Prevalence of Bovine Mastitis at Elkadaro Cross-Sectional Survey

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 (X²= 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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Keywords: fasciolosis, cattle, prevalence, risk factors, abattoir, khartoum, sudan.

1. 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/L², given by (Martin et al., 1988) where:

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P = prevalence  
Q = 1 - P  
L = 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).
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 fascioliosis 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

Fascioliosis is a widespread ruminant health problem and causes significant economic losses to the livestock industry in some areas in Sudan. The abattoir prevalence of fascioliosis 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 play a part (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 fascioliosis in cattle varied with sex, age groups, breeds, weights, sources, size of animals and other diseases concurrent with fascioliosis.

The association between the occurrence of bovine fascioliosis 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 fascioliosis 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 fascioliosis was observed among Ethiopian source animals by postmortem and coprological examinations in consensus 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 fascioliosis with concurrent infections with both coprological and postmortem examinations revealed that there is no significant difference between the concurrent infections with fascioliosis by both examinations.

The prevalence of fascioliosis 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
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.

V. Acknowledgements

Special appreciation to Elkadaro Abattoir officials and all the workers for their willingness and collaboration which was indispensable inputs for the accomplishment of this study. All contributions and supports are gratefully acknowledged.

References Références Referencias


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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency</th>
<th>Relative frequency (%)</th>
<th>Cumulative frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>210</td>
<td>68.4% (210/307)</td>
<td>68.4%</td>
</tr>
<tr>
<td>Positive</td>
<td>97</td>
<td>31.6% (97/307)</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>307</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency</th>
<th>Relative frequency (%)</th>
<th>Cumulative frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>245</td>
<td>79.8% (245/307)</td>
<td>79.8%</td>
</tr>
<tr>
<td>Positive</td>
<td>62</td>
<td>20.2% (62/307)</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>307</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3: Multivariate analysis for the association between fasciolosis (examined by postmortem) and potential risk factors using logistic regression

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

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

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>No. tested</th>
<th>No. positive (%)</th>
<th>Exp (B)</th>
<th>95% C.I. for Exp (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>25</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>2-4</td>
<td>71</td>
<td>1 (1.4%)</td>
<td>1</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>&gt;4</td>
<td>209</td>
<td>61 (28.9%)</td>
<td>28.8</td>
<td>3.9, 34.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>128</td>
<td>1 (0.7%)</td>
<td>1</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Cross</td>
<td>34</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Foreign</td>
<td>145</td>
<td>61 (42.1%)</td>
<td>94.4</td>
<td>13.2, 102.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>137</td>
<td>60 (43.8%)</td>
<td>53.4</td>
<td>7.1, 61.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Medium</td>
<td>139</td>
<td>2 (1.4%)</td>
<td>1</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Heavy</td>
<td>31</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nyala</td>
<td>79</td>
<td>1 (1.2%)</td>
<td>1</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>142</td>
<td>61 (43.0%)</td>
<td>60.2</td>
<td>8.3, 70.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Khartoum</td>
<td>35</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Kordofan</td>
<td>13</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Eljazeera</td>
<td>1</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>White Nile</td>
<td>9</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Kassala</td>
<td>25</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>South Sudan</td>
<td>3</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Animal Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>136</td>
<td>60 (44.1%)</td>
<td>54.9</td>
<td>6.3, 63.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Medium</td>
<td>141</td>
<td>2 (1.4%)</td>
<td>1</td>
<td>- -</td>
<td>1.000</td>
</tr>
<tr>
<td>Large</td>
<td>30</td>
<td>0</td>
<td>- -</td>
<td>- -</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Table 5: The difference in fasciolosis prevalence estimated based on coprology and postmortem examination at Elkadaro abattoir during May -July 2011

<table>
<thead>
<tr>
<th>Test Type</th>
<th>No. Positive</th>
<th>Prevalence</th>
<th>Diff. (95% CI)</th>
<th>X²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coprology</td>
<td>62</td>
<td>20.2%</td>
<td>7.3 (3.1, 9.4)</td>
<td>1.669</td>
<td>0.000</td>
</tr>
<tr>
<td>Postmortem</td>
<td>97</td>
<td>31.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Faecal examination</th>
<th>Presence of <em>Fasciola</em> spp in liver</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluke (+ve)</td>
<td>Fluke (-ve)</td>
</tr>
<tr>
<td>Egg present (+ve)</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>Egg absent (-ve)</td>
<td>35</td>
<td>210</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>210</td>
</tr>
</tbody>
</table>
Effects of Feeding Processed Kidney Bean Meal (*Phaseolus Vulgaris*) by Replacing Soybean Meal on Egg Fertility and Qualities of Chicks of White Leghorn Hens

By Taju Hussein, Mengistu Urge, Getachew Animut & Sisay Fikru

Jigjiga University, Ethiopia

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

**GJMR-G Classification**: NLMC Code: QW 70, WA 390

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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 α, Getachew Animut α & Sisay Fikru ω

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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).

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
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 libitum. 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. Canding 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
drawn from the incubator. Finally eggs found fertile, i.e.
egg 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 18th day.
Hatched chicks were counted and chick quality was
determined. Fertility and hatchability were determined by
using the following formulae, respectively.

%Fertility = \frac{\text{number of fertile eggs}}{\text{total egg set}} \times 100

% Hatchability on fertile eggs base = \frac{\text{number of chick hatched}}{\text{fertile eggs}} \times 100

% Hatchability on total eggs base = \frac{\text{number of chick hatched}}{\text{total eggs set}} \times 100

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

% of early mortality = \frac{\text{total number of early dead embryo}}{\text{total number of fertile eggs}} \times 100

% of mid mortality = \frac{\text{total number of mid dead embryo}}{\text{total number of fertile eggs}} \times 100

% of late mortality = \frac{\text{total number of late dead embryo}}{\text{total number of fertile eggs}} \times 100

% of pip mortality = \frac{\text{total number of pip dead embryo}}{\text{total number of fertile eggs}} \times 100
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 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:

Quality chicks = \[
\frac{\text{total number of quality chicks}}{\text{total number of hatched chicks}} \times 100
\]

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:

\[Y_{ij} = \mu + T_i + e_{ij}\]

Where: \(Y_{ij}\)=Individual observation,
\(T_i\)=Treatment effect, \(\mu\)=Overall mean, \(e_{ij}\)= 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:

\[\ln\left\{\frac{\pi}{1-\pi}\right\} = \beta_0 + \beta_1 x\]

Test H0: No treatment effect (i.e., \(\beta_1 = 0\)) vs.
HA: Significant treatment effect (\(\beta_1 \neq 0\)).

Where, \(\pi\)=probability, \(\beta\)=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) (93.3%) 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 Aria 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 Aria 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 PKB 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 Shewangawzaw 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 to T1 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 hatching 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
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).

IV. Acknowledgement

The authors’ heartfelt appreciation goes to the Ethiopian Ministry of Education (MOE) for fully sponsoring this study and Haramaya University for provision of research facilities.

References Références Referencias


### Table 1: Experiment lay out

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Replications</th>
<th>Layers per replication</th>
<th>Cocks per replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1(100%SBM:0%PKB)</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>T2 (75% SBM:25%PKB)</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>T3 (50% SBM:50%PKB)</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>T4 (25% SBM:75%PKB)</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>T5 (0% SBM:100% PKB)</td>
<td>3</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>56</td>
<td>53</td>
<td>52</td>
<td>48</td>
<td>37</td>
</tr>
<tr>
<td>WS</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>SBM</td>
<td>26</td>
<td>19.5</td>
<td>13</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>PKB</td>
<td>0</td>
<td>6.5</td>
<td>13</td>
<td>19.5</td>
<td>26</td>
</tr>
<tr>
<td>NSC</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>LS</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>VPM</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Feed type</th>
<th>DM (%)</th>
<th>CP (%)</th>
<th>EE (%)</th>
<th>CF (%)</th>
<th>Ash (%)</th>
<th>ME kcal/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>89.5</td>
<td>8.7</td>
<td>4.3</td>
<td>8.0</td>
<td>6.21</td>
<td>3230.5</td>
</tr>
<tr>
<td>WS</td>
<td>90.3</td>
<td>12</td>
<td>3.3</td>
<td>6.2</td>
<td>7.8</td>
<td>3303.1</td>
</tr>
<tr>
<td>SBM</td>
<td>90.2</td>
<td>38</td>
<td>7.0</td>
<td>9</td>
<td>7.8</td>
<td>3215</td>
</tr>
<tr>
<td>PKB</td>
<td>87.5</td>
<td>28</td>
<td>0.9</td>
<td>6</td>
<td>7.0</td>
<td>3182.2</td>
</tr>
<tr>
<td>NSC</td>
<td>91.5</td>
<td>26</td>
<td>6.0</td>
<td>21.0</td>
<td>10</td>
<td>2006.0</td>
</tr>
</tbody>
</table>

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 = Metabolizable energy

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

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

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 replacement to soybean meal

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

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; HTES = hatchability on total egg set; HFES = hatchability on fertile egg set; EEM = early embryonic mortality; MEM = mid embryonic mortality; LEM = late embryonic mortality; PIEM = pip embryonic mortality
Table 6: Chick quality parameters of white leghorn layers fed different levels of processed kidney bean as a replacement to soybean meal

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>SEM</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual (%)</td>
<td>84.93</td>
<td>85.09</td>
<td>82.36</td>
<td>81.02</td>
<td>70.83</td>
<td>3.89</td>
<td>NS</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>15.63a</td>
<td>15.00a</td>
<td>15.33a</td>
<td>15.03a</td>
<td>14.33b</td>
<td>0.20</td>
<td>*</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>34.13a</td>
<td>34.20a</td>
<td>33.13a</td>
<td>33.06a</td>
<td>32.47b</td>
<td>0.28</td>
<td>*</td>
</tr>
</tbody>
</table>

*a-b means in the row without common superscript are significant; NS = Non-significant; SL = significance level; SEM = standard error of mean; cm = centimeter, 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: 100% PKB;
Prevalence of Bovine Mastitis in Lactating Cows and its Public Health Implications in Selected Commercial Dairy Farms of Addis Ababa

By Alebachew Tilahun & Alemu Aylate

Wola Sodo University, Wolaita Sodo University, Ethiopia

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

GJMR-G Classification : NLMC Code: WA 360

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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 \textit{et al}. , 2009). Economic losses caused by mastitis include value of discarded milk (Radosstit \textit{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 \textit{et al}. , 2005; Radosstit \textit{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 \textit{et al}. , 2003). Previous studies conducted in different countries indicate the distribution and economic importance of the disease. Contreras \textit{et al}. (1997) from Spain; Moshi \textit{et al}. (1998) from Tanzania; Ameh and Tari (2000) from Nigeria; Ndewga \textit{et al}. (2000) from Kenya and Kozacinski \textit{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 \textit{et al}. , 2005; Lakew \textit{et al}. , 2009; Gebreyohannes \textit{et al}. , 2010; Megersa \textit{et al}. , 2010). Several of these studies have been shown the occurrence a range of mastitis causing bacteria indicating \textit{Staphylococcus, Escherichia coli} and \textit{Streptococcus} as dominant and pathogenic species. Some authors (Mungube \textit{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 \textit{et al}. , 2009; Megersa \textit{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 km² and is subdivided into ten sub-cites namely, Arada, Bole, Addis Ketema, Nefas Silk Lafto, Koffe Keranio, Akaki Kality, Yeka, Lideta, Kikors 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 average 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% (Mekbib 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 desired 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
were found in 288 (16.2%), 55 (3.1%), 456 (25.7%) quarters respectively.

### Table 1: Prevalence of mastitis

<table>
<thead>
<tr>
<th>Types of mastitis</th>
<th>Total number examined</th>
<th>Positive (%)</th>
<th>χ²</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>444</td>
<td>94 (21.20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclinical</td>
<td>444</td>
<td>208 (46.80%)</td>
<td>52.078</td>
<td>0.000</td>
</tr>
<tr>
<td>Total</td>
<td>444</td>
<td>302 (68.00%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. examined</th>
<th>Positive (%)</th>
<th>χ²</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holstein-Friesian (HF)</td>
<td>343</td>
<td>246 (71.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jersey</td>
<td>20</td>
<td>14 (70.0)</td>
<td>13.786</td>
<td>.003</td>
</tr>
<tr>
<td>Cross (local x HF)</td>
<td>66</td>
<td>32 (48.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>15</td>
<td>10 (66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>444</td>
<td>302 (68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 years</td>
<td>296</td>
<td>175 (59.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-10 years</td>
<td>126</td>
<td>109 (86.5)</td>
<td>32.497</td>
<td>.000</td>
</tr>
<tr>
<td>11-13 years</td>
<td>22</td>
<td>18 (81.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>444</td>
<td>302 (68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>346</td>
<td>213 (61.6)</td>
<td>30.048</td>
<td>.000</td>
</tr>
<tr>
<td>4-7</td>
<td>98</td>
<td>89 (90.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>444</td>
<td>302 (68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 week-4 month</td>
<td>223</td>
<td>113 (50.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-8 month</td>
<td>146</td>
<td>122 (83.65)</td>
<td>62.722</td>
<td>.000</td>
</tr>
<tr>
<td>9-14 month</td>
<td>75</td>
<td>67 (89.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>444</td>
<td>302 (68)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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
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 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
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

**VI. Acknowledgements**

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(c) Up to ten keywords, that precisely identifies the paper’s subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

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

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Approach

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- 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.
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