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Haematobiochemical Changes and Postoperative Complications following Elective Ovariohysterectomy in Dogs

By M. A. Rafee, P. Kinjavdekar, Amarpal, H.P. Aithal, S. A. Wani, & P. Sangeetha

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Abstract- Ovariohysterectomy was performed via ventral midline clinical cases in dogs (n=35) to present haematobiochemical changes and postoperative complications of elective ovariohysterectomy under dexmedetomidine basal anaesthesia in dogs. Total Leukocyte count and Haemoglobin concentration decreased, whereas, glucose increased significantly. There was a no significant change in neutrophil count, packed cell volume, creatinine, insulin and cortisol. Complications were observed in seven out of thirty five animals. Intra-abdominal haemorrhage was observed in three, abdominal wound dehiscence in 3 animals and ovarian remnant syndrome occurred in one dog. Stress response to surgeries was obtunded dexmedetomidine induced basal anaesthesia.

Keywords: ovariohysterectomy, complications, dogs, stress response, dexmedetomidine.

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Haematobiochemical Changes and Postoperative Complications following Elective Ovariohysterectomy in Dogs

M. A. Rafee [α], P. Kinjavdekar [σ], Amarpal [ρ], H.P. Aithal [ω], S. A. Wani [¥] & P. Sangeetha [§]

Abstract- Ovariohysterectomy was performed via ventral midline clinical cases in dogs (n=35) to present haematobiochemical changes and postoperative complications of elective ovariohysterectomy under dexmedetomidine basal anaesthesia in dogs. Total Leukocyte count and Haemoglobin concentration decreased, whereas, glucose increased significantly. There was a no significant change in neutrophil count, packed cell volume, creatinine, insulin and cortisol. Complications were observed in seven out of thirty five animals. Intra-abdominal haemorrhage was observed in three, abdominal wound dehiscence in 3 animals and ovarian remnant syndrome occurred in one dog. Stress response to surgeries was obtunded dexmedetomidine induced basal anaesthesia. Complications after ovariohysterectomy has been seen in surgeries carried out by experienced surgeons. Surgeons must be prepared for such complications.

Keywords: ovariohysterectomy, complications, dogs, stress response, dexmedetomidine.

I. Introduction

Elective sterilisation of female dogs is one of the most common procedures performed in veterinary practice accomplished by removing both the ovaries and uterus (ovariohysterectomy) or by removing the ovaries alone (ovariectomy) but ovariohysterectomy has historically been recommended. It is generally performed for population control, prevention of diseases of the reproductive tract, and elimination of undesirable behaviours associated with hormonal cycling. Mammary tumours are the most common tumours in female dogs, with an overall incidence of 3.4% out of which 41% to 53% of mammary gland tumours are reportedly malignant and metastasis is common [1, 2]. An important time-dependent benefit of elective sterilisation in female dogs is the decreased incidence of mammary gland tumours [3]. Elective ovariohysterectomy also reduces incidence of endometrial hyperplasia – pyometra complex and uterine neoplasia. However, there are many post operative complications reported with ovariohysterectomy, the incidence ranging from 6.2% to 20.6%. The aim of this study was to record the most common complications associated with ovariohysterectomy.

II. Material and Methods

a) Climatic Condition and Experimental Animals

Geographically, Bareilly U.P is located at 28°10′N 78°23′E in northern India, at an altitude of 166 m above mean sea level. Bareilly has extreme climate changes, temperatures range from 4 °C to 44 °C.

The study was conducted on healthy dogs presented to a Referral Veterinary Polyclinic for elective ovariohysterectomy. Complete history of the animal including breed, age, parity and stage of oestrous cycle was recorded. Clinical examination of the animals included general condition, colour of gingival mucous membrane, heart rate, respiratory rate and rectal temperature. Venous blood samples were collected aseptically in dry syringes for estimation of haemoglobin, packed cell volume, total leukocyte count, differential leukocyte count, urea nitrogen, glucose and creatinine.

b) Procedure

The animals were fasted since the previous day in the context of elective surgery. Pre-emptive analgesia and prophylactic antibiotic were administered in all the animals. Surgery was carried out under general anesthesia. Ventral abdomen was prepared for aseptic surgery and mid line incision (via Linea Alba) starting from the umbilicus and extending few centimetres towards pubis was given to provide direct approach and access to the uterine horns and facilitated prehension of the ovaries. The bladder was retracted laterally; one of the horns was exposed and followed cranially up to the ovary bursa. The ovary was grasped and supensory ligament cut (when possible with ease) and a window was created in broad ligament around ovarian artery and vein. The ovarian blood vessels were crushed with hemostat and ligature of absorbable suture material was tied and hemostat was removed, simultaneously, so that the ligature comes into the groove created by hemostat. Two clamps (hemostats) were then placed between this ligature and the ovary

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and the pedicle was sectioned between the two. Hemostat near to ligature was removed and the quality of the hemostats checked; the long ends of the suture material on the ovarian pedicle were cut. The ovarian pedicle was held throughout the procedure with a hemostat.

The broad ligament was torn the middle above the uterine artery. This was followed by sectioning of the uterine cervix after ligation of the uterine arteries and veins separately as well as by trans-fixation suture. The cervix was crushed with artery forceps and another hemostat was placed just above the first and the cervix sectioned with a scalpel between the two hemostats. The sutured stump was returned to the abdominal cavity after checking the quality of hemostats. Peritoneum and Linea Alba were sutured with interrupted pattern and subcutaneous connective tissue with simple continuous pattern, using PGA. Finally, the skin was sutured mattress sutures using nylon. The wound was then disinfected with antiseptic solution and protected with gauze bandage and adhesive tape.

Intravenous fluid therapy was administered with isotonic saline perioperatively. The animals were then placed on antibiotic and analgesic therapy for at least 5 days after surgery. The sutures were removed after 10 days in uncomplicated cases.

c) Statistical Analysis

All data were summarised using descriptive statistics and values reported as mean ±SE. Continuous variables were then categorised to facilitate analysis. Dependent variable was alive (yes/no). Significance was $P<0.05$.

III. Results and Discussion

Thirty five healthy dogs were presented for elective ovariohysterectomy. All animals at the admission were in the age of 6 months to 9 years but 28 dogs were 1 to 3 years old. The most common breeds presented for neutering were Spitz and Pomeranian. The intensity breeds presented may be because of the popularity of such small breeds in the local area. Most of the animals were presented during their pro-oestrus or oestrus phases and the surgery performed few weeks later. Other owners had preset plan to spay their dogs and some among them believed female dogs should have a litter before being spayed. Hygiene issues and the nuisance created by the dog during pro-oestrus and oestrus stages subjected the owners to opt for spaying during these stages. Some owners (apart from these 35 owners) didn't report after taking the scheduled date, may be due to their concern about the risk in anesthesia and surgery for their pet, cost of the surgery and post operative care.

Complications that have been reported secondary to ovariohysterectomy in the dog and cat include hemorrhage, ovarian remnant syndrome, stump pyometra, stump granuloma, fistulous draining tracts, eunuchoid syndrome, accidental ureteral ligation, and oestrogen responsive urinary incontinence [4, 5]. In the previous reports, surgical complication rates associated with ovariohysterectomy in healthy dogs and cats have been reported to range from 6.2% to 20.6%. In the present study, complications were observed in seven out of thirty five animals (7/35). Intra-abdominal hemorrhage is one of the most common complications secondary to an ovariohysterectomy, and can even result in death of the patient if severe [5]. Intra-abdominal hemorrhage was observed in total of three dogs and it occurred only after releasing the ligated ovarian pedicle and cervical stump back into the abdominal cavity. In one animal hemorrhage was observed only during surgery and there was no oozing of blood though incision line after closing the abdomen. Hemorrhage can occur from the ovarian pedicle, uterine pedicle or from the broad ligament but in this study source of location of the bleeding was not ascertainable. Hemocoagulase was sprayed locally as well administered intravenously which successfully controlled the hemorrhage. In another animal there was little oozing of blood through incision line after closure of abdominal cavity which decreased progressively till it stopped after 12 (next day) hours and in the third animal little oozing continued up to 24 hrs. Post operative intra abdominal hemorrhage in these cases was confirmed by abdomenocentesis. In these two animals hemocoagulase administration as well as abdominal pressure bandage was applied till blood stopped oozing through incision line. Hemocoagulase is isolated from venom of Bothrops atorox or Bothrops jararaca contains two different types of enzymes acting on blood coagulation; of which one has thrombin like action and the other one has thromboplastin like effect. It acts by conversion of fibrinogen to fibrin polymer and promotes the interaction of platelets with fibrin clot to coagulate the blood [6]. Abdominal pressure bandage successfully stopped postoperative bleeding in ovariohysterectomy [7].

Abdominal wound dehiscence was observed in 3 animals, out of which two were Spitz and one Labrador. Wound dehiscence is one among the common complications of surgical wounds, involving the breaking open of the surgical incision along the suture. Problems associated with incisional healing following ovariohysterectomy, is sometimes far exceeding the incidence of intraoperative hemorrhage [7]. Malnourishment, sudden increase in abdominal pressure, infection, Obesity, diabetes and hypersensitivity to catgut can be the various factors causing suture dehiscence. These wounds were derided and sutured again. One Spitz in which abdominal suturing done with catgut was presented with wound dehiscence, instead of PGA, was presented three times and every time re-sutured with PGA. Third time all the

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catgut was removed; edges derided and sutured using PGA. The wound healed successful.

Ovarian remnant syndrome occurred in one dog. The dog developed the clinical signs of pro-estrus and oestrus signs like vaginal discharge, vulvar swelling, behavioural changes and even mated with a dog. Residual ovarian tissue most commonly results from incomplete resection of the ovary during the initial surgery or fragments of ovarian tissue can become revascularized through the mesentery or omentum, maintaining functional status indefinitely [8, 9]. This complication is usually attributable to surgical error. Techniques that may predispose to ovarian remnant syndrome include inadequate exposure of the ovarian pedicles resulting in poor visualisation, inaccurate placement of clamps or ligatures, or accidental separation of a portion of the ovary with subsequent loss of the tissue in the abdomen. This syndrome has been observed even after ovriohysterectomies carried out by experienced veterinarian [8, 9]. This syndrome results into signs of pro-estrus, oestrus, and (rarely) false pregnancy and cornification of vaginal epithelial cells during pro-estrus or oestrus demonstrated on cytology as well [10].

a) Haematobiochemical parameters

Haematobiochemical parameters on admission are summarised in table 1. The stress response to surgery is characterized by increased secretion of pituitary hormones and activation of the sympathetic nervous system [11]. Release of corticotrophin from the pituitary stimulates cortisol secretion from the adrenal cortex. In the pancreas, glucagon is released and insulin secretion may be diminished. Blood glucose concentrations increase after surgery begins. Haematology shows alteration under stress. PCV decreased nonsignificantly ($p>0.05$ and Hb decreased significantly ($p<0.05$). The decrease in haemoglobin and PCV levels might be due to due to shifting of fluids from the extravascular compartment to the intravascular compartment in order to maintain the cardiac output in the animals [12], haemodilution in response to fluid therapy [13] and due to dexmedetomidine which has been shown to preserve blood flow to the most vital organs (brain, heart, liver and kidney) at the expense of organs like skin and pancreas [13]. Similar findings were also observed in earlier studies [14, 15]. TLC decreased significantly in the postoperative period from the baseline; however, there was a negligible change in neutrophils count. Negligible changes in neutrophils count can be attributed to dexmedetomidine, which directly (inhibiting neuroendocrine response) or indirectly (sedation and analgesia) obtund the stress response when administered systemically. A significant decrease in TLC might be due to haemodilution.

There was nonsignificant ($p>0.05$) decrease in plasma creatinine concentration. Preservation of blood supply to vital organs by dexmedetomidine [13] and continuous intravenous fluid infusion might have been responsible for adequate renal blood flow and enough glomerular filtration rates to decrease plasma creatinine values but maintaining it near the baseline. Insulin also decreased, although, nonsignificantly ($p>0.05$). The decrease in insulin concentrations may be partly by alpha-2 adrenergic inhibition of beta cell secretion. In addition, there is a failure of the usual cellular response to insulin, the so called 'insulin resistance', which occurs in the perioperative period [16].

Cortisol concentrations have been associated with a variety of surgical procedures conducted under anaesthesia in dogs [17, 18]. Dexmedetomidine obtunds stress response and a delayed ACTH and cortisol response has been recorded in previous studies in dogs undergoing ovariohysterectomy in which medetomidine had been administered preoperatively [19]. In this study cortisol increased but nonsignificantly. Dexmedetomidine prevented the extreme rise in cortisol levels by directly (inhibiting neuroendocrine response) or indirectly (sedation and analgesia) obtunding the stress response. Blood glucose concentrations increased significantly ($p<0.05$) over base values in post operative period. Blood glucose level increases just after the start of surgery due to cortisol and catecholamine mediated gluconeogenesis and glycogenolysis as well as due to decreased peripheral use of glucose [16]. The usual mechanisms that maintain glucose homeostasis are ineffective during perioperative period. Alpha-2 agonists have been reported to induce an increase in serum glucose by suppressing insulin release, stimulating glucagon release [20, 21].

IV. Conclusion

From the present study it can be concluded that Complications after ovariohysterectomy has been seen in surgeries carried out by experienced surgeons. Surgeons must be prepared for such complications. Stress response to surgeries was obtunded to a greater extent by dexmedetomidine when given as a component of basal anaesthesia. This prevented the stress related neutrophilia and extreme increase in cortisol concentration. Blood glucose levels still increased significantly due to direct effects of dexmedetomidine on pancreas. Blood supply to vital organs like kidney was well maintained by dexmedetomidine and fluid therapy and thus prevented the extreme changes in creatinine in blood.

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Table 1: Mean (± SE) haematobiochemical profile before and after elective ovario-hysterectomy in healthy dogs.

Parameters	Base Line	Post Recovery
TLC ($\times 10^9$/L)	9.68±0.83	7.23±0.62*
PCV (L/L)	0.52±0.03	0.48±0.03
Hb (g/L)	144.58±6.57	110.62±4.24*
Creatinine (μmol/L)	103.95±3.24	95.96±3.19
Cortisol (nmol/L)	152.07±14.80	176.88±24.30
Insulin (μIU/ml)	6.84±1.01	9.05±1.58
Glucose (mmol/L)	5.45±0.37	8.23±0.55
Neutrophils (%)	61.26±1.03	61.41±1.15

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Prevalence and Risk Factors of Human and Bovine Tuberculosis at Mymensingh District in Bangladesh

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Abstract- Tuberculosis (TB) is a major global health problem and economically important zoonotic diseases worldwide. This study was conducted to determine the prevalence of tuberculosis and risk factor in human and cattle at Mymensingh district in Bangladesh. In this study, 3085 human and 649 cattle were examined during January 2009 to December 2011 at Dhobaura upazila of Mymensingh district. The overall prevalence of tuberculosis in human and animal was 9.7% and 2.34%, respectively ($p<0.01$). The difference in the prevalence of tuberculosis in human and cattle is statistically significant ($p<0.001$). Statistically significant higher prevalence was found in the age group of ≤29 years and 30≤39 years than 40≤49 years, 50≤59 years and ≥60 years age group of human ($p<0.001$).

Keywords: human, bovine, tuberculosis, prevalence, risk factor, Bangladesh.

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Prevalence and Risk Factors of Human and Bovine Tuberculosis at Mymensingh District in Bangladesh

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Abstract- Tuberculosis (TB) is a major global health problem and economically important zoonotic diseases worldwide. This study was conducted to determine the prevalence of tuberculosis and risk factor in human and cattle at Mymensingh district in Bangladesh. In this study, 3085 human and 649 cattle were examined during January 2009 to December 2011 at Dhobaura upazila of Mymensingh district. The overall prevalence of tuberculosis in human and animal was 9.7% and 2.34%, respectively ($p<0.01$). The difference in the prevalence of tuberculosis in human and cattle is statistically significant ($p<0.001$). Statistically significant higher prevalence was found in the age group of 20 ≤29 years and 30≤39 years than 40≤49 years, 50≤59 years and ≥60 years age group of human ($p<0.001$). The relationship among the prevalence in different age group of cattle was statistically insignificant ($p=0.129$). In human, statistically significant higher prevalence was recorded in female (11.2%) than in male (8.6%) ($p=0.02$). But in cattle, statistically insignificant slightly higher prevalence was recorded in male (2.4%) than in female (2.1%) ($p=0.777$). In human, highest prevalence was found in the April month (15%) and lowest in the July month (5.7%) ($p=0.012$). In cattle, highest prevalence was found in the April and October month (3.8%) and no positive cases were recorded in the July month.

Keywords: human, bovine, tuberculosis, prevalence, risk factor, bangladesh.

I. Introduction

Tuberculosis (TB) remains is a major global health problem (Cosivi *et al.*, 1998; Schiller *et al.*, 2010). Tuberculosis plays a central role in public health and animal health because of its severe disease in humans and significant economic losses to cattle producers related to affected herds (Rodriguez *et al.*, 1999; Ayele, *et al.*, 2004; Zinsstag *et al.*, 2006; Samad, 2008; OIE, 2009). *Mycobacterium tuberculosis, M. bovis* causing disease in humans ((Dankner *et al.*, 1993). *M. bovis* is the most universal pathogen among mycobacteria and affects many vertebrate animals of all age groups although, cattle, goats and pigs are found to be most susceptible, while sheep and horses are showing a high natural resistance. *M. tuberculosis* and *M. bovis* are genetically and antigenically very similar and cause identical clinical disease in humans. ((Radostis *et al.*, 2000; Zinsstag *et al.*, 2006). Transmissions of tuberculosis in humans are mainly by inhalation and ingestion of raw milk or unpasteurized dairy products or meat from an infected animal ((Srivastava *et al.*, 2008). Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection of cattle, but infection by ingestion of a contaminated material also occurs ((Biet *et al.*, 2005). Fever, night sweats, weight loss, poor appetite, weakness, chest pain, swollen glands and breathing problems, a general sick feeling are the general symptom in human. In cattle, the early stages of TB, clinical signs are not visible. In later stages, clinical signs may include: emaciation, lethargy, weakness, anorexia, low-grade fever, and pneumonia with a chronic, moist cough. Lymph node enlargement may also be present ((Radostis *et al.*, 2000). The most common method for diagnosing TB in human worldwide is sputum smear microscopy (developed more than 100 years ago). Chest x-ray also a common method for TB diagnosis in human. In countries with more developed laboratory capacity, cases of TB are also diagnosed via culture methods. TB in Bangladesh is commonly diagnosed by suggestive clinical symptoms and signs coupled with a suggestive chest x-ray and sputum sample (Matin *et al.*, 2011). Bovine TB is difficult to diagnose with clinical signs alone. Many methods are available for diagnosis of tuberculosis in infected animals but the single comparative intradermal tuberculin test (SCITT) is most widely used for diagnosis and eradication of Bovine tuberculosis (OIE, 2009). In Bangladesh, so far the single intradermal (SID) skin test with purified protein derivative (PPD) has been used to detect the prevalence of bovine TB (BTB) ((Pharo *et al.*, 1981; Samad and Rahman, 1986; Islam *et al.*, 2007). Sero-diagnostic tests, ICGA as Antigen Rapid Bovine TB Ab Test Kit was

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used to detect Bovine TB and its effect on milk production in lactating cows in Bangladesh ((Rahman and Samad, 2008).

Tuberculosis causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide. The latest estimates included that there were almost 9 million new cases in 2011 and 1.4 million TB deaths worldwide. About 89% of the world's TB cases are account for 96 countries. Bangladesh ranks 5th Globally (WHO, 2006). In Bangladesh mortality rate in human varies from 19 to 82 (average 45), Prevalence varies from 199 to 698 (average 411), incidence varies from 185 to 268 (average 225) per 100 000 population (WHO, 2012). There is little literature available on the prevalence of tuberculosis in human and animal in Bangladesh. Therefore, the study was conducted to determine the prevalence and risk factors associated with human and bovine tuberculosis.

II. Methodology

This was a prospective, cross-sectional, observational study conducted among the human and animal population simultaneously. The study was conducted for the period of three years starting from January 2009 to December 2011 to determine the prevalence and risk factors associated with human and bovine tuberculosis.

a) Selection of study population

In this study, 3085 patients who were admitted in Dhobaura Health Complex and 649 cattle registered in Dhobarua Upazila Veterinary Hospital were selected. A detailed history, age, sex were recorded in a questionnaire from disease register maintained by the Upazila Tuberculosis and Leprosy Control Unit, Health Complex, Veterinary Hospital, human residence and animals owners houses of Dhobaura upazila in Mymensingh.

b) Diagnoses of cases

The diagnosis of human tuberculosis was based on history, clinical examination, BCG test and X-ray, Sputum examination, tuberculin test, lymph node biopsy and histological or cytological examination at Dhobaura Health Complex, Mymensingh; Mymensingh Medical college Hospital, Mymensingh; Department of Medicine, Bangladseh Agriculturl University, Mymensingh, Bangladesh. Acid fast bacilli were demonstrated in the section of chemical and mediastinal lymphnodes by acid fast staining. Bovine tuberculosis was diagnosed based on history, clinical findings, complete physical examination, Caudal fold tuberculin (CFT) test at Dhobarua Upazila Veterinary Hospital, Mymensingh and Department of Medicine, Bangladseh Agriculturl University, Mymensingh, Bangladesh. To determine the seasonal influence on the clinical prevalence of tuberculosis in human and animals, the data were collected in different months of the year.

c) Caudal fold tuberculin (CFT) test

This is the primary screening test to identify animal potentially infected with bovine TB. The test measures the immune response to *Mycobacterium bovis,* the causative agent of bovine TB. The test was performed by intradermal injection of 0.1 ml bPPD with a hypodermic syringe in the skin of the caudal fold (the fold of skin at the base of the tail). If the animal was exposed to mycobacteria, the immune system responded with inflammatory cells at the injection site to cause swelling and/or discoloration of the skin. After 72 hours, inspection and palpation of the injection site was done to evaluate for a response. Marked edematous swelling, reddening at the injection site classified the animal as a responder. If no response was noted, the animal was classified as CFT test-negative. Responder animals were further tested with CCT test for confirmation.

d) Statistical analysis

The collected data was compiled, tabulated and analyzed in accordance with the objectives of the study. The approximate percentage was calculated for each parameter. The questionnaire-based data was processed in Microsoft Excel and analyzed in SPSS. The z-test for proportions was done to find out the relationship of different factors on the occurrence of tuberculosis in human and cattle. Where Significance was determined in terms of age, sex, year and month of occurrence at 5% level.

III. Results

a) Overall prevalence of tuberculosis in human and cattle

In this study, 3085 human and 649 cattle of different sexes and ages were examined to determine the prevalence and risk factors associated with human and bovine tuberculosis. Out of 3085 human, 300 were shown positive reaction to human tuberculosis and out of 649 cattle, 15 were shown positive reaction to bovine tuberculosis. So the overall prevalence was 9.7% in human and 2.34% in cattle (Table 1). The difference in the overall prevalence of tuberculosis in human and cattle is statistically significant ($p<0.001$).

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Table 1: Comparison between prevalence of human and bovine tuberculosis in Mymensingh District

Species	Total selected	TB positive	Percentage (%)	95% CI (%)	P Value	Level of significance
Human	3085	300	9.7	8.7-10.7	<0.001	S
Cattle	649	15	2.3	1.1-3.5		

S=significant at 1% level of significance

b) Prevalence in human

Age-wise prevalence of tuberculosis in human revealed that the prevalence was 19.4%, 15.5%, 10.1%, 3.7% and 1.7% in 20≤29 years, 30≤39 years, 40≤49 years, 50≤59 years and ≥60 years age group, respectively. Highest prevalence (19.4%) was found in age group 20≤29 years old human. Prevalence was gradually decreasing with higher age group and lowest prevalence was recorded in the ≥60 years age group. Statistically significant higher prevalence was found in the age group of 20≤29 years and 30≤39 years than 40≤49 years, 50≤59 years and ≥60 years age group (p<0.001). Also statistically higher prevalence was found in the age group of ≤40 years than 50≤59 years and ≥60 years age group (p<0.001). (Table 2).

Table 2 : Prevalence of tuberculosis based on different risk factors in human at Dhobaura upazila in Mymensingh

Age group (years)	Selected human	TB positive	Percentage (%)	95% CI (%)	P Value	Level of significance
20≤29	371	72	19.4	15.4-23.4		
30≤39	504	78	15.5	12.3-18.7		
40≤49	1130	114	10.1	8.3-11.9	<0.001	S[a]
50≤59	900	33	3.7	2.5-4.9		
≥60	180	3	1.7	-0.2-3.6		
Sex						
Male	2085	201	8.6	7.4-9.8	0.02	S[b]
Female	1000	99	11.2	9.2-13.2		
Year						
2009	959	100	10.4	8.5-12.3		
2010	1165	101	8.7	7.1-10.3	0.304	NS
2011	961	99	10.3	8.4-12.2		

NS= Not significant at 5% level of significance,

[a]= significant at 1% level of significance

[b]= significant at 5% level of significance

Sex-wise prevalence of tuberculosis in human showed that higher prevalence was recorded in female (11.2%) than in male (8.6%) which is statistically significant (p=0.02) (Table 2). Over the three study years slightly similar prevalence was found in the year 2009 (10.4%) and 2011 (10.3%). Lower prevalence was found in the year 2010 (8.7%) than 2009 and 2011. The difference in the prevalence of three years is not statistically significant (p=0.304) (Table 2).

Monthly distribution of tuberculosis in human is shown in the Figure 1. The distribution revealed that highest prevalence was found in the April month (15%) and lowest prevalence was found in the July month (5.7%). The difference in the prevalence of tuberculosis in April and July months is statistically significant (p=0.012). The difference among the prevalence of tuberculosis in the other months of the year is statistically insignificant (p>0.05).

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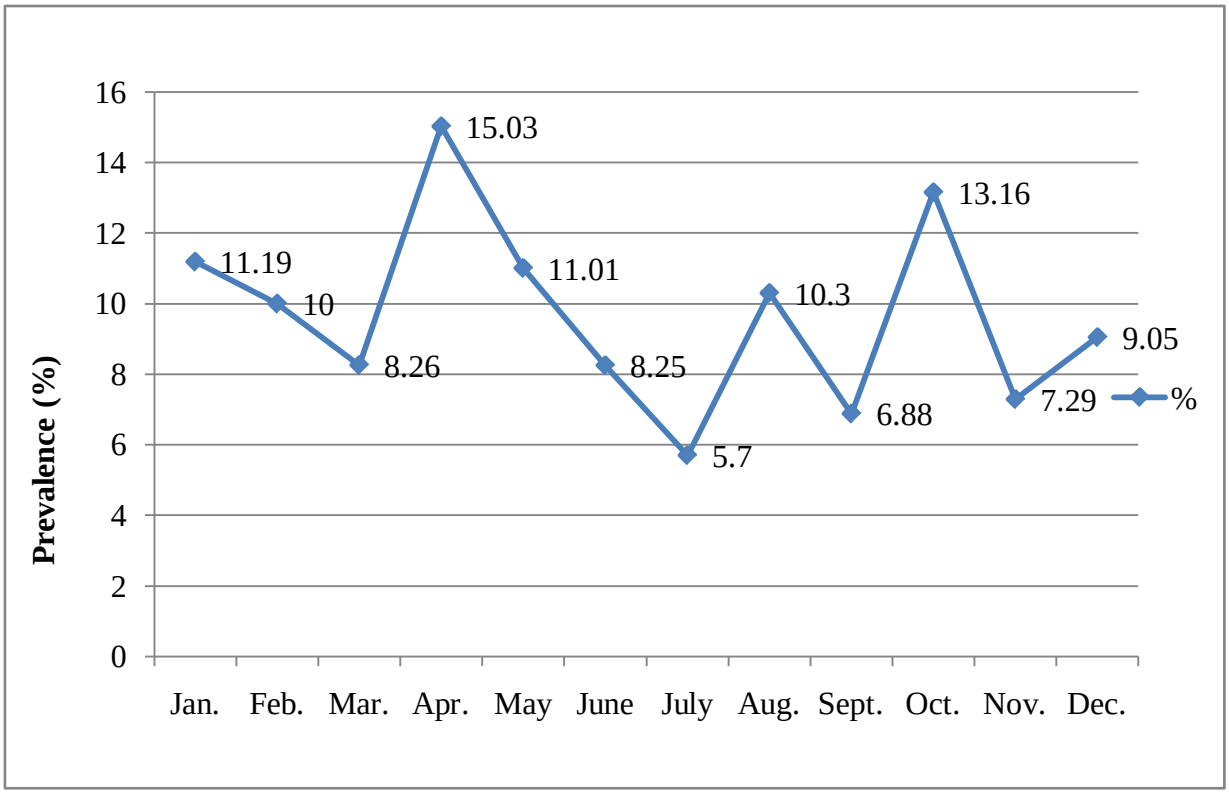

Figure 1 : Monthly distribution of tuberculosis in human

c) Prevalence in animal

Age-wise prevalence of tuberculosis in cattle revealed that the prevalence was 0%, 1.8%, 3.0% and 4.0% in ≤4 years, 4.1≤5 years, 5.1≤6 years and 6.1≤10 years age group, respectively. Highest prevalence (4%) was found in age group 6.1≤10 years old cattle. Prevalence was gradually decreasing with lower age group and no tuberculosis cases were recorded in the ≤4 years age group. The relationship among the prevalence in different age group is statistically insignificant (p=0.129) and ≤4 years age group is not included in the statistical comparison as its proportion is zero (Table 3). Sex-wise prevalence of tuberculosis in cattle showed that slightly higher prevalence was recorded in male (2.4%) than in female (2.1%) but not statistically significant (p=0.777) (Table 3). Over the three study years same prevalence was found in the year 2009 (2.2%) and 2011 (2.2%). Slightly higher prevalence was found in the year 2010 (2.5%) than 2009 and 2011. The difference in the prevalence of three years is not statistically significant (p=0.97) (Table3).

Table 3 : Prevalence of tuberculosis based on different risk factors in cattle at Dhobaura upazila in Mymensingh

Age group (years)	Selected cattle	TB positive	Percentage (%)	95% CI (%)	P value	Level of significance
2≤4	135	0	0	0		
4.1≤5	165	3	1.8	-0.2-3.8	0.129	NS
5.1≤6	198	6	3.0	0.6-5.4		
6.1≤10	151	6	4.0	0.9- 7.1		
Sex						
Male	410	10	2.4	0.9-3.9	0.777	NS
Female	239	5	2.1	0.3-3.9		
Year						
2009	230	5	2.2	0.3-4.1		
2010	240	6	2.5	0.5-4.5	0.97	NS
2011	179	4	2.2	0.1-4.3		

NS= Not significant at 5% level of significance

Monthly distribution of tuberculosis in cattle is shown in the Figure 2. The distribution revealed that highest prevalence was found in the April and October month (3.8%) and no positive cases were recorded in the July

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month. July month was not included in the statistical comparison as its proportion is zero. The difference among the prevalence of tuberculosis in the other months of the year is statistically insignificant (p=0.985).

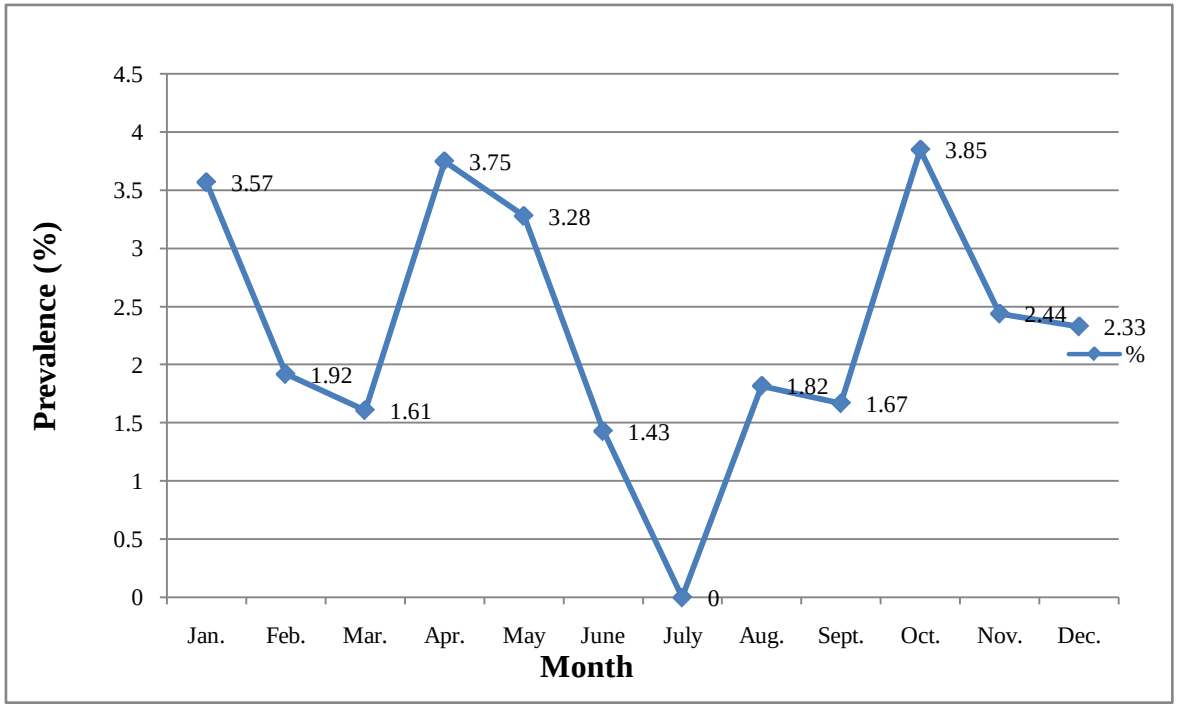

Figure 2 : Monthly distribution of tuberculosis in cattle

d) Comparison between human and bovine tuberculosis

Higher prevalence of tuberculosis was reported in human (9.7%) compared to cattle (2.34%) (Table 1). The difference in the prevalence of tuberculosis in human and cattle is statistically significant (p<0.001). Prevalence was gradually decreasing from comparatively lower age group (20≤29) towards higher age group ≥(60) of human. Where prevalence was gradually increasing from comparatively lower age group (2≤4) towards higher age group (6.1≤10) of cattle. In human, prevalence was higher in female (11.2%) than in male (8.6%) but in cattle, prevalence was higher in male (2.4%) than in female (2.1%). Monthly distribution of tuberculosis revealed that similar strand was found both in human and cattle. Prevalence was gradually decreased from January to March and then peak at April followed by lowest prevalence at July. Prevalence was fluctuating in the rest of the year with an increase prevalence at October in both cases.

IV. Discussion

The Tuberculosis is of paramount importance and public health authorities because of its economic and zoonotic implications (Hermandez and Baca, 1998). It is quite prevalent in Bangladsh (Rahman and Samad, 2008; Samad and Rahman, 1986; Pharo et al., 1981). It is now estimated that every year 300 000 people in Bangladesh develop active tuberculosis (Karim et al., 2012). The paucity of literature on the prevalence of tuberculosis in human and animal in Bangladesh encouraged the authors to report findings. Therefore the study was conducted to determine the prevalence and risk factors associated with the occurrence of tuberculosis in human and animal.

In this study, significant difference was found on the overall prevalence of TB in human (9.7%) and in cattle (2.34%). Where, Ibrahim et al. (2012) found no statistically significant association between reactor cattle (2%) and human TB cases (5%) in the households. This could be due to difference in agro-ecological zones and management system. Age-wise prevalence of tuberculosis in human revealed that highest prevalence (19.4%) was found in age group≤2920 years old human. Prevalence was gradually decreasing with higher age group and lowest prevalence was recorded in the≥60 years age group. The present study corresponds to the study of Biswas et al. (1999) who found more prevalence in young than old. But does not corresponds to the study of Zaman et al. (2012) who reported highest prevalence in the 55-64 years age group and lowest in 15-24 years age group. The present study reveals that the prevalence of female patient (11.2%) is more than that of male patient (8.6%) which are in disagreement with the observation of Baker et al. (1996) and Zaman et al. (2012) who reported more prevalence in male than in female. Weiss et al. (2008) studied on cultural epidemiology of TB with reference to gender in Bangladesh, India and Malawi. They found

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that female patients reported more diverse symptoms and men more frequently focused on financial concerns. Men emphasized smoking and drinking alcohol as causes of TB, and women in Malawi reported sexual causes associated with HIV/AIDS. Over the three study years, slightly similar prevalence was found in the year 2009 (10.4%) and 2011 (10.3%). Lower prevalence was found in the year 2010 (8.7%). Monthly distribution of tuberculosis in human revealed that highest prevalence was found in the April month (15%) and lowest prevalence was found in the July month (5.7%). There is no report available in literature to compare on monthly distribution of TB in human.

In this study the overall prevalence was 2.34% in cattle by caudal fold tuberculin test. The prevalence is slightly higher than the earlier reports of prevalence in indigenous cattle (2.10%) but lower than the prevalence in cross-bred cattle (7.80%) detected with a caudal fold tuberculin test in Bangladesh reported by Samad and Rahman, (1986) and however, these increased prevalence rate of bTB in RCC might be due to differences of the sensitivity of the test used, increased infection rate and different breed tested (Samad and Rahman, 1986). The prevalence is also slightly higher than the prevalence (2.0%) reported in southeast Ethiopia by Gumi et al., (2012). Age-wise prevalence of tuberculosis in cattle revealed that highest prevalence (4%) was found in age group 6≤10 years old cattle and gradually decreasing with lower age group. The present findings support the finding of Chauhan et al., (1974) who reported the incidence of bovine tuberculosis in India was higher in adult (3.599%) against in young stock (0.30%). The finding is also similar with the finding of Kazwala et al., (2001) who found that older cattle were more affected by the disease than yearlings and calves. Sex-wise prevalence of tuberculosis in cattle showed that slightly higher prevalence was recorded in male (2.4%) than in female (2.1%). This finding support the finding of Shehu et al., (1988) who reported that male animal had a higher chance of being positive than female animal in the tuberculin tests. This may be due to the usage of the male cattle in agriculture. Male cattle are mostly used as oxen and therefore are kept in the herd for long thereby having more chances of being exposed to infection than female cattle. Similarly, female cattle have less frequent contact with other cattle except at grazing and watering point (Shehu, 1988). Kazwala et al. (2001) also found significant differences in the prevalence of tuberculosis between male and female cattle. Over the three study years same prevalence was found in the year 2009 (2.2%) and 2011 (2.2%) and slightly higher prevalence was found in the year 2010 (2.5%). Monthly distribution of tuberculosis in cattle revealed that highest prevalence was found in the April and October month (3.8%) and no positive cases were recorded in the July month. There is no report available in literature to compare on monthly distribution of TB in cattle. High prevalence in April and October month may be due more usage of cattle in agriculture in these two months and therefore, more chance of exposure to infection.

a) The zoonotic importance of tuberculosis

Most of the human patients having tuberculosis in this study are poor, having malnutrition. Most of them live with animals in the same damp and overcrowded houses. Most of the time of day they are in close contact with animals. They share the same materials used for animls and man. They did not take proper hygienic measures during milking and processing of milk. They drink unpasteurized milk and eat infected meat with tuberculosis. All these factors help in spread of the disease from animal to human. So people should not eat infected meat, improvement of socioeconomic and housing condition can help to limit spread of disease.

V. Conclusions

Tuberculosis is a zoonotic and economically important disease in Bangladesh. In this study prevalence and risk factors were determined in both human and bovine. The result represents the present status of tuberculosis in Bangladesh. This study will help to take necessary action to control and eradicate tuberculosis in Bangladesh. It is necessary to carry out a routine program of tuberculin testing, for confirmation, combined with interventions to reduce the risk of nosocomial transmission in the workplace. It might be suggested that a well coordination in activities should be taken among the public health and Veterinary public health organelles for complete eradication of the disease from the country.

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Prevalence and Economic Importance of *Stilesia Hepatica* in Small Ruminants Slaughtered at Helmix Abattoir, Bishoftu, Ethiopia

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Abstract- Across sectional study was conducted at HELMEX abattoir, Debrezeit town, central highlands of Ethiopia from October 2010 to march 2011 on 800 young and adult sheep and goats (400 sheep and 400 goats) originated from different areas of Ethiopia. The objectives of the study were to determine the prevalence of *Stilesia hepatica* in young and adult sheep and goats brought to the slaughter house from different parts of Ethiopia and to assess the direct financial loss incurred due to rejection of *Stilesia hepatica* infected livers. Pearson's chi-Square (x2) test was calculated to determine the degree of association of *S.hepatica* infection with species (sheep and goats), origin and age (young and adult) of the animals. P-value less than 0.05 were considered to be statistically significant.

Keywords: goats, sheep prevalence, stilesia hepatica.

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Prevalence and Economic Importance of *Stilesia Hepatica* in Small Ruminants Slaughtered at Helmix Abattoir, Bishoftu, Ethiopia

Zelalem Sisay [α], Dinka Ayana [σ] & Hika Waktole [ρ]

Abstract- Across sectional study was conducted at HELMEX abattoir, Debrezeit town, central highlands of Ethiopia from October 2010 to march 2011 on 800 young and adult sheep and goats (400 sheep and 400 goats) originated from different areas of Ethiopia. The objectives of the study were to determine the prevalence of *Stilesia hepatica* in young and adult sheep and goats brought to the slaughter house from different parts of Ethiopia and to assess the direct financial loss incurred due to rejection of *Stilesia hepatica* infected livers. Pearson's chi-Square (x2) test was calculated to determine the degree of association of *S.hepatica* infection with species (sheep and goats), origin and age (young and adult) of the animals. P-value less than 0.05 were considered to be statistically significant.The overall prevalence of *S.hepatica* in sheep and goats was 32.5% (130/400) and 21.3 %(85/400), respectively. This difference in the prevalence of *S.hepatica* between sheep and goats showed statistically significant (P<0.05) values. The prevalence of S.hepatica in young and adult sheep and goats was 18.7 %(88/471) and 38.6 %(127/329), respectively. Statistical significant difference (P<0.05) was recorded between the respective adult and young age groups of sheep and goats. The prevalence of *S.hepatica* for sheep and goats originated from different areas of the country was Afar 21.3 %(17/80), Arbaminch 32.2 %(29/90), Awash 30.0 %(48/160), Borena 20.0% (10/50), Jinka19.2 %(25/130), Harar 36.7% (22/60), Ogaden 29.1% (32/130), Wolaita 26.7% (32/120). Statistically no significant difference (P>0.05) was recorded in the prevalence of *S.hepatica* in sheep and goats originated from different areas of Ethiopia. The total annual financial loss due to condemnation of stilesia affected livers was estimated to be 50,614.92 USD or 860,453.58 ETB. *S.hepatica* causes significant loss to farmers, butchers and consumers and it is also major cause of concern in the trade of small ruminants. Therefore, the disease should be investigated further on farms to determine the prevalence in animals of various ages, Species and breed and develop economic strategies for disease control at farm level.

Keywords: goats, sheep prevalence, stilesia hepatica.

I. Introduction

Africa has a population of 209 million sheep and 174 million goats representing approximately 17% and 31% of the world total respectively (FAO, 1994). Within Africa the distribution of these small ruminants varies widely with a higher concentration found in dry areas than in humid. Small ruminants (sheep and goats) are important domestic animals in the tropical animal production system (Devendra and Meclorey, 1990). Within Africa society they comprise a great proportion of the total wealth of poor families because of low in put requirements such as small initial capital, fewer resources and maintenance cost and ability to produce milk and meat using marginal lands and poor pasture (Ibrahim, 1998). Furthermore, they need only short periods to reconstitute flocks after disaster and respond quickly to demand (Gatenby, 1991; Steele, 1996).

Ethiopia own huge numbers of small ruminants, about 23.62 million sheep and 23.33 million goats (CSA, 2004). The low land part constitutes 65% of the country area where 25% sheep and close to 100% goats' population exist (PACE-Ethiopia, 2003).

Sheep and goats cover more than 30% of all domestic meat consumption and generate cash income through export of meat and edible organs (Fletcher and Zelalem, 1991). Even though the livestock sub-sector contributes much to the national economy, its development is hampered by different constraints which include rampant animal diseases, poor nutrition, poor husbandry, poor infrastructure, shortage of trained man power, and lack of government policies (Gryseals, 1986).

Diseases cause extensive financial losses as a result of direct and indirect economic impacts; it is the major concern to small ruminant industry (Jibat, 2006). A significant economic loss incurred each year in the different abattoirs in Ethiopia is due to mortality, inferior weight gain and condemnation of edible organs at slaughter (Abebe, 1995; Jobre *et al.*, 1996). This production loss to the livestock industry is estimated to be more than 900 million USD annually (Jacob, 1979).

Various investigations have been conducted through abattoir survey to determine the prevalence and economic importance of organs and carcass condemnation in Ethiopia (Jembere, 2001; Yilma, 2003). However, most of the surveys paid attention to parasitic causes; fasciolosis and hydatidosis especially in cattle. There is lack of information on the causes of organ and carcass condemnations and associated economic losses in small ruminants especially due to *Stilesia hepatica*. *Stilesia hepatica* is a cestode parasite living in the bile ducts of cattle, sheep, goats and occasionally

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camel. It is non-pathogenic but extremely prevalent (90-100%) in sheep in many parts of Africa including Ethiopia (Kaufmann, 1996). The condemnation of large proportion of sheep livers at meat inspection is the major loss due to this parasite for aesthetic reason (Gracey, 1999).

The objectives of this study were:

- To determine the prevalence of Stilesia hepatica in sheep and goats slaughtered at HELMEX abattoir, Debrezeit.
- To estimate the magnitude of direct financial loss due to condemnation of Stilesia hepatica infected livers

II. Material and Method

a) Study Area and Abattoir

The study was conducted at Hashim Nur's Ethiopian livestock and Meat Export (HELMEX) abattoir, Debrezeit, from October 2010 to March 2011. The abattoir is a privately owned export abattoir exporting beef, mutton, lamb, goat meat and edible organs like liver, kidney and brain of sheep and goats to Middle East countries. This abattoir is found in Debrezeit town, which is located at 90N and 400E with an altitude of 1880m a.s.l in the central highlands of Ethiopia at 47km South East of Addis Ababa. It has annual rain fall of 1151.6mm of which 84% falls during the long rainy season that extends from June to September; and the remaining during the short rainy season that extends from March to May. The mean annual minimum and maximum temperature are 8.50C and 30.70C, respectively and the mean relative humidity is 61.3% (NMSA, 2003).

The abattoir has a capacity of slaughtering up to 1500 animals per day, however the average current daily killing capacity was 700 animals due to lack of livestock availability and market infrastructure network. This abattoir has got few numbers of meat inspectors and had a problem to inspect all organs and carcass thoroughly.

b) Study Animals and Sampling

The animals were all males originating from different areas of the country (Ogaden, Arbaminch, Wolaita, Afar, Jinka, Awash, Borena and Harar) representing different agro-ecological zones (highland, semi-arid and arid). Animals were transported to the abattoir using vehicles and on foot. The animals were systematically selected using regular interval during ante mortem inspection. For determination of the sample size, the expected prevalence was decided to be 50%. The desired precision was also decided to be 5% on the confidence interval of 95%. Thus, the formula described by Thrusfield (2005) was used to determine the sample size. Accordingly, the sample size was calculated to be 384 per species but to generate reliable data 400 sheep and 400 goats were taken. Hence, the total sample size for sheep and goats was 800.

To see the effect of age, animals were classified into two groups: young (goats less than 1year; sheep less than 1.25year) and adult (goats more than 1year; sheep more than 1.25 year), based on eruption of one or more incisor teeth.

c) Study Methodology

The animals were identified (selected) systematically using regular interval (every 10th animal) then ropes which have different colors for age and origin of the animals were tied

After the removal of the head, the ropes were tied on the hind leg of the animals and after evisceration the ropes were tied on the liver of the identified animals. Livers which have rope were identified separately and inspected by visualization and making systematic (longitudinal) incision on the bile ducts to detect the presence of stilesia hepatica parasite.

d) Data Analysis

The prevalence of S.hepatica was calculated by dividing the number of positive sheep and goats for S.hepatica by the total number of animals (sheep and goats) examined and multiplied by 100 to express in percentage.

Data generated from post-mortem inspection of the livers was entered to Microsoft excel 2002. Descriptive statistics, such as percentage and chi-Square test were calculated with SPSS software for windows version 15. Pearson's chi-Square (x2) test was used to determine the degree of association of S.hepatica infection with species (sheep and goats), origin and age (young and adult) of the animals. P-value less than 0.05 were considered to be statistically significant.

e) Assessment of Direct financial loss

In assessing the economic losses, only the direct financial loss due to rejection of liver was considered. The analysis was based on annual slaughter capacity of the abattoir considering market demand, average market price on international market and in the town of Debrezeit and the rejection rate of liver. The annual slaughter rates were estimated from retrospective data recorded in the past four years. Average market price of liver was determined from interviews made with personnel of the abattoir and marketing department. Financial loss was then computed mathematically by using the formula of Ogurinade and Ogurinade (1980) for liver rejection as follows:

$$EL = \sum Srx.Coy.Roz$$

Where: -

EL- estimated annual economic loss due to organ and carcass condemnation from international or domestic market.

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Srx- annual sheep/goat slaughter rate of the abattoir
Coy- average cost of each sheep/goats liver /lung/heart/kidney/brain/carcass.
Roz- condemnation rates of sheep/goats liver/lung /heart /kidney/brain/carcass.

III. Results

Totally 800 sheep and goats (400 sheep and 400 goats) were inspected at post-mortem by categorizing them according to species, origin and age of sheep and goats.

The prevalence of *S.hepatica* in sheep and goats was found to be 32.5 %(400) and 21.3% (400), respectively (Table1).

Table 1 : prevalence of *S.hepatica* in slaughtered sheep and goats

Species	No of animals examined	Prevalence N (%)
Ovine	400	130(32.5%)
Caprine	400	85(21.3%)
Total	800	215(26.9%)

X2= 12.880; P=0.000

Statistically significance difference (P<0.05) in the prevalence of *S.hepatica* between sheep and goats was observed.

Among the 800 sheep and goats examined at post-mortem, 329 of them were adult and 471 of them were young. The prevalence of *S.hepatica* was found to be 38.6% (127) and 18.7 %(88) in adult and young respectively (Table 2).

Table 2 : prevalence of *S.hepatica* in slaughtered adult and young sheep and goats

Age category	No of animals examined	Prevalence N (%)
Adult	329	127(38.6%)
Young	471	88(18.7%)
Total	800	215(26.9%)

X^2=39.103; P=0.000

Statistically significant difference (P<0.05) in the prevalence of *S.hepatica* between adult and young age groups was observed.

The animals (Sheep and goats) which were slaughtered during study period had different origin.

Among 800 sheep and goats examined at post-mortem, 80 of them were from Afar, 90 from Arbaminch, 160 from Awash, 50 from Borena, 130 form Jinka, 60 form Harar, 110 from Ogaden and 120 from Wolaita. The prevalence was found to be 21.3% (17), 32.2%(29), 30.0%(48), 20.0%(10), 19.2%(25), 36.7%(22), 29.1%(32) and 26.7% (32), respectively (Table 3).

Table 3 : prevalence of *S.hepatica* in sheep and goats originated from different areas of Ethiopia

Origin of animals	No of animals examined	Prevalence N (%)
Afar	80	17(21.3%)
Arbaminch	90	29(32.2%)
Awash	160	48(30.0%)
Borena	50	10(20.0%)
Jinka	130	25(19.2%)
Harar	60	22(36.7%)
Ogaden	110	32(29.1%)
Wolaita	120	32(26.7%)
Total	800	215(26.9%)

X^2= 11.665; P=0.112

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The prevalence of *S.hepatica* in shoats slaughtered at HELMEX abattoir showed no statistically significant difference (P>0.05) among the different places of origin.

The average annual slaughter rate of the abattoir was estimated to be 177,509 shoats. The average liver condemnation rate of the current study was 26.9% (215/800). The average cost of a kilogram of liver was 4.25USD and on average 4 pieces of liver could weigh 1kg. Thus, the average cost of one liver is 1.06USD or 18.02ETB. Therefore, by substituting these values in the formula of Ogurindae, the annual financial loss due to liver condemnation was estimated to be 50,614.92 USD or 860, 453.58 ETB (Table, 4)

Table 4 : Direct financial loss incurred annually due to rejection of stilesia affected livers

Examined organ	Slaughter capacity of abattoir	Rejection rate	Average price per kg	Annual loss
Liver	177509	26.9%(215/800)	1.06USD or 18.02ETB	50,614.92USD or 860,453.58ETB

IV. Discussion

Abattoirs provide information on the epidemiology of diseases on livestock to know what extent the public is exposed to certain zoonotic diseases and estimate the financial losses incurred through condemnation of affected organs and carcasses (Nfi and Alonge, 1987; Vanlongtesijin, 1993).

The over all prevalence of *S.hepatica* in sheep and goats slaughtered at HELMEX abattoir in the present study was found to be 32.5% (130/400) and 21.3% (85/400), respectively. This prevalence was in agreement with the prevalence reported by Ashenafi (2010) who recorded a prevalence of 31.04% and 27.02% in sheep and goats respectively; Sisay et al., (2008) who reported prevalences of 39% and 36% in sheep and goats, respectively and Mungube et al. (2006) recorded also a prevalence of 28% and 22% in sheep and goats, respectively in Kenya.

The prevalence reported by Sisay et al. (2008) was higher than the prevalence recorded in the current study, where as the prevalence recorded Mungube et al. (2006) in Kenya was lower than the current study. This may be related to differences in the agro-ecology of countries.

The prevalence of S.hepatica in adult and young sheep and goats in the current study was found to be 38.6% (127/329) and 18.7 %(88/471), respectively. This prevalence was in agreement with Ashenafi (2010) who reported a prevalence of 27.5% and 24.5% in adult and young, respectively. The higher prevalence of S. hepatica in adult than young shoats may be attributed to the greater exposure of adult shoats than young ones during life time.

The prevalence of *S.hepatica* in slaughtered sheep and goats at HELMEX abattoir which were brought from different areas of the country was found to be 21.3%(17/80) from Afar, 32.2%(29/90) Arbaminch, 30.0%(48/160) Awash, 20.0%(10/50) Borena,

There was no significant difference in the prevalence of *S. hepatica* among shoats from different sites of origin. This may be due to the similarity in the distribution of intermediate hosts and reservoirs among the different places from which the animals were recruited.

The frequency of occurrence has not been quoted, since little work has been conducted on this parasite. However, S. hepatica prevalence is high (60%) especially considering post-mortem liver inspection (Mungube *et al.* 2006). This estimate is higher than the present study. Losses due to *S.hepatica* liver condemnation were mainly observed in small ruminants rather than in bovines. Out of 5124 and 20226 livers inspected in caprine and ovines 61% and 85% were condemned due to *S.hepatica* in caprine and ovines respectively (Mungube *et al.* 2006).

The direct annual loss in HELMEX abattoir due to rejection of affected livers due to S. hepatica infection was estimated to be 50,614.92 USD or 860,453.58 ETB from international and domestic market. This estimate was higher than the estimate of Seid (2007) and Shiferaw(2002), who recorded annual loss of 57,939.84 and 130,718.49 ETB, respectively due to organ /carcass condemnation in cattle. This may be due to inadequate diagnosis or lack of control of *Stilesia hepatica* at farm level.

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Immunogenicity of Testicular and Epididymal Spermatozoa

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Abstract- Problem: The sequential changes in the immunogenicity of spermatozoa in male reproductive tract and the effect of seminal vesicle secretions are long thought to act as central players in influencing immunological equilibrium in the male reproductive tract.

Method of Study: Popliteal lymph nodes of mice were collected on the 8th day after sensitizing them with the testicular and epididymal spermatozoa of boar, weighed, dissociated into a cell suspension and the white blood cells were counted using haemocytometer.

Keywords: immunosuppression, popliteal lymph node, secondary immune response, seminal vesicle, testicle.

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Immunogenicity of Testicular and Epididymal Spermatozoa

Gaurav Singhal [α σ] & Phillip M Summers [α]

Abstract- ***Problem:*** The sequential changes in the immunogenicity of spermatozoa in male reproductive tract and the effect of seminal vesicle secretions are long thought to act as central players in influencing immunological equilibrium in the male reproductive tract.

Method of Study: Popliteal lymph nodes of mice were collected on the 8th day after sensitizing them with the testicular and epididymal spermatozoa of boar, weighed, dissociated into a cell suspension and the white blood cells were counted using haemocytometer.

Results: The antigenicity of spermatozoa varied in different parts of male reproductive tract; lowest in corpus epididymis and highest in cauda epididymis. Seminal fluid has immunosuppressive effect on spermatozoa and antigenic effect on surrounding tissues. The development of secondary immune response to spermatozoa has also been established through this study.

Conclusion: Our work is the first evidence to suggest thatthere is awell-developed immunological mechanism in the male reproductive tract and immunogenicity of spermatozoa varies in different parts of male reproductive tract.

Keywords: immunosuppression, popliteal lymph node, secondary immune response, seminal vesicle, testicle.

I. Introduction

The spermatozoon has an immune privileged status in the testis [1-4]. Once ejaculated in the female reproductive tract, spermatozoa act as the potential target for the female immune system due to their foreign nature [5]. Females exposed to spermatozoa have shown an increase in the weight of lymph nodes that drain the reproductive tract even though there is an immunosuppressive effect of seminal plasma[6]. However, in spite of the fact that single physiological exposure to semen by natural insemination initiates an immune response involving the lymph nodes which drain the uterus, a significant immune reaction rarely occurs in females even with frequent coital activity[7], the reason for which is still not known. Although, factors like immune insult from bacterial infections[8,9], and female sex hormones [10] have been shown to influence the viability of spermatozoa and immune response against them in females.

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The secretions from the accessory sexual glands also affect the immunogenic property of spermatozoa in each ejaculation. The immunosuppressive components obtained from the seminal fluid have been found to reduce B lymphocyte activity to mitogens11. In addition, seminal proteins coating on sperms is essential for several processes in female reproductive tract, such as formation of the oviductal sperm reservoir, sperm capacitation, oocyte recognition and sperm binding to the oocyte [12]. Indeed, seminal plasma, containing cytokines and prostaglandins, is believed to provide the physiologically protective environment to the highly antigenic spermatozoa in female reproductive tract [11,13-17].

Dostalet al. found reduction in the number of white blood cells and decrease in the activity of plaque-forming cells after injecting the immunosuppressive components of boar seminal plasma into the rectum of female mice18. It has been suggested by researchers that this immunosuppressive effect of seminal plasma may also compromise the immune system in females for viral and bacterial attack [11,18-21]. The immunosuppressive components of boar seminal fluid lead to the suppression of primary and secondary immune response and delay in the production of immunoglobulin G and immunoglobulin M antibodies to boar epididymal spermatozoa and to bacterial antigens [22].Researchers have also demonstrated that seminal leukocytes are responsible for the phagocytosis of morphologically abnormal spermatozoa in the semen[17,23].

In some women, genital secretions and the serum showed the presence of sperm antibodies and this raises the question as to whether these sperm antibodies are produced in response to the immunogenicity of spermatozoa in reproductive tissues or it is a transudate from the serum [24]. However, the titre of the antibodies to spermatozoa is generally lower in serum than in genital secretions which supports the hypothesis that these antibodies are produced in response to spermatozoa in the genital tract and not in the serum [25]. Formation of anti-sperm antibodies has been established as an important cause of both male and female infertility, especially in humans [26,27].

The aim of the current study is to investigate variations in the immunogenicity of spermatozoa, as they move from rete testis to different locations in epididymis, using popliteal lymph node assay in mice. Estimation of the effect of seminal fluid on spermatozoa

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antigenicity and the secondary immune response to spermatozoa were also included during our work.

II. Materials and Methods

a) Animals

Ethics approval to conduct research on animals was taken from the James Cook University (JCU) Animal Ethics Committee prior to the commencement of study (Approval number A 1191).

i. *Boars*

Male pigs were purchased from a pig farmer at 3-4 weeks or 16 weeks of age and grown to 12 months of age using standard husbandry practices within the animal facilities of the School of Veterinary and Biomedical Sciences, James Cook University (JCU), Townsville.

ii. *Mice*

Female Balb/c mice 12-15 weeks of age were used for the lymph node bioassay. The mice were obtained from the rodent facility of the School of Veterinary and Biomedical Sciences at JCU.

b) Surgical procedure for unilateral castration of boars

Food was withheld for 12 hours and the boar pre-medicated with an intramuscular injection of atropine (Apex Laboratories Pty. Ltd., Somersby, New South Wales, Australia) at 5 mg/kg body weight. Surgical anaesthesia was induced with intramuscular injections of xylazine hydrochloride (Ilium xylazil-100; Troy Laboratories Pty. Ltd., Smithfield, New South Wales, Australia) at 1 mg/kg body weight and ketamine (Parnell Laboratories Pty. Ltd., Alexandria, New South Wales, Australia)at 6 mg/kg body weight. Once anaesthesia was induced, the scrotum was prepared aseptically and 5 mls of local anaesthetic (Lignocaine 20; Troy Laboratories Pty. Ltd., Smithfield, New South Wales, Australia)was injected under the scrotal skin along the intended site of incision. A vertical incision of about 8 cm in length was made on the skin of the scrotum. The incision was deepened through the subcutaneous tissue and spermatic fascia to reach the parietal vaginal tunic which was then excised to expose the testicle. The testicle with attached epididymis and spermatic cord was extruded out. A large haemostat was applied to the spermatic cord proximal to the pampiniform plexus and three simple interrupted sutures (6.0 metric chromic catgut) were applied to the spermatic cord. The spermatic cord was cut ventral to the sutures and the testicle removed by incising the spermatic fascia and the scrotal ligament. The testicle was held in a vertical position for 2-3 minutes in order to drain out as much blood as possible. Immediately after that, it was placed in an insulated box containing frozen cold blocks until spermatozoa were collected in the laboratory. Simple interrupted sutures (3.5 metric chromic catgut)were used to suture the parietal vaginal tunic and scrotal muscles and the scrotal skin was closed with mattress sutures (Vicryl 3.0 metric; Johnson and Johnson, North Ryde, New South Wales, Australia). The boar was given an intramuscular injection of 1200 mg oxytetracycline (Engemycin 100; Intervet Australia pty. Ltd., Bendigo, Victoria, Australia) in the neck muscles for preventing any post-operative infections.

c) Collection of the second testicle and seminal vesicles

Each boar was sent to the Charters Towers abattoir four to five weeks after the unilateral castration. The testicle and seminal vesicles were collected immediately after slaughter, placed in an insulated box containing frozen cold blocks and bought back to the laboratory at School of Veterinary and Biomedical Sciences, JCU. The interval between slaughter and collection of seminal vesicle fluid and spermatozoa was between two and two and half hours. Spermatozoa were collected from the caput, corpus and cauda epididymidis, as well as from the rete testis (Fig I) into sterile 15 ml graduated conical tubes (Falcon 2096; Beeton Dickinson Labware, Franklin Lakes, New Jersey, USA). Seminal fluid was also collected into Falcon tube by incising the seminal vesicle and aspirating the contents with a sterile pipette.

d) Collection of spermatozoa from testis and epididymis

Spermatozoa from the caput, corpus and cauda epididymidis, and rete testis were collected and suspended in normal saline at concentrations of 2x103, 2x105, 2x107/ml. The caput, corpus and caudal epididymal spermatozoa were collected by taking incisions on the caput, corpus and cauda, aspirating the contents and placing it into sterile Falcon tubes containing 1 ml of sterile normal saline. Spermatozoa were collected from the rete testis by excision of the mediastinum and aspirating the contents.

e) Determination of the concentration of spermatozoa

The concentration of spermatozoa was determined in each sample using a Hamilton Thorne sperm analyser. Half hour before the analysis, the HTM-IVOS analyser version 10 (Hamilton Thorne; Beverley MA, USA) was turned on in order to acquire the working temperature of 39°C. The temperature of the four compartmented 20 micron deep analysis chamber (Standard count, Leja, Nieuw-Vennep, Netherlands) was set at 39°C and then the chambers were loaded with the semen samples by capillary action. This was followed by the loading of the analysis chamber into HTM-IVOS analyser and the spermatozoa concentration in each sample was determined. The final calculations to obtain the required concentration were done manually using a calculator.

f) Washing of spermatozoa

The samples were then added to sterile normal saline to make a final volume of 14 ml and centrifuged at

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1200 rpm (207.24 g) for 10 minutes. The supernatant was discarded and the sperm pellet re-suspended and washed in 14 ml of normal saline and centrifuged again. The spermatozoa were then re-suspended in normal saline to the required three concentrations.

g) Injection of mice and collection of popliteal lymph nodes

Fifty µl of each sample were injected subcutaneously with a 25 G needle and a 1ml syringe just above the right hock of the mouse. Three mice were used for each sperm concentration, source of spermatozoa, diluent and time period. A control injection of 50 µl of sterile saline was injected subcutaneously above the left hock. At four, eight and twelve days after the injection, the mice were killed with CO2 gas and both popliteal lymph nodes were carefully removed, placed in normal saline, adhering fat removed under a stereomicroscope, blot dried and weighed in Sartorius analytical balance (maximum capacity = 120 g; readability = 0.1 mg; repeatability = 0.1 mg; linearity = 0.2 mg; weighing units = g, mg, kg, oz t, ct).

III. Full Experimental Protocol

a) Primary immune response

Spermatozoa were collected from the rete testis and caput, corpus and cauda epididymidis from ten testes, prepared, re-suspended in normal saline and injected in mice as described in previous sections. The mice were killed eight days later and the popliteal lymph nodes weighed as described above. The lymph nodes were then dissociated into a cell suspension in 1.5 ml conical eppendorf tubes by meshing it with a sterile cell strainer in 1 ml normal saline and the number of white blood cells enumerated using a haemocytometer. The response to the lymph nodes was calculated as a stimulation index based on weights of test and control lymph nodes as well as a stimulation index based on the number of cells in the test and control lymph nodes. The repeatability of the response between the two testes and epididymis of each boar was also examined.

b) Secondary immune response

The secondary immune response to spermatozoa from four boars was examined. Groups of three mice were injected with spermatozoa from the rete testis and caput, corpus and cauda epididymidis. When the boar was slaughtered four to five weeks later, the mice were injected again near the popliteal lymph node and killed eight days later. The stimulation indices based on the weight and cell numbers in the lymph nodes were calculated as above.

c) Influence of seminal vesicle fluid on the primary immune response

Fluid from the seminal vesicles was collected from seven boars and kept at room temperature until sperm samples were being prepared. In the first group of experiments, spermatozoa were prepared in normal saline as well as seminal vesicle fluid and injected into mice as described previously. In a second group of experiments, 2x107 spermatozoa were incubated in 1 ml of seminal fluid for 15 minutes at 390 C. The samples were then centrifuged at 207.24 g for 10 minutes, the supernatant removed and spermatozoa re-suspended in 14 ml of normal saline. The process was repeated twice before suspending spermatozoa in 1 ml normal saline for injection. In the third group of experiments, seminal vesicle fluid from six boars was injected into groups of four mice with sterile normal saline as control to determine the response to seminal vesicle fluid alone. The stimulation indices based on the weight and cell numbers in the lymph nodes were calculated as above.

d) Statistical analyses

A descriptive analysis was carried out on the data obtained using Microsoft excel and SPSS software. A parametric or non-parametric test was performed depending upon the nature of sampling distribution and the satisfaction of basic assumptions of the tests. One way ANOVA or Kruskal-Wallis test were used to find the significant differences among various samples in a group or among groups. Linear regression was used to find the relationship between mean lymph node weight stimulation index and mean cellularity index for all the groups. The results were expressed as Mean ± Standard Error and the p value was calculated at 95 % confidence interval i.e., $p \leq 0.05$.

IV. Results

a) Immunogenic effect of spermatozoa in normal saline

Irrespective of the boar, the overall mean lymph node weight stimulation index value for the four samples declined from rete testis towards the corpus epididymidis before it increased to maximum for the cauda epididymidis (Table I).

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Table 1 : The mean (± SEM) lymph node weight stimulation index of murine popliteal lymph nodes stimulated by porcine spermatozoa from the rete testis, caput epididymidis, corpus epididymidis and caudaepididymidis in five groups

Site	N	Mean±SEM	Minimum	Maximum	Range	Variance
RT+NS	10	2.3965± 0.26724	0.89	3.4	2.51	0.714
CPT+NS	10	2.1699± 0.21106	1.13	3.3	2.18	0.445
CPS+NS	10	1.8752± 0.25619	0.77	3.23	2.46	0.656
CDA+NS	10	2.4773± 0.19306	1.66	3.54	1.88	0.373
RT+SF	7	3.3131 ± 0.51335	2.12	6.28	4.16	1.845
CPT+SF	7	3.3629 ± 0.46752	2.14	5.89	3.75	1.53
CPS+SF	7	3.0621 ± 0.63132	1.5	6.3	4.8	2.79
CDA+SF	7	3.1139 ± 0.44144	1.7	4.88	3.18	1.364
RT+ISF	5	2.1094 ± 0.20466	1.48	2.75	1.28	0.209
CPT+ISF	5	2.3892 ± 0.80524	1.1	5.56	4.46	3.242
CPS+ISF	5	1.7604 ± 0.26991	0.93	2.54	1.62	0.364
CDA+ISF	5	1.5972 ± 0.14358	1.06	1.88	0.82	0.103
RT(SIR)	3	1.6190 ± 0.31072	1.11	2.18	1.07	0.29
CPT(SIR)	4	1.7748 ± 0.19161	1.45	2.27	0.82	0.147
CPS(SIR)	4	1.6608 ± 0.24145	1.05	2.16	1.11	0.233
CDA(SIR)	4	1.9650 ± 0.47160	1	3.23	2.23	0.89
NS and SF	6	2.8367 ± 0.39930	1.54	4.51	2.97	0.957

Similarly, looking into the cellularity index values (Table II), the mean cellularity index increased from the corpus epididymidis to the cauda epididymidis. However, unlike the weight stimulation index values, the cellularity index values for caput epididymidis was higher than the cellularity index values for the rete testis.

Table 2 : The mean (± SEM) cellularity index of murine popliteal lymph nodes stimulated by porcine spermatozoa from the rete testis, caput epididymidis, corpus epididymidis and caudaepididymidis in five groups

Site	N	Mean ± SEM	Minimum	Maximum	Range	Variance
RT+NS	10	25.3610 ± 4.04052	7.47	50.39	42.92	163.258
CPT+NS	10	26.0980 ± 4.46078	11.07	50.63	39.56	198.985
CPS+NS	10	18.9400 ± 3.12247	5.99	34.52	28.53	97.498
CDA+NS	10	24.1870 ± 3.53647	12.03	45.28	33.25	125.066
RT+SF	7	40.3614 ± 10.6637	20.07	102.46	82.39	796.002
CPT+SF	7	39.8729 ± 7.2426	21.86	68.63	46.77	367.187
CPS+SF	7	33.9429 ± 4.37092	19.05	52.88	33.83	133.735
CDA+SF	7	36.1857 ± 10.39975	11.98	94.72	82.74	757.083
RT+ISF	5	19.152 ± 3.40075	10.93	31.42	20.49	57.826
CPT+ISF	5	20.0760 ± 4.40577	9.18	32.24	23.06	97.054
CPS+ISF	5	19.0460 ± 2.50037	10.55	23.95	13.4	31.259
CDA+ISF	5	20.8120 ± 5.31957	4.65	32.57	27.92	141.489
RT(SIR)	3	14.8467 ± 0.77102	13.74	16.33	2.59	1.783
CPT(SIR)	4	17.1850 ± 2.63916	12.61	22.42	9.81	27.861
CPS(SIR)	4	15.4825 ± 2.97052	8.96	22.12	13.16	35.296
CDA(SIR)	4	21.2275 ± 5.13919	12.8	36.17	23.37	105.645
NS and SF	6	33.385 ± 4.76468	14.19	44.86	30.67	136.213

b) Immunogenic effect of spermatozoa in seminal fluid

Irrespective of the boar, the overall mean for the lymph node weight stimulation index was almost the same for the four samples of spermatozoa in seminal fluid (Table I). Still, the highest mean lymph node stimulation index is seminal fluid groups was observed for spermatozoa from the caput epididymidis and the minimum was for spermatozoa from the corpus epididymidis.

The mean cellularity index among spermatozoa in seminal fluid groups decreased from the rete testis to the corpus epididymidis before increasing again for the cauda epididymidis (Table II).

c) *Immunogenic effect of spermatozoa incubated in seminal fluid*

In contrast to all the previous findings, the lymph node weight stimulation index was least for the cauda epididymidis (Table I). It was almost same for the

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rete testis and caput epididymidis followed by a progressive decrease towards the corpus epididymidis and the cauda epididymidis.

On analyzing the mean values for cellularity index in case of four samples of spermatozoa incubated in seminal fluid (Table II), we found that the mean cellularity index was almost same for all the four samples.

d) Immunogenic effect of seminal fluid

Seminal plasma from six boars was used to test the immunogenic effect of seminal plasma alone compared to saline controls.

The mean lymph node weight stimulation index value of seminal plasma alone was higher than for spermatozoa suspended in the normal saline and for spermatozoa incubated in the seminal fluid but lower than for spermatozoa suspended in the seminal fluid (Table I). The mean cellularity index value also followed the same pattern (Table II).

e) Immunogenic effect of spermatozoa in secondary immune response group

Irrespective of the boar, the overall mean for the lymph node weight stimulation index among secondary immune response groups (Table I) was least for the rete testis and increased to highest for the cauda epididymidis.

The mean cellularity index followed the same trend as the mean lymph node stimulation index (Table II) except that corpus epididymidis had lower mean cellularity index value than caput epididymidis.

In all of the above experiments, few findings were similar:

a) The popliteal lymph node weight stimulation index and cellularity index were highly variable for spermatozoa from rete testis but variance was least in case of the spermatozoa from cauda epididymidis being almost half of the rete testis.

b) A positive relationship can be seen between the mean lymph node weight stimulation index and mean cellularity index indicating that the samples with a higher popliteal lymph node weight index also have higher cellularity index.

V. Discussion

The results from the normal saline group suggest maximum immunogenicity of the caudal epididymal spermatozoa and least of the corpus epididymal spermatozoa among 4 groups. The immunogenicity of spermatozoa seems to decrease from the rete testis to corpus epididymidis before increasing for cauda epididymidis which is evident by the mean lymph node weight stimulation index as well as the mean cellularity index. The highly variable immunogenicity of spermatozoa taken from the rete testis indicates that some factors in the process of formation of spermatozoa in testis also determine the immunogenic trait of spermatozoa and this needs further evaluation.It is also clear that the groups with higher lymph node stimulation index also have a higher cellularity index. Some workers however have described the cellularity index attribute as more sensitive, informative and accurate than lymph node stimulation index [28-30].

The role of seminal fluid as an immunosuppressive agent to spermatozoa has been described by many workers in the past [11,15,17,18,20,22,31]. But the extent to which seminal fluid is responsible for the overall immunosuppressive effect on spermatozoa among many other probable factors has not been described before. The increase in the mean lymph node weight stimulation index from the corpus epididymidis to cauda epididymidis again confirms greater immunogenicity of spermatozoa in the cauda epididymidis. The seminal fluid alone does not seem to have any immunosuppressive effect which is clear from the results obtained. Instead, the results suggest that the seminal fluid is responsible for the increase in immunogenicity of spermatozoa.

The higher variability for rete testis spermatozoa further indicates that some factors involved in the formation of spermatozoa are responsible for variable immunogenicity. As these spermatozoa moves from the rete testis towards the cauda epididymidis, the immunogenicity seems to decrease initially until the corpus epididymidis and then it again increases for the cauda epididymidis. One possible cause for this increase might be the metabolic activities that are taking place in spermatozoa while stored in the cauda epididymidis temporarily[32] change the antigenic proteins on the surface of spermatozoa during storage[32-36].

Immunosuppressive fractions of seminal fluid have already been isolated before by some of the workers and their immunosuppressive effect on spermatozoa has been demonstrated[12]. The effect of incubation on spermatozoa is immunosuppressive which is evident from the results obtained. But the values are slightly higher for each location than the normal saline group indicating the residual immunogenic effect of seminal proteins even after two washings with normal saline. However, the values were much lower than for spermatozoa suspended in seminal fluid indicating that two washings of spermatozoa in normal saline removed most of the adherent antigenic seminal proteins.

The results obtained show a high variance value for the caput epididymidis for the spermatozoa incubated in seminal fluid. But the variance for other sites is less following the same decreasing trend from the rete testis to the cauda epididymidis. This perhaps indicates that spermatozoa with highly variable immunogenicity in the rete testis acquire almost the

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same immunogenicity level while stored in the cauda epididymidis though lower than the rete testis and caput epididymidis but higher than the corpus epididymidis. The spermatozoon after incubation in seminal fluid has the least immunogenicity for the cauda epididymidis suggesting that caudal spermatozoa loses maximum immunogenicity, more than corpus spermatozoa in seminal fluid.

The seminal fluid alone seems to be more immunogenic then spermatozoa in normal saline and spermatozoa incubated in seminal fluid by both the mean lymph node weight stimulation index and mean cellularity index. Conversely, the seminal fluid alone is less immunogenic then spermatozoa suspended in the seminal fluid. This could probably be due to the additive effect of immunogenicity of spermatozoa on the immunogenicity of seminal fluid. Since the spermatozoa incubated in seminal fluid are less immunogenic then seminal fluid alone, it indicates that the twice washing with normal saline has probably eliminated most of the immunogenic proteins of seminal fluid. Spermatozoa left after incubation and washed with normal saline were less immunogenic then the spermatozoa in seminal fluid possibly due to the immunosuppressive effect of some of the components of seminal fluid on spermatozoa during incubation.

The secondary immune response could be important for determining the fertility in both males and females. This is because after the first few intercourses, the predominant immune response in females with only one male partner will be the secondary immune response. On the other hand, the primary immune response could be important for the animals with multiple partners.The results obtained for the secondary immune response are contrary to earlier results in terms of the mean lymph node weight stimulation index and mean cellularity index. The immunogenicity of spermatozoa increases from the rete testis to cauda epididymidis; however the highest immunogenicity is for the spermatozoa from caput epididymidis than the spermatozoa from rete testis. However, the results obtained for secondary immune response were not statistically significant and also there was no linear relationship observed between the lymph node weight stimulation index and cellularity index. In addition, a lower immunogenic response was seen for secondary immune response then for spermatozoa in normal saline and seminal fluid. This was probably due to the occurrence of peak immunogenic response in mice at earlier than eighth day so that on the eighth day, the immune response was in the decline phase.

Overall, it is clear that the mean lymph node weight stimulation index and mean cellularity index among five groups are in the following order:Spermatozoa in seminal fluid group > seminal fluid only group > normal saline group > incubated seminal fluid group ≈ secondary immune response

VI. Concluding Remarks

Our study is the first evidence to suggest that there is a well-developed mechanism in the male reproductive tract to suppress the antigenicity of spermatozoa before ejaculation.This is also the first instance when an effort has been made to determine the immunogenicity of spermatozoa in different parts of the testes and epididymis. While higher values for the spermatozoa in seminal fluid group could probably be due to additive effect of antigenicity of seminal proteins and spermatozoon surface proteins, the higher value for the seminal fluid only group could be due to the antigenic effect of only seminal proteins. Similarly, the marginally higher values for spermatozoa incubated in seminal fluid could be due to the residual immunogenic effect of seminal proteins along with the immunogenic effect of spermatozoon surface proteins. Finally, the lowest value for secondary immune response group among all samples could probably be due to the initiation of immunogenic mechanism and recovery phase at the earlier stage than in the primary immune response. Although, decrease in the antigenicity of spermatozoa is evident in the male reproductive tract, substantial evidence are still required to confirm the hypothesis that seminal and spermatozoa surface proteins play a role in this process.

VII. Future Aspects

Further studies are required for determining the type and the strength of immune response in females to spermatozoa during both primary and secondary immune response, the role of humoral and cellular immune system during this process and the factors responsible for altering the immunogenicity of spermatozoa in female reproductive tract. In addition, more studies are required to completely understand the immunogenicity of spermatozoa and its variability as it moves from the cauda epididymidis to the exterior at ejaculation. These studies may play an important role in understanding the exact role of immunological response to spermatozoa on fertility in mammals.

VIII. Conflict of Interest Statement

This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Haematological Studies on West African Dwarf (WAD) Bucks Experimentally Infected with Trypanosoma Vivax and Trypanosoma Brucei and Response to Treatment with Diaminazene Aceturate

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Abstract- This study investigated the haematological changes in West African Dwarf (WAD) bucks experimentally infected with Trypanosoma vivax and Trypanosoma brucei. Each of the group is eight in number while the control experimental group had five bucks. Clinical records (weight, rectal temperature) for the animals were monitored. The haematological parameters accessed include packed cell volume (PVC) estimation of Haemoglobin (HB) White and Red Blood Cell count (WBC and RBC) mean corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin concentration (MCHC) and were calculated accordingly.

Keywords: *haemalotogical changes, parasitemia, trypanosoma, anaemia.*

GJMR-G Classification : *NLMC Code: WC 7*

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Haematological Studies on West African Dwarf (WAD) Bucks Experimentally Infected with Trypanosoma Vivax and Trypanosoma Brucei and Response to Treatment with Diaminazene Aceturate

Amadi [α],A.N.C. [σ], Okore [ρ], I. B. [ω] & Amajuonwu[¥]

Abstract- This study investigated the haematological changes in West African Dwarf (WAD) bucks experimentally infected with Trypanosoma vivax and Trypanosoma brucei. Each of the group is eight in number while the control experimental group had five bucks. Clinical records (weight, rectal temperature) for the animals were monitored. The haematological parameters accessed include packed cell volume (PVC) estimation of Haemoglobin (HB) White and Red Blood Cell count (WBC and RBC) mean corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin concentration (MCHC) and were calculated accordingly.

The PVC valves varies from the pre-infection levels of 21.4 – 23.0 and 24.0 – 1.9.9 in the 1st – 3rd for T. brucei and T. vivax respectively to infective stage 21.5 – 20.3 and 22.8 –13.5 as the anaemia progressed. The effect of the infection for T. vivax and T. brucei was acute and chronic respectively as the infection was more severe in T. vivax than T. brucei. Knowing that the WAD bucks are trypano tolerant however, the effect of the parasite on the haematological features showed that anaemia was normocytic and normochronic for most periods. The intensity of the anaemia was related to the degree of parasitemia and in case where the animals are infected adequate dietary measures and proper sanitation need to be taken to ensure productivity is not hindered.

Keywords: haemalotogical changes, parasitemia, trypanosoma, anaemia.

I. Introduction

Trypanosomiasis is an infective disease which affects domestic and game animals including man. It is caused by flagellated protozoan parasite of the genus Trypanosoma and transmitted mainly by different species of tsetse fly of the genus Glossina [9]. Trypanosoma vivax, Trypanosoma congolense and Trypanosoma brucei are the main species of trypanosome of importance in livestock, that cause Animal Africa Trypanosomaisis (AAT) [1]. Trypanosomiasis is a major constrain on livestock production in Africa and of all the livestock diseases endemic on the African continent, trypanosomisis has been regarded as the single factor which limits the number and productivity of ruminant; sheep, goat and cattle. It is known to render approximately a quarter of African arable land mass unsuitable for profitable livestock farming [18]. Reminants; cattle, goat and sheep represent an important source of animal protein in many countries of world. Supplying a good percentage of the daily meat and dairy products in cities and villages in many countries including Nigeria [22]. Apart from being a source of animal protein, their waste are also very important in agriculture [23]. Ruminants like goat and sheep are used in special ceremonies such as weddings and burial in Nigeria. However, parasitic diseases like trypanosomiasis coupled with inadequate management practices, hamper the productive husbandry of these animals [25]. In infected areas, the disease may result in severe reduction in animal productivity reflected in poor growth, low milk production and meat yields, reduced capacity for work and financial loss in terms of veterinary controls. If these infected animals are left untreated animals may die of anaemia, heart failure, and inter-current bacterial infections that take advantage of the animals weakened resistance or suppressed immune system. The economic impact of the disease trypanosomiasis on these animals has been shown to be substantial [17]. Response to infection by trypanosomiasis may be influenced by the stress of work, intercurrent disease, poor nutrition etc. [21]. Drug treatment remains the only means of intervention, there is no vaccine against trypanosomiasis and prospects of vaccine are very poor owing to the significant antigenic variation exhibited by the trypanosome [13]. There were initial suggestions that indigenous sheep and goats are more resistant than imported exotic breeds to syringed or needle passed Trypanosoma vivax as well as field challenged [8]. Various breeds of livestock have been re-cognized as having degrees of tolerance to trypanosomiasis enabling them to survive and produce in areas where

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other breed could succumb [12]. [15] reported that Trypanosoma vivax and trypanosoma congolense were the most prevalent species encountered in sheep and goat because of their grazing requirement which compels the animals to traverse different vegetation zones especially during the dry season to the Southern areas of Nigeria many of which are tsetse fly infected. Infection in these animals causes symptoms manifested by intermittent fever, anemia, pyrexia, lymphatic enlargement with hepatomegly and a progressive cachexia [5] .However, the severity of the infection in a host animal is influenced by a number of factors: virulence of the different special of trypanosoma, environment of the host, age, nutritional status, weight etc. [20]. This work was carried out to investigate the etiology of the disease trypanosomiasis and the haematogical changes in the West African Dwarf (WAD) bucks when infected with Trypanosoma vivax and trypanosome Bruce their susceptibility to the infection and response to treatment with diaminazene aceturate.

II. Materials and Methods

a) Study Area

The Study was carried out at the experimental house of the Animal science Department of Michael Okpara University of Agriculture, Umudike Abia State. The University is located at about longitude 7032'East and latitude 5029; North 129 M2 above sea level.

It has warm hound climate and temperature that ranges from above 290C in the wet season to slightly over 250C in the hot season Umudike falls within the rainforest zone of south Eastern Nigeria with a mean altitude of 123m.

b) Experimental Design

21 West African Dwarf (WAD) bucks were divided into 3 groups as follows.

Group A: 8 WAD bucks were infected with trypanosome brucei

Group B: 8 Wad Bucks were infected With trypanosome vivax

Group C: 5 WAD bucks were uninfected (control)

To infect the designated bucks in group A 4ml of blood was obtained from mice inoculated with Trypanosoma brucei and diluted with 1ml of normal saline, ml of the diluents was used to infect the WAD bucks through the jugular vein. To infect the designated bucks in group B 3ml of blood was obtained from a WAD buck inoculated with Trypanosoma vivax and diluted with Iml of normal saline, 1ml of the diluents was used to infect the WAD bucks in group B through the jugular vein. The animals were intensively maintained on Dry hay, water and concentrate adlibidum throughout the experiment. During the period of acclimatization which lasted for 21 days the animals were dewormed with levamisole, vaccinated against PPR (Peste des petil Ruminant virus) and treated with diaminazene aceturate (Berenil R) at 0.3 0.25ml to clear any possible protozoan infection, haemoparasite and trypanosome. Clinically, the rectal temperature was taken twice daily (morning and evening), respiratory rate, heart rate and body weight was recorded weekly. Other treatment were given appropriately after this period, 8 of the WAD bucks in Group A and Group B were infected into the jugular vein with 1ml of the diluents. Animals in both groups were treated with diaminazene aceturate (Berenil R) 0.30-035ml at the 8th week and 13th week respectively.

III. Sample Collection

A total of twenty one (21) West African Dwarf (WAD) bucks all makes were bled from the jugular vein after sterilizing with methylated spirit using cotton wool, Iml of Blood was collected with a 4ml vaccutainer and a disposable hypodermic syringe blood was drawn from the jugular vein into the EDTA (Ethylene diaminetertra acetic acid) vaccutainer container already prepared EDTA overnight and allowed to evaporate. These blood were thoroughly mixed to prevent clotting and lysing of Red blood cells. The samples were then transferred to the laboratory for further investigation. Samples were collected once a week between the months of June to October.

IV. Haematological Methods and Parameters Studied

Animal were examined before and during infection Packed Cell volume (PCV) was determined by micro-haematocrit method, Red and White Blood Cell (RBC and WBC) Count were estimated by the use of Neubauer-ruled haemonytometer and haemoglobun concentration (Hb) by the Acid haematin Concentration Method. Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin concentration (MCHC) and Mean Corpuscular Volume (MCV) were monitored weekly and Calculated according to [26]. Weight, rectal temperature, colour of mucous membrane were also monitored.

V. Results

Trypanosomes were first detected in the blood of the WAD bucks infected with T. vivax followed by the WAD bucks infected with T. brucei. The control WAD bucks remained trypanosome free throughout the period of investigation as no trypanosome was detected in their blood. As the infection progressed, the T. vivax and T. brucei showed acute and chronic form of the disease trypanosomiasis respectively.

Clinical signs: following infection of the WAD bucks with T. vivax and T. brucei, trypanosomes were detected in blood by microscopic examination of the bufty coat within the first 5th week of infection in Group B. No infection was detected in Group A. The clinical disease was characterized by marked pyrexis at an average of

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390C. The temperature fluctuated daily during the period of infection, infected WAD bucks were emaciated with very pale mucous membranes anorexic with facial and sub mandibular oedema, ocular discharges and they showed signs of dullness. All animals infected showed a decreases in total body weight.

VI. Haematolagical Changes

With the onset of parasitemia, all the infected WAD bucks developed anaemia with a drop in erythrocyte (PCV, RBC, HB Values) Table 1-4. These reflected in the 5th-6th week when the animals become recumbent or reached the critical erytrocyte levels. The PCV value varied from 25-5-21.9 for the control, 25.4-19-3 for the T. brucei and 18.6-12.9 for T. vivax. The Hb value varied as follows 8.5-7.3 for the control, 8.46-6.44 for the T. brucei and 6.2-4.3 for the T. vivax (Figs 1,2,&3).

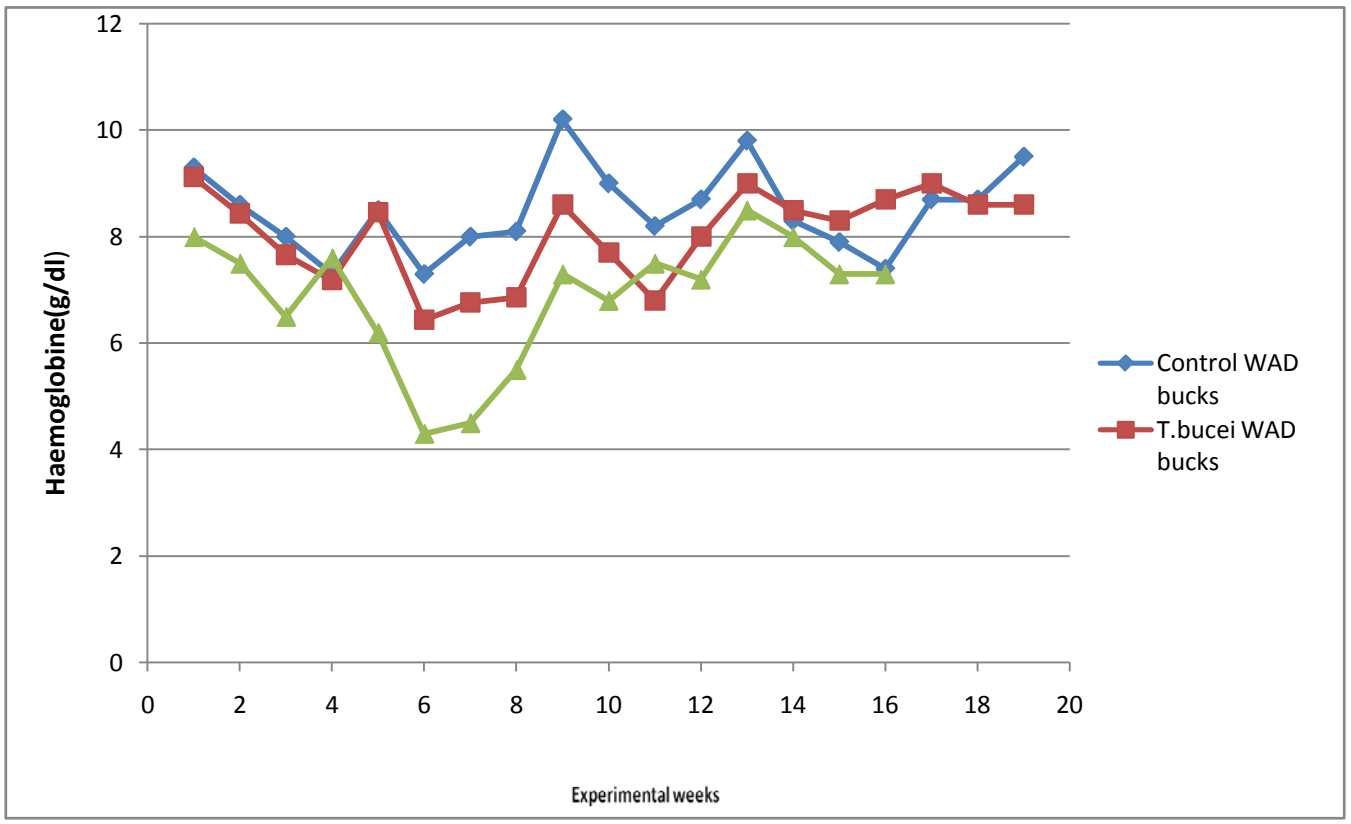

Figure1 : A Line graph showing values of Haemoglobin concentration for the period experiment

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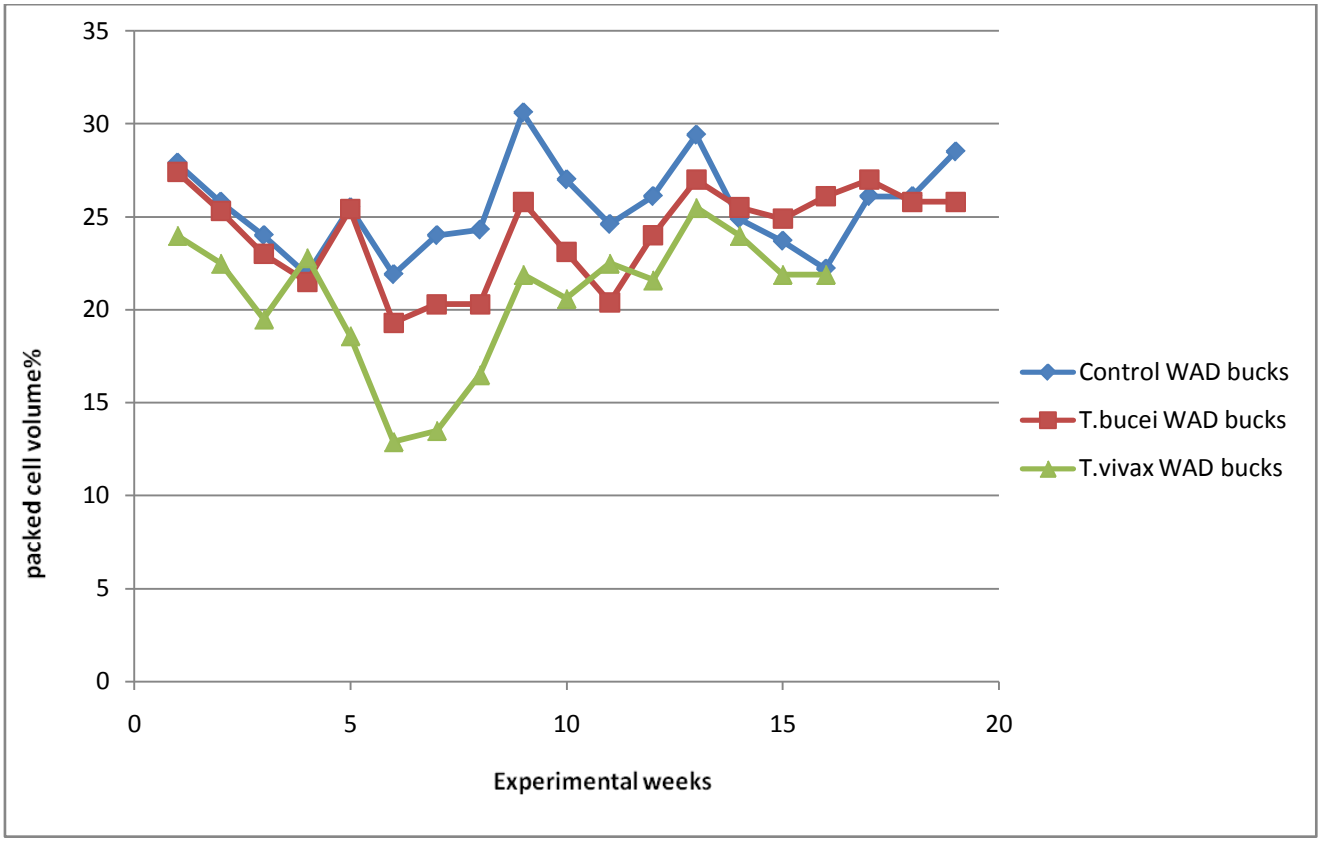

Figure 2 : A line graph showing values of packed cell volume for the period of experiment

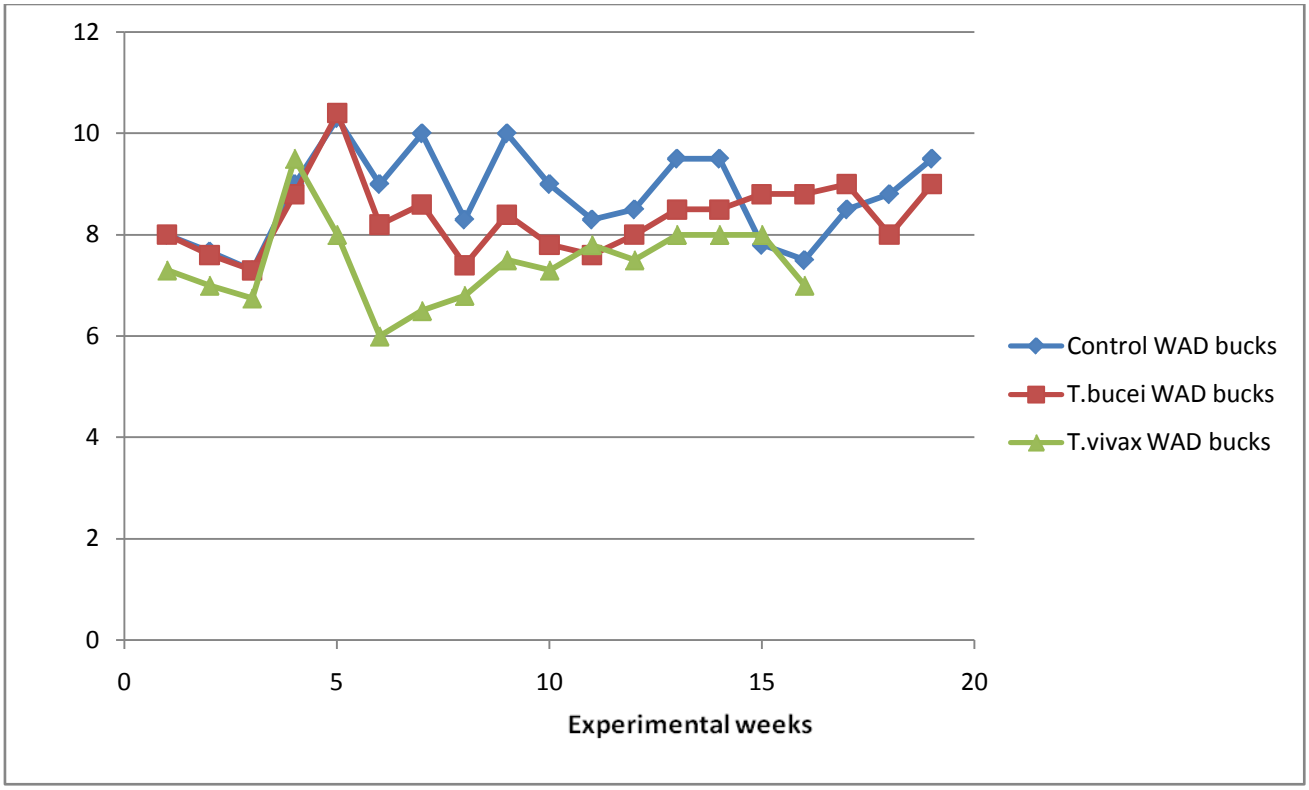

Figure 3 : A line graph showing values of White blood cell counts for the period of experiment

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The anaemia developed progressively during the experiment. There were no appreciable variations in the erythrocyte values and with T. brucei but there were appreciable variation in the erythrocyte values of the WAD bucks infected with T. vivax. However, the mean MCV values of infected WAD bucks fluctuated but did not vary significantly for the normal values before infection. MCH values during infection relatively followed the pattern of MCV changes. There was significant variation in the MCHC values during the experiment, the mean total of WBC counts during the infection fluctuated but increased during the week of infection of the WAD bucks. By the end of 7th week of infection the animals that survived were treated with 0.3-0.35 mi/kg of diaminazene aceturate (berenil R) in group B, while that of group A were treated and the end of 13th week of infection and rapidly recovered. Parasite were not detectable in the blood following treatment and relapses were not encountered following an observation period of 12weeks and 6 weeks respectively for the WAD bucks in group B and A. Parasites were not encountered following an observation period of 12weeks for WAD bucks in group B and 6 weeks group A.

Table 1 : Mean values of red blood cells RBC (x106ul) and estimation of haemoglobin (Hb) for the period of experiment

Weeks	Mean values of RBC (x106ul)			Mean values of Hb g/dl		
	C	Tb	Tv	C	Tb	Tv
1	5.75	5.65	4.95	9.3	9.12	8.0
2	5.30	5.25	4.65	8.6	8.44	7.5
3	4.90	4.50	4.05	8.0	7.66	6.5
4	4.55	4.15	4.40	7.3	7.18	7.6
5	4.95	4.85	3.60	8.5	8.46	6.2
6	4.55	3.75	2.55	7.3	6.44	4.3
7	4.95	3.9	2.65	8.0	6.76	4.5
8	5.00	4.25	3.4	8.1	6.86	5.5
9	6.25	4.95	4.45	10.2	8.6	7.3
10	5.55	4.40	4.15	9.0	7.7	6.8
11	5.05	3.95	4.55	8.2	6.8	7.5
12	5.35	4.60	4.35	8.7	8.0	7.2
13	6.05	5.40	5.25	9.8	9.0	8.5
14	5.15	5.15	4.85	8.3	8.5	8.0
15	4.85	5.05	4.85	7.9	8.3	7.3
16	4.55	5.25	3.65	7.4	8.7	7.3
17	5.35	5.60	---	8.7	9.0	---
18	5.35	4.85	---	8.7	8.6	---
19	5.85	5.60	---	9.5	8.6	---

	Pre-infective phase	Infective phase	Treatment phase	
Tb -	*Trypanosoma brucei*	Week 1-3	Week 4-12	Week 13-19
Tv -	*Trypanosoma vivax*	Week 1-3	Week 4-7	Week 8-19
C -	*Control.*	------	-------	-------

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Table 2 : Mean values of packed cell volume (PCV) Mean corpuscular haemoglobin (Hb) g/dl for the period of experiment

Mean values of PCV						
				Mean values of MCH		
Weeks	TC	TB	TV			
1	27.9	27.4	24.0	16.2	16.1	16.2
2	25.8	25.3	22.5	16.2	16.1	16.1
3	24.0	23.0	19.5	16.3	17.0	16.0
4	21.9	21.5	22.8	16.0	17.3	17.3
5	25.5	25.4	18.6	17.2	17.4	17.2
6	21.9	19.3	12.9	16.0	17.2	16.9
7	24.0	20.3	13.5	16.2	17.3	17.0
8	24.3	20.3	16.5	16.2	16.1	16.2
9	30.6	25.8	21.9	16.3	17.4	16.4
10	27.0	23.1	20.6	16.2	17.5	16.5
11	24.6	20.4	22.5	16.2	17.2	16.5
12	26.1	24.0	21.6	16.2	17.4	16.6
13	29.4	27.0	25.5	16.2	16.7	16.2
14	24.9	25.5	24.0	16.1	16.5	16.5
15	23.7	24.9	21.9	16.3	16.4	15.1
16	22.2	26.1	21.9	16.3	16.6	20
17	26.1	27.0	----	16.3	16.1	----
18	26.1	25.8	----	16.3	15.4	----
19	28.5	25.8	----	16.2	15.4	----
		Pre-infective phase	Infective phase	Treatment phase		
Tb - *Trypanosoma brucei*		Week 1-3	Week 4-12	Week 13-19		
Tv - *Trypanosoma vivax*		Week 1-3	Week 4-7	Week 8-19		
C - *Control.*		------	-------	-------		

Table 3 : Mean values of mean corpuscular volume (MCV) in femto litres (FL) and haemoglobin (Hb) in percentage (%) for the period of experiment

Mean values of MCV			Mean values of HB (%)			
C	Tb	Tv	C	Tb	Tv	
1	48.9	48.6	48.9	63.7	62.5	54.9
2	48.6	48.5	48.4	58.9	57.8	51.4
3	48.5	48.4	48.2	54.8	52.5	44.5
4	48.3	51.8	52.0	50.0	49.2	52.1
5	48.6	52.1	52.0	58.2	57.9	42.5
6	48.3	52.0	50.2	50.0	44.1	29.5
7	48.5	51.9	51.2	54.8	46.3	30.8
8	48.5	51.2	48.7	55.5	47.0	37.7
9	48.8	52.3	49.4	69.9	58.9	50.0
10	48.7	52.1	47.5	61.6	52.7	46.9
11	48.5	52.1	49.4	56.2	46.6	51.4
12	48.6	52.2	47.5	59.6	54.8	49.3
13	48.7	51.5	49.5	67.1	61.6	58.2
14	48.5	48.1	48.8	56.8	58.2	54.8
15	48.5	49.2	49.5	54.1	56.8	50.0
16	48.4	49.7	49.3	50.6	59.6	50.0
17	48.6	50.0		59.6	61.6	
18	48.6	49.5		59.6	58.9	
19	48.7	50.0		65.1	58.9	
		Pre-infective phase	*Infective phase*	*Treatment phase*		
Tb - *Trypanosoma brucei*		*Week 1-3*	*Week 4-12*	*Week 13-19*		
Tv - *Trypanosoma vivax*		*Week 1-3*	*Week 4-7*	*Week 8-19*		
C - *Control.*		------	-------	-------		

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Table 4 : Mean values of Clinical parameters monitored

Clinical parameters	Tv	Tb	C
Weight (kg)	8.0 ± 2.0^{a}	6.0 ± 1.6^{b}	10.0 ± 2.0^{c}
Rectal temperature (°C)	39.16 ± 0.27^{a}	39.16 ± 1.0^{a}	30 ± 0.05^{b}
Respiratory rate (cpm)	40 ± 10^{b}	30 ± 10^{a}	30 ± 10^{a}
Heart rate (1pm)	90 ± 30^{a}	90 ± 20^{b}	90 ± 30^{a}

Means in the same row with different superscripts are Significantly different ($P<0.05$)
Tv Trypnosoma vivax
Tb Trypnosoma brucei
C control

VII. Discussion

The haemotological Values of the parameters monitored revealed that Trypnosoma vivax and Trypanosoma brucei infected WAD bucks showed acute and chronic course of trpanosomiasis respectively while values of the control animals remained within the normal levels (Tables 1-3).

There was a rapid development of anaemia in T. brucei and T. vivax infected WAD bucks with the PVC dropping as low as 27.9-23.0 and 24.0-19.5 respectively.

This was a more serious anaemia than that previously recorded by [19], he observed 0.25 to 0.30 in T. brucei infection but less severe than PVC value of 0.11 recorded in naturally T. brucei infected bucks [16]. Although clinical symptoms associated with trypanosomasis observed in this study include high rectal temperature, ocular discharge, decrease in weight and anaemia severity of the disease and more in T. vivax infected WAD bucks and more pathogenic than those of T. brucei infected bucks. This is similar to work of previous researchers [16][27][2][5] and [14]. They observed such symptoms as rectal Temperature fluctuation, pale mucous membrane, weakness, anaemia among others also infection with T. brucei had nervous system disorder. Anaemia which is a major consequence of the disease contributed more to the outcome of the infection than any other pathological entity and was characterized by depressed erythrocyte values. This result is in agreement with observation of [16] and [3]. They recorded that if the infection is left untreated could lead to death of the animal.

From the Pre-infection levels of 27.4-23.0 and 24.0-19.5 in the 4^{th} to 7^{th} and 1^{st} to 3^{rd} week for T. brucei and T. vivax respectively and as it progressed was found to be normacytic and normochronic for most periods and its intensity was related to the degree of the parasitemia. There was an increase within 4^{th}-5^{th} week in the MCH Values of infected bucks and this is correlated with an increase in the MCV values within the same period (table 4). It is noteworthy that the rise in MCH values was observed at the onset of anaemia and similar observation was made by Naylor (1971) in T. Congolese infected cattle. The increase in MCH and MCV values were observed due to increased erythropoiesis indicating that erythorid response peaks as the anaemia enrages.

The failure of the bone marrow to generate sufficient erythrocytes was partly responsible for persistent anaemia as indicated by low PCV values during the 4th-7th week (fig 3) of infection. The level of Parasitemia is concurrent with a relatively stable reduction in Hb and RBC levels during the chronic phase of infection. This is in keeping with the development of anaemia which was more pronounced during this period and also presumptive evidence of possible damage to the host cells and tissues by the invading trypanosomes [4], [7], [6].

Animals given good nutrition and rest are more likely to recover rapidly than undernourished and stressed animals. No vaccines are available against trypanosomiasis and prospect of vaccines are very poor owning to the significant antigenic variation exhibited by trypanosome [13]. Therefore a tsetse fly eradiation campaign can be conducted to help reduce the transmission of trypanosomiasis. The use of drugs or chemoprophylaxis and chemotherapy for the prevention and treatment of trypanosomiasis has also been effective [11].

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Comparison of two Methods in the Detection of *Cryptosporidium* in Pigs in Ogun State, Nigeria

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Abstract- Two diagnostic methods, a modified Kinyoun's acid-fast staining technique and an enzyme-linked immunosorbent assay (ELISA), for the detection of *Cryptosporidium* spp. in porcine faeces were compared regarding their sensitivities. Of the 209 faecal samples examined, *Cryptosporidium* spp. was detected significantly higher ($p<0.05$) by ELISA (31.1%) than the acid-fast staining method (16.3%). The sensitivities of the ELISA and acid-fast staining techniques were 100.0% and 52.3% respectively. The ELISA is therefore a preferable method than microscopy for detection of *Cryptosporidium* spp.

Keywords: *cryptosporidium, elisa, nigeria, pigs.*

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Comparison of two Methods in the Detection of *Cryptosporidium* in Pigs in Ogun State, Nigeria

Akinkuotu Olufemi Ambrose [α], Jacobs Eniope Bamidele [σ] & Okwelum Ngozi [ρ]

Abstract- Two diagnostic methods, a modified Kinyoun's acid-fast staining technique and an enzyme-linked immunosorbent assay (ELISA), for the detection of *Cryptosporidium* spp. in porcine faeces were compared regarding their sensitivities. Of the 209 faecal samples examined, *Cryptosporidium* spp. was detected significantly higher ($p<0.05$) by ELISA (31.1%) than the acid-fast staining method (16.3%). The sensitivities of the ELISA and acid-fast staining techniques were 100.0% and 52.3% respectively. The ELISA is therefore a preferable method than microscopy for detection of *Cryptosporidium* spp. in faeces of pigs and will be useful in routine diagnosis and screening of large number of samples in epidemiological surveys.

Keywords: cryptosporidium, elisa, nigeria, pigs.

I. Introduction

Cryptosporidium species are ubiquitous and infect a wide range of vertebrate hosts, including humans and various domestic animals (Wang et al., 2010) and they cause enteric infections and severe diarrhoea in these host species. *Cryptosporidial* infections in pigs were first described by Bergeland (1977) and Kennedy *et al.* (1977), and in contrast to the numerous studies on bovine *cryptosporidiosis* (Ibrahim *et al.*, 2007; Xiao and Fayer, 2008; Ayinmode and Fagbemi, 2010), there are relatively fewer epidemiological studies on porcine *cryptosporidiosis* (Chen and Huang, 2007; Kvac *et al.*, 2009; Chen *et al.*, 2011).

Different methods are used for diagnosis of *cryptosporidiosis* and these vary in their sensitivities, need for experienced staff and cost (Kuhnert-Paul *et al.*, 2012). A conventional method of identification is the microscopic examination of faecal smears stained with acid-fast stains (Yatswako *et al.*, 2007; Ayinmode and Fagbemi, 2010) and other staining methods (Mahdi and Ali, 2004; Hamedi et al., 2005; Kuhnert-Paul *et al.*, 2012). In some studies, it was determined that the sensitivity of the ELISA was higher than those of various staining methods (El-Shazly et al., 2002; Yilmaz *et al.*, 2008). El-Shazly *et al.* (2002) stated that the acid-fast staining technique showed the lowest sensitivity when compared to ELISA and the polymerase chain reaction (PCR) for diagnosis of C. parvum in cattle.

In Nigeria, very few studies have been carried out to detect *Cryptosporidium* spp. in pigs (Kwaga *et al.*, 1988; Yatswako *et al.*, 2007; Maikai et al., 2011) with the acid-fast staining method being utilized in majority of these studies. To the best of our knowledge, the comparison of an acid-fast staining technique and an ELISA to diagnose porcine cryptosporidiosis has not been previously reported in Nigeria. The results of this study will therefore highlight which of these diagnostic methods is more sensitive and suitable for routine diagnosis and epidemiological studies on *Cryptosporidium* infections in pigs in Nigeria.

II. Materials and Methods

a) Study period and area

A total of 209 faecal samples were obtained from five piggeries and one slaughter slab in Ogun state, southwestern Nigeria. The collection of faecal samples was initiated in September, 2012 and ended in April, 2013.

b) Sample collection

Faecal samples were collected per rectum from individual pigs. For pigs in which rectal sampling was not possible, such as neonates, freshly voided faeces were collected by the use of wooden tongue depressors which were used to scoop up the superficial layer of faeces without contacting the floor. The faeces were then dropped into individual universal sample bottles and labeled appropriately. These were then transported, in cold packs, to the laboratory where analysis was carried out immediately. When analysis was delayed, the samples were stored at 4oC until they were processed.

c) Detection of Cryptosporidium oocysts by microscopy

Faecal sample concentration: This was achieved using the formalin-ethylacetate sedimentation method as previously carried out by Ayinmode and Fagbemi (2010) with few modifications. Briefly, 1g of solid faeces or 3ml of watery stool was washed in 8ml of 10% formalin and centrifuged at 650x g for 10 minutes. The supernatant was decanted, after which the sediment was re-suspended with 7ml of 10% formalin. 3ml of ethylacetate was thereafter added, the mixture vigorously shaken and allowed to stand for 3 minutes. This was then centrifuged at 650x g for 10 minutes and the supernatant discarded. A small portion of the sediment was evenly spread on a microscopic slide and air dried for acid-fast staining.

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d) Acid-fast staining

Modified Kinyoun's acid-fast staining method was carried out. Briefly, the faecal smears were fixed with absolute methanol for 1 minute after which they were flooded with carbolfuscin for 15 minutes. The slides were then rinsed briefly with distilled water. The smears were immediately decolorized by flooding them with 10% sulphuric acid for 1 minute and then rinsed with distilled water. Counterstaining of the smears was done by flooding the smears with 0.4% Malachite green for 1 minute and rinsing with distilled water. The smears were air dried and examined initially at x400 and then at x1000 magnification for confirmation of the oocyst morphology.

e) Detection of Cryptosporidium parvum antigens by ELISA

The detection of *Cryptosporidium* parvum coproantigens in the samples was done using a commercially available ELISA kit for faecal samples (RIDASCREEN® *Cryptosporidium*; R-Biopharm AG, Germany). The procedure was carried out according to manufacturer's instruction.

The optical densities (OD) of the samples were read at 450nm using an ELISA reader (Model: ELx800, Biotex Instruments, USA). Samples were analyzed using the manufacturer's cut-off calculations in the instruction manual.

III. Statistical Analysis

Data were analyzed on Statistical Package for Social Sciences (SPSS) on Windows 7. The Chi-squared test was used to compare the detection rates of the ELISA and microscopy at 5% level of significance.

IV. Results

The detection rate of *Cryptosporidium* in the samples was significantly higher ($p<0.05$) with ELISA, which detected the coproantigens in 31.1% (65/209), when compared to the detection rate by microscopy, which detected *Cryptosporidium* oocysts in 16.3% (34/209) of the samples (Table 1).

The sensitivities of the ELISA and MZN techniques were 100% and 52.3% respectively (Table1).

Table 1: Comparable performance of ELISA and microscopy for the diagnosis of *Cryptosporidium* in pigs

	Microscopy Positive	Microscopy Negative	Total (ELISA)
ELISA Positive	34	31	65
ELISA Negative	0	144	144
Total (Microscopy)	34	175	209

Sensitivity:

a. *ELISA: (34/34) X 100 = 100%*
b. *Microscopy: (34/65) X 100 = 52.3%*

V. Discussion

While acid-fast staining of faecal smears may help identify Cryptosporidium oocysts, there is the need for experienced staff (Kuhnert-Paul *et al.*, 2012). In contrast, ELISA, an antigen-based technique is easy to perform and its evaluation does not require considerable experience.

The higher sensitivity of the ELISA than the modified Kinyoun's acid-fast staining technique in detecting Cryptosporidium infection in faeces of pigs corroborates previous reports by Yilmaz *et al.* (2008), Kuhnert-Paul *et al.* (2012) and Chalmers *et al.* (2011). In contrast, similar sensitivities were reported by El-Moamly and El-Sweify (2011) and Ignatius *et al.* (1997).

As reported by Johnston *et al.* (2003), faecal samples containing only a few Cryptosporidium oocysts often yield a false-negative ELISA result. The lack of false-negative ELISA result observed in this study may therefore imply that the faeces of infected pigs contained at least 17.6 oocysts/µl of Cryptosporidium (Johnston *et al.*, 2003).

The ELISA detects a high molecular, soluble glycoprotein that is secreted by the parasite during replication (Kuhnert-Paul *et al.*, 2012). This antigen may also appear in the faeces before and after the end of patency (oocysts excretion) (Ungar, 1990). This may therefore account for the false-positive results of ELISA observed in this study. The lesser detection of oocysts in stained faecal smears may be related to several aspects of the staining procedure, especially decolourization, which causes some of the oocysts to lose their stain (Baxby and Blundell, 1983). Furthermore, storage of the samples at 4oC may reduce the sensitivity of microscopy in detecting *Cryptosporidium* oocysts (Kuhnert-Paul *et al.* 2012).

From our study, the ELISA, though more expensive than the acid-fast staining method, is more sensitive, easier to perform and evaluate, therefore more suitable for routine screening of porcine faecal samples in laboratories. It has however been suggested that ELISA should be carried out together with one of the staining techniques to increase the accuracy of diagnosis (Godekmerdan *et al.*, 1999).

The high prevalence rate of *Cryptosporidium* coproantigens observed in this study necessitates routine examination of symptomatic and asymptomatic

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pigs. Thus, *Cryptosporidium* antigen screening of porcine stools by ELISA should be regularly carried out in laboratories in Nigeria.

Ethical consideration

The manuscript does not contain clinical studies or patient data.

Conflict of interest

The authors declare that they have no conflict of interest.

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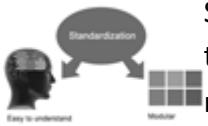

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Note :

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You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

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(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

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Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

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- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

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22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

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28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

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· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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Content

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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