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Seroprevalence of bovine leukemia virus infection in contrasting farming systems in Kenya

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Abstract

Enzootic bovine leukosis (EBL) is a worldwide disease of cattle caused by the bovine leukemia virus (BLV) and clinically characterised by occurrence of multiple lympho-sarcomas. In Kenya, cases of bovine lympho-sarcomas have been reported but limited information available on prevalence and distribution of BLV infection in the country. The objectives of this cross-sectional study were to estimate the seroprevalence of BLV infection in Kenya and how the seroprevalence is affected by different livestock farming systems. In 2016, 1383 bovine serum samples were randomly collected from 14 counties which were purposively selected to represent 3 livestock farming systems in the country. The sera were tested for the presence of antibodies against BLV using the IDEXX anti–BLV indirect ELISA test. An overall seroprevalence of 7.6% (95% CI: 6.3% - 9.1%) BLV infection was estimated. A multivariable mixed logistic regression model, with county as a random variable controlling for clustering, identified age and farming system as significant risk factors associated with BLV seropositivity. Zero-grazing (0.6%), ranching (4.4%) and pastoral systems (18.3%) differed in seroprevalence. Cattle under 1 year of age had a prevalence of 6.4%, while cattle over 1 year of age had a prevalence of 7.9%. BLV infection was present across the three farming systems but in only five of the fourteen counties assessed. This information contributes to designing effort on control programs of BLV infection in Kenya. Further research should be carried out to determine the frequency of clinical cases of EBL and the impact on the livestock industry in Kenya.

Keywords: Bovine Leukemia Virus; Cattle, Seroprevalence; Kenya

Introduction

Enzootic bovine leukosis (EBL) is a lympho-proliferative disease of cattle caused by the bovine leukemia virus (BLV) which is a delta retrovirus in the retroviridae family and is notifiable according to the World Organization for Animal Health (Stear, 2005). Natural BLV infection has been confirmed in cattle and water buffaloes (Hald and Baggesen, 2013). Trans-placental transmission has been reported to account for 10–25% of infections; natural transfer of BLV infected cells between animals during processes such as parturition is fairly common. Artificial transmission frequently occurs through blood contaminated instruments such as needles, surgical equipment and rectal gloves (Mekata *et a*l., 2015). Large numbers of blood-sucking insects, such as Tabanids, have been shown to transmit the virus mechanically (Kobayashi *et al*., 2014). Pro-viral DNA can be isolated in semen and milk of the infected animals (Santos *et al*., 2007).

Bovine leukemia virus infects B-lymphocytes which proliferate through mitosis. About 70% of the infected animals act as carriers and do not manifest clinical signs or a change in the circulating lymphocyte counts. At this stage, antibodies against the virus and the pro-viral DNA can be detected using serology and Polymerase Chain Reaction (PCR), respectively (Rola and Kuzmak, 2002; Hald and Baggesen, 2013). With time, about 30 to 50% of the infected animals develop persistent lymphocytosis which is a polyclonal proliferation of B-cells (Stear, 2005)**.** Elevated numbers of circulating B-lymphocytes (over 10,000/mm$^{3)}$ can be observed at this stage using hematology (Hald and Baggesen, 2013). About 5% of the infected animals develop the clinical form of bovine leukosis, with clinical signs including; enlarged lymph nodes, in appetence, weight loss and general weakness (Stear, 2005). On post-mortem, malignant lympho-sarcomas are observed in multiple organs of the body, especially lymph nodes, spleen, mesentery and uterus (Stear, 2005).

Enzootic bovine leukosis has no treatment or vaccine available at present (Stear, 2005). Approaches to control and eradicate the disease involve testing cattle serologically, and then eliminating, segregating or managing the infected cattle (Rodriguez *et al*., 2011). Zoonotic importance of bovine leukemia virus has been studied widely but no conclusive evidence of transmission has been established (Baltzell *et al*., 2009; Buehring *et al*., 2014).

The economic losses associated with BLV positivity include; increased heifer replacement, condemned carcasses, decreased reproductive efficiency, decreased milk production, cattle deaths and inability to export cattle and their products to regions with strict enzootic bovine leukosis control measures, such as the European Union (Ott *et al*., 2003; Rhodes *et al*., 2003).

Presently, BLV infection affects cattle herds globally, with herd-level prevalence being as high as 88% and 91% in dairy herds in the United States and Canada, respectively, and about 30% of animals infected in those herds (Ott *et al*., 2003; Nekouei *et al*., 2015). In Uganda, a 17% animal-level prevalence has been reported (Azuba *et al*., 1994), while other African countries, such as South Africa, Namibia, Nigeria and Tanzania, reported animal-level prevalences of 12.6%, 12.3%, 4.2% and 36%, respectively (Adu and Olson, 1981; Kaura and Hbschle, 1994; Schoepf *et al*., 1997; Ndou *et al*., 2011).

In Kenya, 35 cases of bovine lympho-sarcoma were reported on post-mortem in the department of Pathology, Microbiology and Parasitology at the Faculty of Veterinary Medicine in the University of Nairobi over the last 28 years (Wandera *et al*., 2000). However, prevalence, distribution and risk factors of BLV infection in Kenya have not been described. This study was aimed at estimating the seroprevalence and identify potential risk factors associated with the presence of BLV infection in different livestock farming systems in Kenya.

Materials and methods

Study area

Bovine sera used in this study were collected from cattle in 14 counties in Kenya namely: Laikipia, Garissa, Marsabit, Narok, Nyeri, Nakuru, Nyamira, Kiambu, Kakamega, Homabay, Nandi, Kwale, Murang'a and Machakos.

Study design

The Bovine serum samples were collected as part of a larger cross-sectional surveillance study for trade sensitive diseases that was carried out between July and October 2016 jointly by the Department of Veterinary Services in the Ministry of Agriculture, Livestock and Fisheries in Kenya, and the Inter-Governmental Authority on Development (IGAD) across the 47 counties of Kenya.

Sampling technique and sample size determination

Briefly, stratified multi-stage sampling method was adopted whereby administrative locations (lower than divisions and headed by a chief) in each of the counties were purposively selected. From these locations, households were randomly selected, and animals randomly sampled from each of the households. The number of households selected per location was dependent on the number of households in the location and the number of animals per household. Each location was considered a site, and a total of 14 bovine samples were collected from each site.

For this study, a minimum sample size of 384 per farming system (a total of 1152 samples) was determined using the formula described by (Dohoo *et al.*, 2009) using a hypothetical BLV prevalence of 50%, 95% confidence, and 5% precision. However, there were enough kits to test 1383 samples. A total of 1383 cattle were randomly selected from 14 counties that were purposively selected to represent zero-grazing, ranching and pastoral livestock farming systems in the country. Cattle kept in other livestock farming systems were excluded from the study.

Data collection

In addition to the cattle identification, breed, age, sex, farming system and county of origin of each cattle were recorded. Farming systems were categorized into: 1) zero-grazing, where cattle do not graze, and are often segregated within a farm to ensure different and appropriate management to different animal cohorts (young stock, milking cattle, dry cattle, bulls); 2) ranching, where cattle graze together but do not mingle with cattle from other farms; and 3) pastoral, where cattle migrate long distances to graze on individual and communal lands, co-mingling with cattle from other farms.

Laboratory analysis

The serum samples were tested for the presence of antibodies against BLV using the IDEXX anti–BLV indirect ELISA test (IDEXX Laboratories, Inc., Westbrook, Maine, USA), in the serology laboratory at the Central Veterinary Investigation Laboratories in Kabete, Nairobi. The test was conducted according to the manufacturer's instructions. The optical density was measured using the Halo LED 96 DYNAMIC ELISA Reader at 450nm. The ELISA kit used

in this study had a sensitivity of 100% and a specificity of 97% (Tirziu *et al*., 2014) indicating good reliability of the testing method.

Data handling and analysis

The results were entered into Microsoft Excel® 2013 (Microsoft, Sacramento, California, USA) and analysed using Stata 14 (StataCorp, College Station, Texas, USA). Seroprevalence of BLV infection (expressed as a percent positivity) was described in the different categories of age, sex, breed, farming system and the county of origin. Logistic regression was used to determine the possible risk factors for the presence of BLV infection in cattle in Kenya, initially in univariable analyses for each possible risk factor. Mixed logistic regression was used to determine the possible risk factors of BLV seropositivity in a multivariable model, controlling for confounding and with county of origin analysed as a random effect to account for clustering of the data at this level. Pearson's correlation coefficient was used to determine correlations between risk factors in order to guide the model-building process by eliminating collinear predictors ($r \geq 0.5$). Total variation and intra-class correlation (ICC), which provided the proportion of variance at the county level, were calculated using the latent variable approach (Dohoo *et al*., 2009). Only main effects were retained in the final model at a significance level of $P \leq 0.05$, and interactions between main effects were investigated in the final model. Testing for confounding by variables not in the final model was explored by comparing changes in model coefficient estimates with and without the confounder and a change of ≥30% on the coefficient was used to indicate a confounder. Goodness-of-fit of the model was assessed and a likelihood ratio test was used to determine the superiority of a mixed logistic model over a fixed effect logistic model.

Results

The overall seroprevalence was 7.6% with 105 of the 1383 sera samples testing positive for BLV antibodies (95% CI: 6.3 - 9.1). The prevalence was only slightly higher in older cattle versus yearlings and in male cattle versus females ($p > 0.05$). Indigenous cattle appeared to have a higher prevalence than exotic breed cattle on univariable analyses. The prevalence of BLV infection in the farming systems ranged from 18.3% in the pastoral system to 0.6% in the zero-grazing system. Five counties in the study (Nakuru, Nandi, Laikipia, Marsabit and Garissa) had a prevalence that ranged from 2.9% (Nakuru) to

37.5% (Garissa), while the other counties had no positive animals for BLV infection, with an average, minimum and maximum of 70, 14 and 210 animals tested per county in those counties with no positives, respectively.

Table 1: Seroprevalence of BLV infection in cattle kept in three contrasting farming systems in Kenya in 2016.

Factor	Category	No. Positive/ No. Tested	Prevalence (%)	Odds Ratio	95% Confidence Interval	p-value
Age (years)	≤1 year	19/291	6.5	Reference	Reference	Reference
	>1 year	86/1092	7.9	1.12	0.84, 1.51	0.442
Sex	Female	78/1069	7.3	Reference	Reference	Reference
	Male	27/314	8.6	1.11	0.85, 1.44	0.444
Breed	Exotic	4/444	0.9	Reference	Reference	Reference
	Indigenous	101/939	10.8	4.40	2.47, 7.82	<0.0005
Farming system						<0.0005*
	Zero-grazing	4/638	0.6	Reference	Reference	Reference
	Pastoral	90/492	18.3	7.73	4.34, 13.93	<0.0005
	Ranching	11/253	4.3	3.10	1.60, 6.01	0.001
County						<0.0005*
	Laikipia	12/350	3.4	Reference	Reference	Reference
	Garissa	57/152	37.5	5.05	3.46, 7.39	<0.0005
	Marsabit	32/126	25.4	3.65	2.44, 5.46	<0.0005
	Nakuru	2/70	2.9	0.90	0.38, 2.14	0.808
	Nandi	2/56	3.6	1.02	0.43, 2.45	0.957
	Other 9 counties	0/629	0.0		n/a	n/a
Total		1383	7.6		6.3, 9.1	n/a

**Global p-value for categorical variables*

Breed was highly and significantly correlated with farming system (r=0.622) and county of origin (r=0.563). The likelihood ratio test indicated that the mixed logistic model was better than the multivariable logistic regression model ($p \leq 0.0005$). After accounting for clustering at the county level, and adjusting for confounding by other variables in the final multivariable model, the presence of BLV infection in Kenya was significantly associated with the cattle age and the farming system that the cattle were kept in (Table 2). The odds of testing positive for BLV infection was about 2 times higher in older cattle (>1 year) than young ones. The odds of BLV infection occurring in ranched cattle were 10 times higher than in zero-grazed cattle, while the risk of BLV

infection in pastoral cattle was over 5 times higher than in zero-grazed cattle. The probability of zero-grazed adult cattle testing positive for BLV infection ranged from 0.01% to 2.42% (95% CI for the baseline risk among zero-grazed cattle). Given that cattle in a specific county tested positive for BLV infection, the probability of a randomly selected cattle from the same county testing positive for BLV was 64.2% (ICC=0.642). The interaction variable between the two main effects of the model was not significant and there was no additional confounding by other variables not in the final model.

Table 2: Multivariable mixed logistic regression model of risk factors associated with seropositivity of BLV infection in cattle in Kenya in 2016.

Factor	Category	Odd ratio	95% CI	p-value
Age (years)	≤1	Reference	Reference	Reference
	>1	1.79	(1.26,2.56)	0.001
Farming system				0.063*
	Zero-grazing	Reference	Reference	Reference
	Pastoral	5.44	(0.98,30.27)	0.05
	Ranching	10.38	(1.48,72.97)	0.02

**Global P-value*

Discussion

This is the first study on seroprevalence of bovine leukemia virus infection in livestock farming systems in Kenya. The overall prevalence of 7.6% was low compared to other African countries such as South Africa, Namibia, Uganda and Tanzania that reported prevalences of 12.6%, 12.3%, 17% and 36%, respectively (Adu and Olson, 1981; Azuba *et al.*, 1994; Kaura and Hbschle, 1994; Schoepf *et al*., 1997; Ndou *et al*., 2011). In South Africa, EBL outbreaks had been reported before this study was carried out, which could explain the high prevalence (12.6%) seen there (Ndou *et al*., 2011) in comparison to the 7.6% prevalence reported in the present study. Schoepf et al (1997) stipulated that the high prevalence of BLV infection in Tanzania (36%) was due to the high sensitivity of the ELISA test (CHEKIT Leukotest) used in the study; however our test sensitivity was reported at 100% (Tirziu *et al*., 2014) and the prevalence was lower thus the reason could not be used the lower prevalence in the present study. Lower infection rates have been reported in Sudan (1.5%) and Nigeria (4.2%) where the less sensitive AGID test was used (Adu and Olson, 1981; Osheik *et al*., 1988; Schoepf *et al*., 1997).

The higher risk of adult cattle (OR=1.79) testing positive for BLV infection could be attributed to the persistent nature of the infection once animals are infected and due to cumulative exposures of cattle over a lifetime (Watanabe *et al*., 2015). Similar results were observed in a study done in cattle in Iran where older cattle had a higher risk of BLV infection (Mohammadi *et al.*, 2011). A study on Yaks (*Bos mutus*) in China also indicated that the prevalence of BLV infection increased with age (Ma *et al*., 2016).

The resulting higher prevalence of BLV infection in indigenous breeds (10.8%) than in exotic breeds (0.9%) seen in our univariable results was similar to reports made from other studies in Venezuela and Argentina (Trono *et al*., 2001; Nava *et al*., 2011) . In Kenya, cattle kept in pastoral systems are mostly indigenous breeds, while zero-grazed cattle are mostly exotic breeds, which could explain the correlation between breed and farming system (r=0.622), and the removal of breed in the final model when farming system was in the model. The high prevalence (18.3%) and odds of infection (OR=5) observed in cattle kept in pastoral farming systems could be due to unrestricted contact between infected and uninfected cattle among different owners during communal grazing, which eases the transmission of BLV among these cattle, where the infection exists. Consequently, in Garissa and Marsabit Counties where pastoral farming is frequently practiced, the highest BLV prevalences of 37.5% and 25.4% were observed, respectively. Movement of pastoralists with their animals in search of pasture and water could facilitate spread of the BLV. In ranches, cattle have unlimited contact with cattle in the same ranch but not cattle from other ranches, which could explain the lower prevalence (4.4%) in comparison to pastoral system. The unlimited contact between animals within a ranch could explain the higher prevalence of BLV infection when compared to zero-grazing farms which only had a prevalence of 0.6%. In the zero-grazing farming system, the risk of BLV transmission between cattle in the same herd appears to be very low, likely due to the segregation of cattle cohorts within farms (e.g. dry cattle and young stock are usually kept away from milking cattle), which likely lowers the rate of contact between the cattle in the same farm. On zero-grazing farms, there is usually no exposure to cattle from other farms. Zero-grazing livestock systems usually have higher inputs and better management than ranching and pastoral systems, and thus, cattle in this system may have better resistance to diseases.

Laikipia county, with a high number of ranches, had a moderately high prevalence of 3.4%. Of the 10 tested counties that practiced zero-grazing livestock farming, only Nandi (3.6%) and Nakuru (2.9%) had cases of BLV infection.

A limitation of this study is that we only had limited data at the animal level and at the county/system level, since we obtained serum samples from another study. We did not have herd level data at all on the cattle tested; therefore, we cannot say anything about herd-level prevalence or herd-level risk factors. We also could not adjust the prevalence for sampling weights without this herd-level information. With the limited data, we could only control for confounding for those variables with data. Future studies should collect and interpret herd level data and more animal level data to allow testing of hypotheses on the important mechanisms of BLV transmission.

Conclusion

Bovine leukosis infection was found to be present across the three farming systems assessed and in five of the fourteen selected counties in Kenya. Cattle kept in pastoral farming systems had the highest prevalence, while zero-grazed cattle had the lowest prevalence. Similarly, in counties where pastoral farming is largely practiced, a larger number of cattle tested positive for BLV relative to the other counties. The Kenyan local breeds had a higher prevalence than the exotic breeds; however, this finding is likely a function of zero-grazing farmers preferring exotic breeds over local breeds. Further research should be carried out to determine: a) BLV seroprevalence in other Kenyan counties; b) the frequency of clinical cases of enzootic bovine leukosis; and c) the economic impact of BLV in the livestock industry in Kenya, along with important management risk factors to determine recommendations for reduced transmission relevant to Kenyan management systems.

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Informed consent

Informed consent was obtained from all individual participants included in the study.

Compliance with ethical standards

All applicable international, national and institutional guidelines for the care and use of animals were followed.

Conflict of interest statement

The authors declare they have no financial or personal relationship(s) that may have inappropriately influenced them to write this article.

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