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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 anoestrus (46.4%). The sero-prevalence of neosporosis was 24.1 % (n = 552) and Bovine Viral Diarrhea Virus52.3% (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 Diarrhoea, 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-Sahara Africa (Odero-Waitituh, 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 (GoK 2012). 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 *et al.*, 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, oestrus, 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 (Abraham, 2017; Mutavi *et al.*, 2016; Hafez and Hafez 2000).

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Reproductive diseases have also been implicated in resulting to reproductive wastage among these, Bovine Viral Diarrhoea 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 (Mutavi *et al.,* 2016; Mungube *et al.,* 2014; Wambugu *et al.,* 2011).

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 (anoestrus) and cycling (follicular or luteal phase).

Anoestrous cows were treated with Gonadotropin Releasing Hormone – GnRH (GONAbreed® 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 Diarrhoea virus was determined using enzyme linked immune-sorbent assays (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 *et al*., (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 probability values of < 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).

Table 1: Farm and animal description in Study Counties

County	Dairy cattle sampled	Mean Cows/herd	Range	BCS (1- 10)
Kakamega	229	3	1-12	4.6
Nandi	226	3	1-6	4.9
Makueni	187	3	1-8	5.2

Table 2: Breed diversity in the Counties

Breed	Kakamega	Nandi	Makueni	Total
Friesian	86	112	110	308
Ayrshire	121	76	27	224
Guernsey	1	13	3	17
Jersey	2	4	1	7
Guernsey/Ayrshire	0	6	7	13
Other crosses	4	3	12	19

Table 3: Pregnancy diagnosis results

County	Cows examined	Pregnant	% pregnancy
Kakamega	229	70	30.6 (24.9 – 36.8)[a]
Nandi	226	54	23.9 (18.7 - 29.8)[a]
Makueni	187	72	38.5 (31.7 – 45.6)[b]
Total	642	196	30.5 (27.1- 34.2)

Values with different letter superscripts are significantly different (p<0.05) along the column of comparison; parentheses represent 95% confidence intervals.

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

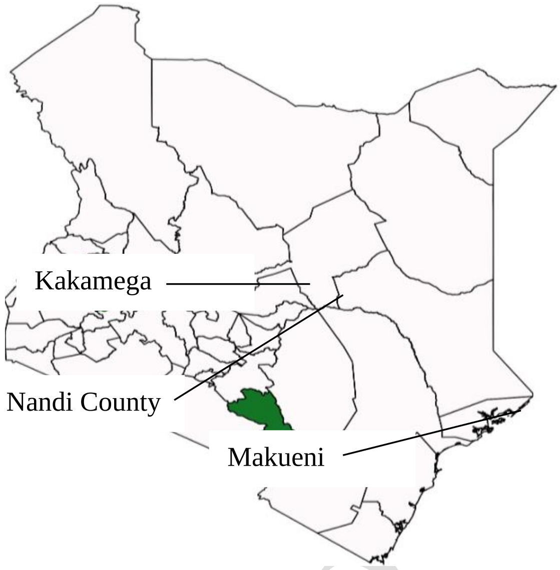

Fig. 1: A map showing the study counties in the context of Kenya

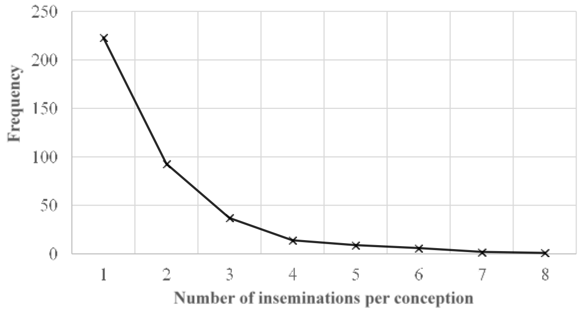

Fig. 2: Number of services before conception and their frequencies

Sero-prevalence results for BVD and Neosporosis disease

Sero-prevalence of BVD and *Neospora caninum* was determined for all animals sampled during the study. The BVD antibodies were detected animals in the three study counties, with anoverall sero-prevalence of 52.3%.

The overall the sero-prevalence of *N. caninum* across the three counties was 24.1%. The variation in prevalence of antibodies across the counties was not statistically different (P>0.05) (Table 5).

Co – infection sero-prevalence was detectable in 14.6% (79/541) of the samples. Chi square tests of 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.

DISCUSSION

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

Table 4: Ovarian findings of non-pregnant cows

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

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

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

(Souames *et al*., 2018). The goal of the current study was investigate infertility levels and possible causes in the small holder dairy farms in three counties of Kenya namely Nandi, Makueni and Kakamega. The results of the study clearly indicate cases of infertility as measured through long suboptimal calving intervals, higher portion 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 *et al.,* 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 accountsfor 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 sero-prevalence 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 faeces. This results to the dogs being infected, the shedding of faecal 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 (Björkman *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*., 2012).

The current study reported CI of about 19 months which was longer than 14 months reported by Mungube *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, foetal/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 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 anoestrus. A proper recording system with details of reproductive status of each animal at a certain period of time could be of great use in troubleshooting infertility in farms, thus taking appropriate intervention to minimise losses due to reduced production.

The current study confirmed the presence of sero-positive animals with antibodies to *N. Caninum* BVD. The presence of antibodies indicates exposure of the animals to the diseases. The two diseases have been documented to cause reproductive wastage in herds although this could not be directly confirmed in the study. Therefore, there is need for further studies to determine the impact of the two diseases on reproductive indices.

Authors 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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