A Cross-sectional Study on Infertility and its Causes in Small Holder Dairy Cattle in Selected Counties of Kenya

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ABSTRACT

Dairy production in Kenya is one of the most developed in sub-Saharan Africa. Despite this, it is still faced by challenges attributed to poor nutrition, inappropriate breeding practices, reproductive inefficiency, reproductive diseases among others. A study to establish the infertility rates in selected dairy herds was conducted in three Counties of Kenya from October 2017 to July 2018. A total of 216 herds were purposively sampled from Nandi, Makueni and Kakamega Counties. Data on reproductive performance was collected and pregnancy and ovarian status determined by per-rectal examination. Other reproductive indices such as calving interval, repeated inseminations were calculated from the records. Blood samples were also collected to screen for Bovine Viral Diarrhea (BVD) and Neospora caninum antibodies. Overall, the percentage of pregnant animals across the examined herds was 30.5\% (n=642). Reproductive indices were suboptimal with 2.1 inseminations per conception and a calving interval of 18.8 months; an indication of infertility. About half of the population of animals were anestrus (46.4\%). The sero-prevalence of neosporosis was 24.1\% (n=552) and Bovine Viral Diarrhea Virus 52.3\% (n=545) across all the counties. Additionally, there was no significant association between BVD infection (P=0.575) and neosporosis (P=0.626) on pregnancy status. The findings of this study strongly indicate reproductive wastage in the dairy herds which in turn affects the overall productivity of farms. There is therefore a need for a holistic approach to address infertility as a way of improving dairy farm productivity and profitability.

Key words: Bovine Viral Diarrhea, Pregnancy, Dairy cattle, Infertility, Neospora caninum, Infertility.

INTRODUCTION

Dairy farming is not only the single largest sub-sector of agriculture in Kenya but also accounts for 3\% of the 18\% milk production in sub-Saharan Africa (Odero-Waititu, 2017). Apart from providing milk for consumption, the dairy enterprise has been estimated to earn farmers over one hundred billion shillings annually from milk sales as well as providing employment to over 350,000 people at farm level and over 400,000 people in the informal sector. The true contribution by the sub-sector to the economy is likely to be even higher if unrecorded slaughter and home consumption is considered (GoK, 2012).

Reproductive performance is one of the determinants of the productivity of dairy farm. A dairy farm’s optimal reproductive performance translates to less farm inputs and more output. Fertility of a dairy cow is the ability of the animal to conceive and maintain pregnancy if served at the appropriate time (Evans and Zeng, 2017). Reproductive management aims to have cows become pregnant at a biologically optimal time and at an economically profitable interval after calving. Successful reproduction starts with oogenesis, fertilization of the ova, successful implantation, carrying pregnancy to term, and the birth of a calf. Resumption of ovarian activity, estrus, and ovulation are all reproductive events that need to precede conception (Garnsworthy et al., 2008). Failure at any one stage results in infertility. Additionally, poor nutrition, ovarian dysfunction, poor and inaccurate heat detection methods, failed artificial insemination and pregnancy wastage also disrupt reproductive activities in dairy cattle (Hafez and Hafez, 2000; Mutavi et al., 2016; Abraham, 2017).
Reproductive diseases have also been implicated in resulting to reproductive wastage among these, Bovine Viral Diarrhea and Neosporosis in pregnant cows could result to early embryonic deaths, abortions, still birth or newborns with congenital defects (Abraham, 2017).

The small-holder dairy system is growing rapidly within Kenya with the changing population and land structure. Studies elsewhere have indicated reproductive challenges in small scale holder dairy production system (Moges, 2012). Most of these farmers are resource poor with no ability to efficiently manage profit making dairy farms due to reproductive efficiency. Few studies have been published on reproductive management of these farms. There is thus a dearth of information on the reproductive performance indicators of dairy cattle despite the management challenges faced by these farms (Wambugu, 2011; Mungube et al., 2014; Utuk and Eski, 2017).

MATERIALS AND METHODS

Study areas

The study was conducted in Nandi, Makueni and Kakamega Counties (Fig. 1) of Kenya and had field and laboratory phases. Nandi and Kakamega were selected to represent the high rainfall areas. Although Nandi has an almost similar ecosystem to Kakamega, it was included to represent a semi-intensive production system given its larger land sizes compared to Kakamega where land sizes are small and appropriate for the intensive small-scale dairy production system. Kakamega represented a transition zone between non-dairy and dairy areas. Makueni was included to represent the semi-arid zones where dairy farming is an emerging enterprise.

Study design and selection of herds and individual cattle

This was a cross-sectional study conducted between April 2017 and July 2018. Multi-stage sampling approach was used to select three sub-counties in the target counties. The sub-counties were purposively selected based on high dairy cattle population and the high reports of reproductive inefficiency and other infectious diseases. This was arrived at following key informant interviews with county veterinary officials.

After selection of the sub-counties, a list of dairy farmers in each of the three sub-counties per Study County was drawn to form a sampling frame from where farmers with cows 2 years and above were randomly sampled.

Reproductive Examination

The reproductive history of the selected animals was recorded followed by per rectal palpation to determine their pregnancy and ovarian status. The age of pregnancy for pregnant cows was estimated. Non-pregnant animals were classified into non – cycling (anestrus) and cycling (follicular or luteal phase).

Anestrus cows were treated with Gonadotropin Releasing Hormone – GnRH (GONAbred® Australia) to stimulate ovarian activity while those with corpus luteum (CL) on their ovaries were treated with prostaglandins - PGF2 alpha (estroPLAN® Australia) to induce estrus. Farmers observed for heat signs in the animals and advised to inseminate appropriately.

Sample collection and analysis

After rectal palpation, blood samples were collected from the selected cows irrespective of their reproductive status. Approximately 10ml of whole blood was collected from the jugular vein using venoject needles (BD vacutainer® UK) in plain vacutainer tubes (BD vacutainer® UK). The blood was left to clot before the serum was separated by centrifugation and stored into 2ml cryo-vials. The serum samples were transported in ice packed cool boxes to the laboratory at the Bacteriology Laboratory of the Veterinary Research Institute Muguga for serological analysis.

Presence of serum antibodies against reproductive diseases; Neosporosis and Bovine Viral Diarrhea virus was determined using ELISA. Indirect ELISA tests to detect Neospora caninum (ID Screen® Neospora caninum Competition NCC – 5P) in all samples were carried out. For BVD, Competitive ELISA for the detection of Bovine Viral Diarrhea anti-p80-125 (anti-NSP2-3) antibodies in serum were run using the – BVD kitID Screen® BVD p80 Antibody Competition BVDC – 5P. All the tests were run as per the user manuals provided by the kit manufacturers and the standard operating procedures of the hosting laboratory.

Herd management data

Herd reproductive history which included heat detection methods, type of breeding and timing of insemination, number of inseminations per conception, calving interval was collected through structured questionnaires. Individual animal bio data and reproductive history was also recorded. In addition, body condition scores (BCS) of individual animals were determined by visual examination and scored in the scale of one – ten, method described by Holmes (1987) (where one is emaciated and ten a grossly obese animal). The data was captured into mobile phone application Epicollect 5 for storage.

Data analysis

The data was entered in Microsoft excel spreadsheet program (Microsoft office Excel 2007; Microsoft Corporation, 2007) and then transferred to STATA statistical software (STATA Corp., Version 12, College station USA) for analysis. The data was cleaned for general errors in data entry, outliers, missing values and double entries. Significant differences in antibody titres for NCD and BVD, pregnancy status, stage of estrous cycle in cows across the three counties animals were obtained by Analysis of Variance (ANOVA) and Student t test statistics. The effect of different variables on the fertility of animals was also assessed by ANOVA. Statistical significance was set at P<0.05.

RESULTS

A total of 216 dairy farms participated in the study with 73, 71 and 72 farms located in Nandi, Makueni and Kakamega Counties, respectively. Reproductive examination was performed on a total of 642 cows; 229 from Nandi, 226 from Kakamega and 187 from Makueni Counties (Table 1). Each study farm/household had on average three cows with average BCS ranging between 4.6 and 5.2 (Table 1).
Various breeds of cattle were kept in the three counties. Farmers in Nandi and Makueni Counties preferred the Holstein-Friesian whereas those in Kakamega County preferred Ayrshire breed of cattle (Table 2). Other dairy cattle breeds kept by the farmers included Guernsey and Jersey. Among the crosses, Guernsey/Ayrshire was the most common in the three study counties.

Reproductive status of the cows examined

The total number of pregnant animals across the counties ranged between 23.9 and 38.5% (Table 3). Overall, the percentage of pregnant animals for the three counties was 30.5% with 69.5% of the cows examined being open. Makueni County had a significantly (P<0.05) higher number of pregnant animals compared to both Kakamega and Nandi.

Ovarian palpation was done in non-pregnant cows to determine the cyclicity status. Cycling animals had either follicles or corpora lutea on the ovary. Non cycling (anestrous) animals had inactive ovaries. Proportions of cycling and non-cycling animals were calculated (Table 4). Half (53.6%) of the total open animals across the counties were cycling.

Although Makueni County had the highest pregnancy rates, it also had the highest proportion of cows in anestrus (58%) followed by Kakamega County (46%) and Nandi (38%). Ovarian cysts were detected in seven animals.

Reproductive indices

Some farmers using artificial inseminations reported cases of non-conception following repeated services. Nevertheless, some farmers reported 1 or 2 inseminations per conception (Fig. 2).

Forty six percent of the respondents reported that the cows conceived after 1 or 2 services. On average, 1.6, 1.2 and 2.1 inseminations were needed before cows conceived in Kakamega, Nandi and Makueni, respectively.

Overall, the average calculated calving interval for cows in Kakamega was 19.10 (95% CI: 17.1-21.1) and Makueni was 18.5 (95% CI: 15.8-21.2). The average calving interval for cows in the two Counties was 18.8 months (95% CI: 17.1-20.5) and there was no non-significant (P>0.05) difference between two estimates. No data on calving interval were recorded in Nandi County.

Sero-prevalence results for BVD and Neosporosis disease

Seroprevalence of BVD and Neospora caninum was determined for all animals sampled during the study. The BVD antibodies (52.3%) were detected animals in the three study counties. The overall the seroprevalence of N. caninum across the three counties was 24.1%. The variation in prevalence of antibodies across the counties was non-statistically (Table 5).

Seroprevalence of the two diseases was detectable in 14.6% (79/541) of the samples. Association between prevalence and county was significant for BVD (P=0.000) but not for N. caninum (P=0.626). Further, there was no association between BVD (P=0.575) and Neosporosis (P=0.626) on pregnancy status.
Successful reproductive management is crucial in profitability of dairy farms. Delayed conception, higher proportion of open dry cows and suboptimal reproductive performance leads to decreased milk yield per time unit (Souames, 2018). The goal of the current study was to investigate infertility levels and possible causes in the small holder dairy farms in three counties of Kenya. The results of the study clearly indicate cases of infertility as measured through long suboptimal calving intervals, higher proportion of open dry cows, suboptimal conception rates and presence of reproductive diseases antibodies amongst the herds of cattle.

The percentage of pregnant animals across the herds in the three counties was lower than recommended optimal with only about a third of the animals being positive for pregnancy. The low percentage of pregnancy animals indicate delayed conception. Scientists have documented delayed patterns of resumption of post-partum cyclicity in dairy cattle due postpartum conditions (Souames, 2018). In the current study, the animals examined were mature cows either primiparous or multiparous, there is a possibility of failure of resumption of ovarian cyclicity after calving resulting to prolonged calving intervals. The accuracy of heat detection is crucial in successful conception of dairy cattle (Saint-Dizier, 2012). Poor heat detection methods which entirely depend on observation of heat signs, are subjective, unreliable since the AI technicians depend on farmers’ information to serve the cows. If the farmer did not observe for start of heat, then they would give unreliable information resulting to failed artificial insemination. Herds which practice good heat detection are able to attain impressive reproductive performance indicators (Saint-Dizier, 2012). Beyond heat detection, exposure of animals to high environmental temperatures following a dry season in the tropics is likely to have a negative impact on conception due to animals being heat stressed. Decreases in conception rates of 20 to 30 percentage points attributed to heat stress have been reported (de Rensis et al., 2015; Young, 2015).

While reproductive diseases account for less than 5% of the causes of infertility in cattle, their impact on production and reproduction are significant. Indeed, BVD and NC antibodies were found to be present across all the counties indicating previous exposure to infection or active infections. Neosporosis accounts for 12-42% of abortions in dairy cattle; (Xu et al., 2012) and lowers reproductive performance (VanLeeuwen et al., 2010). Yavru et al., (2012) documented lower conception rates, reduced fertility in herds infected with BDV.

The variation in seroprevalence across the counties could be due to differences in dairy cattle population density and preventive measures to control diseases (Mainar-Jaime et al., 2001) as is the case in Nandi County where livestock population density is higher.

The high individual animal seroprevalence and the fact that all the dairy herds had at least one positive animal, indicates that N. Caninum infection is widely spread among dairy cattle in study counties. This could be due to the presence of stray dogs that consume contaminated aborted material and placentas on farms and shed the oocysts in feces. This results to the dogs being infected, the shedding of fecal oocysts in the environment pose the risk of infection to cattle (VanLeeuwen et al., 2010).

Cases of co-infections between BVD and NC have been reported in other studies as is the case in this study (Björkmann et al., 2000). However, there was no association found between the infections unlike earlier studies where positive association between sero-positivity to N. caninum and BVDV in cows was reported in smallholder herds in Vietnam (Lassen et al., 2014). The optimal CI in well managed dairy herds ranges between 12-13 months (Roberts, 1986). Length of the CI is influenced by the time to post-partum resumption of ovarian cyclicity, the occurrence and detection of oestrus, and fertility at service (Nakami et al., 2017). Long inter-calving intervals of 450 to 500 days have been reported to cause estimated loss of milk worth over Ksh 4 billion in Kenya (MoLD, 2010). The abnormally long inter calving interval in the study farms could be attributed to delayed resumption of postpartum ovarian cyclicity, fetal/embryo losses caused by infections amongst others.

It has been shown that the calving to conception period is longer in N. caninum seropositive cows and have therefore speculated that N. caninum could be a cause of

**DISCUSSION**

**Table 4: Ovarian findings of non-pregnant cows**

<table>
<thead>
<tr>
<th>Ovarian status</th>
<th>Kakamega (n=158)</th>
<th>Nandi (n=170)</th>
<th>Makueni (n=114)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus albicans</td>
<td>23 (14.6)</td>
<td>21 (12.4)</td>
<td>9 (7.9)</td>
<td>53 (12.0)</td>
</tr>
<tr>
<td>Corpus luteum</td>
<td>28 (17.7)</td>
<td>16 (9.4)</td>
<td>27 (23.7)</td>
<td>71 (16.1)</td>
</tr>
<tr>
<td>Cystic ovarian disease</td>
<td>1 (0.6)</td>
<td>4 (2.4)</td>
<td>2 (1.8)</td>
<td>7 (1.6)</td>
</tr>
<tr>
<td>Follicular phase</td>
<td>30 (19.0)</td>
<td>63 (37.1)</td>
<td>10 (8.8)</td>
<td>103 (23.3)</td>
</tr>
<tr>
<td>Inclusive</td>
<td>2 (1.2)</td>
<td>1 (0.6)</td>
<td>0 (0)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Not cycling</td>
<td>72 (45.6)</td>
<td>65 (38.2)</td>
<td>66 (57.9)</td>
<td>205 (46.4)</td>
</tr>
<tr>
<td>Total</td>
<td>158 (100)</td>
<td>170 (100)</td>
<td>114 (100)</td>
<td>442 (100)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate percentage.

**Table 5: Sero-prevalence of BVD and Neospora caninum in Study counties**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Kakamega (n=218)</th>
<th>Nandi (n=151)</th>
<th>Makueni (n=176)</th>
<th>Total (n=545)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive for BVD</td>
<td>82</td>
<td>103</td>
<td>110</td>
<td>285</td>
</tr>
<tr>
<td>Prevalence</td>
<td>37.6 (31.4-44.2)</td>
<td>68.2 (60.5-75.3)</td>
<td>62.5 (55.2-69.4)</td>
<td>52.3 (48.1-56.5)</td>
</tr>
<tr>
<td>Neospora</td>
<td>n=219</td>
<td>n=156</td>
<td>n=177</td>
<td>N=552</td>
</tr>
<tr>
<td>No. positive for Neospora</td>
<td>59</td>
<td>40</td>
<td>34</td>
<td>133</td>
</tr>
<tr>
<td>Prevalence</td>
<td>26.9 (21.4-33.1)</td>
<td>25.6 (19.3-32.9)</td>
<td>19.2 (13.9-25.5)</td>
<td>24.1 (20.7-27.8)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate range values.
early fetal death (Reichel and Ellis, 2002; Dubey et al., 2007; Kamga-Waladjo et al., 2010). Nevertheless, these increases have not always reached the level of statistical significance (Romero et al., 2005).

Nutritional stress caused by negative energy balance in postpartum cows that do not get enough nutrients from feed also plays a significant role in infertility. Under nutrition results to anoestrus state thus prolonging the calving interval (Garnsworthy et al., 2008). Cattle in the study areas depend majorly on rainfall dependent pastures for nutrition. With climate change, long dry seasons, with little or no rainfall result to loss of pastures thus cattle have little to feed on. This study was conducted following a dry season; it is therefore possible that lack of adequate nutrition partly explains the low conceptions. Makueni County which falls within the arid and semiarid areas was adversely affected (Njarui et al., 2011).

The average number of inseminations per conception is 1.5 in Kenya (MoLD, 2010) which is comparatively lower than the 2.1 inseminations per conception recorded in the study. This is however lower than India where an average of 3.1 inseminations per conception has been reported (Sattar et al., 2005).

These statistics of failed inseminations resulting to low conception rates impact negatively on efforts by the county government to provide AI services at a subsidized rate of 7 US dollars as opposed to 20 dollars charged by private veterinarians. As a consequence, some farmers across the study counties halted the use of the subsidized semen citing poor quality semen as a reason for the failed repeated inseminations. This negatively affects the success of county government breeding programs.

Reproductive performance in dairy cows determines the overall productivity of dairy farms. Availability of milk is dependent on lactation which occurs after calving. Reproductive wastage through; repeat breeding, failed insemination, long calving interval leads to reduced lifetime productivity of cows. Reports from the current study indicate reproductive inefficiency in the dairy herds in study areas. Farmers in the study areas did not have proper herd records that are important in tracing infertile animals that have prolonged postpartum open days or are anoestrous. A proper recording system with details of animals that have prolonged postpartum open days can be directly confirmed in the study. Therefore, there is need for further studies to determine the impact of the two diseases on reproductive indices.

Author’s contribution
All the authors have contributed equally to this manuscript and are in agreement with its contents. The manuscript is not being considered for publication elsewhere.

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