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Cross-Sectional Survey

Bovine Fasciolosis at Elkadaro

Highlights

Prevalence of Bovine Mastitis

Implications in Selected Commercial

Discovering Thoughts, Inventing Future

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A Cross-Sectional Survey of Bovine Fasciolosis at Elkadaro Abattoir, Khartoum State, Sudan

By Mohammed B. Badreldeen & Abdelhamid A. Elfadil
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Abstract- A cross-sectional study was conducted from May to July 2011. The multivariate analysis exposed significant associations by postmortem examination: age >4 years (P= 0.000, OR= 18.5, 95% CI= 3.1, 21.7), foreign breed (P= 0.000, OR= 77.6, 95% CI= 9.3, 81.6), light weight (P= 0.000, OR= 3.0, 95% CI= 1.9, 5.3), Ethiopian cattle (P= 0.000, OR= 76.1. 95% CI= 8.4, 83.7) and small size (P= 0.000, OR= 3.1, 95%CI= 1.9, 5.3). while, the coprology demonstrated significant associations among: age >4 years (P= 0.000, OR= 28.8, 95% CI= 3.9, 34.2), foreign breed (P= 0.000, OR= 94.4, 95% CI= 13.2, 102.6), light weight (P= 0.000, OR= 53.4, 95% CI= 7.1, 61.3), Ethiopian cattle (P= 0.000, OR= 60.2, 95% CI= 8.3, 70.1) and small size (P= 0.000, OR= 54.9, 95% CI= 6.3, 63.8). A higher prevalence was recorded by postmortem examination (X^2= 1.669, P= 0.000). This study determined the prevalence of bovine fasciolosis.

Keywords: *fasciolosis, cattle, prevalence, risk factors, abattoir, khartoum, sudan.*

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A Cross-Sectional Survey of Bovine Fasciolosis at Elkadaro Abattoir, Khartoum State, Sudan

Mohammed B. Badreldeen [α] & Abdelhamid A. Elfadil [σ]

Abstract- A cross-sectional study was conducted from May to July 2011. The multivariate analysis exposed significant associations by postmortem examination: age >4 years (P= 0.000, OR= 18.5, 95% CI= 3.1, 21.7), foreign breed (P= 0.000, OR= 77.6, 95% CI= 9.3, 81.6), light weight (P= 0.000, OR= 3.0, 95% CI= 1.9, 5.3), Ethiopian cattle (P= 0.000, OR= 76.1. 95% CI= 8.4, 83.7) and small size (P= 0.000, OR= 3.1, 95%CI= 1.9, 5.3). while, the coprology demonstrated significant associations among: age >4 years (P= 0.000, OR= 28.8, 95% CI= 3.9, 34.2), foreign breed (P= 0.000, OR= 94.4, 95% CI= 13.2, 102.6), light weight (P= 0.000, OR= 53.4, 95% CI= 7.1, 61.3), Ethiopian cattle (P= 0.000, OR= 60.2, 95% CI= 8.3, 70.1) and small size (P= 0.000, OR= 54.9, 95% CI= 6.3, 63.8). A higher prevalence was recorded by postmortem examination (X^2= 1.669, P= 0.000). This study determined the prevalence of bovine fasciolosis.

Keywords: fasciolosis, cattle, prevalence, risk factors, abattoir, khartoum, sudan.

I. Introduction

Fasciolosis is among the most neglected important tropical diseases. Although it has significant economic impact on livestock industry, particularly cattle and sheep and occasionally can infects human beings (CDC, 2013). Among the estimated 91.1 million humans at risk for infection worldwide, as many as 17 million may be infected (Tolan, 2011). However, the disease is also consider as one of the major parasitic diseases contributing to loss in productivity estimated at over 200 US$ million per annum worldwide (Sturat, 1998). The infection is due to the food- and water-borne route. The two species most commonly implicated, as the etiological agents of fasciolosis are *F. hepatica* and *F. gigantica* (CDC, 2013).

Sudan possesses one of the highest livestock populations in Africa but productivity is low as a result of diseases, malnutrition and other management problems. Fasciolosis is one of these diseases which is responsible for considerable economic losses in livestock production (Boray, 1985). The disease is proved to be endemic in certain districts that characterized by intensive sheep or cattle production, in addition to the existence of favorable habitats for the snails host, like: White Nile, Eljazeera and Sennar regions (Ali, 1983). Another negative economic impacts on indigenous livestock result from inefficient conversion of feed, retarded growth, death, condemnation of infected livers, cost of preventive and treatment programs, reduced production, predisposition to other diseases, restricted use of infested lands and protein deficiencies among livestock dependant people (Saad, 2004).

Diagnosis is made serologically most often, although fecal examination for the eggs is fruitful if obtained when the adult worm is laying eggs (Tolan et al., 2011).

Therefore, the present study was designed to estimate the prevalence of bovine fasciolosis among cattle slaughtered at Elkadaro Abattoir, to identify potential risk factors associated with the occurrence of fasciolosis and to evaluate the accuracy of fecal examination.

II. Materials and Methods

a) Study Abattoir

Elkadaro Abattoir was designed for a processing capacity of 30 cattle per hour, with shifting work system every 10 hours (two shifts per day), in five days weekly. The abattoir was classified as code no.1, certified via O.I.E categories. The plant is situated within an open free disease area (recognized as free zone referring to O.I.E scientific terms). The main task of the abattoir is to process fresh and frozen meat, mainly for export orders. The abattoir is managed directly by the Federal Ministry of Animal Resources and Fisheries.

b) Duration of Study

A field investigation was launched in Sunday 21⁄ May and completed in Saturday 23⁄ July 2011.

c) Study Animals

According to the latest estimation of the livestock population in Khartoum State 2010, about 33800 of cattle are raised. The target animals were provided from different sites in and out of Sudan. The local cattle were fetched from: Nyala, Kordofan, Kosti, Kassala, Eljazeera, Khartoum and Upper Nile (South Sudan). While, the foreign ones were from Ethiopia (eastern neighboring country).

d) Sample Size Determination and Sampling Methods

The sample size was calculated using the formula: $4PQ/L^2$, given by (Martin et al., 1988) where:

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P≡ prevalence
Q≡ 1-P
L^2≡ allowable error, for systematic random sampling with 7.4% reported prevalence (Eldoush, 1995) and 3% allowable error. Accordingly, the sample size was determined to be 307.

The sampling procedure was carried out in such a way that from daily 120 slaughtered cattle, 10 were randomly selected. There are five slaughter days a week and accordingly, 50 cattle were examined weekly. Hence, 307 cattle were examined within two months of the study period. A fresh feces was collected instantly after slaughtering of the selected animals. Then livers were subjected to detailed postmortem examination.

e) Study Methodology

Coprology: Fecal samples for parasitological examination were collected directly from the rectum of each animal immediately after slaughter using disposable plastic gloves and placed in new plastic bags. Prior to slaughter, each selected animal was given an identification number. Then each fecal sample was clearly labeled with the cattle identification number. Samples were kept at the room temperature and examined fresh. In laboratory, coproscopic examinations were performed to detect *Fasciola* eggs using standard sedimentation technique as previously described (Coles, 1986).

Liver Inspection: Liver of each cattle was strictly examined for the presence of liver flukes separately to correlate the coprology and postmortem examination of each animal. Examination of livers for *Fasciola* was carried out immediately after removal of liver from abdominal cavity. The inspection was made according to the procedures certified by FAO (2003).

f) Sensitivity and Specificity of the Fecal Examination Method

One of the objectives of this study was to evaluate the accuracy of the direct coprological examination method, which is routinely employed at field to examine the presence of *Fasciola* species eggs in feces. The sensitivity and specificity of the method was computed by taking liver inspection at postmortem as gold standard for the diagnosis of fasciolosis. Kappa statistic was used to determine the degree of agreement between the two methods of liver fluke diagnosis. The kappa value was interpreted as: slight agreement (K <0.2); fair agreement (K= 0.2→0.4); moderate agreement (K= 0.4→0.6); substantial agreement (K= 0.6→0.8); and almost perfect agreement (K >0.8) (Thrusfield, 2005).

g) Data Management and Analysis

Both fecal examination and liver inspection results were recorded on specially designed forms and preliminary analysis was done in Microsoft Excel. The outcome variable was the cases of fasciolosis detected during routine postmortem inspection (positive or negative) and fecal examinations for *Fasciola spp* eggs (positive or negative). Descriptive statistics were carried out to summarize the prevalence and proportion of infection in each category of investigated potential risk factors. Univariate and multivariate logistic regression analysis were conducted to see the significance and strength of association between potential risk factors and the occurrence of the infection. 95% confidence interval and p-value (P=<0.05) were used to notice the significance of association. Also, Odds Ratios (Exp B) was employed to assess the strength and direction of this association using SPSS statistical software (SPSS 16.0).

III. Results

a) Abattoir (Postmortem) Prevalence

Of the total 307 slaughtered cattle that subjected to detailed postmortem examination at Elkadaro Abattoir, 31.6% (97/307) were found positive for fasciolosis (Table I). The highest prevalence was recorded in age greater than 4 years (44.5%), foreign breed (64.1%), light weight (47.4%), Ethiopian source (65.5%) and small size (47.8%) (Table III).

b) Risk Factor Analysis for the postmortem results

The occurrence of fasciolosis significantly varied with age, breed, weight, source and animal size (P=<0.05). The likelihood of fasciolosis occurrence was significantly higher in age >4 years (P=0.000, OR= 18.5, 95% CI= 3.1, 21.7), foreign breed (P=0.000, OR= 77.6, 95% CI= 9.3, 81.6), light weight (P=0.000, OR= 3.0, 95% CI= 1.9, 5.3) Ethiopian source (P=0.000, OR=76.1, 95% CI=8.4, 83.7), Kassala source (P=0.027, OR= 1.6, 95% CI= 1.1, 3.1), small size (P=0.000, OR=3.1, 95% CI= 1.9, 5.3) (Table III).

c) Prevalence by Coprology

Of the total 307 collected fecal samples 20.2% (62/307) were positive for coprological examination by sedimentation technique (Table II). The highest prevalence was recorded in age >4 years (28.9%), foreign breed (42.1%), light weight (43.8%), Ethiopian source (43.0%) and small size (44.1%) (Table IV).

d) Risk Factors Analysis for the Coprological Results

The results of coprological examination revealed significant association (P=<0.05) between the occurrence of fasciolosis and the risk factors: age, breed, source, weight and animal size. The likelihood of fasciolosis occurrence was significantly higher in age >4 years (P= 0.000, OR = 28.8, 95% CI = 3.9, 34.2), foreign breed (P= 0.000, OR = 94.4, 95% CI= 13.2, 102.6), Ethiopian source (P= 0.000, OR = 60.2, 95% CI = 8.3, 70.1) light weight (P= 0.000, OR = 53.4, 95% CI= 7.1, 61.3) and small size (P= 0.000, OR = 54.9, 95% CI= 6.3, 63.8) (Table IV).

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e) *Difference in Prevalence between the Two Diagnostic Methods*

Based on the proportions comparison test there was significant difference (X^2=1.669, P=0.000) between fasciolosis prevalence estimated by coprology and postmortem examinations. Hence, in this study, higher prevalence of infection was observed by postmortem examination (31.6%) than by coprology (20.2%) (Table V).

f) *The Sensitivity and Specificity of the Fecal Examination Technique Considering the Presence of Fasciola spp in the liver as a Gold Standard Test*

As indicated in (Table VI), no animal that was positive with fecal examination and negative during postmortem examination. This revealed that postmortem examination was the golden test for diagnosis of fasciolosis when compared with coprology. The table set out the number of positive and negative tests in animals with and without flukes in their livers (Smith, 1995). The sensitivity and the specificity of fecal examination were found to be 63.9% and 100%, respectively. The calculated Kappa value (Kappa= 0.69) indicated substantial agreement between the two techniques.

IV. Discussion

Fasciolosis is a wide spread ruminant health problem and causes significant economic losses to the livestock industry in some areas in Sudan. The abattoir prevalence of fasciolosis obtained from the present study (31.6%) is very high compared to 7.4% (Eldoush, 1995) and nearly similar with 30% (Elmannan, 2001) and slightly lower than 34.4% (Abu-rigaila, 1983). These differences within the country could be attributed mainly to variations such as altitude, rainfall and temperature, although differences in livestock management system and the ability of the meat inspectors to detect the infection may playapart (Abu-rigaila, 1983). From African countries, a higher prevalence of 63.8% from Tanzania (Keyyu et al., 2006) and 53.9% from Zambia (Phiri et al., 2006) were reported. The observed prevalence may reflect suitable ecological and climatic conditions for the snail intermediate host in the areas from which the study animals came from.

Regarding the risk factors analysis, the results of this study indicate that the occurrence of fasciolosis in cattle varied with sex, age groups, breeds, weights, sources, size of animals and other diseases concurrent with fasciolosis.

The association between the occurrence of bovine fasciolosis and sex of the animals by both coprological and postmortem examinations revealed that the prevalence of *Fasciola* infection was found to be higher in males in agreement with a previous report (Shiferaw et al., 2009). The significant effects of sex on the prevalence of bovine fasciolosis might be attributed to the management system in which males are kept outdoor while females are kept indoor at the beginning of lactation (Balock et al., 1985).

Higher *Fasciola* infection rate was recorded among older cattle (>4 years) with both postmortem and coprological examinations in agreement with a previous report (Andrade et al., 2002). The higher prevalence in older animals by both examinations could be associated with the degree of exposure to the parasite which is normally greater in old animals than young animals. In contrast to our finding, higher prevalence in young cattle than older ones has been reported (Mulugeta et al., 2011).

The current study determined a higher prevalence of *Fasciola* infection among Ethiopian zebu cattle by both postmortem and coprological examinations in agreement with previous reported (Kassaye et al., 2012) and (Chakiso et al., 2014) This might be attributed to the difference in resistance to parasitic infection between different breeds (Tasawar et al., 2007).

In this study a higher prevalence of fasciolosis was observed among Ethiopian source animals by postmortem and coprological examinations in consistence with another finding (Yilma et al., 2000). The higher prevalence of the disease among Ethiopian source animals could be associated with the existence of suitable ecological conditions for the intermediate snail host in the areas where animals graze (Abebe et al., 2010).

We determined a higher prevalence of *Fasciola* infection rate among light weight animals with both postmortem and coprological examinations in agreement with previous reports (Kassaye et al., 2012) and (Nega et al., 2012). This signifies that the light weight animals are more susceptible to the infection.

The results of our study also showed a higher infection rate among small size animals by postmortem and coprological examinations in agreement with a previous report (Bekele et al., 2012). This attributed to low resistance against the disease.

In this study the association between the occurrence of fasciolosis with concurrent infections with both coprological and postmortem examinations revealed that there is no significant difference between the concurrent infections with fasciolosis by both examinations.

The prevalence of fasciolosis reported by using coproscopy was lower than that obtained by the abattoir results indicating that the latter is more sensitive in detecting the disease. The detection of *Fasciola* eggs can be unreliable as the eggs are expelled intermittently, depending on the evacuation of the gall bladder (Briskey et al., 1998). Similar study suggested that about 36% infected animals may pass undetected with single fecal examination technique. This might be attributed partly to the fact that *Fasciola* eggs only appear in feces 8-15

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weeks post infection, so most of pathological lesions had already occurred (Mulugeta et al., 2011).

The present sensitivity value (63.9%) is comparable to other reports: 65.9% from Ethiopia (Regassa et al., 2012) and 69% from Switzerland (Rapsch et al., 2006). The latter stated that traditional coproscopy can be very efficient if there is repeated sampling, resulting in sensitivity of approximately 92%. Therefore, worm counts at liver necropsy can only be considered as a gold standard if the livers are sliced and soaked. Even then very light or prepatent infections could still be missed, affecting the calculated sensitivity and specificity of the evaluated tests.

The current study revealed that the infection with fasciolosis varies according to different regions in Sudan and demonstrated that the disease had a high prevalence among Ethiopian cattle. It also elucidated that coprological examination for the parasite eggs has significant limitations in detecting the presence or absence of fasciolosis in animals. On the other hand, it has helped to illustrate the usefulness of meat inspection in monitoring disease situation and demonstrating possible long term trends. This study also showed that bovine fasciolosis is significantly associated with age, breed, weight, source and size of animal.

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Table 1 : Prevalence of fasciolosis in 307 cattle examined by postmortem examination at Elkadaro abattoir, Khartoum, Sudan

Disease	Frequency	Relative frequency (%)	Cumulative frequency (%)
Negative	210	68.4% (210/307)	68.4%
Positive	97	31.6% (97/307)	100%
Total	307		

Table 2 : Prevalence of fasciolosis in 307 cattle examined by coproscopic examination at Elkadaro abattoir, Khartoum, Sudan

Disease	Frequency	Relative frequency (%)	Cumulative frequency (%)
Negative	245	79.8% (245/307)	79.8%
Positive	62	20.2% (62/307)	100%
Total	307		

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Table 3 : Multivariate analysis for the association between fasciolosis (examined by postmortem) and potential risk factors using logistic regression

Risk Factors	No. tested	No. positive (%)	Exp (B)	95% C.I. for Exp (B)	p-value
Age (years)					
<2	25	0	- - -	- - -	1.000
2-4	71	3 (4.2%)	1	- - -	.999
>4	211	94 (44.5%)	18.5	3.1, 21.7	0.000
Breed					
Local	128	3 (2.3%)	1	- - -	1.000
Cross	34	1 (2.9%)	1.3	0.4, 3.2	0.641
Foreign	145	93 (64.1%)	77.6	9.3, 81.6	0.000
Weight					
Light	137	65 (47.4%)	3.0	1.9, 5.3	0.000
Medium	139	32 (23.0%)	1	- - -	1.000
Heavy	31	0	---	- - -	1.000
Source					
Nyala	79	2 (2.5%)	1	- - -	1.000
Ethiopia	142	93 (65.5%)	76.1	8.4, 83.7	0.000
Khartoum	35	0	- - -	- - -	1.000
Kordofan	13	0	- - -	- - -	0.999
Eljazeera	1	1 (100%)	- - -	- - -	1.000
White Nile	9	0	- - -	- - -	0.999
Kassala	25	1 (4 %)	1.6	1.1, 3.1	0.027
South Sudan	3	0	- - -	- - -	1.000
Animal Size					
Small	136	65 (47.8%)	3.1	1.9, 5.3	0.000
Medium	141	32 (22.7%)	1	- - -	1.000
Large	30	0	- - -	- - -	1.000

Table 4 : Multivariate analysis for the association between fasciolosis (examined by coprology) and potential risk factors using the logistic regression

Risk Factors	No. tested	No. positive (%)	Exp (B)	95% C.I. for Exp (B)	p-value
Age (years)					
<2	25	0	- - -	- - -	1.000
2-4	71	1 (1.4 %)	1	- - -	1.000
>4	209	61 (28.9%)	28.8	3.9,34.2	0.000
Breed					
Local	128	1 (0.7%)	1	- - -	1.000
Cross	34	0	- - -	- - -	1.000
Foreign	145	61 (42.1%)	94.4	13.2,102.6	0.000
Weight					
Light	137	60 (43.8%)	53.4	7.1,61.3	0.000
Medium	139	2 (1.4%)	1	- - -	1.000
Heavy	31	0	- - -	- - -	1.000
Source					
Nyala	79	1 (1.2%)	1	- - -	1.000
Ethiopia	142	61 (43.0%)	60.2	8.3,70.1	0.000
Khartoum	35	0	- - -	- - -	1.000
Kordofan	13	0	- - -	- - -	1.000
Eljazeera	1	0	- - -	- - -	1.000
White Nile	9	0	- - -	- - -	1.000
Kassala	25	0	- - -	- - -	1.000
South Sudan	3	0	- - -	- - -	1.000
Animal Size					
Small	136	60 (44.1%)	54.9	6.3,63.8	0.000
Medium	141	2 (1.4%)	1	- - -	1.000
Large	30	0		- - -	1.000

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Table 5 : The difference in fasciolosis prevalence estimated based on coprology and postmortem examination at Elkadaro abattoir during May -July 2011

Test Type	No. Positive	Prevalence	Diff. (95% CI)	X^2	p-value
Coprology	62	20.2%	7.3 (3.1, 9.4)	1.669	0.000
Postmortem	97	31.6%			

Diff = difference

Table 6 : The presence or absence of *Fasciola spp* eggs in the feces of cattle with and without *Fasciola* in the liver for measuring the sensitivity, specificity and agreement level of the two tests

Faecal examination	Presence of *Fasciola spp* in liver		Total
	Fluke (+ve)	Fluke (-ve)	
Egg present (+ve)	62	0	62
Egg absent (-ve)	35	210	245
Total	97	210	307

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Effects of Feeding Processed Kidney Bean Meal *(Phaseolus Vulgaris)* by Replacing Soybean Meal on Egg Fertility and Qualities of Chicks of White Leghorn Hens

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Abstract- The study was to evaluate feeding processed kidney bean meal (PKBM) instead of soybean meal (SBM) on fertility, hatchability, embryonic mortality and chick quality of white leghorn (WL) hens. Replacement of SBM with PKBM in the diet did not affect the fertility, hatchability and embryonic mortality. Chick length (15.63, 15.00, 15.33, 15.03 and 14.33 (SEM $=\pm 0.02$)) and chick weight (34.13, 34.20, 33.13, 33.06 and 32.47(SEM$=\pm 1.39$)) for T1, T2, T3, T4 and T5 respectively, were significantly ($P<0.05$) lower for T5 than the rest treatments. Visual assessment of chick quality is lower for treatments containing higher proportion of PKBM than treatments containing lower proportion of PKBM. Therefore, as it affects the quality of chicks at 100% replacement, it is only up to 75% replacement of SBM by PKBM (dosed at 195 g/kg concentrate diet) is possible without having significant negative effect on chick quality.

Keywords: embryonic mortality, fertility, hatchability, kidney bean, soybean meal.

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Effects of Feeding Processed Kidney Bean Meal (*Phaseolus Vulgaris*) by Replacing Soybean Meal on Egg Fertility and Qualities of Chicks of White Leghorn Hens

Taju Hussein [α], Mengistu Urge [σ], Getachew Animut [ρ] & Sisay Fikru [ω]

Abstract- The study was to evaluate feeding processed kidney bean meal (PKBM) instead of soybean meal (SBM) on fertility, hatchability, embryonic mortality and chick quality of white leghorn (WL) hens. Replacement of SBM with PKBM in the diet did not affect the fertility, hatchability and embryonic mortality. Chick length (15.63, 15.00, 15.33, 15.03 and 14.33 (SEM =±0.02)) and chick weight (34.13, 34.20, 33.13, 33.06 and 32.47(SEM=±1.39)) for T1, T2, T3, T4 and T5 respectively, were significantly (P<0.05) lower for T5 than the rest treatments. Visual assessment of chick quality is lower for treatments containing higher proportion of PKBM than treatments containing lower proportion of PKBM. Therefore, as it affects the quality of chicks at 100% replacement, it is only up to 75% replacement of SBM by PKBM (dosed at 195 g/kg concentrate diet) is possible without having significant negative effect on chick quality.

Keywords: embryonic mortality, fertility, hatchability, kidney bean, soybean meal.

I. Introduction

Chick quality is affected by pre-incubation storage conditions, time in the Hatcher, and size of egg. Tona et al., (2004) Found that increased incubation storage produced poor quality chick. Larger eggs tend to have significantly poor quality chicks as compared to other egg size Tona *et al.,* (2004).

The fertility of an egg is affected by several factors originating from the hen such as her ability to mate successfully, to store sperm, to ovulate an egg cell, and to produce a suitable environment for the formation and development of the embryo. The fertility also depends on the cock's ability to mate successfully, quantity and quality of semen deposited Brillard (2007). When fertility is low, it can affect other categories because of the lack of uniformity of embryo temperature inside the egg set (not as much heat provided by the developing embryos).

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Hatchability is a process that has several critical points that can be monitored and controlled to produce consistently healthy and mature hatchlings. These includes assessing hatching egg, fertility, egg storage and care, evaluation of hatch residue, poultry processing, sanitation, and poultry health and viability Hullet (2007).

The cost of poultry feed is very high and it accounts for 60-70% of the layer production cost Wilson and Beyer (2000). In recent years, the price of conventional or basic feed ingredients has tremendously increased. This has made poultry and live-stock production very expensive. In Ethiopia where soybean and its meal are in short supply and very expensive, the use of soybean meal as protein source of poultry ration is limited. Thus, an alternative protein source should be assessed and used. Sisay et al., (2015b) suggested that 100% replacement of SBM with PKBM is possible at 10% (100 g/kg) of soybean in layer ration. Therefore, the present research was initiated to evaluate the effect of feeding processed kidney bean meal (*Phaseolus vulgaris*) by replacing soybean meal at 19.5% (195g/kg) on egg fertility and qualities of chicks of white leghorn hens.

II. Materials and Methods

a) Management of Experimental Animals

Experimental house which were partitioned into 15 pens with wire-mesh and covered with grass litter material of 10 cm depth were used for experiments. Before the commencement of the actual experiment, the experimental pens, (2.5*2m), watering equipment, feeding troughs and laying nests were disinfected, sprayed against external parasites and thoroughly cleaned. Disinfectant was placed at the gate of the experimental house for workers and other visitors to step on before they enter into the house, which is in addition to the one placed at the main gate of poultry farm, for prevention of disease introduction.

The birds were vaccinated against Newcastle disease according to the vaccination program of the farm. The birds were offered with experimental diets for 7 days as period of adaptation before actual data

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collection takes places. The feed was measured and given to the birds in groups twice a day at 8 am and 2 pm on *ad libitum* basis by dividing the daily offer into two equal portions. Feed refusals were collected every morning at 7:30 am before providing new feed, external contaminants were removed by visual inspection, weighed and recorded for each pen separately. Feed were offered into two metal tubular feeders per pen that was hanged approximately at a height of the backs of the birds. Water was provided in a plastic fountains placed on a flat stone at the center of the pen. The watering trough was cleaned every morning before feeding. Clean and fresh water was available to the birds' *ad libtum*. The experimental duration lasted for 12 weeks.

b) Treatments and Experimental Design

The ration of the experiment consists of PKB, which replaced SBM at five levels, namely 0, 25, 50, 75 and 100% PKB for T1, T2, T3, T4 and T5, respectively (Table 1). Initially weighed two hundred ten (210) birds consisting of 180 White leghorn layers and 30 cocks of mature white leghorn breeds of similar age and weight, which were obtained from the University's poultry farm, were randomly assigned to the five treatment rations. Thus, the design employed was a completely randomized design (CRD) with five treatments and three replications (pen) per treatment (Table 1).

c) Chemical Composition of Experimental Feeds

Representative samples of feed ingredients was taken and analyzed before formulating the actual dietary treatment rations. The results of the analysis were used to formulate the treatment rations (Table 2). The feed ingredient offer samples were analyzed for dray matter (DM), ether extracts (EE), crude fiber (CF) and ash following the procedure of Weende (proximate analysis method) of the AOAC (1990). Kjeildhal procedure was employed to determine the nitrogen (N) content of the feeds and crude protein (CP) was determined by multiplying the N value with 6.25. The total metabolizable energy (ME) contents were calculated indirectly by using the formula presented by Wiseman (1987). ME (Kcal/kg DM) = 3951 + 54.4 EE – 88.7 CF – 40.8 Ash.

d) Data Collection and Measurements

i. *Fertility and hatchability*

Hundred ninety-five, that is 39 and 13 eggs from each treatment and replication, respectively, of medium sized, non-defected and normal shaped eggs were collected in three consecutive days. The eggs were kept at room temperature until incubated within 4 days after the first day of collection. The eggs were lightly coded by marker before they were placed into the incubator. The incubation temperature and relative humidity during the 18 days of incubation was auto fixed at 37-38°C and 65-70%, while that of hatchery unit was adjusted to 38.5-39°C and 90% relative humidity.

The eggs kept in the tray with small end down and turned automatically by slanting the tray at 45°. The incubator is equipped with the turner that facilitates the turning operation at an interval of two hours. Fertility was determined by candling the incubated eggs on the 7th day of incubation. Candling was done at dark room with egg Candler powered by electricity. Eggs found to be infertile, which are characterized by clear appearance, egg with blood adhering to one sides of the eggs were drown from the incubator. Finally eggs found fertile, i.e. eggs having small dark spot, numerous blood vessels arising from those dark spot of yolk at day of candling, clearly visible thick and dark and well fill structure was further kept in the incubator for hatching North (1984). Eggs with living embryo were transferred to the hatching section of the incubator at the end of the 18thday. Hatched chicks were counted and chick quality was determined. Fertility and hatchability were determined by using the following formulae, respectively.

$$\%\text{Fertility} = \frac{\text{number of fertile eggs}}{\text{total egg set}} x100$$

$$\%\ \text{Hatchability on fertile eggs base} = \frac{\text{number of chick hatched}}{\text{fertile eggs}}\ x100$$

$$\%\ \text{Hatchability on total eggs base} = \frac{\text{number of chick hathced}}{\text{total eggs set}} x100$$

ii. *Embryonic mortality*

Embryonic mortality was determined by candling eggs at 7th, 14th and 18th days of incubation and at hatching. Eggs that had a structure encircled with blood ring, absence of blood vessels, adhering to the shell membrane and absence of clear demarcation between embryo and air cell was considered as dead embryo and removed from the incubator North (1984). The dead embryo further categorized in to early, mid, late dead and pip embryo, based on the classification criteria set by Butcher (2009). The embryonic mortality was computed by using the formulae indicated by Rashed (2004).

$$\%\ \text{of early mortality} = \frac{\text{total numbr of early dead embryo}}{\text{total number of fertile eggs}} x100$$

$$\%\ \text{of mid mortality} = \frac{\text{total number of mid dead embryo}}{\text{total number of fertile eggs}}\ x100$$

$$\%\ \text{of late mortality} = \frac{\text{total number of late dead embryo}}{\text{total number of fertile eggs}}\ x100$$

$$\%\ \text{of pip mortality} = \frac{\text{total number of pip dead embryo}}{\text{total number of fertile eggs}} x100$$

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iii. *Chick quality*

Chicks' quality were measured using two different methods, which includes visual scoring and measuring of day old chick weight and length. Visual scoring of chicks was done by visual examination based on the quality standards described by North (1984). Accordingly, a chick that was not malformed, physically active, stands up well, and looks live has been taken as good quality chicks. The researcher and two technicians were under taken the visual quality assessment. Based on common decisions, the chicks were classified in to poor and normal chicken. After the chicks were classified in to two groups, namely quality (chicks with dried body, stand well, and active) and non-quality chicks (chicks with wet body, not firm, non-straight, and not having perpendicular leg to the ground), five chicken from normal chicken were taken randomly from each replicate and their weight and length were measured using sensitive balance and ruler for further quality assessments respectively. The chick's length was taken by stretching the chicks on the table and taking the length from the tip of the beak to the middle toe by a ruler. Percentage of quality chicks were calculated as

$$\text{Quality chicks} = \frac{\text{total number of quality chicks}}{\text{total number of hatched chicks}} x100$$

e) Statistical Analysis and Models

The data collected during the study period were subjected to statistical analysis using SAS computer software version 9.1.3. SAS (2008). during data analysis, chick weight and length were analyzed following one way analysis of variance procedure. When the analysis of variance indicated the existence of significant difference between treatment means, list significant difference (LSD) method was used to locate the treatment means that were significantly different from the other Gomez and Gomez (1984).

The model used for statistical analysis was:

$$Yij = \mu + Ti + eij$$

Where: Yij=Individual observation,
Ti=Treatment effect, μ=Overall mean, eij= Error term

General logistic regression analysis was employed for analysis of data recorded on fertility (fertile/infertile), hatchability (hatched/un-hatched), embryonic mortality (alive/dead), and visual scoring (normal/poor). The general logistic regression model used is given below:

$$\text{Model: } 1n\left\{\frac{\pi}{1-\pi}\right\} \beta 0+\beta 1^{*} (x)$$

Test H0: No treatment effect (*i.e.*, $\beta 1=0$) vs.
HA: Significant treatment effect ($\beta 1 \neq 0$).
Where, π=probability,
β=slope, x=treatment

III. Results and Discussion

a) Nutrient Composition of Ingredients and the Treatment

The results of the chemical analysis of ingredients used and nutritional composition of the ration–for each treatment are given in Table 3 and 4 respectively. The DM contents of PKB obtained in the present study was slightly lower than reported by Marzo et al., (2002) (93.2%) and Audu and Aremu (2011) (96.8) but very close to Sisay et al., (2014) and Sisay et al. (2015a) (88) while CP contents is slightly higher than reported by Marzo et al., (2002), Audu and Aremu (2011), Emiola (2011), Sisay et al., (2014), Sisay et al., (2015b) and Emiola and Olghobo (2006) which were 20.9%, 23.6%, 24.7%, 25.8% and 26.8%, respectively. The EE content of kidney bean is the same with that reported by Emiola (2011) but Marzo et al., (2002) reported lower than the current results. The CF content of kidney bean used in the present study was comparable to that reported by Arija et al., (2006), Emiola (2011), Sai-ut et al., (2009), Audu and Aremu (2011), Sisay et al., (2014) and Sisay et al., (2015b) who reported 5.1, 5.0, 6.0, 4.7% and 4.5% respectively. The result of chemical composition of kidney bean used in the present experiment showed comparable ME contents to that noted by Ofongo and Aloghobo (2007) and Arija et al., (2006) who reported 3342.2 and 3365 kcal/kg, respectively.

In general, as different report showed the processing (treatments) could cause drop or increase in nutrients compared to the raw seed. The treatment method (boiling) employed was suggested increased total carbohydrates and decrease the CP, EE, CF and ash contents Marzo et al., (2002), Audu and Aremu (2011). Similarly, Akaerue and Onwuka, (2010) and Mubarak (2005) reported reduced CP, EE, CF and ash for Mung bean (*Vigna radiata*). The low content of CF of PKB and the further reduction as a result of boiling might favored the feed intake of the layers by decreasing the problem of feed digestibility.

SBM and NSC are rich in CP content that make these ration to be ideal source of protein supplement for poultry. The NSC used to formulate experimental ration composed 26% CP and 2006 kcal ME. The CP values are comparable with that of Shewangzaw et al., (2011) and Meseret et al., (2011) but low in ME. Previously under taken studies indicated that the CP content of SBM to be in the range of 41 to 50% Waldroup (2002), Ekeren et al., (2006). However, similar to the value obtained in this study, a 38% CP content of SBM were reported in Ethiopia Meseret et al., (2011).

When SBM and PKB are compared, SBM contain by far higher CP, EE and ME. On the other hand, PKB has lower CF and ash than SBM. As a result, the CP of the formulated ration ranged between 16% (diet 5) and 18% (diet 1). Crude fiber increased, EE

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decreased because of total replacement of SBM by PKB. The energy content of SBM is higher than that of PKB. For this reason, the energy content decreased with increasing level of processed kidney bean. Nevertheless, energy and protein content of all rations ranged within the recommended level for layers. Lesson and summer (2001) recommended 16-18% of CP and 2500-3300 kcal/kg ME, respectively for white leghorn layers. Furthermore, the energy of compound ration is the same with that used by Zebiba (2012) and Senayt et al., (2011) in their experiment for the same bird in the same farm.

b) Fertility and Hatchability of Eggs

Mean values of fertility and hatchability for the treatments are presented in Table 5. The logistic regression results for fertility and hatchability of eggs showed no significant difference among treatments. However, there was a numerical decrease in fertility and hatchability percentage for T5 as compared toT1 which is similar with Sisay et al., (2015b) finding. The numerical decrease in fertility and hatchability from T1 to T5 could be the result of increased, level of kidney bean in the ration that caused reduction of level of protein, calcium and phosphorus found in the rations. The diets of breeder poultry should be adequate in both quantity and quality to meet the recommended levels of feed standard Brillard (2007). The level of dietary protein significantly affected egg fertility and hatchability Gareil et al., (2006). Poor hatching happen when nutritionally deficient feed is used for layers Hocking et al., (2002). The authors have shown that low calcium levels tended to decrease percent of fertility and hatchability. On the contrary, El-Ghamry et al., (2010) reported improved fertility (83% and 77%) and hatchability (78% and 71%) for the group fed 2.4% and 2.6% calcium, respectively.

c) Embryonic Mortality

The mean values of embryonic mortality at different stages of development are presented in Table 5. The logistic regression result showed no significant differences among treatments for early, mid, late and pip embryonic mortality. Nevertheless, the mortality tends to numerically increase with increased level of PKB compared to control diets that do not contain PKB. This might occurred because of the decreased crude protein level with increased level of PKB. Embryonic mortality of eggs of breeder hens' fed low protein is reported to be higher than hens fed high protein diets. Low-protein rearing rations were associated with higher rates of food intake, higher mortalities and lower rates of egg production than the conventional protein ration Hocking et al., (2002).

d) Chick Quality

i. *Visual Scoring (Observation)*

The visual scoring of chicks is presented in Table 6. Wald chi-square statistics indicated that visual scoring of chicks was insignificant (0.64) at (α = 0.05) among the treatments, but Sisay et al., (2015b) indicated that visual scoring of chicken was not significant (*pr>chisq* 0.641 α=0.05). The visual assessment showed that the quality of chicken is better in the order of T2>T1>T3>T4, while the chick quality of T5 is inferior or poor on visual scoring. The chicken in T5 were not well standing, they are dehydrated and seems inactive at their day old age. This assessment was supported by length and weight of chicks that indicated significant differences among treatments. Utilization of visual score parameters such as naval quality, firmness of leg, size of beak, eye and vital chick are recommended ways of determining highest quality chicks Petek et al., (2010).

ii. *Chick Length*

The length of chicks hatched from eggs of hens fed diet containing 0, 25, 50 and 75% of PKB are not significantly different which is similar with Sisay et al., (2015b) finding that there was no significant difference ($p>0.05$) among treatments in chick weight and chick length. However, the chicken hatched for eggs harvested from layers fed 100% PKB (T5) had significantly lower length as compared to that from the layers fed SBM at different levels. This occurred either because of the egg size that accommodate larger embryo of chicken compared to the small eggs that has less environment to hold large chicken or the nutrition of the layers that promote better growth of the chicks. Mukhtar et al., (2013) Found similar result and stated that hatching length is an easy and repeatable quality evaluation parameter for newly hatched chicks. This important trait has a positive correlation with the size of the egg and the chick's weight. It is an important economic trait to predict chick development because it is positively related to yolk-free body mass at hatch and potential of chicks for optimum future performance.

Furthermore, Petek et al., (2010) pointed out that each extra cm of hatchling length at day of hatch meant an increase of 18 g BW at seven days of age. Chicks with longer hatchling length have better-feed efficiency and survival rates as compared to smaller chicks. Petek et al., (2009) Classified length intervals in to short, middle and long for a day old chicks. Accordingly, layer chick with a length of < 17.8, 17.8-18.2 and > 18.2cm, respectively are grouped as short, medium and long chicks. As to this classification, chick lengths in the present experiment for all treatments fall within a short category. On other hand, Petek et al., (2008) reported that the body weight and chick length uniformity in long group in all poultry to be better than the shorter group

iii. *Chick Weight*

The chick weight of layers were higher (P<0.05) in ration that do not contain SBM as compared to the ration containing 100%PKB (Table 6). The variation in

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chick weight may be due to the weight of eggs, which is slightly decreasing across treatment as well as the amino acid content of the rations, which is higher in control diet while decreasing across treatment because of decreased SBM. The present finding is in agreement with many previous of findings. For instance the chicks injected with amino acids invariably had higher plasma protein and lower plasma glucose on the day of hatch, because methionine and threonine are critical for the growth of chicken embryo Subrat et al., (2012). Egg weight has a direct impact on the weight of chick and there is a positive correlation between egg and chick weight Petek et al., (2010). Chicken hatched from large eggs are heavier than those hatched from comparatively smaller eggs Al-Murrani (1978). A heavy chick indicates a good development. However, this is sometimes not true and evaluating chick quality by measuring only body weight can be misleading Molenaar (2009).

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Table 1: Experiment lay out

Treatments	Replications	Layers per replication	Cocks per replication
T1(100%SBM:0%PKB)	3	12	2
T2 (75% SBM:25%PKB)	3	12	2
T3 (50% SBM:50%PKB)	3	12	2
T4 (25% SBM:75%PKB)	3	12	2
T5 (0% SBM:100% PKB)	3	12	2

SBM=Soybean meal; PKB=Processed kidney bean; 100% PKB represents replacement of maximum SBM (260g/kg) as recommended by earlier study Senayt et al. (2011)[6]

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Table 2 : Proportion of feed ingredient used in formulating experimental rations

	Treatments				
Feed (%)	T1	T2	T3	T4	T5
CG	56	53	52	48	37
WS	7	7	7	7	16
SBM	26	19.5	13	6.5	0
PKB	0	6.5	13	19.5	26
NSC	4	7	8	12	14
LS	5.5	5.5	5.5	5.5	5.5
Salt	0.5	0.5	0.5	0.5	0.5
VPM	1	1	1	1	1
Total	100	100	100	100	100

CG= corn grain; WS= wheat short; SBM= soybean meal; PKB= processed kidney bean; NSC= noug seed cake; LS= limestone; VPM= vitamin pre mix; T1 100%SBM: 0%PKB; T2= 75%SBM: 25%PKB; T3= 50%SBM: 50%PKB; T4=25%SBM: 75%PKB; T5= 0%SBM: 100%PKB

Table 3 : Ingredient used in the study and its nutrients compositions

	Nutrient composition (% for DM and % DM for others)					
Feed type	DM	CP	EE	CF	Ash	ME kcal/kg
CG	89.5	8.7	4.3	8.0	6.21	3230.5
WS	90.3	12	3.3	6.2	6.8	3303.1
SBM	90.2	38	7.0	9	7.8	3215
PKB	87.5	28	0.9	6	7.0	3182.2
NSC	91.5	26	6.0	21.0	10	2006.0

CG=Corn grain; WS=Wheat short; SBM=Soybean meal; PKB=processed Kidney bean; NSC=Noug seed cake; DM=dry matter; CP=Crude protein; EE=ether extract; CF=crude fiber; ME=methabolizable energy

Table 4 : Nutritional composition of treatment diets containing different levels of processed kidney bean as a replacement for soybean meal

	Nutrient composition (% for DM and % DM for others)							
Treatments	DM	CP	EE	CF	Ash	Ca	P	ME kcal/kg
T1	91.85	18	5.64	6.26	9.96	3.4	0.39	3296.20
T2	91.56	17.8	5.63	6.36	9.97	3.26	0.38	3286.40
T3	91.17	17.6	5.58	6.52	9.98	3.28	0.38	3269.00
T4	90.21	16.3	5.40	6.56	9.98	3.01	0.36	3255.70
T5	89.86	16.0	4.90	6.86	10.20	2.79	0.32	3192.90

DM= dry matter; CP= crude protein; EE = ether extract; CF= crude fiber; SBM= soybean meal; PKB processed kidney bean; T1 100%SBM: 0%PKB; T2= 75%SBM: 25%PKB; T3= 50%SBM: 50%PKB; T4=25%SBM: 75%PKB; T5= 0%SBM: 100%PKB

Table 5 : Fertility and hatchability of eggs and embryonic mortality in layers fed different levels of processed kidney bean as a replacementz to soybean meal

	Treatments						
Parameter	T1	T2	T3	T4	T5	SEM	SL
Fertility (%)	94.87	94.87	94.87	94.87	92.3	2.29	NS
HTES (%)	71.79	71.79	71.79	64.10	61.54	4.59	NS
HFES (%)	75.43	75.64	75.43	69.44	66.67	3.50	NS
EEM (%)	5.56	5.56	5.56	8.33	8.33	2.14	NS
MEM (%)	5.56	8.12	8.12	11.11	8.33	1.76	NS
LEM (%)	5.56	5.56	5.56	8.30	13.89	2.48	NS
PIEM (%)	10.89	8.12	10.89	11.11	11.11	3.07	NS

SBM= soybean meal; PKB= processed kidney bean;T1=100% SBM:0% PKB; T2=75%SBM:25%PKB; T3=50%SBM:50%PKB; T4=25%SBM:75%PKB; T5=0%SBM:100PKB; HTES= hatchability on total egg set; HFES= hatchability on fertile egg set; EEM =early embryonic mortal; MEM= mid embryonic mortality; LEM = late embryonic mortality; PIEM =pip embryonic mortality

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Table 6 : Chick quality parameters of white leghorn layers fed different levels of processed kidney bean as a replacement to soybean meal

	Treatment						
Parameters	T1	T2	T3	T4	T5	SEM	SL
Visual (%)	84.93	85.09	82.36	81.02	70.83	3.89	NS
Length (cm)	15.63[a]	15.00[a]	15.33[a]	15.03[a]	14.33[b]	0.20	*
Weight (g)	34.13[a]	34.20[a]	33.13[a]	33.06[a]	32.47[b]	0.28	*

[a-b] means in the row without common superscript are significant; NS = Non- significant, SL= significance level; SEM= standard error of mean ; cm = cent meter, g = gram; % = percent; PKB = processed kidney bean meal; SBM= soybean meal; T1=100% SBM:0% PKB; T2=75%SBM: 25%PKB; T3=50%SBM: 50%PKB; T4=25%SBM: 75%PKB; T5=0%SBM: 100PKB;

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Prevalence of Bovine Mastitis in Lactating Cows and its Public Health Implications in Selected Commercial Dairy Farms of Addis Ababa

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Abstract- A cross sectional study was conducted in Addis Ababa from October 2011 to May 2012 to determine prevalence of bovine mastitis and discuss its public health implications. A total of 444 systematically selected lactating cows of different cattle breed from thirty seven (37) dairy farms were investigated. The herds were visited and the farmers interviewed about the management, housing, feed and feeding, and milking conditions. California Mastitis Test (CMT) was applied on milk samples collected from 1776 individual quarters. The overall prevalence of bovine mastitis was 68.0% (302/444) of which sub-clinical mastitis accounted for 46.8% (208/444) while 21.2% (94/444) were found to be clinical forms. There was significant difference ($P<0.05$) in the prevalence of mastitis among the different breeds, age groups, parity and lactation stage. Relatively higher number of farmers interviewed (20.8%) replied that they do not withhold milk from cows treated for mastitis and continue to avail it to the public without interruption. As it is economically damaging, the need to establish diagnostic facility to enable early detection for screening large number of samples was emphasized. Further work on identification of the causative agents and conducting public awareness creation about major zoonotic diseases were also recommended.

Keywords: *california mastitis test, interview, prevalence, mastitis, zoonotic.*

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Alebachew Tilahun [α] & Alemu Aylate [σ]

Abstract- A cross sectional study was conducted in Addis Ababa from October 2011 to May 2012 to determine prevalence of bovine mastitis and discuss its public health implications. A total of 444 systematically selected lactating cows of different cattle breed from thirty seven (37) dairy farms were investigated. The herds were visited and the farmers interviewed about the management, housing, feed and feeding, and milking conditions. California Mastitis Test (CMT) was applied on milk samples collected from 1776 individual quarters. The overall prevalence of bovine mastitis was 68.0% (302/444) of which sub-clinical mastitis accounted for 46.8% (208/444) while 21.2% (94/444) were found to be clinical forms. There was significant difference ($P<0.05$) in the prevalence of mastitis among the different breeds, age groups, parity and lactation stage. Relatively higher number of farmers interviewed (20.8%) replied that they do not withhold milk from cows treated for mastitis and continue to avail it to the public without interruption. As it is economically damaging, the need to establish diagnostic facility to enable early detection for screening large number of samples was emphasized. Further work on identification of the causative agents and conducting public awareness creation about major zoonotic diseases were also recommended.

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I. Introduction

Despite many years of research, mastitis subclinical remains the most economically damaging and zoonotic potential disease for dairy industry and consumers worldwide irrespective of species of animal (Ojo *et al*., 2009). Economic losses caused by mastitis include value of discarded milk (Radostits *et al*., 2007). Bacterial contamination of milk from affected cows may render unsuitable for human consumption by causing food poisoning or interference with manufacturing process or in rare cases provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw cow milk include brucellosis, caseous lymphadenitis, leptospirosis, listeriosis, melioidosis, Q-Fever, Staphylococcal food poisoning, toxoplasmosis and tuberculosis (Mungube *et al*., 2005; Radostits *et al*., 2007).

The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, sub-clinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al*., 2003). Previous studies conducted in different countries indicate the distribution and economic importance of the disease. Contreras *et al*. (1997) from Spain; Moshi *et al*. (1998) from Tanzania; Ameh and Tari (2000) from Nigeria; Ndegwa *et al*. (2000) from Kenya and Kozacinski *et al*. (2002) from Croatia reported different prevalence rates of mastitis in dairy cattle. The disease has been reported by several authors in different parts of the Ethiopian country (Mungube *et al*., 2005; Lakew *et al*., 2009; Gebreyohannes *et al*., 2010; Megersa *et al*., 2010). Several of these studies have been shown the occurrence a range of mastitis causing bacteria indicating *Staphylococcus*, *Escherichia coli* and *Streptococcus* as dominant and pathogenic species. Some authors (Mungube *et al*., 2005) reported a substantial economic loss in Ethiopian highland crossbred dairy cows due to subclinical mastitis.

Subclinical mastitis can be recognized indirectly by several diagnostic method including the California mastitis test (CMT), the Modified White Side test, Somatic cell count, pH, and catalase tests. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Joshi and Gokhale, 2006).

In some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects (Mekebib *et al*., 2009; Megersa *et al*., 2010).

This study aimed (i) to evaluate the prevalence of subclinical mastitis in apparently healthy dairy cows in Holeta district, (ii) to determine the most frequency of intramammary infection, causative agents, and (iii) to evaluate associated risk factors affecting on subclinical mastitis.

II. Materials and Methods

a) Study area

The study was conducted in Addis Ababa city administration, the capital of the Federal Democratic

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Republic of Ethiopia. The city covers an area of 530.14 km^2 and is sub divided into ten sub-cites namely, Arada, Bole, Addis Ketema, Nefas Silk Lafto, Kolfe Keranio, Akaki Kality, Yeka, Lideta, Kirkos and Gulele sub-cites. Addis Ababa lies at an altitude of 2000-3000 meters above sea level and is a grass land biome located between 9.03 North latitude and 38.74 East longitudes. The city has alternating dry and rainy seasons with the long rainy season that extends from June to September and short rainy season that lasts from March to May. The mean annual minimum and maximum temperatures range between 14°C and 21°C respectively with an overall overage of 17°C. The mean relative humidity is 61.3% (CSA, 2003).

b) Study Animals and Sample Size Determination

The study was conducted on 444 lactating cows (local, Holstein-Friesian, Jersey and cross breeds) from 37 dairy farms in Addis Ababa. The farms were purposively selected based on the availability of lactating cows within the farm and the owners' willingness. Systematic random sampling method was applied for the selection of individual animals (lactating cows) in the farms. The sample size was determined by the formula given by Thrusfield (2007) considering an expected prevalence of 71% (Mekibib *et al*., 2009), 95% confidence level and 5% desired precision. Adding a few more samples to improve on the accuracy, a total of 444 lactating cows were considered for the study.

c) Study Design

A Cross sectional study was conducted. Three dairy farms were purposively selected for their ease accessibility. Simple random sampling technique was followed to select the study animal and the desire sample size was calculated according to the formula given by Thrusfield (2007).

The study was carried out from November 2011 to April 2012 by collection of events associated with mastitis in lactating cows from 37 small holder's dairy farms in Addis Ababa.

d) Study Methodology

i. *Clinical inspection of udder*

The udder was first examined visually and then by palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue and swellings of supra mammary lymph nodes. The teat condition (color changes, swelling at or near the teat base, swelling or firmness at or near the teat end, openness of the teat orifice, teat skin condition, signs of vascular damage like petechial hemorrhage, etc.) was evaluated during clinical examination (More, 1989). Upon palpation, one can feel hot, painful swelling on udder and ventral abdomen and was manifested by loss of appetite, depression, recumbence and blood mixed milk in acute mastitis. In chronic mastitis, continuous or intermittent discharge of pus, clots, flakes or watery secretion will be seen from the udder (Chauhan and Agarwal, 2006).

e) California mastitis test (CMT)

The California Mastitis Test (CMT) was performed according to the manufacturer's instruction. In brief, a small sample of milk (approximately ½ teaspoon) was collected from each quarter into a plastic paddle that has 4 shallow cups marked A, B, C and D. An equal amount of CMT reagent was added to the milk and the paddle rotated to mix the contents. After approximately 10 seconds, the score was read while continuing to rotate the paddle. Results were recorded as T (trace), 1, 2 or 3 based on the level of precipitation (coagulation) (Mellenberger and Carol, 2000).

i. *Risk factor assessment*

Information on animal and farm-based risk factors was collected in two separate pre-designed questionnaires, by observation, and by interviewing of the different farm attendants and owners. A check-list was used to record such information as the cows' age, breed, parity, lactation stage, and body condition, problems of leaking milk and previous history of mastitis. Farm-based risk factors considered were teat drying, teat cleaning, floor types, teat dipping, milkers, bedding and treatment history.

ii. *Assessment of public health risks*

This was done by asking respondents weather they adapt the behavior of boiling milk before consumption, stripping of the foremilk at the start of milking, and by asking them the time duration of time they withheld milk before distribution to the public if the animals were treated for mastitis.

f) Statistical analysis

The data was compiled and analyzed with SPSS statistical package version 17. Prevalence estimation of commonly isolated pathogens in Holeta town dairy farms was determined using standard formulae (i.e., the number of positive animals/samples divided by the total number of animals/samples examined). Descriptive statistics such as percentages and frequency distributions was used to describe/present the nature and the characteristics of the data.

III. Results

a) Prevalence of Mastitis at Individual Cow and Quarter Level

Three hundred forty three Holstein-Friesian (HF), 20 Jersey, 15 local and 32 cross (HF X Local) breeds were included in the study. Of the total 444 lactating cows, 302 (68%) were found to be affected with clinical or sub clinical mastitis based on clinical examination of the udder and CMT results. From these, 94 (21.2%) was clinical and 208 (46.8%) was sub-clinical mastitis (Table 1). Out of 1776 quarters examined, clinical, non-functional and sub-clinical abnormalities

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were found in 288 (16.2%), 55 (3.1%), 456 (25.7%) quarters respectively.

Table 1 : Prevalence of mastitis

Types of mastitis	Total number examined	Positive (%)	χ^2	P- Value
Clinical	444	94 (21.20)		
Subclinical	444	208 (46.80)	52.078	0.000
Total	444	302 (68.00)		

b) Prevalence of Bovine Mastitis across Different Categories of Cows

Breed, age, parity and lactation stages have significant influence ($P<0.05$) on the prevalence of bovine mastitis. There was a significant difference in prevalence between animals of different age categories ($P<0.05$). The highest prevalence (86.5%) was found in lactating cows of ages 7-10 years, followed by cows of ages 11-13 years (81.8%), and the lowest prevalence (59.1%) was recorded in cows of ages 3-6 years. Higher prevalence (90.8%) was recorded in cows which gave birth to 4-7 calves and the lower prevalence (61.6%) was recorded in cows that gave birth to 1-3 calves. The difference was statistically significant ($P<0.05$) (Table 3).

The effect of lactation stage on the current prevalence of mastitis was studied and analyzed and the result revealed that lactation stage had significant effect ($P<0.05$) on the prevalence of mastitis. Higher prevalence (89.3%) of mastitis was observed and recorded in cows of late lactation stage (9-14 month) followed by cows in mid (83.65%) lactation (5-8 month) and early lactation stage (3 week-4 month) that had a prevalence of 50.7%. The effect of breed on the prevalence of mastitis was also studied and analyzed and the result revealed that breed had significant effect ($P<0.05$).

Among the different breeds studied, the highest mastitis prevalence was observed in Holstein-Friesian breeds (71.8%) followed by Jersey (70.0%), local (66.7%), and cross (48.5%) breeds (Table 3).

Table 3 : The prevalence of bovine mastitis in association with potential predisposing factors

Variables	No. examined	Positive (%)	$\chi 2$	P-Value
Breed				
Holstein-Friesian (HF)	343	246 (71.7)		
Jersey	20	14 (70.0)	13.786	.003
Cross (local x HF)	66	32 (48.5)		
Local	15	10 (66.7)		
Total	444	302 (68)		
Age				
3-6 years	296	175 (59.1)		
7-10 years	126	109 (86.5)	32.497	.000
11-13 years	22	18 (81.8)		
Total	444	302 (68)		
Parity				
1-3	346	213 (61.6)	30.048	.000
4-7	98	89 (90.8)		
Total	444	302 (68)		
Lactation stage				
3 week-4 month	223	113 (50.7)		
5-8 month	146	122 (83.65)	62.722	.000
9-14 month	75	67 (89.3)		
Total	**444**	**302 (68)**		

c) Questionnaires Survey, Observation and Interviewing

Questionnaires were distributed to 24 farms among the 37 farms included in the study. One questionnaire per farm owner/attendant was distributed. The entire farms included in the study followed manual milking (hand milking) system and most (80%) of the milkers were males. No specific sequence is followed during milking in many (87.5%) of the farms. Rather, it depends on the placement of the animal in the shed. Fifty four percent of the farm owners were educated to

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high school level while 12.5% were educated up to university level. The remaining (33.5%) attended elementary schools. Overall, educated people had better know how about the zoonotic implications of consuming raw milk, predisposing factors for mastitis and drug residue effect post treatment of mastitic animals. A few (12.5%) farmers emphasized the need to milk healthy cows first and the diseased cows later to prevent transmission of disease. Most (66.7%) of the milkers used disinfectant before milking only while 8 (33.3%) milkers said that they use disinfectant both before and after milking. Tap water is the primary source of water to clean teats and hands in many (91.7%) of the farms while few (8.3%) milkers use river water for teats and hands cleansing.

Eighteen (75%) farms strip the foremilk first while few undertake direct milking to the material used for milking. Among the 24 farms, 8 (33.3%) used individual towels, 10 (41.7%) communal towel and 6 (25%) did not use towel for drying of teats before or after milking. Among the 24 farms, 14 (58.3%) milkers disinfect their hands before proceeding to milk the next cow while 10 (41.4%) milkers disinfect their hands only at the beginning of milking. Most (75%) of the farmers boil milk before consumption while few (25%) milkers consume raw milk. In almost all of the farms, `animals were previously treated for mastitis while few animals (heifers that gave the first and second calf) were not treated for mastitis cases. Few (20.8%) farms distribute the milk for public consumption starting from the same day the animals were treated while most (79.2%) withhold the milk depending on the withdrawal period of the drug as prescribed by veterinarians. The management (housing, bedding, feeding, etc.) and the degree of sanitation were also observed. Among the 24 farms, there were leakage of urine, feces and milk during milking in 7 (29.2%) while in the remaining (14 farms), the bedding, housing and other degree of sanitary measures like milking procedures, use of disinfectant etc. were good.

IV. Discussion

A total of 444 dairy cows, from which 343 HF, 20 Jersey, 66 cross (HF x local), and 15 local breeds from Addis Ababa were investigated in a cross sectional study conducted between November 2011 and April 2012. The current prevalence of mastitis was 68.0%. The finding in this study is greater than that of Girma (2010), who reported 44.1% and Nibret *et al.* (2011), who reported 32.6% in different parts of Ethiopia. The high prevalence of sub-clinical mastitis may be attributed to improper milking hygiene, lack of post milking teat dipping and contact labors used, absence of order in milking cows of different ages and milking of mastitic animals before the healthy ones all of which might have increased the prevalence (Radostits *et al.,* 2007). The quarter level prevalence was 41.9% (744/1776). This finding was greater than that of Benta *et al.* (2011), who reported 31.4% (349/1112). This difference in the observed prevalence of mastitis among studies may be attributed to various factors like management, environmental, animal risk factors and causative agents (Radostits *et al*., 2007).

This study revealed a higher prevalence of sub-clinical mastitis (46.8%) than clinical mastitis (21.2%). A similar result was found by Mekibib *et al*. (2009) who reported 22.4% and 48.6% for clinical and sub-clinical mastitis respectively, in dairy farms of Holeta town, central Ethiopia. In case of sub-clinical mastitis, the cow-level prevalence (46.8%) obtained in this study was greater than the finding by Girma (2010) and Nibret *et al.* (2011) who reported 33.8% and 31.67%, respectively, in different parts of Ethiopia. However, it was lower than that reported by a study carried out in Sudan (88.1%) (Abdelrahim *et al.*, 1989). This may be attributed to the difficulty of detecting sub-clinical mastitis by the owners compared to the easily detectable clinical cases which prompt owners seek treatment for their animals (Radostits *et al*., 2007).

Increasing age, lactation stage, parity and poor management increased the risk of mastitis. This is line with previous reports on mastitis in Ethiopia (Kerro Dego and Tareke, 2003) and industrialized countries (Schukken *et al.*, 1989). Stage of lactation was a risk factor for mastitis (Mungube *et al*., 2004). In late lactation the risk of mastitis increased. Two reports on Ethiopian conditions found higher prevalence of mastitis during early lactation than late lactation (Hussien, 1999). The reason may be due to excluding of lactating cows below 3 weeks to avoid false positive result since SCC increases during early lactation (Tesfu *et al*., 1999).

Manual milking methods in the entire farms that included in this study was the major predisposing factors to increase the prevalence of mastitis. Most of employed milkers have little educational background and have limited knowledge about the mechanism/s of disease transmission. Often, they do not disinfect their hands and teats during and between milking of different cows, use of communal towel for drying of teats and also, they have no special preference between tape and river water. This study also noted a high prevalence of mastitis in farms that use river water for sanitation. Sequence of milking cows also seemed to have a role on the prevalence of mastitis. For example, in farm A which employ a specific sequence (first milk healthy heifers, healthy cows and last diseased cows), the prevalence was lower as compared to the other farms in which they apply random milking procedures in the placement of cows in the shed.

In this study, 33.3% of the farm attendants reported to consume raw milk. This practice can be said as risky as raw milk can contain a variety of disease-causing pathogens, as demonstrated by numerous

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scientific studies. These studies, along with numerous milk borne out breaks, clearly demonstrated the risk associated with drinking raw milk. For instance, in the US alone, there were 85 reported outbreaks of human infections over the years 1998-2008 due to the consumption of contaminated milk, 1614 illness, 187 hospitalizations and two deaths (Thorne, 2011).

There is also concern that small amounts of certain antimicrobial agents (residue) may significantly shift the resistance patterns in the microbial population in human intestinal tract, allergy from residue of penicillin etc. (Jones, 1999). The present study also found reluctance in 79.2% of the farm owner's to withhold milk from mastitic cows after treatment. Pasteurization effectively kills raw milk pathogens without any significant impact on milk nutritional quality. Stripping of the foremilk is also necessary as it contains many microbes that affect human health negatively.

V. Conclusion and Recommendations

In a spite of a large research efforts aimed to gain prevalence and to develop a new control tools for mastitis, the subclinical occurrence of the mastitis remains a substantial problem for dairy producers. The result of the present study indicated a relatively high prevalence of subclinical mastitis in dairy cattle of the study area. The relatively high prevalence reported in this study was clearly indicated lack of strategic control measures against the disease as well as poor surveillance measures. Lack of maintenance of strict hygiene and good sanitary environment may be contributory factors in the cause of subclinical mastitis. It is therefore important that farmers should ensure strict personal hygiene and that of animals and general sanitary condition of the farms should be improved and maintained. Furthermore, all dairy producers know that early detection of intramammary infection is important for selecting and implementing proper therapy. Unfortunately, most infections are not detected until they become clinical, and by then extensive and costly damage can result. Routine milk cultures should be an ongoing part of any mastitis control program. The sampling strategies for any ongoing program require the input of the herd veterinarian as well as herd management.

Conflict of Interest

The authors have no declared any conflict of interest

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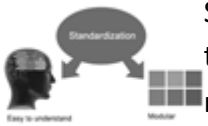

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3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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

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27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

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.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

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.)

· Keep on paying attention on the research topic of the paper

· 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

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

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Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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