cell cycle (Gustafson and Page, 2013). The toxicity profiles associated with anticancer agents include immediate and evident toxicities (e.g., those that develop within 24–48 hrs after treatment), acute delayed effects (e.g., those that develop within 2–14 days after treatment), and/or cumulative/chronic toxicity (effects extending over weeks,

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months, or years). Immediate toxicity may result from infusion hypersensitivity due to histamine release associated with allergic reactions or vehicle-induced mast cell de-granulation (Gustafson and Page, 2013). Routine management of these effects with antihistamines and steroids may significantly reduce or eliminate these side effects. Acute nausea and vomiting may also occur with specific agents or if an infusion is performed too rapidly. Delayed acute effects from chemotherapy often include bone marrow suppression and nausea, vomiting, and diarrhea. In the majority of instances, these effects are self-limiting and the incidence of hospitalization for such problems is rare (Vail, 2009; Gustafson and Page, 2013; Vols, 2016). Examples of potential cumulative and/or chronic toxicity include hepatic dysfunction after multiple doses of Chloroethyl Cyclohexyl Nitrosourea (CCNU, also known as lomustine), cardiac abnormalities after exceeding a safe cumulative dose of doxorubicin, and renal disease after cisplatin use (Gustafson and Page, 2013).A consensus currently exists in veterinary oncology regarding the quantification and rating of adverse treatment effects in dogs and cats in response to chemotherapy agents. This grading system is referred to as Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events (VCOG-CTCAE) (Veterinary Co-operative Oncology Group, 2016). The purpose of this retrospective case series was to investigate the delayed acute effects of chemotherapy drugs in dogs receiving cancer treatment and their owners' opinions regarding chemotherapy acceptance by their pet.

MATERIALS AND METHODS

This retrospective study involved 292 dogs treated with chemotherapy between August 2011 and August 2016, in Rio de Janeiro, Brazil. All of the dogs had been previously diagnosed with malignant neoplasia and chemotherapy was prescribed as a definitive and/or adjuvant treatment.

Medical records were reviewed to determine if the chemotherapy agents used had any observed delayed acute toxicity effects (between 12 h and 21 d after the administration of chemotherapy) or not. The chemotherapy agents administered included vincristine, vimblastine, compounded lomustine, cyclophosphamide, doxorubicin, mitoxantrone, and carboplatin, according to neoplasm histologic type, treatment protocol, and each animal´s concomitant diseases.

The reported effects included: hematologic effects (e.g., neutropenia, thrombocytopenia, increases in liver enzymes, and azotemia), gastrointestinal effects (e.g., vomiting, diarrhea, and inappetence), and sepsis. A summary of the reported adverse events is provided in Table 1. Late, cumulative, and/or chronic toxicity (e.g., hepatic dysfunction, cardiac abnormalities, and chronic renal disease) were not studied.

Table 1. Common terminology criteria for adverse events following chemotherapy or biological antineoplastic therapy in dogs, of veterinary cooperative oncology group

Adverse Event	Grade				
	1	2	3	4	5
Neutropenia (μL^{-1})	1500 to <LLN	1000–1499	500–999	<500	Death
Thrombocytopenia (μL^{-1})	100 000 to <LLN	50 000–99 000	25 000–49 000	<25 000	Death
Creatinine	>1–1.5× bl	>1.5–3× bl	>3× bl	>3× bl	-
ALT	>ULN to 1.5× ULN	>1.5–4.0× ULN, transient (<2 weeks)	>4.0–10× ULN	>10× ULN	-
Anorexia	Coaxing or dietary change required to Maintain appetite	Oral intake altered (≤3 days) without significant weight loss; oral nutritional supplements/appetite stimulants may be indicated	Of >3 days duration; associated with significant weight loss (≥10%) or malnutrition; IV fluids, tube feeding or force feeding indicated	Life-threatening consequences; TPN indicated; >5 days duration	Death
Vomiting	<3 episode in 24 h, medical intervention not indicated	3–10 episodes in 24 h; <5 episodes/day for ≤48 h; parenteral fluids (IV or SC) indicated ≤48 h; medications indicated	Multiple episodes >48 h and IV fluids or PPN^{+}/TPN^{+} indicated >48 h	Life-threatening (e.g.haemodynamic collapse)	Death
Diarrhea	Increase of up to 2 stools per day over bl; no increase in frequency, however, consistency decreased	Increase of 3–6 stools per day over bl; medications indicated; parenteral (IV or SC) fluids indicated ≤48 h; not interfering with ADL	Increase of >6 stools per day over baseline; incontinence >48 h; IV fluids >48 h; hospitalization; interfering with ADL	Life-threatening (e.g.haemodynamic collapse)	Death

LLN = lower limit of normal; ULN = upper limit of normal; bl = baseline; ADL = activities of daily living (eating, sleeping, defecating and urinating); PPN = partial parenteral nutrition; TPN = total parenteral nutrition

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The following factors were statistically examined to determine their relationship to the chemotherapy agents used and their impact on the owners' opinions about treatment: age (< 7 y, 8–11 y, or > 12 y), breed (divided into divided into mixed breed, retrievers, daschunds, companion, guard, terriers, pastor, greyhound, spitz and pitbull dogs), neoplasm histologic type (Lymphoma, carcinoma, mast cell tumor, melanoma, sarcoma, cerebral tumors and transmissible venereal tumor), and presence of concomitant disease (hemoparasitosis, endocrine, cardiovascular, renal and gastrointestinal).

Side effects were classified according to VCOG-CTCAE grading of adverse effect severity (Veterinary co-operative oncology group, 2016) as follows: grade 1; asymptomatic, or mild symptoms, clinical signs or diagnostic observations only, intervention not indicated, grade 2; moderate, minimal, outpatient, or noninvasive intervention indicated, moderate limitation of daily living activities, grade 3; severe or medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling, significantly limited daily living activities, grade 4; life-threatening consequences, urgent interventions indicated, and grade 5; death related to adverse events.

All of the dogs in this study were treated according to the same protocol. Animals receiving chemotherapy received ondasetron, 0.5 mg/kg (Plumb, 2015) orally twice daily, and omeprazol, 1 mg/kg orally once daily, in the first five days following chemotherapy administration, independent of the chemotherapy drug administered. If vomiting occurred with oral medication, then maropitant, 2mg/kg (Plumb, 2015), was administered subcutaneously for three consecutive days, as oral medication was maintained. In addition, the animals were fed a special diet in case of vomiting/anorexia (Hill´s prescription a/d or royal canin gastrointestinal). When necessary, feeding was forced, and in some cases, an e-tube was recommended. If diarrhea (> grade 2) occurred, probiotics were administered for seven days. Hematologic examination was performed 6–14 days after chemotherapy. If neutropenia (> grade 3) occurred, filgrastim, 5 ug/kg, was administered subcutaneously for three consecutive days together with prophylactic antibiotics.

The dog owners' opinions were collected 7–21 days after each chemotherapy. The owners were surveyed about their perception of their animal's tolerance to chemotherapy, changes in activities of daily living (eating, sleeping, defecating, and urinating), and quality of life during oncologic treatment. No classification or graduation was presented and owners were free to state their opinion. The owners were asked to categorize their overall perceptions regarding the tolerance of their pets to chemotherapy as: good, regular, bad, or poor.

A database exploratory analysis was performed and comparisons between variables were made with the Pearson Chi-square, Kruskal-Wallis, and Mann-Whitney tests as appropriate. Descriptive statistics served as a basis for interpretation of the results. The level of statistical significance was 5%. Tests and studies were performed with the software, Statistical Package for the Social Sciences (SPSS; version 20.0, SPSS Inc., Chicago, IL, USA).

Ethical approval

The authors did a retrospective cohort study of oncologic patients treated with chemotherapy. They did not seek informed consent or ethical committee approval for their study, as the paper does not report on primary research and all data analysed were collected as part of routine oncologic treatment.

RESULTS

A total of 292 oncologic records of various canine breeds were reviewed. The breeds included: mixed (n=51), retrievers (n=92), dachshund (n=18), companion (n=57), guard (n=24), terriers (n=29), pastor (n=6), greyhounds (n=1), spitz (n=5) and pitbull (n=9).Most of the animals were8-12 years old (126; 43%), while 105 (36%) were older than 12 years and 60 (21%)were1-7 years old. One animal had unknown age.

The neoplasm histological types were highly variable and were categorized as: carcinoma [mammary (39/292), squamous cell (2/292), transitional cell (5/292), pharyngeal (2/292), sinonasal (1/292), perianal (2/292), sebaceous (4/292), pulmonary (1/292) and hepatocellular (3/292)], sarcoma [soft tissue (12/292), osteosarcoma (3/292), hemangiosarcoma (14/292), intestinal leiomyosarcoma (1/292) and sinonasal (1/292)], lymphoma (131/292), mast cell tumor (43/292), melanoma (20/292), sertolioma (1/292), thymoma (3/292), malignant trichoepithelioma (1/292), cerebral tumors (1/292) and TVT (2/292). Concomitant diseases were present in 33 (11%) animals, and these included renal (n=4), cardiovascular (n=14), endocrine (n=13), gastrointestinal (n=4) and hemoparasites (n=2).

Various chemotherapy agents were administered. The chemotherapy agent was compounded lomustine (CCNU) in 46 (16%) cases, carboplatin in 58 (20%) cases, vincristine in 43 (15%) cases, doxorubicin in 54 (18%) cases, cyclophosphamide in 47 (16%) cases (40 conventional therapy and 7 metronomic therapy), mitoxantrone in 22 (8%) cases and vimblastine in 22 (7%) cases. These doses are consistent with those previously published, although the author's preference determined the dose. Doses were 70 mg/m^2 for lomustine, 230-250 mg/m^2 for carboplatin, 0.75 mg/m^{2}for vincristine, 2 mg/m^{2}for vimblastine, 30 mg/m^2 for doxorubicin and 5.5 mg/m^2 for mitoxantrone.

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Cyclophosphamide was administered at a dose of 250 mg/m^2 in conventional therapy and 15 mg/m^2 in metronomic therapy.

Blood exams, including hematologic examination and biochemistry [Blood Urea Nitrogen (BUN), creatinine, Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP)] were performed 7-21 days after chemotherapy, according to chemotherapy agent nadir and author's previous experience. For example, the animals that received lomustine (CCNU) underwent their exams on day 6 and day 21. For the animals that received carboplatin, blood exams were performed between days 10–14.For the animals that received vincristine, vimblastine, mitoxantrone, or doxorubicin, their exams were performed between days 7–10.

Vomiting (despite prophylactic oral administration of antiemetics) was observed in62 (21%) cases, with severity grade 1 in 56 (19%), grade 2 in 4 (1%) and grade 3 in 2 (1%). Associations between the chemotherapeutic agents and vomiting did not exhibit statistically significant differences (P=0.078). However, most of these cases occurred with cyclophosphamide (n=15) and vincristine (n=15), followed by doxorubicin (n=12), carboplatin (n=10), mitoxantrone (n=4), lomustine (n=3), metronomic cyclophosphamide (n=2) and vimblastine (n=1) (Figure 1).

Diarrhea was observed in 58 (20%) cases, with severity grade 1 in 50 (17%) and grade 2 in 8 (3%). A comparison between the presence of diarrhea of various grades and chemotherapeutic agents revealed a statistically significant difference (p<0.001). Most of these cases occurred with cyclophosphamide (n=16) and vincristine (n=14), followed by doxorubicin (n=12), carboplatin (n=8), lomustine (n=3), mitoxantrone (n=2), metronomic cyclophosphamide (n=2) and vimblastine (n=1) (Figure 2).

Various grades of inappetence/anorexia occurred in 58 (20%) cases. Four cases were grade 1 (1%), 50 were grade 2 (17%), three cases were grade 3 (1%) and one case was grade 4 (1%).A statistically significant difference was observed when the presence of inappetence/anorexia was compared to chemotherapeutic agents (P =0.035). The agents that caused most cases of inappetence were conventional cyclophosphamide (13/40), carboplatin (12/58), doxorubicin (12/54) and vincristine (10/43) (Figure 3).

Neutropenia is a major side effect of chemotherapy and it was only observed in63 (22%) cases. The severity of neutropenia included grade 1 in 5/63 cases, grade 2 in 37/63 cases, grade 3 in 20/63 cases and grade 5 in 1/63 cases. There was no statistically significant difference when the presence of neutropenia and chemotherapeutic agents were compared (P =0.088). However, lomustine was the chemotherapy agent responsible for most of the cases of neutropenia (18/46), and grades 1–3 had an incidence of 1case, 8cases and9cases, respectively. Carboplatin also caused neutropenia in 45/58 dogs (including nine cases of grade 2, three cases of grade 3 and one case of grade 5), as did vincristine in 8/43dogs (including seven cases of grade 2 and one case of grade 3). Mitoxantrone caused neutropenia in six dogs (one case of grade 1, one case of grade 2 and four cases of grade 3). Doxorubicin administration leads to neutropenia in seven cases and vimblastine in two cases (Figure 4). Sepsis secondary to chemotherapy occurred in four dogs of this study (three had received carboplatin and 1mitoxantrone), and three of them died despite treatment.

Thrombocytopenia was observed in 24 (8%) cases, with grades 1–4 affecting18cases, 3cases, 3 cases and 1 case, respectively. There was no statistically significant difference when the presence of thrombocytopenia and chemotherapeutic agents were compared (p =0.37). In most cases, It was caused by lomustine (n=8), carboplatin (n=5) and cyclophosphamide (n=4), followed by mitoxantrone (n=3), doxorubicin (n=2) and vincristine (n=2) (Figure 5).

Azotemia occurred in three dogs, including one case of grade 2, one case of grade 3 and one case of grade 5. All dogs had been previously diagnosed with renal disease, and two cases had received carboplatin and one case metronomic cyclophosphamide. Elevated levels of ALT and/or ALP were observed in 16 dogs (including nine cases of grade 2 and seven cases of grade 3), and 13/16 occurred after lomustine administration.

According to the current VCOG-CTCAE system for grading the adverse effects of chemotherapy, general tolerance to chemotherapy was at grade 1 in 242(83%) cases, and grade 2-5 in 22 (8%) cases, 17 (6%) and11 (4%), respectively. VCOG was classified as grade 1 in 78-90% of cases in most chemotherapies (Figure 6)**.**There was no statistically significant difference when the VCOG-CTCAE grading and chemotherapeutic agents were compared (P =0.83).

For the cases examined, owner perception was positive in most of the cases; with 224 (77%) owners reporting that the treatments received were well tolerated by their dogs. Treatment was considered as regular in 44 (15%) cases and bad in 11 cases (4%) cases. For the 13 (4%) dogs that had poorly tolerated their treatments, a negative impact on quality of life was observed. Chemotherapy was considered well tolerated in most agents (67-96%) (Figure 7). There was no statistically significant difference when the owner perception and chemotherapeutic agents were compared (P =0.22)

A statistical association between neutropenia, vomiting, inappetence, azotemia, and owner's opinion was identified for the chemotherapy agents. For example, the Kruskall-Wallis test confirmed the presence of a statistically significant difference between the chemotherapy agents and the incidences of neutropenia, vomiting, diarrhea, azotemia and hepatopathy (P<0.05). To further identify these differences, the Mann-Whitney statistical test was applied. In this analysis, statistical association between azotemia and chemotherapy agents was not maintained.

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In paired comparisons, a statistical difference (P<0.05) was observed for hepatopathy with the administration of lomustine versus administration of the other chemotherapeutic agents. Lomustine contributed significantly to the occurrence of hepatopathy in dogs. Similar results were observed with the occurrence of neutropenia with this agent (P=0.02).

There was a significant statistical difference in the occurrence of diarrhea when comparing cyclophosphamide with carboplatin (P= 0.006), lomustine (P= 0.000), mitoxantrone (P= 0.01), and vimblastine (P= 0.003). The treatment with vincristine was also associated with the occurrence of diarrhea, with a statistically significant difference compared to the chemotherapeutics carboplatin (P= 0.04), lomustine (P= 0.002), and vimblastine (P= 0.008).

The occurrence of vomiting was statistically significant in dogs treated with cyclophosphamide when compared to the chemotherapeutics carboplatin (P = 0.03), lomustine (P= 0.000) and vimblastine (P= 0.005). The use of vincristine was also statistically associated with vomiting, compared to the use of lomustine (P= 0.001) and vimblastine (P= 0.01). The use of doxorubicin and lomustine also showed a significant statistical difference (P= 0.03), in this case, we observed a tendency of lomustine to be related to grade 1 vomiting, whereas doxorubicin with grade 1, 2 and 3 vomiting.

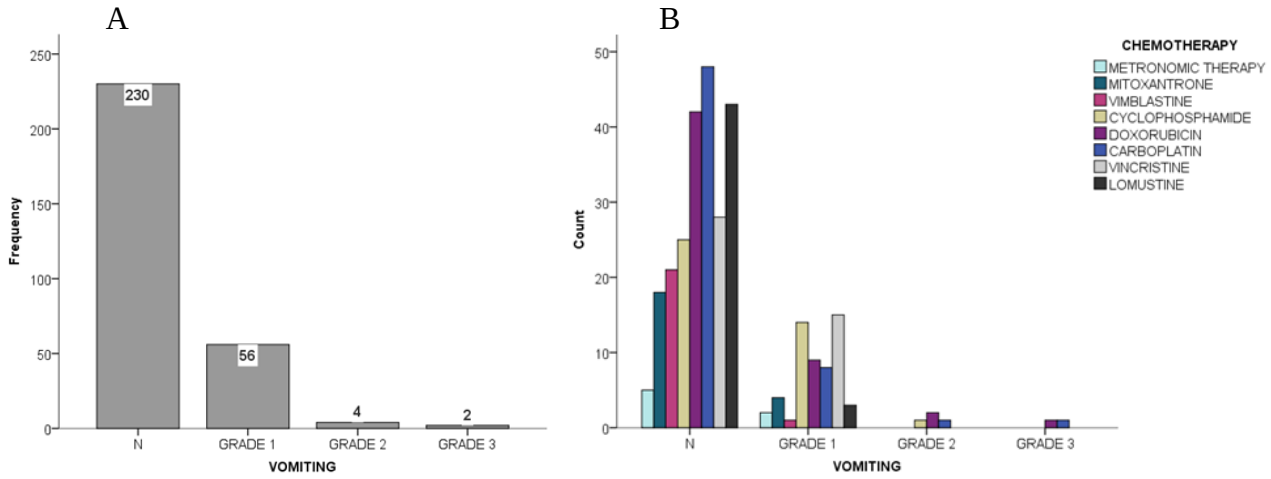

Figure 1. Adverse events of chemotherapy in dogs - vomiting. A: Presence of vomiting and its veterinary cooperative oncology group toxicity criteria grade in studied dogs. B: Presence of vomiting according to chemotherapy in studied dogs

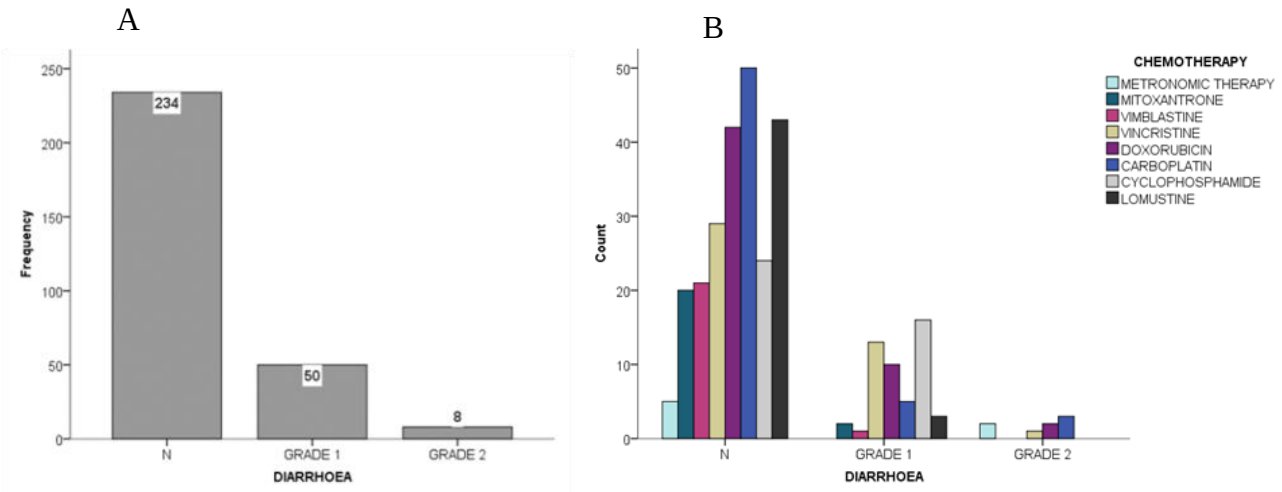

Figure 2. Adverse effects of chemotherapy in dogs - diarrhea. A: Presence of diarrhea and its veterinary cooperative oncology group toxicity criteria grade in studied dogs. B: Presence of diarrhea according to chemotherapy in studied dogs

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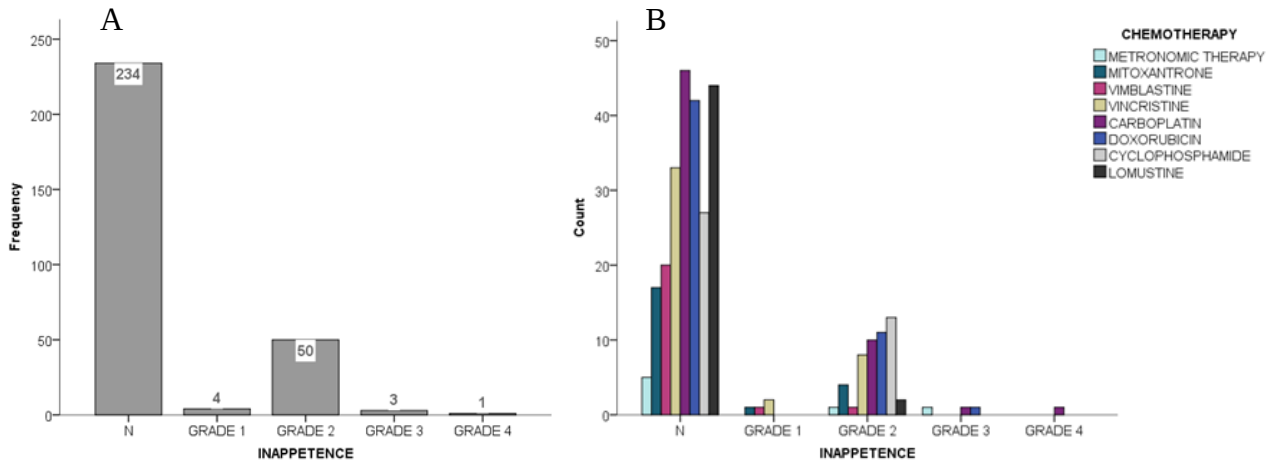

Figure 3. Adverse effects of chemotherapy in dogs - inappetence. A: Presence of inappetence and its veterinary cooperative oncology group toxicity criteria grade in studied dogs. B: Presence of inappetence according to chemotherapy in studied dogs

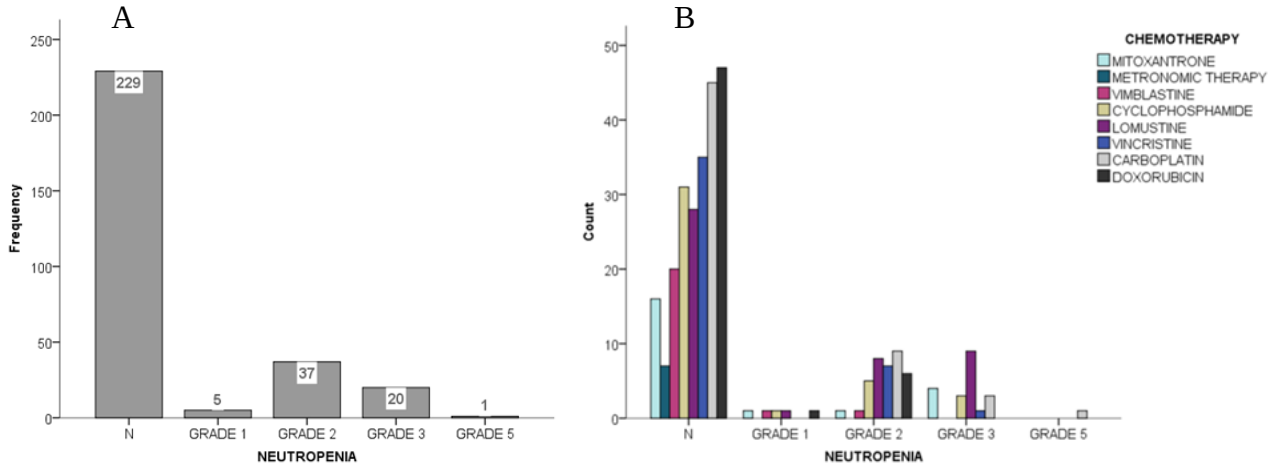

Figure 4. Adverse effects of chemotherapy in dogs - neutropenia. A: Presence of neutropenia and its veterinary cooperative oncology group toxicity criteria grade in studied dogs. B: Presence of neutropenia according to chemotherapy in studied dogs

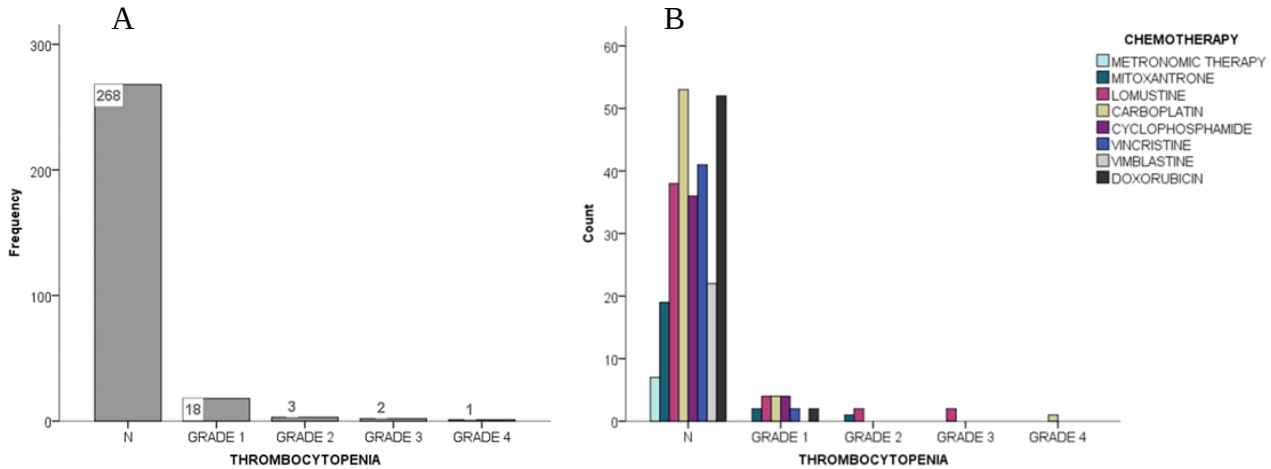

Figure 5. Adverse effects of chemotherapy in dogs - thrombocytopenia. A: Presence of thrombocytopenia and its veterinary cooperative oncology group toxicity criteria grade in studied dogs. B: Presence of thrombocytopenia according to chemotherapy in studied dogs.

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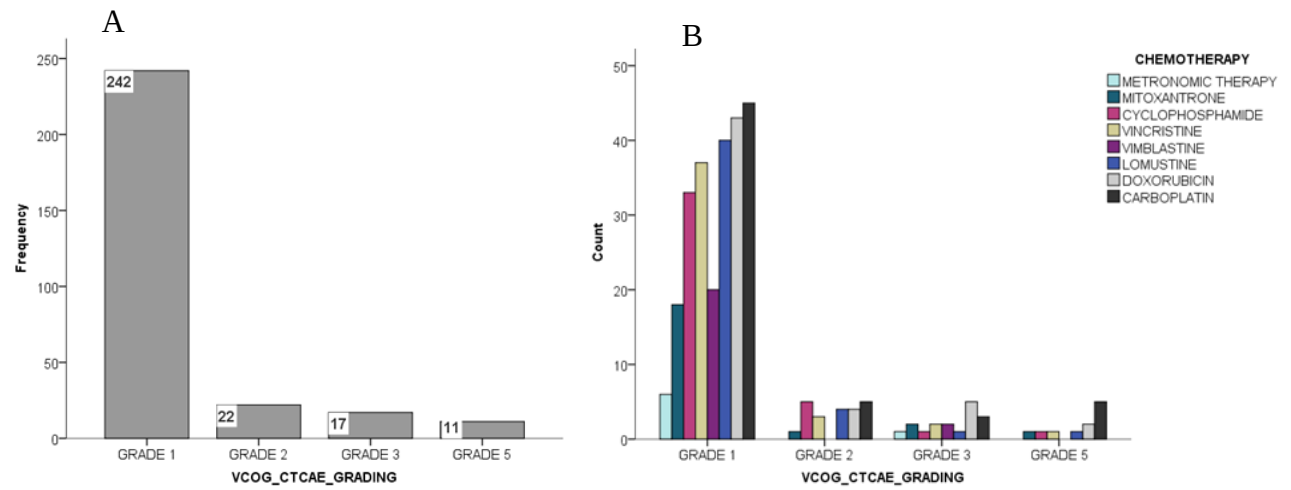

Figure 6. Grading system of adverse events to chemotherapy according to veterinary oncology group in studied dogs. A: General chemotherapy veterinary cooperative oncology group toxicity criteria grade in studied dogs. B: Veterinary cooperative oncology group toxicity criteria grade according to chemotherapy in studied dogs

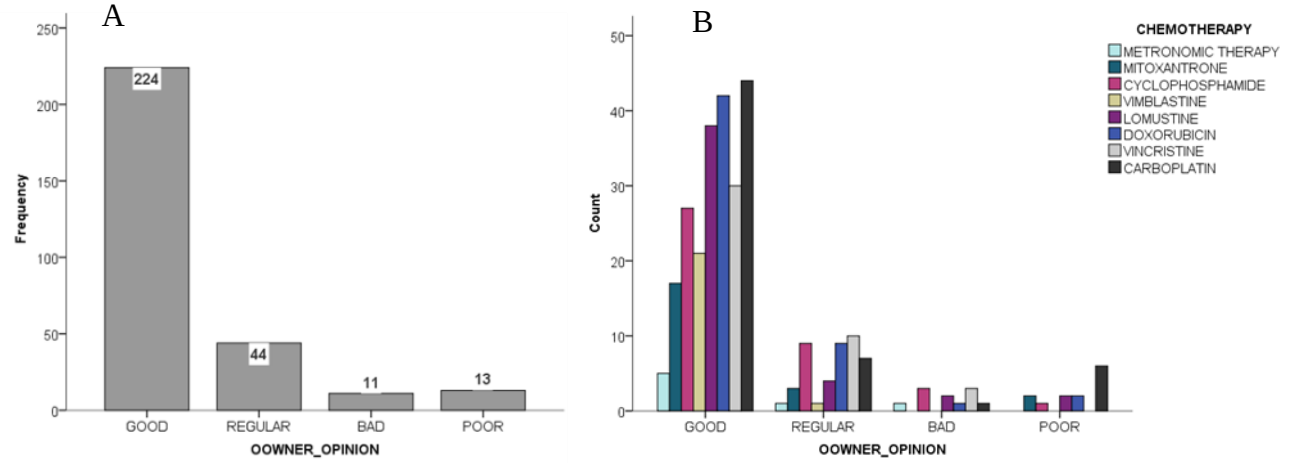

Figure 7. Owner's perception of chemotherapy in studied dogs. A: General owner perception. B: Owner perception according to chemotherapy

DISCUSSION

This retrospective study studied adverse effects of 292 dogs treated with chemotherapy. In a general manner, chemotherapy was very well tolerated in most dogs of this study. According to the current grading system of chemotherapy adverse effects of VCOG-CTCAE (Veterinary Co-operative Oncology Group, 2013), general tolerance to chemotherapy was at grade 1 in 83% dogs, which means most dogs had only mild, asymptomatic or mild symptoms, and medical intervention was not needed. In the previous studies with dogs and cats, authors believed that less than 1 in 4 animals will have an unpleasant adverse event following chemotherapy, and only approximately 3% to 5% have a serious adverse event leading to hospitalization (Bowles et al., 2010; Vols et al., 2016). In the present study, only 4%of the dogs had experienced serious and life threatening adverse events.

Cyclophosphamide in conventional therapy and vincristine were the agents that caused more gastrointestinal adverse events (vomiting, diarrhea and inappetence), while lomustine was the drug that caused more hematologic events (neutropenia, thrombocytopenia and hepatopathy). Carboplatin and doxorubicin lead to intermediate gastrointestinal and hematologic adverse events. Vimblastine was the agent that caused less adverse events. Cyclophosphamide is a nitrogen mustard, commonly included in multi-agent protocols for lymphoma in both dogs and cats (Gustafson and Page, 2013; Matsuyama et al., 2017). Even with antiemetic preventive administration, inappetence and nausea were observed in some dogs (24% and 33% respectively). For those dogs, maropitant was administered subcutaneously, together with oral ondasetron and omeprazol, and provided a good antiemetic effect, as previously reported by Mason et al. (2014). The

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high percentage of vomiting and diarrhea after vincristine administration was also observed in a study by Mason et al. (2014). Lomustine is a drug with known myelotoxicity (Burton et al., 2015), and in a previous study, 25% of dogs given compound lomustine (similarly to this study) had neutropenia (Burton et al., 2015).

Neutropenia, a major concern of chemotherapy, was observed in only 22% cases, most of them following lomustine administration. Carboplatin, doxorubicin, mitoxantrone and cyclophosphamide caused intermediate neutropenia in some dogs. According to Vail (2009), neutropenia is likely to be seen 7 to 10 days after the administration of most chemotherapy drugs. Exceptions to this rule includes vinblastine and paclitaxel, which can cause neutropenia as early as 4 to 5 days after administration, and carboplatin, which can occasionally cause neutropenia as late as 2 to 3 weeks after administration in dogs and cats. However, in this study, some dogs receiving lomustine and carboplatin had neutropenia (some of them severe) in the sixth day. Most companion animals have a low risk of infection if their neutrophil count remains greater than 1000/L. The severity of neutropenia and associated sepsis can be extremely variable, ranging from clinically silent to overwhelming and fatal (Vail, 2009).Sepsis occurred only in 4 animals but could have occurred in more cases if hematology was not performed in the 6-7 day of these chemotherapies.

Azotemia occurred in 3 dogs which had been previously diagnosed renal disease, and 2 had received carboplatin. This drug is considered nephrotoxic in dogs, and should have been avoided in these patients. However, it was considered for chemotherapy because it was the best oncologic treatment for these histologic types of tumor.

Elevation of ALT and/or ALP was observed 13/16 after lomustine administration. This result is similar to previous studies, which lomustine was considered to cause acute and chronic hepatotoxicity in dogs (Kristal et al., 2004).

Owner opinion was positive in most cases, and 77% of the owners evaluated that the treatment was well tolerated by their dogs. Bad and poor tolerance, and negative impact in quality of life, corresponded only to 8% of cases. This result agrees with other authors experience in dogs, where most canine patients tolerate chemotherapy very well (Bronden et al., 2003; Mellanby et al., 2003; Vail, 2009; Giuffrida and Kerrigan, 2014). In a study of the owners' perception about chemotherapy in dogs and cats, 62 of the 69 the owners thought that the anticancer chemotherapy was worthwhile for cats and dogs in general.

Chemotherapy was very tolerated well in most dogs, with positive owner opinion and minimal impact on the dog´s quality of life. This result may encourage veterinarians to perform chemotherapy in canine patients. The most common adverse effects were neutropenia (22%), vomiting (21%), diarrhea (20%) and in appetence (20%). Gastrointestinal adverse events must be prevented with antiemetic medication, especially in dogs receiving cyclophosphamide, vincristine, carboplatin and doxorubicin. Hematologic profile must be performed as early as in the 6-7th day after lomustine and carboplatin, as neutropenia can occur. Adverse chemotherapy effects may occur in about 20-25% of canine patients.

Competing of interests

The authors have no competing interests to declare.

Author`s contribution

The authors Simone Cunha and Katia Corgozinho were responsible for the clinical, oncological, and chemotherapeutic treatment of the cats, as well as the article writing. The authors Kassia Silva, Franciele Silva and Ana Ferreira performed the statistical analysis and review of the manuscript.

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Incubation Duration of Broiler Breeder Egg and Post Hatch Performance

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ABSTRACT

Incubation duration is a most essential factor to achieve standard hatchability, water loss and chick yield.Ross-308 different breeder flocks which were 42-46 weeks old and standard eggs that weighed up to 55-60g were selected. In the current study, two experimental groups each consisting of (n= 538560) eggs were selected to investigate the effect of eggs incubation duration on hatchability and post-hatch performance. Therefore, this experiment was conducted in order to evaluate the exact duration of incubation and its effects on broiler performance at the farm. Group A was incubated for 444hrs in setter and 62 hrs in hatcher. Hatch pulling for A was performed twice 1st after 494 hrs and remaining un-hatch eggs were again shifted to hatchers for next 12 hrs for 2nd pulling (conventional method of hatch pulling in Pakistan). For B hatch pulling was performed after 456hrs in setters and 50 hrs in hatcher, complete hatch pulling only once. Eggs weight at transfer was (53.9±0.8gm and 54.9 ±0.6gm), water loss at transfer was (11.67±0.7% and 10.6±0.7%) and chick weight was at (41.6±0.3gm and 42.7±0.3gm) on day one were significantly better for group B than A respectively. Similarly, hatchability (86.16±1.02% and 85.16±1.02) and dead in shell (5.10 ±0.8% and 6.61±1.5%) were also significantly better for group B then A respectively. Candling (8.23±0.9% and 8.23±1.33%) was same for both groups. Mortality was estimated to be at (1.80±0.06and 2.47±0.23%), weight gain was determined to be at (2001.33±24.33gram and 1955.66±25.02gram), feed intake (g/bird) was at (3245.02±18.03 and 3260.51±13.47) and feed conservation ratio at day 35(1.44±0.02 and 1.716±0.03) were found to have been significantly better for B than A respectively. So, incubation of eggs for 456 hrs in setters and 50 hrs in hatchers, along with single pull is better in term of water loss, chick yield, hatchability and post hatch performance.

Key words: Broiler, Chicks quality, Incubation duration, Post hatch performance

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INTRODUCTION

Poultry is the second largest industry in Pakistan, which plays a key role in the grass domestic production of country (Hussain et al., 2015). Poultry farming has been widely adopted in Pakistan and almost every farmstead keeps some poultry mainly for consumption and cash sales. The science and technology which have contributed widely to the expansion of poultry industry and a number of strategies have been adopted to modulate the quality of poultry products (Abel et al., 2014) Pakistan contain about 25000 poultry farms, about 400 hatcheries,150 feed mills, 8.5 million broiler breeders (Anonyms, 2011; FAO, 2011).

Hatching egg quality and incubation conditions influence broiler performance (Jabbar and Yousaf, 2017). The incubation period of chicken (*Gallus gallus*) embryo is approximately (506 hours) 21.08 days including drying down, and the gap among first to last chick hatch time is approximately 12 to 24 hours (Tong et al., 2013; Van de Ven et al., 2011). This time interval between the first and the last chick hatch is called "Hatch Window" (Molenaar et al., 2011). In commercial hatcheries incubation times of chicken is approximately 504 hours (Almeida et al., 2006). The large scales of chicks pulling have been extended up to 510 to 526 hours (Laughlin et al., 2007). Pulling of the chicks from hatchers is started when almost 90-95% chicks are complete dry. The incubation practices may also influence the length of

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incubation. Such as, storage temperature (Tona et al., 2003) and also incubator temperature (Yildirim et al., 2004), the position of eggs placement in tray (Van de Ven et al., 2011), turning and turning angle (Tona et al., 2005) are recorded with great care in terms of good hatchability. Gaseous exchange and CO_2 concentration also affect the hatchability (Everaert et al., 2007). Post hatching fasting impairs the chick's weight gain and breast muscles deposition capacity (Halevy et al., 2000). The current experiment was performed to find out the exact hatch time and its effect on broiler performance.

MATERIALS AND METHODS

Ethical approval

This experiment was performed according to all ethics and animal rights (University of veterinary and animal sciences).

Site selection

The study was carried out at Sadiq Poultry (Pvt) limited, Chakri Hatchery Rawalpindi which is situated five km from chakri interchange on motorway-2 (Islamabad to Lahore), Pakistan. The hatchery contains the latest in Heating Ventilation and Air Conditioning (HVAC) automation, having ISO (International Standard Organization) 1900-2000 certified. This is largest eggs capacity hatchery of south Asia which is producing the best quality of chicks through the single stage incubation system (Avida G4, Chick Master USA).

Selection of breeds

Eggs (55-60 g of weight) from broiler breeders (Ross-308, 45 weeks of age) were divided into two groups, such as A for twice pulling (conventional method) and B single pull.

Selection of eggs

Each experimental group was consisting of n=538560 eggs, which were graded upon their quality, poor shell, elongated eggs, cracks were isolated, only normal eggs (having 60-65 gram weight, good quality shell, oval shape without any crack and contamination) were set in the incubator machine Advida4 chick master USA having capacity of 1,34,640 eggs (Khan et al., 2016). These eggs were collected farm at 20°C and 75% relative humidity until used in hatching trial.

Eggs fumigation

Before being tested, trial eggs were fumigated with 20g $KMnO_4$and 40ml formalin (40%) and 40 ml of water for 100ft three areas for 15 minutes through automatic fumigation process provided by chick master.

Incubation programme

Both groups were pre-heated at 82^{o}F for five hours inside incubators. After completion of pre-warming the setter started automatically the incubation stage profile (Ross prime age recommended by Chicks Master USA).

Incubation durations in setters

Group A was incubated for 444 hrs in setter and group B was incubated for 456 hrs in separate setter. The eggs were then shifted to hatchers.

Egg's water loss

Before being transferred to the hatchers, water loss of both groups was measured. Water loss was measured for group A after 444 hrs, while for group B it was measured after 456 hours as given formula:

$$\text{Water Loss} \quad \frac{\text{full tray weight at setting (gm) - full tray weight at transfer (gm)} \times 100}{\text{Full tray weight at setting (gm)-empty tray weight (gm)}}$$

Candling

Eggs from group A were transferred to hatchers after 444 hrs of incubation in setter while eggs for group B were transferred to hatchers after 456 hrs of incubations. For both groups during transfer from setter to hatchers candling was performed through candling tables.

Incubation durations in hatchers

The duration of incubation in hatchers was short for A and B as compared to incubation duration in setters. For A it was 62 hrs and for B it was 50 hrs.

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Hatch pulling

Conventional hatch pulling. Hatch pulling for both groups was different. For group A hatch pulling was performed through the conventional method of hatch pulling in Pakistan. First pull at 494hours (444 in setters and 50 hrs in hatchers, for second hatch pull the remaining pips and eggs that had not hatched were again shifted to hatcher for next 12 hrs. After 12 hrs again pulling of un-hatched eggs from group A was performed.

Single hatch pulling. Group B was pulled out only one time after 506 hours (456 hrs in setters and 50 hrs in hatchers). Nothing was left behind inside hatchers. Hatch pull out was performed through shell separator (KUHL). Body weights of chick were determined immediately after chick collection. Single hatch pull after 506 hours was performed first time in Pakistan to best of our knowledge.

Chick grading. Grading of chicks was performed on the automatic conveyer, grading table. Only stranded (shining eyes, soft legs and nose, healed naval and healthy chicks) were shifted to chick's box after counting, while under weight, weak, and unhealed naval chicks were removed as international standard.

Chick yield measure. As for hatch out the chick's weight and yield was immediately measured through electrical weight balance by using following formula:

$$\text{Chick Yield} \quad \frac{\text{Weight of chicks (gm)}}{\text{Egg weight (gm)}} \times 100$$

Dead in shell analysis. To investigate the reason of embryo's mortality inside the eggs, dead in shell (DIS) analysis was necessary. For this purpose analysis of un-hatch eggs was performed (Table 3).

Delivery to poultry house

The total n=56,000 (A n=28000, B n=28000) day old chicks were send to Sadiq broiler farm Khilari-Chakri, Rawalpindi, Pakistan. Environmentally control vehicles (75 ^{0}F temperatures, 65% humidity) are used to deliver the chicks to control poultry house in the 102 chicks/box with dimensions of plastic chick's box (27×19.5×6.5 inches). Poultry house condition was remained same for both groups. At farm, chicks of both groups were offered water and feed *ad libitum*. Sadiq feed was offered to both groups. Feed with starter diets from 1 to 12 day (3020 Kcal ME/kg, 22% CP), grower diets from 13 to 22 day (3185 Kcal ME/kg, 20% CP) and finisher diets from 23 days to 35 days of age (3230 Kcal ME/kg, 18% CP). The diet was formulated according to the recommendations of the NRC (1994) using windows user-Friendly feed formulation (WUFFDA) software program. Intake of feed and water was record daily, while body weight and total feed consumed were recorded on a weekly basis. After 35 days trial the chick's weights were measured for both group. For ventilation Viper Touch (Big Dutchman, Co., Germany) system was installed (Table 1).

Statistical analyses

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). All means were compared using Duncan's Multiple Range test and results were presented as mean ± SEM (standard error of mean). Result were considered significant if $P<0.05$.

RESULTS

Initial eggs weight for both groups before setting was significantly same (same weight eggs were selected for both groups) ($P<0.05$) (60.2±0.7, 60.1±0.8gm). Eggs weight of group A at transfer was significantly high ($P<0.05$) (54.9 ±0.6gm) as compare to group B ($P<0.05$) (53.9±0.8gm) due to significantly more water loss from group B ($P<0.05$) (11.67±0.7%) compared to group A (10.6±0.7%). This water loss from eggs is major source of variation in chick quality and weight at day one. Chick weight of group A ($P<0.005$) (42.7±0.3gm) was significantly higher than group B ($P<0.05$) (41.6±0.7gm) due to high water loss from group B (Table 2). According to Tong et al. (2013) water loss for good quality chicks should be at11-12%.Water loss less than 11% causes ascites. So, group B which had been incubated for 456 hours in the setter was found to be better for water loss at transfer.

Hatchability was significantly ($P<0.05$) better for group B (86.16±1.02%) as compared to A (85.16±1.02%). Similarly, dead in shell was significant ($P<0.05$) better for group B (5.10±0.8) as compared to group A (6.61±1.5%) while Candling was same for both groups (Table 2).

Chicks from both groups were shifted to broiler farms into separate houses through environmental control vehicles. During the 35 days' trial period, mortality, feed intake, weight gain and FCR were recorded weekly and the results have been presented in table 3. Interestingly, the effect of 506 hours incubation and single hatch pulling on broilers performance was found better as compared to incubation of 506 hours and twice hatch pulling.

Feed conversion ratio (FCR) was found significantly better ($P<0.05$) in group B (1.44±0.02) then A (1.716±0.03). Feed intake (g/bird) was better for group B (3245.02±18.03) then A (3260.51±13.47). Weight gain was recorded to have

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been at maximum for group B (2001.33±24.33) as compare to A (1955.66±25.02) and mortality was reduced significantly (P<0.05) for B (1.80±0.06) as compare to A (2.47±0.23) (Table 3).

Table1. Poultry house condition at Sadiq broiler farm Khilari-Chakri Rawalpindi, Pakistan (February-May2016)

Parameters	1st Week	2nd Week	3rd Week	4th Week	5th Week
Temperature (^{0}F)	95-86	86-83	83-77	77-75	75
Humidity (%)	65	65	65	65	65
Ventilation (m^3/hour/bird)	0.07	0.25	0.40	0.59	0.87

Table 2. Effect of single and twice hatch pull on Mortality, Weight gain(g), Feed intake(g) and FCR at Sadiq Hatchery Chakri Rawalpindi, Pakistan (February to May 2016)

Parameters	A	B
Mortality %	2.47±0.23	1.80±0.06[b]
Weight gain (g)	1955.66±25.02[a]	2001.33±24.33[b]
Feed intake(g)	3260.51±13.47[a]	3245.02±18.03[b]
FCR	1.716±0.03[a]	1.44±0.02[b]

Table 3. Effect of single and twice hatch pull on Weight at setting, Egg weight at transfer , Water loss , Chick weight , Hatchability, Candling and Dead in shell at Sadiq Hatchery Chakri Rawalpindi, Pakistan (February to May 2016)

Parameters	A	B
Weight at setting (g)	60.2±0.7[a]	60.1±0.8[a]
Egg weight at transfer (g)	54.9 ±0.6[a]	53.9±0.8[b]
Water loss (%)	10.6±0.7[a]	11.67±0.7[b]
Chick weight (g)	42.7±0.3[a]	41.6±0.3[b]
Hatchability (%)	85.16±1.02[a]	86.16±1.02[b]
Candling (%)	8.23±1.33[a]	8.23±0.9[a]
DIS (%)	6.61±1.5[a]	5.10±0.8[b]

DIS: dead in shell

DISCUSSION

It is a common practice in Pakistan for the first hatch pulling to be done first after 494 hours then the remaining un-hatch eggs were shifted again to same hatchers for next 12 hours for second hatch pull or balance. First time in Pakistan single pull at international standard (hatch pull only once after 506 hours) was performed and better results were achieved. Transfer of both groups from the setter to the hatcher was different. Transfer after 456hours was found to be better due to proper water loss (Table 3).

11-12% water loss is standard to achieve better chick quality and carcass yield (Tong et al., 2013). Proper water loss helps to avoid dehydration during the transportation of chicks to farm. Pulling of chicks for a second time disturbs the temperature and humidity for chicks which are under process of hatching becomes a source of increased dead in shell (DIS) (Van de Ven et al., 2011).

Pulling of chicks after complete incubation duration improves proper grading and post hatch performance (Yousaf, 2017). Dead in shell analysis also indicate that early (0-7days), mid (8-14 days) and late (15-21 days) embryonic mortality were fond better in group B (Figure 1). The chicks produced through proper incubation have better ability to perform at the farm table 3. So, it's better to incubate eggs for 456 hours in setter and 50 hours in hatcher and single hatch pull after 506 hours to get better water loss, hatchability, dead in shell (DIS) and post hatch performance.

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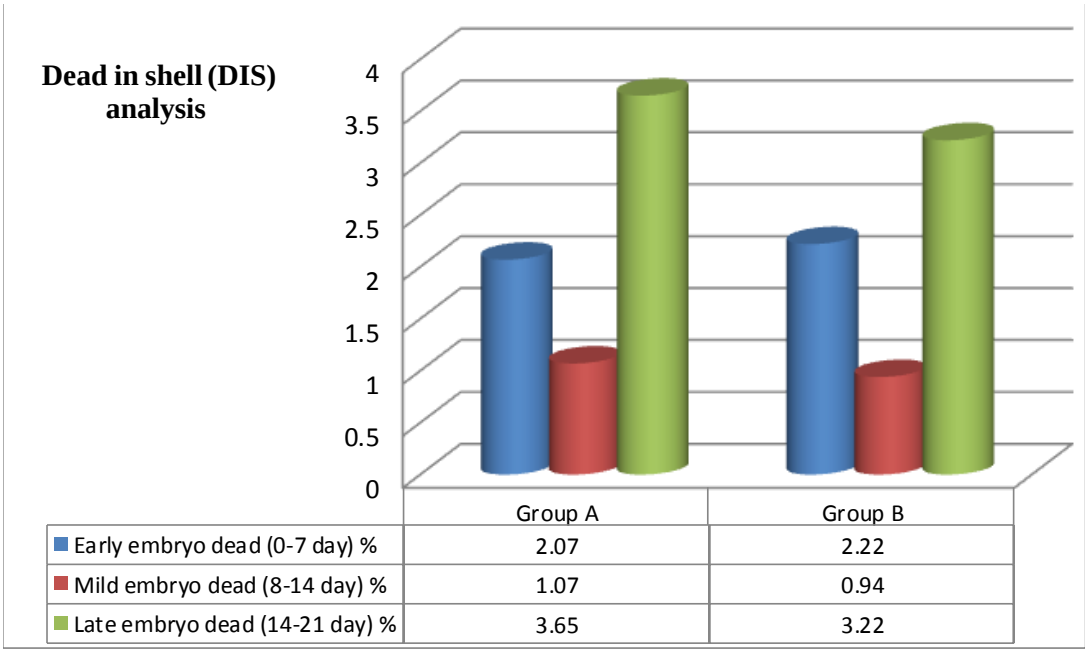

Figure 1. Effect of single and twice hatch pull on early, mid and late embryonic mortality at Sadiq Hatchery Chakri Rawalpindi Pakistan (February to May, 2016)

CONCLUSION

In summary, the findings of current study tended to show that full time 506 hours incubation and single hatch pulling to broiler breeder eggs provides better quality chicks and enhances the post hatch performance.

Author's contribution

Dr. Adnan Jabbar Ansari was main author responsible for tabulation of experimental data and article writing. Dr. Yasir Allah Ditta helped in statistical application while Dr. Adnan Yousaf helped in write up.

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Conflict of interest

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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Haematological and Biochemical Changes in Nigerian Dogs with Short Bowel Syndrome

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ABSTRACT

The purpose of this study was to evaluate the haematological and biochemical changes in Nigerian dogs with short bowel syndrome. Thirty adult dogs each weighing approximately 12.4kg (range 7-18kg) were used in this study. The dogs were randomized into five groups of six dogs each. Group 1 is the control group. The dogs here were not placed on any treatment. Group 2 dogs were supplemented with glutamine. Group 3 dogs were supplemented with honey. Group 4 dogs were supplemented with ascorbic acid and group 5 dogs were supplemented with glutamine, honey and ascorbic acid combination. Haematological parameters, serum electrolytes (Sodium, potassium, bicarbonate and, chloride) and enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) were also evaluated. There was no depletion in sodium, potassium, bicarbonate and chloride in all the animals as the value of these electrolytes had remained at normal range in all five groups. There was a significant decrease in the value of alkaline phosphatase in the five groups and non significant changes in the value of alanine aminotransferase in all the animals. It was therefore, concluded that patients with resection of proximal small intestinal tract have better chances of survival than patients with a resected distal small intestinal tract.

Keywords: Adaptation, Alanine aminotransferase, Electrolytes, Haematology, Alkaline phosphatase, Short bowel syndrome

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INTRODUCTION

Patients with short bowel syndrome experience changes in physiological variables until the adaptation of the remnant small intestinal length takes place during which the digestive and absorptive capability of the residual bowel length improves (Ziegler et al., 2002; Cisler and Buchman., 2005; Efsen and Jeppesen., 2011; Rowland et al., 2012; Herath and Kulatunga, 2017).This happen in patients that have reason to undergo extensive small intestinal resection that will resects over 50% of the small intestine leaving the patient with less absorptive surface area that cannot support or meet the nutritional requirement of such patients (Sukhotnik et al., 2004; Gorman et al., 2006; Shaw et al., 2012). These patients experience nutritional malabsorption leading to malnutrition, diarrhea, steatorrhea, fluid and electrolyte imbalance and specific nutrient imbalance (Sundaram et al., 2002; Donohoe and Reynolds, 2010; Thompson et al., 2012; Cunha-melo and Costa, 2014). These patients therefore, require total parenteral nutrition (TPN) for survival (Messing et al., 2006; Joly et al., 2009; Han et al., 2015; Mayer and Kerner, 2017).

The severity of these signs depends on the remnant small bowel length, part or section of the intestine that was resected and the presence or absence of the colon and ileocecal valve (Sundaram et al., 2002; Storch, 2014). The amount of small intestinal absorptive surface area required for the survival of patients with short bowel syndrome varies between individuals (Liu et al., 2014; Tappendene, 2014). This study evaluates the nature and the types of physiological changesthat present themselves in Nigerian dogs with short bowel syndrome.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the ethical committee of the college of veterinary medicine, university of Agriculture, makurdi, Nigeria with reference no.001.

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Experimental Animals

Thirty apparently healthy dogs with average age of 15 months (range 6-24 month) and approximate mean weight of 12.4kg (range 7-18kg) were used for this study. The dogs were bought from breeders and on arrival each dog was subjected to clinical evaluation where vital parameters (temperature, pulse and respiratory rates), blood and faecal samples were evaluated. They were dewormed and those with ectoparasites were treated. The dogs were stabilized for 4 weeks by being boarded in kennels within the veterinary teaching hospital. They were fed daily and water was provided *ad libitum*. Each animal was fasted for 12 hours prior to surgery. They were premedicated with Atropine sulphate (Jiangsu Huayang pharmaceutical, China) at a dose rate of 0.04mg/kg and xylazine hydrochloride (XYL-M2®, VMD, Belgium) at a dose rate of 1mg/kg body weight intramuscularly. Induction was done with thiopentone sodium (Rotexmedica, Germany) at a dose of 10mg/kg body weight intravenously.

Surgical procedure

Each animal was aseptically prepared and a ventral midline abdominal incision was made. The intestinal tract was exteriorized. A sterile drip infusion set with both end cut was used to measure the small intestinal length insitu. The measurement was done beginning from the duodenum, just at the distal end of the pancreas to the ileocolic junction. After each measurement, the drip set used was placed on a sterile calibrated ruler and the value of each measurement was determined in centimetres (cm) and recorded. Four measurements were done in each animal and the average intestinal length was determined for each animal. The crown-rump length of each dog was also measured and the average value for each determined. The average small intestinal length was divided by the average crown-rump length to get 3.4cm as the proportion. The crown-rump length value obtained was multiplied by 3.4 cm (mean of index of small intestinal length) and this gave the average total intact small intestinal length in each dog. This was then recorded. Seventy (70%) per cent of the small intestinal tract was resectioned from a point 7cm from the duoduno-jejunal flexure (treitz ligament). The residual intestinal tract was sutured using end to end anastomosis with polyglactin 910 (vicryl® Ethicon, USA) size "0" using horizontal mattress suture pattern. A full thickness biopsy sample of the small intestinal tract (Jejunum and ileum) were collected and fixed in 10% formalin (pretreatment sample). Two mls of normal saline was injected tangentially close to the anastomostic site to check for leakage and patency. Viability was assessed using arterial pulsations, peristalsis and colour. The anastomostic site was covered with omentum and then returned to the abdominal cavity. The abdominal incision was closed using a standard surgical technique (Fossum, 2014). Procain penicillin (Shuazhuang co ltd, China) (20,000 iu/kg) and Streptomycin (North China pharmaceutical co ltd, China) (10 mg/kg) was administered intramuscularly for five days post operation. Pentazocin (Bharat Parenterals ltd, India) was administered intramuscularly at the dose rate of 3mg/kg for seven days to relieve pain.

Blood samples were collected from the animals post-operatively on days 4, 6, 8, 10 and 12 for Complete Blood Count (CBC) and blood serum chemistry (AST, ALT, ALP, Na, K, Cl and HCO_3 ions) analysis using the haemocytometric and flame photometric methods respectively.

The dogs were given 5% dextrose infusion intravenously at 10mls/kg/hr on the second and third day post operation. They were fed bland diet gruel on the fourth post operation day and were then returned healthy to normal solid diet on day five post surgery.

Statistical analysis

Data were expressed as descriptive statistics. Differences among the groups were evaluated using one way analysis of variance (ANOVA) followed by a two tailed student's t-test. P values ≤ 0.05 were considered statistically significant.

RESULTS

In group 1 (control), the Red Blood Cells (RBC), Haemoglobin (Hg), White Blood Cells (WBC), Monocytes (MON), Granulocytes (GNC), Mean Corpuscular Volume (MCV), Red cell Distribution Width (RDW), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) all had shown (non-statistically) significant decrease while lymphocytes (LYM), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet Crit (PCT) and Platelets (PLT) had also shown (non-statistically) significant increase from their base line values 12 days post surgery (Table 1).

In group 2, PCV, Hg, RBC all had shown a statistically significant decrease. WBC and GNC showed statistically significant increase. MCV, PCT and PDW all had shown anon statistically significant increase (Table 2). In group 3, RBC, MON, MCV, MCH, and RDW showed no statistically significant decrease. PCV, WBC, LYM and GNC showed statistically significant decrease. MCHC, PLT, MPV and PDW had shown no statistically significant increase (Table 3). In group 4, RBC, PCV, Hg, MON, GNC, MCV, MCH, RDW, MPV and PDW showed no statistically significant decrease while WBC, LYM, GNC, MCHC had shown (non-statistically) significant increase. PLT and PCT showed statistically significant increase (Table 4). In group 5, RBC, PCV, Hg, MCV, MCH, MPV and PDW had shown (non-

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significant) decrease. PLT and PCT showed significant increase while WBC, LYM, GNC, MCHC and RDW had shown an insignificant increase (Table 5).

There was no statistically significant difference in the values of sodium in group 1,2,3,4 and 5. The value of sodium in group 3 was significant. There was no statistically significant difference in the values of potassium in groups 1,2,3,4 and 5. There was no statistically significant difference in the values of chloride in groups 1, 3 and 5. The values in group 2 and 4 are significant. There was no statistically significant difference in the values of bicarbonate in groups 1 and 3 while the values in groups 2, 4 and 5 were significant.

There was no statistically significant difference in the values of Alkaline Phosphatase (ALP) in groups 1, 4 and 5. The values for groups 2 and 3 are significant. There was no statistically significant difference in the values of Aspartate Amino Transferase (AST) in groups 1, 3 and 4 while the values for groups 2 and 5 were significant. There was no statistically significant difference in the values of Alanine Aminotransferase (ALT) in groups 1, 2, 3, 4 and 5.

Table 1. Haematology of six dogs with post 70% small intestinal resection and anastomosis.

Parameter	Mean (Days)						Min-Max	Overall Mean ± SEM	P-value
	0	4	6	8	10	12			
RBC %	6.23	5.6	4.9	5.3	5.2	5.1	4.9-6.23	5.39±0.19	0.35
PCV (%)	44.48	40.5	33.4	37.3	36.1	34.8	33.4-44.48	37.76±1.67	0.27
HB (g / dl)	16.58	16.1	15.1	13.8	13.7	13.9	13.7-16.58	14.86±0.51	0.06
WBC (x 103/L)	16.85	21.3	17.9	15.0	13.6	14.7	13.6-21.3	16.56±1.14	0.53
LYM ($x10^3$)	3.35	6.5	29.7	4.5	3.5	4.4	3.35-29.7	8.66±4.23	0.33
MON ($x10^3$)	1.58	2.6	1.5	1.6	1.2	1.1	1.1-2.6	1.60±0.22	0.11
GNC ($x10^3$)	11.92	12.3	13.7	8.9	8.9	9.2	8.9-13.7	10.82±0.85	0.74
MCV (fl)	71.33	68.8	64.2	70.8	69.5	68.1	64.2-1.33	68.78±1.04	0.76
MCH (pg)	26.03	50.5	37.3	26.3	26.6	27.4	26.03-50.5	32.36±4.03	0.51
MCHC (g/dl)	36.50	42.3	87.8	37.1	38.7	40.1	36.50-87.8	47.08±8.19	0.46
RDW (%)	15.53	18.1	18.2	15.4	15.5	15.3	15.3-18.2	16.34±0.57	0.39
PLT (ml)	261.83	387.5	324.8	285.2	348	368	261.83-387.5	329.22±19.79	0.88
PCT (%)	0.22	0.4	0.4	0.3	0.3	0.3	0.22-0.4	0.32±0.03	0.66
MPV(fl)	8.12	9.2	8.0	8.2	8.5	8.3	8.12-9.2	8.39±0.18	0.52
PDW(gsd)	13.1	124.8	124.4	12.3	12.9	12.2	12.2-124.8	49.95±23.61	0.56

RBC: Red blood cell; PCV: Packed cell volume; HB: Haemoglobin; WBC: White blood cell count; LYM: Lymphocytes; MON: Monocytes; GNC: Granulocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelets PCT: Platelet crit; MPV: Mean platelet volume; PDW: Platelet distribution width

Table 2. Haematology of six dogs with post 70% small intestinal resection and anastomosis and treated with glutamine

Parameter	Mean (Days)						Min – Max	Overall Mean ± SEM	P-value
	0	4	6	8	10	12			
RBC	5.8	5.2	5.2	4.6	4.8	4.5	4.5-5.8	5.02±0.20	0.01
PCV (%)	40.2	38.9	34.6	32.1	35.3	34.7	32.1-40.2	35.97±1.23	0.02
HB (g / dl)	14.4	12.9	12.6	10.8	11.8	10.7	10.8-14.4	12.20±0.57	0.03
WBC (x 103/L)	10.4	18.7	14.0	14.5	14.1	11.6	10.4-18.7	13.88±1.17	0.01
LYM ($x10^3$)	4.6	3.2	3.4	2.9	2.3	2.5	2.3-4.6	3.15±0.34	0.07
MON ($x10^3$)	1.1	1.2	1.5	1.2	1.1	1.0	1.0-1.5	1.18±0.07	0.01
GNC($x10^3$)	6.4	14.3	9.1	10.5	10.8	8.1	6.4-14.3	9.87±1.11	0.02
MCV(fl)	71.7	68	66.7	73.1	78.0	82.0	66.7-82.0	73.25±2.40	0.15
MCH(pg)	24.9	25.1	24	23.6	24.2	23.8	23.6-25.1	24.27±0.25	0.77
MCHC(g/dl)	35.7	38.3	36.4	33.7	33.2	31.3	31.3-38.3	34.77±1.03	0.21
RDW (%)	15.6	15.1	15.1	15.2	15.4	15.5	15.1-15.6	15.32±0.09	0.24
PLT(ml)	234	272.8	357.5	183.3	201	187.7	187.7-357.5	239.38±27.32	0.56
PCT (%)	0.2	0.22	0.21	0.17	0.26	0.22	0.17-0.26	0.21±0.01	0.81
MPV(fl)	9.1	9.2	9.6	9.6	10	8.9	8.9-10	9.4±0.17	0.70
PDW(gsd)	11.5	30.9	15.4	14.1	15.4	13.5	11.5-30.9	17.8±2.83	0.45

RBC: Red blood cell; PCV: Packed cell volume; HB: Haemoglobin; WBC: White blood cell count; LYM: Lymphocytes; MON: Monocytes; GNC: Granulocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC:Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT:Platelets; PCT: Platelet crit; MPV: Mean platelet volume; PDW: Platelet distribution width

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Table 3. Haematology of six dogs with post 70% small intestinal resection and anastomosis and treated with honey

Parameters	Mean (Days)						Min-Max	Overall Mean ± SEM	P-value
	0	4	6	8	10	12			
RBC %	6.9	5.6	5.5	5.4	5.2	5.6	5.2-6.9	5.70±0.25	0.47
PCV (%)	39.9	37.4	35.0	34.1	32.7	35.9	32.7-39.9	35.83±1.04	0.01
HB (g/dl)	13.9	13.6	13.4	12.8	12.4	13.2	12.4-13.9	13.22±0.22	0.55
WBC (x 10^3/L)	17.3	20.3	21.9	13.6	20.3	12.5	12.5-21.9	17.65±1.58	0.001
LYM (x$10^{3)}$	4.4	6.0	4.9	3.8	2.9	2.5	2.5-6.0	4.08±0.53	0.001
MON (x10^3)	1.8	1.6	1.6	0.9	1.0	0.7	0.7-1.8	1.27±0.19	0.65
GNC (x$10^{3)}$	11.1	12.7	15.4	8.9	7.9	9.3	7.9-15.4	10.88±1.14	0.00
MCV (fl)	67.7	66.8	63.4	63.2	62.4	64.3	62.4-67.7	64.63±0.87	0.17
MCH (pg)	23.7	24.2	24.2	23.3	23.7	23.5	23.3-24.2	23.77±0.15	0.92
MCHC (g/dl)	32.8	34.4	36.2	35.3	35.7	34.1	32.8-36.2	34.75±0.51	0.95
RDW (%)	16.0	15.8	15.8	16.2	15.8	15.7	15.7-16.2	15.88±0.08	0.96
PLT (ml)	258	384	316	275.7	270.8	267.7	258-384	295.37±19.5	0.51
PCT (%)	0.2	0.3	0.3	0.2	0.2	0.2	0.2-0.3	0.23±0.02	0.25
MPV(fl)	8.2	8.5	8.6	8.1	8.2	8.4	8.1-8.6	8.33±0.08	0.73
PDW (gsd)	12.1	11.8	12.9	12.7	12.6	13.4	12.1-13.4	12.58±0.23	0.79

RBC: Red blood cell; PCV: Packed cell volume; HB: Haemoglobin; WBC: White blood cell count; LYM: Lymphocytes; MON: Monocytes; GNC: Granulocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC:Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelets PCT: Platelet crit; MPV: Mean platelet volume; PDW: Platelet distribution width

Table 4. Haematology of six dogs with70% small intestinal resection and anastomosis and treated with ascorbic acid

Parameters	Mean (Days)						Min-Max	Overall Mean±SEM	P-value
	0	4	6	8	10	12			
RBC (%)	5.8	5.9	5.6	5.7	5.6	5.6	5.6-5.9	5.70±0.05	0.79
PCV (%)	38.0	40.1	38.5	36.3	35.5	35.8	35.5-40.1	37.37±0.74	0.51
HB (g/dl)	13.8	14.2	13.2	13.5	13.2	13.3	13.2-14.2	13.53±1.16	0.87
WBC (x103/L)	100.9	115.9	129.7	114.4	89.1	108.9	89.1-129.7	109.82±5.67	0.28
LYM (x103)	28.1	23.4	25.7	27.8	20.5	29.2	20.5-29.2	25.78±3.31	0.48
MON (x103)	1.6	1.3	1.4	1.2	4.1	1.2	1.2-4.1	1.80±0.45	0.08
GNC (x103)	10.6	14.2	15.9	13.3	9.9	12.1	9.9-15.9	12.67±0.92	0.17
MCV (fl)	89.9	66.7	68.5	64.3	65.1	65.2	64.3-89.9	69.95±4.04	0.88
MCH (pg)	23.9	23.8	23.6	23.7	23.6	23.9	23.6-23.9	23.75±0.06	0.88
MCHC (g/dl)	35.3	35.9	34.9	37.2	36.8	37.1	34.9-37.2	36.2±0.40	0.88
RDW (%)	17.6	17.4	17.2	17.6	17.2	17.1	17.1-17.6	17.35±0.09	0.99
PLT (ml)	204.8	294	377.2	442.7	421.5	402	204.8-442.7	357.03±37.0	0.00
PCT (%)	0.02	0.24	0.28	0.33	0.32	0.32	0.02-0.33	0.25±0.05	0.00
MPV (fl)	7.8	7.9	7.4	7.4	7.3	7.6	7.3-7.9	7.57±0.10	0.49
PDW (fl)	10.5	10.4	10.0	9.6	10.1	9.6	9.6-10.5	10.03±0.16	0.88

RBC: Red blood cell; PCV: Packed cell volume; HB: Haemoglobin; WBC: White blood cell count; LYM: Lymphocytes; MON: Monocytes; GNC: Granulocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC:Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelets PCT: Platelet crit; MPV: Mean platelet volume; PDW: Platelet distribution width

Table 5. Haematology of six dogs with post 70% small intestinal resection and anastomosis and treated with glutamine/ honey/ ascorbic acid

Parameter	Mean (Days)						Min – Max	Overall Mean ± SEM	P-value
	0	4	6	8	10	12			
RBC %	6.2	5.6	5.3	5.1	6.2	5.1	5.1-6.2	5.58±0.21	0.03
PCV (%)	39.2	34.8	33.1	32.3	33.2	33.7	32.3-39.2	34.38±1.02	0.13
HB (g / dl)	14.8	13.2	12.8	12.6	12.7	12.4	12.4-14.8	13.08±0.36	0.63
WBC (x 10^3/L)	14.8	19.4	15.1	14.4	14.3	12.7	12.7-19.4	15.12±0.92	0.03
LYM (x10^3)	3.2	4.2	3.3	2.9	1.9	2.3	1.9-4.2	2.97±0.33	0.04
MON (x10^3)	1.4	1.3	1.1	1.0	0.7	0.9	0.7-1.4	1.07±0.11	0.21
GNC(x10^3)	10.0	13.9	10.8	10.1	11.7	9.5	9.5-13.9	11.0±0.66	0.14
MCV(fl)	63.4	62	63.6	63.7	63.4	67.3	62-67.3	63.90±0.73	0.98
MCH(pg)	23.7	23.5	24.8	24.8	24.3	24.7	23.5-24.8	24.30±0.24	0.99
MCHC(g/dl)	37.6	37.6	38.5	38.8	38.0	36.6	36.6-38.8	37.85±0.32	0.86
RDW (%)	16.3	16.5	16.7	16.9	16.7	16.8	16.3-16.9	16.65±0.09	0.99
PLT(ml)	151.7	169.7	244.7	264.3	252.2	211.7	151.7-264.3	215.72±18.94	0.11
PCT (%)	0.12	0.14	0.20	0.22	0.21	0.18	0.12-0.22	0.18±0.02	0.15
MPV(fl)	8.1	8.0	8.2	8.4	8.2	9.7	8.0-9.7	8.43±0.26	0.33
PDW(fl)	12.4	121.7	13.4	125.6	12.7	125.8	12.4-125.8	68.60±24.95	0.02

RBC: Red blood cell; PCV: Packed cell volume; HB: Haemoglobin; WBC: White blood cell count; LYM: Lymphocytes; MON: Monocytes; GNC: Granulocytes; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC:Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelets PCT: Platelet crit; MPV: Mean platelet volume; PDW: Platelet distribution width

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Table 6. Serum biochemistry values of dogs post 70% small intestinal resection and anastomosis

Substance	Days	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Sodium	0	138.6	140.0	140.0	133.0	132.2	142.0	137.63±1.66	0.33
	4	140.0	137.2	138.6	138.6	129.0	138.9	137.05±1.65	
	6	135.8	126.0	137.2	137.2	122.0	137.6	132.6±2.79	
	8	130.2	137.2	135.8	140.0	130.2	138.6	135.33±1.72	
	10	137.2	187.6	134.9	138.8	131.1	138.4	144.67±8.66	
	12	126.0	134.4	133.0	137.2	131.7	137.7	133.33±1.75	
Potassium	0	3.8	4.2	3.2	3.6	4.2	4.0	3.83±0.16	0.27
	4	4.3	4.1	3.1	3.4	4.0	3.8	3.78±0.19	
	6	4.3	2.3	3.0	3.3	3.6	3.8	3.38±0.28	
	8	4.2	4.6	3.0	2.7	3.8	4.0	3.72±0.30	
	10	4.8	4.4	3.0	3.0	4.0	4.2	3.90±0.30	
	12	2.3	2.8	3.0	3.0	4.0	3.8	3.15±0.26	
Chloride	0	100	88.5	100	100	102.4	103.2	99.02±2.18	0.30
	4	88.5	92.3	92.3	103.8	96.1	98.1	95.18±2.20	
	6	88.5	100	100	100	88.2	89.6	94.38±2.52	
	8	103.8	103.8	107.9	96.2	94.3	96.2	100.37±2.25	
	10	100	100	121.2	100	97.1	100	103.05±3.67	
	12	92.3	103.8	123.1	92.3	97.3	100	101.47±4.69	
Bicarbonate	0	21	25	24	25	25	24	24.00±0.63	0.18
	4	24	24	22	28	23	23	24.00±0.86	
	6	23	23	22	24	22	23	22.83±0.31	
	8	21	25	23	23	24	23	23.17±0.54	
	10	26	28	24	28	24	22	25.33±0.99	
	12	22	21	26	22	24	24	23.17±0.75	
ALP	0	23.1	24.1	36.1	36.2	37.1	32.3	31.48±2.59	0.31
	4	23.0	25.7	56.0	48.1	44.4	32.6	38.30±5.39	
	6	18.9	24.1	46.3	38.5	40.1	40.1	34.67±4.36	
	8	24.5	20.7	34.1	32.0	34.2	38.3	30.63±2.72	
	10	30.2	27.6	42.2	27.4	28.3	34.1	31.63±2.35	
	12	12.0	29.3	34.4	28.1	24.2	30.3	26.38±3.18	
AST	0	61	23	62	53	59	63	53.50±6.27	0.82
	4	39	35	32	81	36	48	45.17±7.51	
	6	28	41	99	16	64	45	48.83±12.02	
	8	93	38	74	19	62	40	54.33±11.05	
	10	15	29	98	38	49	36	44.17±11.70	
	12	19	44	255	29	48	58	75.50±36.35	
ALT	0	36	67	38	28	40	43	42.00±5.41	0.58
	4	18	48	48	41	38	40	38.83±4.50	
	6	47	48	35	27	38	38	38.83±3.20	
	8	51	42	45	10	45	41	39.00±5.97	
	10	52	29	45	39	48	50	43.83±3.50	
	12	48	58	45	43	48	48	48.33±2.11	

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

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Table 7. Serum biochemistry values of dogs with post 70% small intestinal resection and anastomosis and supplemented with glutamine

Substance	Days	Number of dogs (n = 6)						Mean ±SEM	P value
		1	2	3	4	5	6		
Sodium	0	141.4	135.8	138.0	135.7	140.0	144.0	139.15±1.34	0.14
	4	140.0	182.0	137.6	148.9	138.6	142.6	148.28±6.94	
	6	138.6	190.4	138.2	157.3	133.7	141.2	149.90±8.75	
	8	133.0	135.8	136.0	135.7	135.9	135.6	135.33±0.47	
	10	135.8	131.6	138.0	131.5	137.1	138.4	135.40±1.27	
	12	137.0	144.2	136.0	144.1	139.1	139.6	140.0±1.42	
Potassium	0	4.3	3.8	4.2	3.9	4.0	4.2	4.07±0.08	0.18
	4	3.9	4.0	3.8	4.1	3.6	3.8	3.87±0.07	
	6	3.8	4.4	3.6	4.5	3.5	3.7	3.92±0.17	
	8	4.1	2.7	4.0	2.8	3.8	4.0	3.57±0.26	
	10	4.5	3.0	4.4	3.1	3.9	4.3	3.87±0.27	
	12	4.5	4.0	4.4	4.1	4.2	4.3	4.25±0.08	
Chloride	0	92.3	103.8	100	88.3	103.7	102.0	98.35±2.66	0.001
	4	92.3	84.6	100	88.1	84.5	83.4	88.82±2.60	
	6	107.7	96.2	115.2	105.2	95.3	94.3	102.32±3.44	
	8	76.9	92.3	88.2	78.6	91.4	93.3	86.78±2.95	
	10	96.2	107.7	98.1	98.3	106.6	103.1	101.67±1.97	
	12	106.4	96.1	107.3	108.6	97.2	98.2	102.30±2.33	
Bicarbonate	0	22	23	22	23	24	22	22.67±0.33	0.003
	4	24	23	24	23	23	22	23.17±0.31	
	6	23	26	23	25	26	23	24.33±0.61	
	8	23	24	23	24	24	23	23.50±0.22	
	10	26	20	24	22	24	23	23.33±0.84	
	12	26	26	24	26	26	26	25.67±0.33	
ALP	0	18.7	18.9	21.2	21.7	17.6	38.3	22.73±3.18	0.0001
	4	30.1	29.3	32.3	32.1	31.2	42.3	32.88±1.94	
	6	43.3	43.1	45.4	45.3	45.1	34.6	42.80±1.69	
	8	22.4	22.4	23.1	24.6	23.2	20.2	22.65±0.59	
	10	31.6	18.9	32.7	21.7	32.4	26.1	27.23±2.43	
	12	36.2	25.8	37.1	27.8	38.1	31.6	32.77±2.11	
AST	0	27	21	27	25	26	32	26.33±1.45	0.0001
	4	59	62	58	66	58	56	59.83±1.47	
	6	37	45	37	49	36	36	40.00±2.28	
	8	50	50	49	54	49	55	51.17±1.08	
	10	51	54	52	58	50	57	53.67±1.33	
	12	53	46	54	50	52	60	52.50±1.89	
ALT	0	42	44	45	46	41	48	44.33±1.05	0.15
	4	162	42	43	44	158	54	83.83±24.16	
	6	18	53	54	55	20	65	44.17±8.15	
	8	49	32	31	34	50	44	40.00±3.55	
	10	100	37	39	39	101	49	60.83±12.66	
	12	86	42	43	44	88	56	59.83±8.84	

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

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Table 8. Serum biochemistry values of dogs with post 70% small intestinal resection and anastomosis and supplemented with honey

Substance	Days	Number of dogs (n = 6)						Mean ±SEM	P-value
		1	2	3	4	5	6		
Sodium	0	133.0	144.2	133.0	140.0	134.0	140.0	137.37±1.92	0.04
	4	133.0	137.2	133.0	138.0	136.2	137.0	135.80±0.93	
	6	130.2	140.0	138.6	138.0	138.0	134.0	136.47±1.50	
	8	140.0	131.6	133.0	136.0	138.0	138.0	136.10±1.32	
	10	135.8	126.0	134.4	137.0	139.0	137.8	135.00±1.91	
	12	186.2	140.0	196.0	138.2	134.0	140.2	155.77±11.28	
Potassium	0	4.2	3.7	3.8	3.8	4.2	4.3	4.00± 0.11	0.55
	4	4.8	3.7	4.5	4.0	4.6	4.1	4.28±0.17	
	6	4.3	4.5	4.8	3.8	4.3	3.8	4.25±0.16	
	8	4.3	4.4	3.0	3.7	4.3	4.0	3.95±0.22	
	10	4.6	3.0	4.8	4.0	4.4	4.2	4.17±0.26	
	12	4.2	4.4	4.8	4.2	4.3	4.2	4.35±0.10	
Chloride	0	88.5	111.5	88.5	100	111.2	98.1	99.63±4.18	0.35
	4	92.3	96.2	92.3	92.2	98.3	93.6	94.15±1.04	
	6	84.6	92.3	80.8	88.3	94.1	100.2	90.05±2.84	
	8	92.3	88.5	92.3	88.1	88.3	99.1	91.43±1.73	
	10	111.5	84.6	100	84.2	84.2	98.3	93.80±4.62	
	12	88.5	103.8	96.2	84.2	107.1	103.6	97.23±3.78	
Bicarbonate	0	24	22	23	23	24	23	23.17±0.31	0.06
	4	24	22	25	23	23	21	23.00±0.58	
	6	20	22	22	20	22	21	21.17±0.40	
	8	24	28	22	24	22	21	23.50±1.02	
	10	22	26	22	21	22	23	22.67±0.71	
	12	24	28	24	23	24	23	24.33±0.76	
ALP	0	38	34.1	45	38	46	36.2	39.55±1.98	0.0001
	4	41.7	43.5	36.6	43.8	40.1	45.4	41.85±1.29	
	6	31.6	21.2	32.0	33.5	36.2	29.6	30.68±2.10	
	8	13.8	18.9	18.9	15.4	32.1	26.7	20.97±2.87	
	10	15.5	13.8	25.9	17.4	18.3	26.4	19.55±2.18	
	12	17.2	13.8	50	17.8	38	27.2	27.33±5.79	
AST	0	26	95	32	28	34	62	46.17±11.14	0.75
	4	36	38	33	36	36	53	38.67±2.94	
	6	32	26	49	32	47	36	37.00±3.72	
	8	25	29	41	27	40	66	38.00±6.24	
	10	22	28	39	22	39	82	38.67±9.21	
	12	55	22	82	53	60	32	50.67±8.69	
ALT	0	38	165	41	41	42	44	61.83±20.65	0.68
	4	41	31	41	44	41	48	41.00±2.29	
	6	35	42	41	37	42	38	39.17±1.19	
	8	32	108	27	34	28	35	44.00±12.87	
	10	29	36	55	31	43	32	37.67±4.01	
	12	35	31	121	37	41	42	51.17±14.02	

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

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Table 9. Serum biochemistry values of dogs with post 70% small intestinal resection and anastomosis and supplemented with ascorbic acid

Substance	Days	Number of dogs (n = 6)						Mean ±SEM	P-value
		1	2	3	4	5	6		
Sodium	0	133.0	144.2	133.0	140.0	134.0	140.0	146.90±9.96	0.79
	4	133.0	137.2	133.0	138.0	136.2	137.0	135.57±1.50	
	6	130.2	140.0	138.6	138.0	138.0	134.0	147.17±8.99	
	8	140.0	131.6	133.0	136.0	138.0	138.0	154.05±10.64	
	10	135.8	126.0	134.4	137.0	139.0	137.8	143.73±9.91	
	12	186.2	140.0	196.0	138.2	134.0	140.2	143.70±8.58	
Potassium	0	4.2	3.7	3.8	3.8	4.2	4.3	4.25±0.06	0.37
	4	4.8	3.7	4.5	4.0	4.6	4.1	3.93±0.25	
	6	4.3	4.5	4.8	3.8	4.3	3.8	3.57±0.23	
	8	4.3	4.4	3.0	3.7	4.3	4.0	4.07±0.12	
	10	4.6	3.0	4.8	4.0	4.4	4.2	3.63±0.37	
	12	4.2	4.4	4.8	4.2	4.3	4.2	3.93±0.30	
Chloride	0	88.5	111.5	88.5	100	111.2	98.1	93.78±3.04	0.02
	4	92.3	96.2	92.3	92.2	98.3	93.6	94.75±3.77	
	6	84.6	92.3	80.8	88.3	94.1	100.2	104.25±2.37	
	8	92.3	88.5	92.3	88.1	88.3	99.1	101.30±1.97	
	10	111.5	84.6	100	84.2	84.2	98.3	90.22±1.45	
	12	88.5	103.8	96.2	84.2	107.1	103.6	96.12±4.06	
Bicarbonate	0	24	22	23	23	24	23	24.17±0.40	0.006
	4	24	22	25	23	23	21	23.00±0.52	
	6	20	22	22	20	22	21	21.33±0.42	
	8	24	28	22	24	22	21	24.00±0.89	
	10	22	26	22	21	22	23	22.33±0.42	
	12	24	28	24	23	24	23	23.167±0.32	
ALP	0	38	34.1	45	38	46	36.2	32.22±4.31	0.76
	4	41.7	43.5	36.6	43.8	40.1	45.4	37.52±6.19	
	6	31.6	21.2	32.0	33.5	36.2	29.6	31.62±7.12	
	8	13.8	18.9	18.9	15.4	32.1	26.7	27.27±3.42	
	10	15.5	13.8	25.9	17.4	18.3	26.4	31.43±1.68	
	12	17.2	13.8	50	17.8	38	27.2	28.700±4.80	
AST	0	26	95	32	28	34	62	51.50±5.93	0.18
	4	36	38	33	36	36	53	40.50±2.99	
	6	32	26	49	32	47	36	34.00±3.27	
	8	25	29	41	27	40	66	39.67±6.08	
	10	22	28	39	22	39	82	38.00±9.51	
	12	55	22	82	53	60	32	31.17±1.47	
ALT	0	38	165	41	41	42	44	68.67±12.10	0.66
	4	41	31	41	44	41	48	61.83±7.76	
	6	35	42	41	37	42	38	64.67±5.52	
	8	32	108	27	34	28	35	54.83±2.59	
	10	29	36	55	31	43	32	53.67±2.84±	
	12	35	31	121	37	41	42	74.167±18.35	

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

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Table 10. Serum biochemistry values of dogs with post 70% small intestinal resection and anastomosis and supplemented with glutamine/ honey/ ascorbic acid

Substance	Days	Number of dogs (n = 6)						Mean ±SEM	P-value
		1	2	3	4	5	6		
Sodium	0	116.2	134.4	182.0	136.0	145.4	140.0	142.33±8.89	0.82
	4	131.6	194.6	137.2	135.4	138.0	137.0	145.63±9.84	
	6	133.0	187.6	134.4	135.2	135.3	135.2	143.45±8.84	
	8	134.4	137.2	135.8	134.6	136.2	136.4	135.77±0.44	
	10	190.4	133.0	134.4	150.2	137.4	138.0	147.23±8.98	
	12	186.2	140.0	138.6	162.4	144.0	138.6	151.63±7.84	
Potassium	0	4.1	4.4	4.0	4.0	4.2	4.0	4.12±0.65	0.33
	4	4.1	4.2	2.1	3.8	3.6	3.8	3.60±0.31	
	6	4.2	4.2	2.8	3.8	3.6	3.9	3.75±0.21	
	8	4.2	2.4	4.0	3.9	3.9	3.9	3.72±0.27	
	10	4.2	2.9	2.8	4.0	4.0	4.0	3.65±0.26	
	12	4.0	4.2	4.2	4.1	4.2	4.2	4.15±0.03	
Chloride	0	84.6	103.8	96.2	97.2	100	102	97.30±2.79	0.92
	4	84.6	103.8	88.5	94.3	93.1	100	94.05±2.90	
	6	92.3	96.1	103.8	100.5	89.3	98	96.67±2.17	
	8	100	96.2	88.5	102.3	97.2	98	97.03±1.92	
	10	88.5	107.7	61.5	98.1	102.5	101.2	93.25±6.86	
	12	88.5	96.2	100	98.3	101.2	102	97.70±2.03	
Bicarbonate	0	22	22	22	23	22	23	22.33±0.21	0.01
	4	22	24	23	22	22	23	22.67±0.33	
	6	21	21	23	21	21	22	21.50±0.34	
	8	21	26	21	21	23	22	22.33±0.80	
	10	26	26	28	22	24	23	24.83±0.91	
	12	21	21	25	23	24	22	22.67±0.67	
ALP	0	58.6	22.4	50	45.2	52.5	59.7	48.07±5.59	0.75
	4	72.4	31.0	108.6	62.1	70.1	62.1	67.72±10.18	
	6	48.3	25.9	75.9	48.4	62.3	58.2	53.17±6.87	
	8	34.5	17.2	82.8	41.2	58.9	60.1	49.12±9.39	
	10	74.1	17.2	43.1	65.1	60.2	76.4	56.02±9.16	
	12	77.6	17.2	58.6	52.5	53.1	80.1	56.52±9.27	
AST	0	33	23	19	34	42	34	30.83±3.42	0.005
	4	47	31	36	40	55	48	42.83±3.59	
	6	36	30	73	36	50	38	43.83±6.42	
	8	34	29	32	35	41	36	34.50±1.65	
	10	52	34	24	49	36	54	41.50±4.88	
	12	97	23	85	70	40	98	68.83±12.70	
ALT	0	44	59	22	49	56	45	45.83±5.35	0,40
	4	188	73	35	51	70	64	80.17±22.31	
	6	88	57	79	65	54	53	66.00±5.91	
	8	80	49	78	62	50	47	61.00±6.09	
	10	112	55	69	57	49	57	66.50±9.48	
	12	179	51	140	52	52	52	87.67±23.27	

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

DISCUSSION

In patients suffering from short bowel syndrome, the absorption of electrolytes is impaired due to loss of mucosal absorptive surface area and large amounts of these electrolytes are also lost in the ensuing diarrhea (Shaw et al., 2012; Winter and Shah, 2013; Walters, 2017; Gillard et al., 2017). Moreover, in this study, the predominant portion of the small intestine where absorption takes place (Jejunum) was completely lost with some portion of the ileum. It was therefore, expected that the amount of these electrolytes (Sodium, potassium, bicarbonates and chlorides) would fall below the normal values due to impaired absorption. But the result showed no impaired absorption as the electrolyte values were all within the normal ranges in all the animals including the control group. Though there were significant

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changes or differences in the values of some of these electrolytes in some groups such as group 2 (chloride and bicarbonate), group 3 (sodium only), group 4 (chloride and bicarbonate) and group 5 (bicarbonate only). However, these changes were still within the normal values of these electrolytes in dogs. This was due to the fact that significant fluid and electrolyte absorption also occurs in the colon which helps to compensate for small intestinal disease (German, 2005; Navarro et al., 2009; Vagholkar et al., 2016). The colon is particularly effective in absorbing sodium; therefore, sodium deficiency hardly occurs in short bowel syndrome patients with an intact colon (Vanderhoof, 2010; Cunha-Melo and Costa, 2014; Mayeur et al., 2016; Rodriguez-Montes et al., 2016). Those patients with their colon resected suffer from water and sodium deficiency and are susceptible to a number of disease conditions especially hypotension and kidney failure (Sundaram et al., 2002; Nightingale and Woodward, 2006; Sriram and Lonchyna, 2009; Matarese, 2013). Also when a significant portion of the jejunum is lost the remaining ileum can undergo morphological and structural adaptation to compensate for many of the jejuna functions including the absorption of electrolytes (German, 2005; Cunha-Melo and Costa, 2014).

The observed significant decrease in ALK value in glutamine and honey treated animals and non-significant changes in ALT in all the groups are an indication that there was no associated liver pathology in these animals. This is in agreement with the report of other researchers that placing patients with short bowel syndrome on enteral nutrition (oral feeding) reduces significantly the incidence of liver disease in such patients compared to its high incidence in patients placed on total parenteral nutrition (Le et al., 2010; Vipperia and O'keefe, 2014). The absence of thrombocytopenia- platelet count below normal gives further credence to the observation that there was no liver pathology.

Vagholkar et al. (2016) who had observed hypochromic microcytic anaemia and megaloblastic anaemia in human patients with short bowel syndrome but in this study, normocytic normochromic anaemia was observed as the PCV decreased from its baseline values in all animals in the five groups while the RBC, Hg, MCV and MCHC remained within the reference values. However, this decrease in the PCV is significant only in group 2 and 3 animals. This might be due to surgical haemorrhage during the surgery and hemodilution from intravenous fluid infusion during and after the surgery. This shows that iron absorption had not been impaired as anaemia due to iron deficiencies are microcytic hypochromic anaemia (Latimer, 2010; Thrall, 2012).This is because the duodenum where predominant absorption of iron takes place had not been resectioned (Seetharam and Rodrigues, 2011). The fact that the haemoglobin values had remained within the reference range is an indication that the animals did not become dehydrated since there was no depletion of fluids and electrolytes. The WBC count had increased in control and all the treated groups at day four post resection. These increases in white blood cell count were immune responses to inflammation that had followed bowel resection. The above findings had also been observed by Shaw et al. (2012).

CONCLUSION

Preservation of the colon in patients suffering from Short Bowel Syndrome improves the outcome and survival of these patients as the colon takes over the function of absorption of water, electrolytes and ensures that these patients attains enteral autonomy by reducing or eliminating the need for Total Parenteral Nutrition and its attendant consequences.

Competing interests

The authors have declared that no competing interest exists.

Author's contribution

AIK and JBA performed the surgery and writing of the manuscript. LMS analyzed the blood samples and reviewed the manuscript

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An Abattoir based Study on Bovine Tuberculosis in Debre Zeit, Ethiopia

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ABSTRACT

Members of the Mycobacterium complex group cause tuberculosis, it recognized as one of the most important threats to humans and animals causing mortality, morbidity and economic losses in many countries of the world, particularly in developing nations. Therefore, a cross-sectional study on bovine tuberculosis conducted in order to determine its prevalence in cattle at the ELFORA export abattoir in Debre-Zeit in the period from November 2014 to April 2015. Routine and detailed meat inspection methods used to detect lesions. Three hundred cattle inspected; their body condition scores and ages recorded before slaughtering. Of the total animals, 5.7% (17/300) had lesions of tuberculosis. Out of these, routine abattoir inspection had detected only 2.7% (8/300) with visible lesions and there was poor agreement (kappa=0.09) between routine and detailed inspection methods. The proportion of lesions found in the lung and associated lymph nodes, mesenteric lymph nodes and lymph node around head were determined to have been at 12.3%, 2% and 3.3%, respectively. The prevalence of the disease was significantly ($P < 0.05$) varying with body condition scores but it did not significantly ($P > 0.05$) vary with age groups of the animals. This study demonstrated the prevalence of bovine tuberculosis in cattle slaughtered at ELFORA export abattoir and low sensitivity of routine abattoir inspection. Hence, the carcass must thoroughly examine well to reduce the chance of missing lesions of tuberculosis.

Key words: Bovine tuberculosis, Meat inspection, Prevalence, Public health, Zoonosis

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INTRODUCTION

Bovine Tuberculosis (BTB) is a chronic bacterial disease characterized by progressive development of tubercles in any tissue/organ of the body (Hlokwe et al., 2013; Pal et al., 2014; Terefe, 2014). It has recognized from 176 countries as one of the important bovine diseases causing great economic loss (Awah-Ndukum et al., 2013). Tuberculosis (TB) remains a major global health problem and causes ill health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide after the human immunodeficiency virus (HIV) (WHO, 2013 and 2014). Tuberculosis can be difficult to diagnose based only on the clinical signs. Regular surveillance by skin test, bacteriology and molecular methods is not feasible due to lack of resources. Thus, Routine Abattoir (RA) inspection will continue to play a key role for national surveillance. We evaluated the efficiency of RA inspection for diagnosis of *M. bovis* infection and discussed its public health implications in light of a high risk of human exposure. TB has been widely distributed throughout the world affecting all age groups. In humans, TB is being responsible for more deaths than any other bacterial disease ever today (Bhatia and Ichpujanti, 1994). Ethiopia has the largest livestock population in Africa, with an estimated 44.3 million cattle, 23.6 million sheep, 26.3 million goats and 2.3 million camels (CSA, 2005). Ethiopia's increasing human population, coupled with expanding urbanization and higher average income is putting an increasing pressure on the meat supply. To meet this demand, millions of food animals slaughtered every year

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throughout the country. In 2007, for example, a total of 18.8 million cattle, sheep, goats and camels slaughtered at municipal abattoirs, primarily for domestic consumption (FAO, 2009). For this reason, close monitoring of meat hygiene, including proper implementation of meat inspection procedures during slaughter, should be a vital part of the national public health protection program.

BTB characterized by the formation of nodules called tubercles whose location depends largely on the route of infection. In calves, BTB usually transmitted by ingestion and lesions involve the mesenteric lymph nodes with possible spread to other organs. In older cattle, infection usually transmitted by the respiratory tract with lesions in the lung and dependent lymph nodes (Carter and Wise, 2004). Recently, there have been increasing reports of human cases due to *M. bovis* especially in patients with HIV (Russell, 2003). Thus, a greater degree of transmission of infection with bacteria to human and domestic farm animals could occur (Taracha et al., 2003). In industrialized countries, animal tuberculosis is controlled and eliminated with milk pasteurization, which has in turn drastically reduced the incidence of the disease caused by *M. bovis* in both cattle and human. In developing countries however, animals' tuberculosis is widely distributed, control measures not applied or applied sporadically and pasteurization rarely practiced. In Ethiopia, animals are kept in the same dwelling with their owners and use of cow dung for wall plaster, floor and as a source of energy for cooking, do exacerbate chance of spread of tuberculosis to human (Asseged, 1999). Thus, BTB is endemic and has reported from different regions of the country (Asseged et al., 2000). The disease in the country is associated with decreased productive efficiency and carcass or organ condemnation in abattoir (Shitaye et al., 2006). The nationwide distribution of the disease and the economic loss associated with it has not been fully determined due to lack of good diagnostic facilities (Asseged, 2004). The primary reason for post mortem examination of carcasses at slaughterhouse is for the protection of public health. TB status of slaughter cattle provides useful information and is a proxy indicator for the prevalence of TB-positive slaughter animals, and therefore likelihood of the human exposure through consumption of infected meat. Apart from providing data for regulatory programmatic awareness of the true prevalence of TB infection, carcass examination also provides clues as to whether the infection is in its early stage or has reached the transmissible stage (ADARDO, 2008). This provides better programmatic awareness with subsequent development of targeted guidance on how to reduce the risk of TB spread within the specific geographic area, as well as opportunities to trace back the source of infection to the herds. Hence, having the knowledge of distribution, prevalence and risk factors of the disease are fundamental to look for effective control strategy. Therefore, the objectives of this study were to determine the prevalence of bovine tuberculosis at the ELFORA export abattoir, in DebreZeit and to evaluate the efficiency of abattoir inspection for the diagnosis of *M. bovis* infection as well as to assess the distribution of tuberculous lesions in slaughtered animals.

MATERIALS AND METHODS

Ethical considerations

This research work conducted according to Organization International Epizootics (OIE, 2010) principles of the use of animals in research and education**.** As much as possible we prevent, alleviate and minimize pain, suffering, and distress and enhance welfare for the animals used for research during ant mortem.

Study area

This study conducted at the ELFORA export abattoir in DebreZeit, Ethiopia from November 2014 to April 2015. DebreZeit is located at 45 kms southeast of Addis Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 meters above sea level in central high land of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 14°C, respectively, with mean relatively, humidity of 61.3% (ADARDO, 2007).

Animals

Animals used for the study were those, which were ready to slaughter at ELFORA export abattoir, in DebreZiet, came from surrounding areas and all of them were from local breeds (*Boss indicus*) and male. Most of the cattle kept in fattening for variable period, so their body condition was almost medium or good (Nicholson and Butterworth, 1986).

Study design

A cross-sectional study conducted to determine the prevalence of BTB. The efficiency of routine abattoir meat inspection to diagnose TB lesions also evaluated. A systematic random sampling procedure used to choose animals in the study. In general, 300 cattle and their carcasses examined.

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Ante-mortem inspection

Those cattle selected for the study had been examined physically before slaughtering; age and Body Condition Score (BCS) recorded. The body condition of each of the study animals scored using guideline established by Nicholson and Butterworth (1986) during ante-mortem examination. In the meantime, the age of the study animals had also been determined according to De-Lahunta, and Habel (1986). The age was categorized as young less than two years old, young adult between two to six years old and adult greater than six years old (Pace and Wakeman, 2003). All animals slaughtered during the study period were older than two years of age so they categorized as young adult and adult.

Routine abattoir inspection

Routine inspection for tuberculosis at the abattoir conducted according to the method developed by the meat inspector and quarantine division of the Ministry of Agriculture, Ethiopia (MOA, 1973). The method involves palpation and incision of the bronchial, mediastinal and pre-scapular lymph nodes, as well as visual inspection and if necessary incision of the lungs, liver, kidneys and lymph nodes around these organs.

Detailed post-mortem examination

Detailed post-mortem examination performed in such a way, that the lungs and lymph nodes removed for the investigation of tuberculous lesions. The seven lobes of the two lungs, including the left apical, left cardiac, left diaphragmatic, right apical, right cardiac, right diaphragmatic and right accessory lobes, inspected externally and palpated. Each lobe then sectioned into about two centimeter thick slices to facilitate the detection of lesions. Similarly, lymph nodes, namely, the mandibular, medial right apical, right cardiac, right diaphragmatic and right accessory lobes, were inspected externally and palpated, each lobe was then sectioned into about two centimeter thick slices to facilitate the detection of lesions. Similarly, lymph nodes, namely, the mandibular, medial retropharyngeal, cranial and caudal mediastinal, left and right bronchial, hepatic and mesenteric lymph nodes, were sliced into thin sections (23 millimeter thick) and inspected for the presence of lesions (Teklu et al.,2004).

Data analysis

The raw data was fed into Microsoft excel and the prevalence of bovine tuberculosis was calculated in percentage. The variation between different factors assessed by using Chi-square (X^2), and all statistical analyses conducted by SPSS statistical software version 20. A p-value less than 0.05 considered statistically significant.

RESULTS

Prevalence of bovine tuberculosis

The overall prevalence of BTB in cattle slaughtered at ELFORA export abattoir during the study period was at 5.7% (17/300) based on the detailed post-mortem examination. Macroscopically, the most common changes seen in the affected organs and/or lymph nodes were the presence of circumscribed yellowish white lesions of various sizes and numbers. However, only 2.7% (8/300) head of cattle found to have detectable tuberculosis lesions by the routine abattoir inspection. Thus, the proportion of lesion detected by detailed examination to that of routine abattoir inspection procedure was in the ratio of 2.1:1. The results of this study have indicated that the probability of missing an animal with tuberculosis during routine abattoir inspection was 3%.

Thus, a poor agreement (Kappa=0.09) was recorded between these two procedures. Distribution of tuberculosis lesions in organs of cattle slaughtered is higher in bronchial ln (3%) and lower in hepatic ln (Table 1). The lymph node and lung regions contribute a higher percentage of tubercle lesions than any other organs (Table 2). The prevalence of tuberculosis in abattoir slaughter cattle in relation with age and body condition score has been presented in table 3. There was no statistically significant (P> 0.05) difference between age groups. However, there was a variation in the occurrence of tuberculous lesions across body condition scores (medium and good), with a considerably higher prevalence recorded in medium scored cattle.

Distribution of tuberculous lesions

The distribution of tuberculous lesions in different tissues of cattle has presented in table 1. Eight organs and/or lymph nodes were containing tuberculous lesions. About 5.3% of the lesions observed in the lung. The lung region contributes a higher percentage of tubercle lesions than the lymph node around head and the gastrointestinal area (Table 2). The prevalence of tuberculosis in abattoir slaughter cattle in relation with age and BCS presented in Table 3 and 4, respectively. There was no statistically significant (P> 0.05) difference between age groups. However, there was a

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variation in the occurrence of tuberculous lesions across body condition scores (medium and good), with a considerably higher prevalence recorded in medium scored cattle.

Table 1. Distribution of tuberculosis lesions in organs of cattle slaughtered in central Ethiopia, during November 2014 to April 2015

Organs	Lung Tissue	Bronchial	Mediastinal	Retropharyngeal	Mandibular	Mesenteric	Liver And Hepatic	Carcasses
No. of lesions (%)	16 (5.3)	8 (3)	14(5)	7 (2.3)	3 (1)	6 (2)	2 (0.7)	5 (1.6)

*ln= lymph node

Table 2. Observation of tuberculosis lesions distributed in organs and lymph nodes of cattle carcasses collected in the central Ethiopia, during November 2014 to April 2015

Anatomical sites	lung tissues and lymph node	Mesenteric ly mph node	Liver and hepatic lymph node	Head lymph nodes	Carcasses	Total
No. of Lesions	37	6	1	10	5	59
Infected animal (%)*	12.3	2	0.3	3.3	1.7	--
Percentage of infected organ (%)**	62.7	10.2	1.7	17	8.5	--

*Percentage from total animal=lesion divided by/number of animal examined (300) multiplied by 100 **Organ proportion=lesion in the region divided by overall lesions (59) multiplied by 100

Table 3. Prevalence of bovine tuberculosis with respect to age of infected animals

Age	Young	Adult	Total
No. of Animals examined	123	177	300
Positive (%)	9 (7.3)	11 (6.2)	
P-value	0.99	0.99	

Table 4. Prevalence of bovine tuberculosis with respect to body condition score of infected animals

Body condition score	Good	Medium	Total
No. of Animals examined	237	63	300
Positive (%)	5 (2.1)	14 (22.2)	
P-value	0.00	0.00	

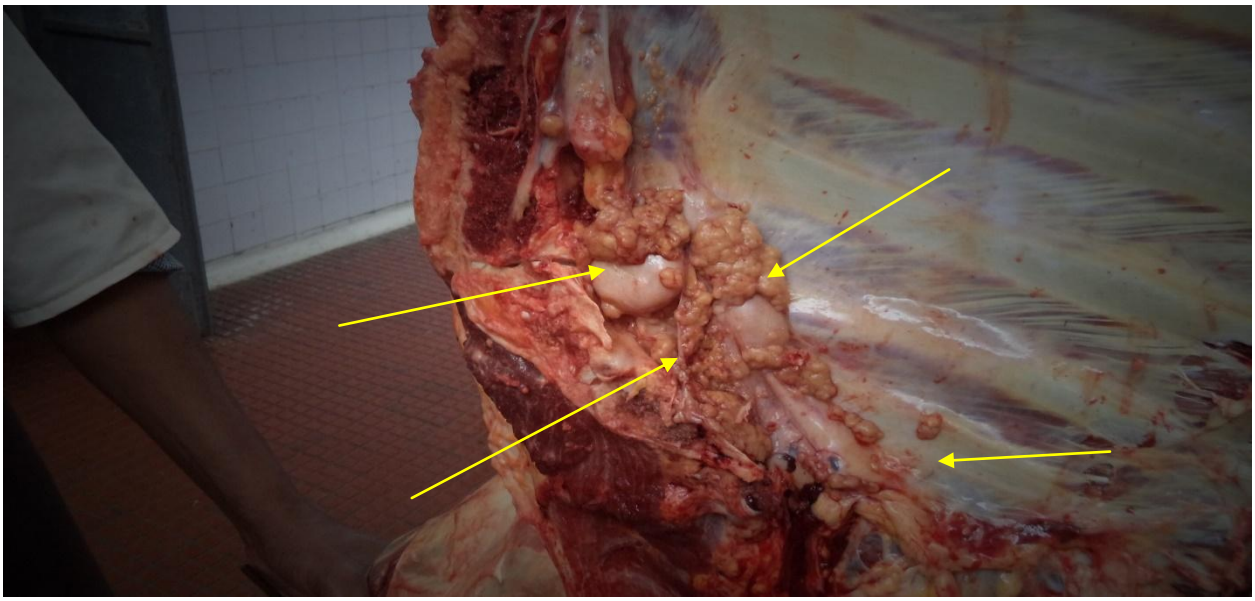

Figure 1. Tuberculosis lesions in the thoracic cavity of adult cattle at abattoir, in central Ethiopia, September 2015

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DISCUSSION

This study has documented the prevalence and distribution of lesions of BTB in cattle slaughtered at ELFORA export abattoir. The findings of overall prevalence of 5.7% tuberculosis reported in detailed post-mortem examination and 2.7% in routine abattoir inspection are in agreement with the results reported by Demelash et al. (2009; Teklu et al., 2004; Nasaka J, 2014) in Yabello municipal abattoir and Hosanna Ethiopia and Masaka district Uganda 4.2% 4.5% and2.7%respectively.

On the contrary, the present results are less than of the previous reports from Ghana 34% (Atiadeve et al., 2014), Camerron 12-17% (Egbe et al., 2016), Ethiopia 8.4% (Aylate et al., 2013), Butajira municipality abattoir 11.50% (Abdurrahman, 2009), Addis Ababa and Adama 10.10% (Demelash et al., 2009), Adama municipal abattoir 24.70% (Tefera, 2009) and 19.8% record from cattle slaughter in rural Tanzania (Cleaveland et al., 2007). This could indicate the endemic nature of the disease and the high infection rate prevailing in the general population of slaughter cattle in Ethiopia. This study has revealed that the probability of missing an animal with TB lesion during routine abattoirinspection is 95.24%. Previous studies have also indicated probabilities of 84.85% (Tefera, 2009), 84% (Corner, 1994.) and 70.59%, (Teklu et al., 2004). Therefore, detailed post-mortem examination can considered as a better procedure to detect tuberculous lesion. This study had also revealed a much low sensitivity of routine meat inspection to detect carcasses with tuberculous lesion, implying that large proportion (95.24%) of tuberculosis infected carcasses pass undetected and the meat is approved for human consumption. The most probable explanation for the failure of standard meat inspection correctly detect tuberculosis infection could be due to the manner of examination (Corner, 1994). It noticed that in standard meat inspection procedure only few sites (organs) often inspected at a glance due to heavy duty of inspecting large number of animals each day. Argued that in abattoir smaller lesion could missed due to limited time available for the examination of each tissue (Corner, 1994). Furthermore, a lack of competence in meat inspection training could be another reason for inefficiency of the service as most of the lack of adequate trained personnel in the area of meat inspection. In the present study, gross tuberculosis lesions were found most frequently in the lymph nodes of lung (12.3%), lymph nodes of the head (3.3%) followed by mesenteric lymph node (2%). This finding is consistent with previous studies done by (Firdissa 2006; Abdurrahman, 2009 and Tefera, 2009) that reported 11.7, 14 and 14.7% TB lesions in lungs and associated lymph nodes, respectively. However, Corner (1994) described that up to 95% of cattle with visible TB lesions could identified by the examination of the lungs and associated lymph nodes. This finding indicated that inhalation might be the principal route of TB infection in cattle. Therefore, during post-mortem examination, focus should give on lungs and its associated lymph nodes. The presence of lesions in mesenteric lymph nodes indicated that the infection occurs through ingestion (Radostits et al., 2007). The prevalence of the diseases was statistically insignificant in age categories, which agree with previous reports (Bekele and Belay, 2011). This might be due to type of animals slaughtered in the abattoir in which both were greater than two years of age, which is enough to develop the lesions once infected. It should noticed that, there was a statistically significant difference ($P< 0.05$) in the prevalence of the disease between BCS. The prevalence was higher in medium (22.2%) than good (2.1%) body conditioned animals. Our findings run parallel with previous reports, which had indicated that animals with good BCS have relatively strong immunological response to the infectious agent than animals with medium BCS and the results could indicate the wasting nature of the disease (Radostits et al., 2007). Our current work is a continuation of the work published earlier describing prevalence of BTB in Ethiopian slaughter cattle *(*Demelash et al., 2009). With generation of new additional data from laboratory work (culture and microscopy and molecular analysis), we wanted to evaluate how well abattoir meat inspection protocols in Ethiopia are performing to detect cattle infected with *M. bovis*. Public health implications of the findings have also discussed.

CONCLUSION

This study demonstrated the limited capacity of current meat inspection procedures in Ethiopia to detect carcasses infected with *M. b*ovis.in Ethiopia and other countries where dietary preferences mean that a significant proportion of meat customarily consumed raw, lack of effective slaughter inspection protocols represents significant risk. Thus, meat borne zoonotic TB continues to be an ongoing and an important threat to public health in a nation that has significant populations of vulnerable HIV-infected citizens. Therefore, it is necessary to understand that the detailed abattoir meat inspection prevails than routine meat inspection, giving safe meat consumption risk and minimize the other risk factors. It deduced that BTB is prevalent in cattle slaughtered at ELFORA export abattoir, Debre-Zeit, Ethiopia. Tuberculous lesions were detected using detailed meat inspection. This should practice giving more attention to the lungs and associated lymph nodes.

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Recommendations for improving the service within the context of the prevailing cultural and socio-economic situation generated as an important outcome of these efforts. Further studies needed to characterize the organism by cultural isolation and implementing molecular techniques.

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Competing interests

The authors have declared that no competing interest exists in relation to this manuscript.

Author's contribution

NigusZenebe designed the experiment, collected data, Tsedale Amare collected data, TeferaWoldemariam helped in manuscript writing, commenting and approval, Mahendra Pal designed the experiment, wrote, commenting and approval of the paper. All authors have read and approved the final manuscript.

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Influence of Enzymatic and Mechanical Liquefaction of Seminal Plasma on Freezability of Dromedary Camel Semen

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ABSTRACT

This study aimed to investigate the efficiency of mechanical and enzymatic elimination of semen viscosity in adult dromedary camel bulls' semen on cryopreservation potential of spermatozoa during the breeding season. Bulls showed reaction time 40.0±8.23 seconds and 251±24 seconds mating duration. Physical properties of raw semen showed volume mean value 5.28±0.66 ml, initial viability 2.5±0.6, initial raw motility 59.34±4.99%, livability 95.3±2.36%, first and second abnormalities 4.13±0.88% and 7.01±1.254%, respectively and acrosomal integrity 5.03±1.05%. The researcher examined three different treatments for viscosity elimination; namely; Amylase Enzymatic Treatment (AET), Syringe Mechanical Treatment (SMT) and Amylase Syringe Mixed Treatment (ASMT). The results revealed that, a significant deleterious effect of the ASMT on the post-thaw motility (M_{PT}) 25.00±3.69% was observed, with sperm Recovery Rate (RR) 35.02±5.02%, contrary to a clear superiority of AET treatment on (M_{PT}) 49.00±4.87%, followed by the SMT treatment (M_{PT}) 41.67±6.72%, with significantly higher RR% (76.86±4.63% and 62.10±6.65%) respectively. The AET recorded the highest acrosomal reaction (10.17±1.11%), followed by the mixed treatment (8.33±0.14%), with the least significant effect ($P<0.05$) on the mechanically treated group (7.33±0.99%). The results also showed the same trend for first and second abnormalities. Computer assisted semen analysis showed a significant superiority for the AET on mostly all sperm kinetics (DCL, DAP, VAP, VSL), except for DSL, VCL that showed highest significant value for SMT treatment. Conversely, the study recorded the lowest significant values for LIN, STR and WOB in the SMT. These results clarified that both enzymatic and mechanical methods have a positive influence on dromedary camel semen cryopreservation.

Key words: Amylase, Cryopreservation, Dromedary, Semen syringing, Viscosity

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INTRODUCTION

Semen processing and cryopreservation are becoming a prerequisite in the application of assisted reproductive technologies in camelids, especially with the increasing of genetically prized valued camels involved in camel racing and beauty contests, although, the technology is yet being sub-optimal in camels due to several challenges. The steady development of Artificial Insemination (AI) with frozen-thawed sperm requires efforts to improve the quality of semen processing techniques. One of the major challenges restricting the development of assisted reproductive technologies (ART's) in camelids is the high viscous nature of seminal plasma (Skidmore et al., 2013; Rateb, 2016). Under natural conditions, complete liquefaction for dromedary camel semen was observed to be 23.89 ± 1.49 hours, varying in a wide range from 18 to 41 hrs (Mal et al., 2016).

Several studies were performed during the last decade for the elimination of camelids' semen viscosity, with various degrees of success. Enzymatic treatments were the most used technique. The cause of the viscous nature of camels' seminal plasma used to be a controversial issue among different studies and authors, either depending on the

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usage of proteolytic enzymes like pepsin, trypsin, alfa-chemotripsin (Ccallo et al.,1999; El-Bahrawy and El-Hassanein, 2009), and lately, the use of Papaine, Bromilaine and Ficin (Desantis et al., 2016; Keshavarz et al., 2016; Monaco et al., 2016), as their theory based on referring viscosity to proteoglycans (protein molecules) (Kershaw-Young and Maxwell, 2012; Mal et al., 2016). Although the superior effect of proteolytic enzymes on seminal plasma viscosity elimination, but still the drastic effect of such enzymes on sperm parameters is to be taken into consideration, (Monaco et al., 2016) observed sperm head agglutination, as well as a direct effect on the sperm's acrosomal integrity, these effects have limited the wide usage of these enzymes in a routine work protocol. Other researchers have depended on using poly-saccharides degradation active enzymes (amylase, collagenase, hyaluronidase). As semen viscosity has been postulated that it is caused by glycosaminoglycans (GAGs), which are defined as long un-branched polysaccharides consisting of a repeating disaccharide, (Ali et al., 1976). Lately, (Kershaw-Young et al., 2012) reported in alpaca seminal plasma, a fifteen times higher concentration of GAGs compared to that of rams. Also the claim that camel semen viscosity is attributed to the presence of mucopolysaccharides from the secretions of the bulbourethral gland or the prostate gland (Skidmore et al., 2013). For decades, amylase has been routinely used for the liquefaction of semen hyper-viscosity in humans with different and varied concentrations (Dougherty et al., 1978; Agostini et al., 1996; Henkel and Schill, 2003) with controversial results for its effect. In Camels, (EL-Bahrawy, 2010) and (Monaco et al., 2016) used amylase to eliminate seminal plasma viscosity with different rates of success. In general, enzymatic treatment also affects the diluent components for their long acting effect during the equilibration period of semen processing for cryopreservation as well as sperm cells.

Mechanical methods were also used for seminal plasma viscosity elimination; for Bactrian camel semen, (Niasari-Naslaji et al., 2007) recommended mechanical stirring for viscosity elimination. The WHO manual in 2010 recommended gently running of human semen with hyper viscosity through a 5–10 ml syringe fitted with an 18-gauge needle for several times before initial raw semen parameters examination prior to any processing steps. Kussler et al., (2014) used the process of expulsion of semen through a 10 ml syringe and an18-gauge (18G) needle to reduce the seminal viscosity but reported a doubt on the safety of that procedure. In Camelids, early trails were used by Santiani et al. (2005) using the syringe technique prior to cryopreservation of *Lama Pacos* semen. Morton et al., (2008) reported that gentle pipetting of raw semen reduced semen viscosity with a moderate effect on semen parameters after processing. Although semen liquefaction by syringing is easy to be performed, with a quick, effective, and cheap methodology, but it still has not been confirmed whether the process is entirely harmless to the semen samples especially; after cryopreservation (Mendeluk et al., 2000; Esfandiari et al.,2008).

In the view of these facts, and as the etiology and the impact of seminal hyper viscosity elimination procedures on camel semen characteristics and functional capacity have not yet been fully understood with contradictory results, this investigation aims to figure out the effect of combining both methods of enzymatic and mechanical treatment or using each solely to investigate their effect on the physical characteristics of cryopreserved semen.

MATERIALS AND METHODS

Location and experimental animals

The experiment was carried out in an arid area, in the reproduction camel center of the Tharb camel hospital, Qatar. Semen was collected from five dromedary camel bulls of 9 to 15 years of age and 622 ± 40.12 kg average body weight during December 2016 to February 2017. Animals were daily fed at 10 am on a pelleted concentrate mixture (crude protein, 14%), and were further supplemented with barley and dried dates as sources of energy. Also, dry Berseem hay was offered *adlib.* as roughage, and the animals were allowed to drink twice daily.

Ethical approval

This experiment was a routine field work in animal reproduction considering all rules and regulations in conformity with the European Union Directive for the protection of experimental animals (2010/63/EU).

Semen collection

Semen samples were collected three times a week at 6:30 am in a clean area adjacent to the reproduction laboratory. Female teaser was used for semen collection, where it was physically restrained in sternal recumbency position, using a 42 cm bovine Artificial Vagina (AV) adjusted to 40-45^{0}C (El-Bahrawy, 2010)**.** The AV latex inner liner was lubricated from inside using sperm-friendly vaseline assigned for semen collection (Minitube Vaseline, 1000 g, REF.: 11905/0100) to avoid direct contact of spermatozoa with latex inner liner to overcome latex toxicity suspicious. Any contaminated, oligospermic or azoospermic specimens were discarded from processing.

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Semen diluents preparation and sample processing

Unless stated otherwise, all chemicals and reagents were obtained from Sigma (Sigma-Aldrich) for preparation of Tris-lactose egg yolk extender composed of Tris buffer (3.025%), lactose (5.5%), Citric acid (1.67%), glucose (1%), and supplemented with fresh egg-yolk (20%), the diluent was then divided into two portions A and B. Portion A represented the cooling extender which was added initially to the collected ejaculate with a ratio of 1:1, while portion B was supplemented with 6% glycerol to be added after 2 hours from equilibration (at 5^0C) with a ratio 1:1prior to freezing to make the diluted semen reach a final glycerol level of 3% and a full equilibration period of 4 hours at 5^0C, and a final dilution rate 1:3 according to El-Bahrawy (2010).

Experimental design

Semen samples were allocated to three groups [Amylase Enzymatic Treatment (AET), Syringe Mechanical Treatment (SMT) and Amylase Syringe Mixed Treatment (ASMT)], with equal fractions for each group.

Enzymatic Procedure [Amylase Enzymatic Treatment (AET)]

After primary check in a pre-test study of the enzyme power units. A concentration of 2.5 ul/ml TERMAMYLSUPRA enzyme, [(a trademarked, amylase available from Termamyl Supra) by Novozymes (Novo Nordisk), Denmark] extracted from, *Bacillus licheniformis* was set up to be used in this experiment. Immediately after collection, Tris lactose extender (Portion A) supplemented with 2.5ul/ml amylase was added to the semen with a 1:1 ratio, according to EL-Bahrawy (2010).

Mechanical procedure [Syringe Mechanical Treatment (SMT)]

Soon after the semen was collected, Tris lactose diluent (Portion A) was added with a ratio 1:1, then diluted ejaculates were immediately submitted to a physical process of expulsion of the semen and extender using a 10- or 20-mL (according to the ejaculate volume) for two or three times. This process was repeated two to three times depending on viscosity and visual observation for mixing of semen with the extender.

Mechanical /Enzymatic Procedure [Amylase Syringe Mixed Treatment (ASMT)]

Immediately after collection, Tris lactose extender (Portion A**)** supplemented with 2.5 µl/ml amylase was added to the semen with a 1:1ratio, according to EL-Bahrawy (2010), then the diluted ejaculates were immediately submitted to a physical process of expulsion of the semen using a 10- or 20 ml (according to the ejaculate volume) for two or three times.

Semen cryopreservation

An automatic filling and sealing machine (Minitube, model MPP UNO) and a computer controlled cryo-freezer, with comfortable data input and automatic recording of the freezing curve (Minitube type: Ice Cube 14S) was used for cryopreservation before transporting the cryopreserved straws to the storage tanks for further investigations. Semen straws were subjected to slow thawing temperatures of 40^0C for 40 seconds in water bath.

Samples assessment schedule

Immediately after collection, viscosity, viability, motility, abnormalities, livability and acrosomal integrity were initially assessed in raw semen, 10 minutes after initial dilution and performing the treatments (either mechanical, enzymatic or mixed treatments) the initial motility (M_I) was assessed, the pre-freezing motility (M_{PF}) was assessed after 4 hours of equilibration at 5^0C and prior to the cryopreservation of samples, and finally post-thawing motility (M_{PT}), recovery rate post thawing (RR%), abnormalities and acrosomal integrity were examined post-thawing.

Semen characteristics assessment

Semen volume was recorded using graduated collecting glass tubes for semen collection. A phase-contrast microscope (Carl ZEISS, AX10_Lab.A1, Germany) with a warm stage adjusted at 37°C was used for the assessment of sperm motility in five different fields at 400X magnification. Both mass motility (viability) in raw semen on a scale from 1 to 3 and motility of freely moving sperm were assessed to the nearest 5%. Sperm livability (live and dead sperm, %) and abnormalities (1^{st} and 2^{nd} abnormalities) were examined using eosin-nigrosin differential staining technique. Acrosomal reaction was examined following the procedure reported by Johnson et al. (1976).

Semen viscosity was examined according to the (Bravo et al., 2000a) method, using a scale of 1 to 3, where 1 represented low viscosity, 2 for intermediate viscosity and 3 represented the highest viscous samples.

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Computer-assisted sperm analysis

Sperm kinetics were measured by a computer-assisted semen analysis (CASA) system [Sperm Vision Lite, a registered trademark of Minitube, USA] attached to a Zeiss warm stage microscope. For samples' evaluation, a 3 µl aliquot of the sperm sample was placed in a Lica disposable capillary counting chamber; three fields were analyzed. Kinematic parameters were recorded representing; distance curved line (DCL, µm), distance average path (DAP, µm), distance straight line (DSL , µm), sperm velocity curved line speed (VCL, µm /sec.), velocity average path (VAP µm/sec.), velocity straight line (VSL, µm/sec.), sperm linearity movement (LIN=VSL/VCL), sperm straightness movement (STR=VSL/VAP), sperm balanced movement, Wobble (WOB=VAP/VCL).

Statistical analysis

The obtained data results were statistically analyzed using one-way analysis of variance using SAS® (1999) software program. ANOVA procedure of SAS was used. Mean differences were tested by Duncan's Multiple Range tests (Duncan, 1955) when significant P value was obtained.

RESULTS

Fifty ejaculates were collected from male camels during this study. The males had a very good body condition with an average body weight of 622±40.12 kg and body condition score exceeding 2.5±0.5 (Faye et al., 2001).

Reaction time was of a mean value 40.0± 8.23 seconds, with an approximate mating duration of 251±24 seconds, ejaculate volume 5.28±0.66 ml, initial viability 2.5±0.6, initial raw motility 59.34±4.99%, livability 95.3±2.36%. First and second abnormalities were 4.13±0.88% and 7.01±1.254 %, respectively, while acrosomal integrity was 5.03±1.05%. Results shown in figure [1(A) (B)] revealed that, soon after the treatments, the initial motility (M_I) showed that the 3rd group the ASMT exhibited significantly ($P<0.05$) the highest recorded (M_I) reaching 70.00±4.26% compared to the AET (group 1) and SMT (group 2) being 56.00±4.00% and 50.00±3.25%, respectively, the same trend was recorded for the pre-freezing motility (M_{PF}) after 4 hour equilibration period, as it showed the superiority of ASMT treatment prior to cryo-preservation followed by the AET and finally the SMT. Contrarily to the (M_I) and (M_{PF}) values, a significant deleterious effect of the ASMT was observed in post-thaw motility (M_{PT}) 25.00±3.69%, with sperm RR 35.02±5.02%. Superiority of AET on (M_{PT}) 49.00±4.87% followed by SMT 41.67±6.72% was observed, with significantly higher RR, 76.86±4.63% for the AET group and 62.10±6.65% for the SMT group.

Although the highest post thaw motility (M_{PT}) was recorded for AET, but this was accompanied with the highest reacted acrosome (10.17±1.11%), followed by the ASMT group (8.33±0.14%), with the least significant effect for the mechanical treated group (7.33±0.99%) at $p<0.05$. Almost the same trend referred to the treatments effect was observed regarding the first and second abnormalities [Figure 1A and 1B)].

Results illustrated in Figures [2A , 2B, 2C] showed a significant superiority for the AET group on mostly all sperm kinetics. However, the SMT showed the highest distance straight line (DSL, µm), also recorded high sperm track speed, velocity curved line (VCL, µm /sec.), as compared with other treatments. Velocity average path (VAP, µm/sec.) and velocity straight line (VSL, µm/sec.) were significantly higher in AET group [Figure 2A, 2B and 2C)]. Moreover, the lowest values for linearity of sperm movement (LIN=VSL/VCL), straightness (STR =VSL/VAP) and sperm balance movement (wobble) percentage (WOB=VAP/VCL) were recorded in SMT.

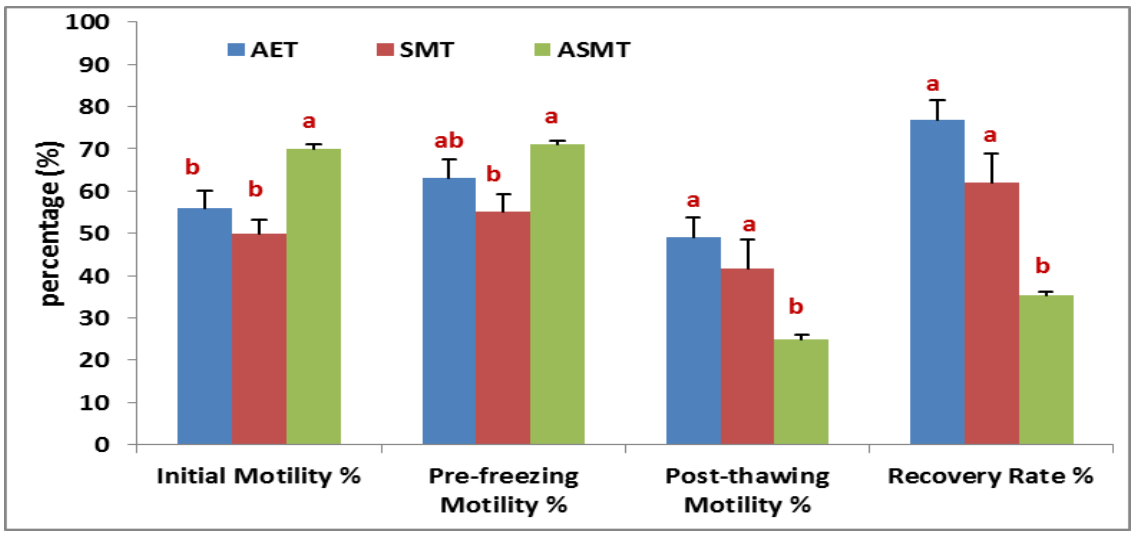

Figure 1. (A)

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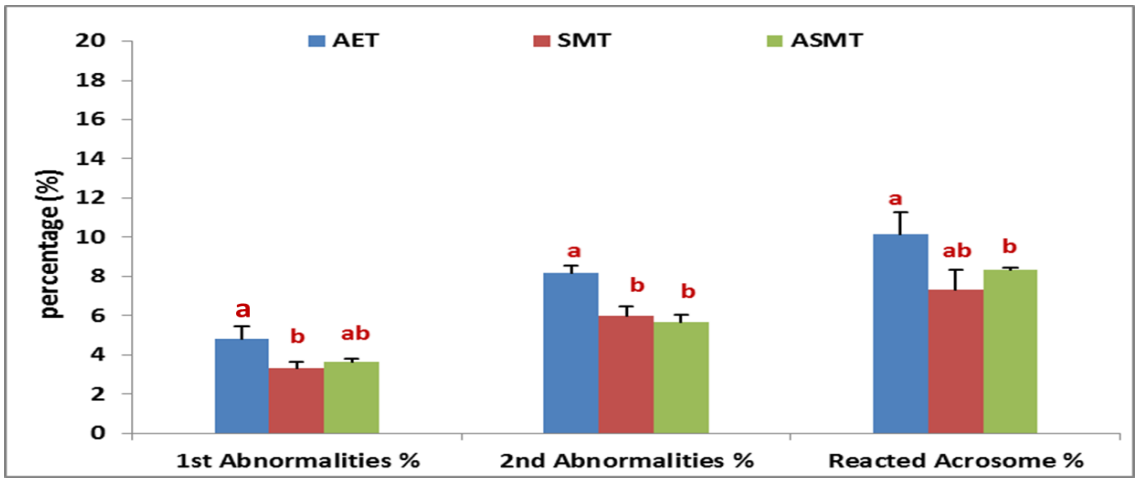

Figure 1. (B)

Figure 1. **(A) and (B):** Effect of Amylase Enzymatic Treatment (AET), Syringe Mechanical Treatment (SMT) and Amylase Syringe Mixed Treatment (ASMT) on sperm physical properties.

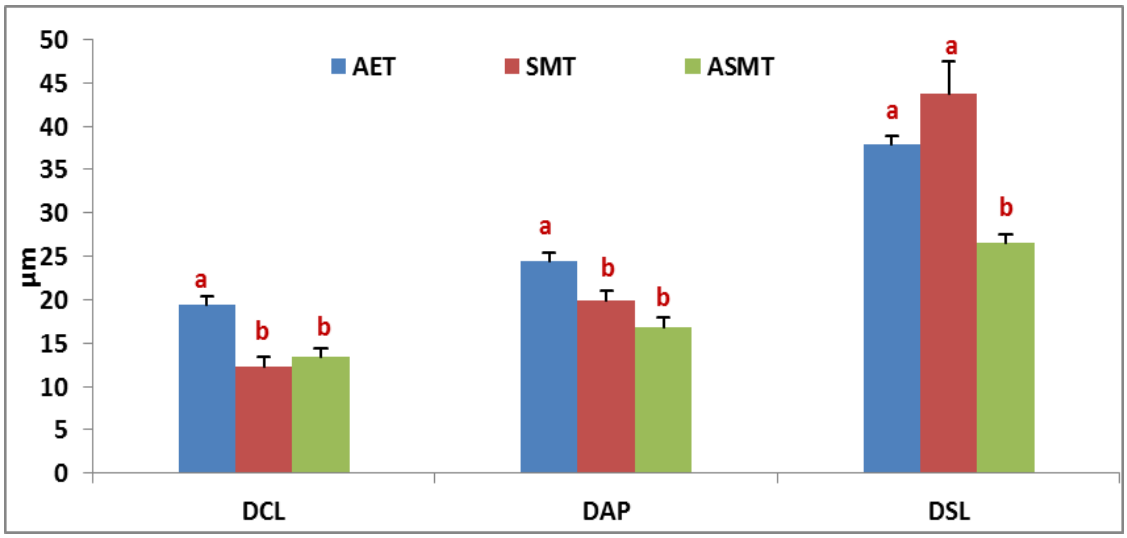

Figure 2. (A)

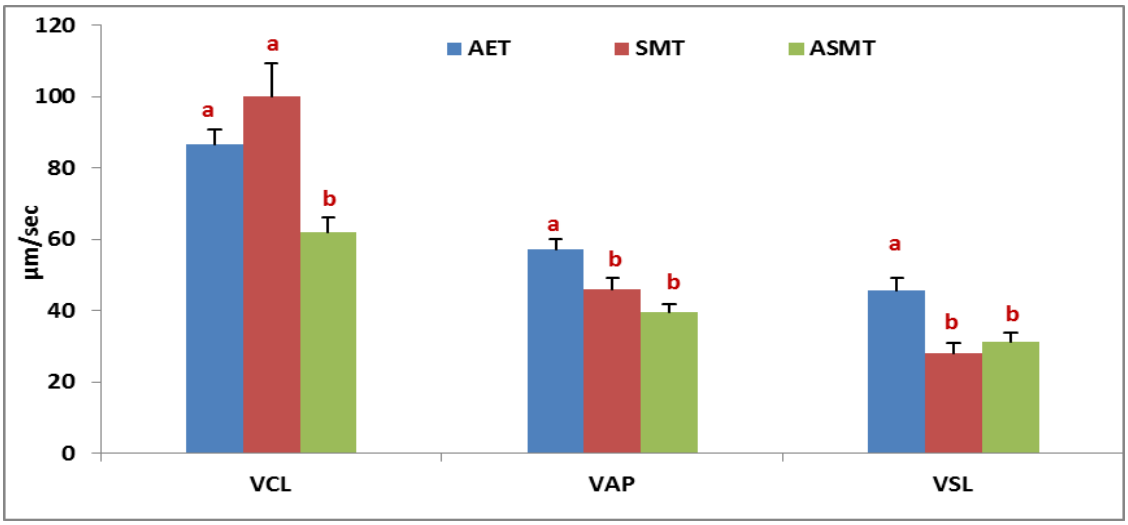

Figure 2. (B)

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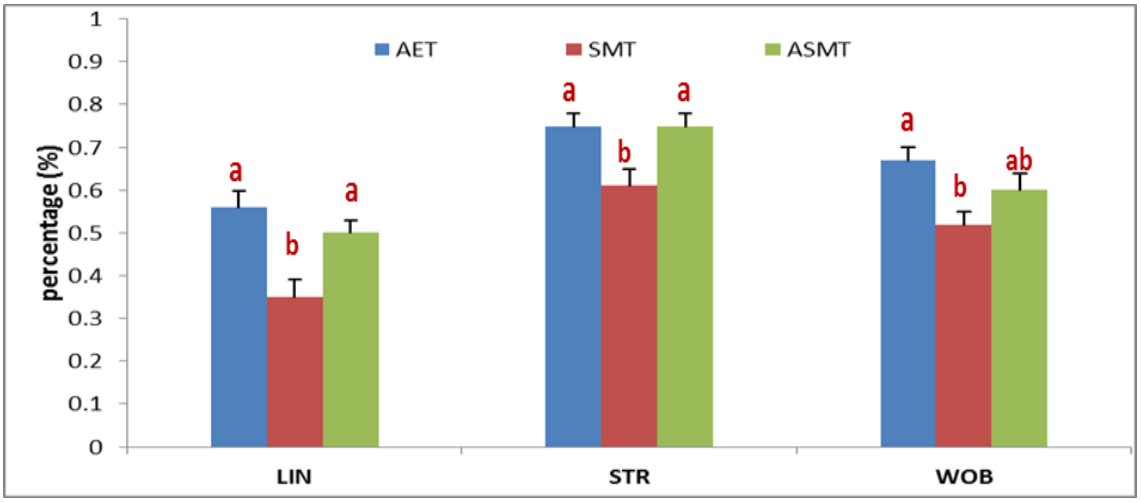

Figure 2. (C)

Figure 2. Effect of amylase enzymatic treatment (AET), syringe mechanical treatment (SMT) and amylase syringe mixed treatment (ASMT) on: Distance curved line (DCL, µm), distance average path (DAP, µm), distance straight line (DSL, µm) **(A)**; Velocity curved line speed (VCL, µm /sec), velocity average path (VAP µm/sec), velocity straight line (VSL, µm/sec) **(B);** Linearity movement (LIN =VSL/VCL), straightness movement (STR =VSL/VAP), balanced movement, wobble (WOB= VAP/VCL) **(C)**.

DISCUSSION

There is a general agreement that the difficulty of manipulating hyper viscous semen samples has been a reason to identify several methods being proposed to decrease viscosity for either initial evaluation or further processing steps of ARTs application. In mostly all species, semen rheological properties radically change immediately after collection. In this regard, viscous semen liquefaction is of fundamental importance for the application of a wide range of assisted reproductive technologies (ARTs) in different species, especially camelids (Crichton et al., 2015; Mal et al., 2016; Malo et al., 2017).

The aim of this investigation was to evaluate the sole enzymatic effect or the sole mechanical effect or a mixed in between treatment serving to reduce viscosity in dromedary camel ejaculates to attain the ability for further semen processing. Up to date, there has been a controversy regarding the reason of semen viscosity in camels.

Although camels have no seminal vesicles, but still mucin-like glycoproteins are generally known to be secreted from the bulbourethral glands (More´, 1991**).** As reported by Owen and Katz (2005**)** and later by Dissanayake et al. (2010)**,** the liquefaction factors responsible for clot lysis are derived from the prostate (plasminogen activator, a-amylase and prostate-specific antigen PSA). (Behr et al., 2009) recommended the usage α-amylase and collagenase without any effect on the quality parameters of spermatozoa, reporting that such enzymatic treatment allows better use of semen ejaculates in application sex sorting using flow cytometry technique. On the same basis, (Rateb, 2016)**,** based his study of using high-power low-frequency ultrasound efficiency in eliminating camel semen viscosity since it causes particle size reduction and viscosity alteration in different substances, this was attributed to modification in the microstructure and functional properties of carbohydrates as well as hydrolysis and cleavage of di- and polysaccharides (Kardos and Luche, 2001; Kbbani et al., 2011; Kunaver et al., 2012). Also, (Mendeluk et al., 2000) assumed that proteins are the main components responsible for the rheological behavior of the semen in its normal form. Hence, the rheological properties of hyper viscous semen samples indicated the existence of a highly organized network in which disulfide bonds and oligosaccharide chains complexes to the peptide core. Consequently, this may play a key role affecting the spermatozoa physiology and the sperm motility (Mendeluk et al., 2000; Skidmore et al., 2013).

As reported in this study, a superiority in using amylase as an enzymatic treatment for viscosity elimination, not only is succeeding in improving the motility and the kinetic characteristics of the sperm, but also the remarked softness of the rich fraction sperm clot, that allows easy packing in the French straws. The superiority of amylase for routine work examination of viscous semen specimens was reported earlier by (Dougherty et al., 1978)**,** as they noted that, the lowest level of amylase did not significantly alter semen parameters, with an 80% sufficient rate to liquefy viscous samples, but with careful regard to the level of the enzyme and the interval between addition and analysis must be controlled carefully. Based on the present data, the results elucidate that a high value of detached acrosome and abnormalities were observed when using amylase as compared to other treatments, but with no observation of sperm agglutination as reported lately by (Monaco et al., 2016) using proteolytic enzymes.

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Despite the fact that the results of enzymatic treatments surpass those obtained by mechanical methods, but still have acceptable post thaw motility compared to enzymatic treatment. Moreover, the SMT showed the highest distance straight line (DSL, µm), also recorded high sperm track speed, velocity curved line (VCL, µm/sec.), this is an indicative value for the ability of the mechanical effect to eliminate viscosity. Reduction of the seminal plasma viscosity may be attributed to the changes in the molecular behavior of semen protein fibrils (Amelar, 1962), making them less viscous and more liquefied without an observed reduction in sperm motility or morphological disorders. (Henkel and Schill, 2003) did not recommend the force elimination of viscous seminal fluid through expulsion of the semen ejaculates through a narrow-gauge needle as it caused sperm immotility. The aforementioned were totally contrary to the present data in this study showing an acceptable post-thaw motility after mechanical treatment, this finding was similar to those reported by (Kussler et al., 2014) who recently recommended the physical process of expulsion of semen through a syringe, but with a great attention to the idea that; this methodology may increase sperm DNA fragmentation. The mechanical stress (passage of diluted ejaculate through the syringe) may play a significant role in sperm DNA breaking. Unfortunately, the effect of the treatments on sperm DNA fragmentation in this study was not investigated. Taking into consideration that, although different studies had recorded high post thaw motility for eliminated viscosity with different methods, thus, still the pregnancy rates attempts depending on camelids semen processing show a very limited success (Aller et al., 2003; Deen et al., 2003; Vaughan et al., 2003; Miragaya et al., 2006; Crichton et al., 2016), as compared with fresh semen insemination, to the best of our knowledge the best of pregnancy rates using frozen semen was reported by Bravo et al. (2000b) when using collagenase for viscosity elimination or even for cryopreserved semen in different species. Literature surveys did not clarify any information regarding the reason of low pregnancy rates using frozen semen. Since the stages of embryonic development that depend on paternal genomes begin after the four-to eight-cell stage, the impact of sperm DNA fragmentation in the embryo is usually not observed in *in-vitro* embryo transfer if carried out preferably up to day three. Worth to mention that, the blastocyst stage development rate and implantation till pregnancy may be affected (Borini et al., 2006), in addition to, expected pregnancy losses (Tarozzi et al., 2007; Rougier et al., 2013). Probably the significant deleterious effect on post thaw motility when mixing between enzymatic and mechanical treatments (ASMT) in the current work was associated with the high stress that may lead to a significant DNA breaking. Lack of consistency in information should be addressed by more investigations concerning DNA fragmentation in cryo-preserved camel semen.

CONCLUSION

From this study, it could be concluded that the viscosity of dromedary camel seminal plasma could be successfully reduced by the use of either mechanical or enzymatic effect, as both may be considered a reliable alternative with different rates of success, with care to the concentration and power of the enzyme and handling and semen ejaculate manipulation during mechanical treatment.

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Competing interests

The author declares that he has no conflict of interest with respect to the research, authorship, and/or publication of this article, the author declares that he has no competing interests.

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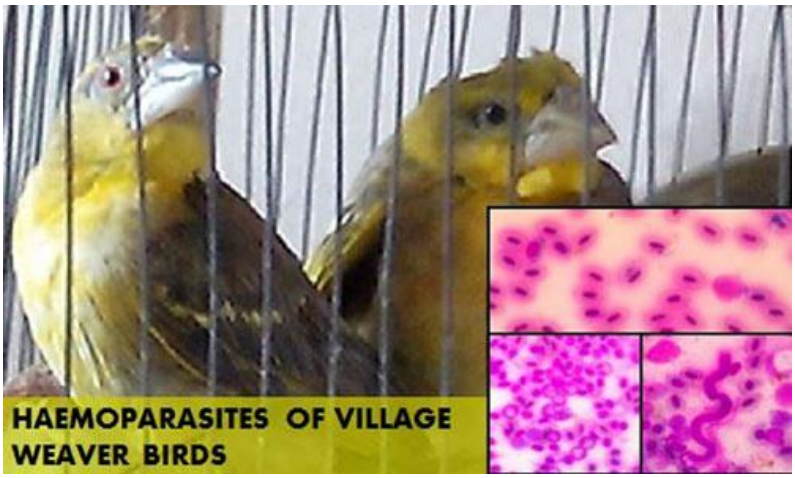

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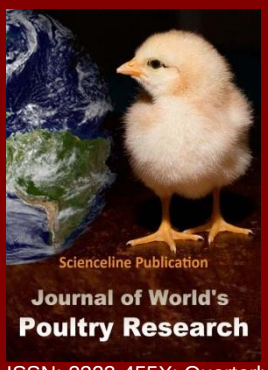

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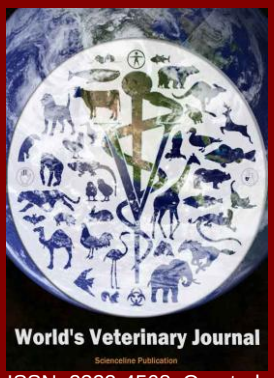

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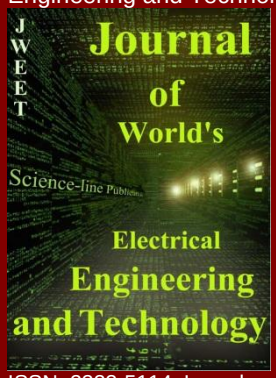

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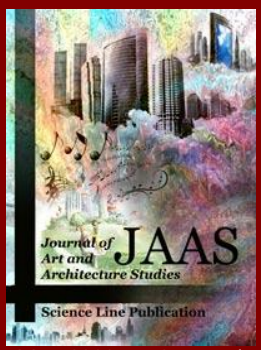

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