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Full Length Research Paper

Prevalence of bovine trypanosomosis and associated risk factor in Jimma Horro District, Kellem Wollega Zone, Western Ethiopia

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Trypanosomosis is a serious disease that causes a significant production loss in cattle. A cross sectional study was conducted in Jimma Horro District of Kellem Wollega Zone, Western Ethiopia to determine prevalence and associated risk factors of bovine trypanosomosis from October 2016 to October 2017. Blood samples from randomly selected 384 cattle of both sex and different age group were collected and examined with parasitological techniques. The overall prevalence of bovine trypanosomosis was 3.7% (14/384) in the study areas. The infection was highest due to Trypanosome congolense (50%) followed by Trypanosome vivax (28.6%) and *Trypanosoma brucei* (21.4%). Multivariable logistic regression analysis identified body condition as risk factors (P<0.05) for trypanosomosis in the district. However, there were no statistically significant difference observed among age groups, sex, skin color and different peasant associations (P> 0.05). The overall mean Packed Cell Volume (PCV) value was statistically significant difference between aparasitaemic and parasitaemic cattle (P< 0.05).The study showed that bovine trypanosomosis is one of the constraints to cattle production in Jimma Horro District. Hence, there is a need to create awareness about impact of disease on cattle production and appropriate control methods of trypanosomosis should be designed and implemented.

Key words: Bovine, Jimma Horro district, prevalence, risk factors, trypanosomosis.

INTRODUCTION

About 85% of the Ethiopian populations are engaged in the agricultural sector (Benti and Zewdie, 2014). The livestock subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP. It also contributes 15% of export earnings and 30% of agricultural employment (Leta and Mesele, 2014). The country has the largest livestock population in Africa. In spite of the presence of huge ruminant population (59.5 million cattle, 30.7 million sheep and 30.2 million goats) (CSA, 2017), Ethiopia fails

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to optimally exploit resources due to a number of factors such as diseases, poor nutrition, poor husbandry practices and lack of government policies for disease prevention and control (Bekele et al., 2010). Among the animal diseases trypanosomosis is one of parasitic disease that hampering the livestock development in Ethiopia (Dumesa and Demessie, 2015).

Trypanosomosis is caused by unicellular, flagellated protozoan parasites which belong to the genus *Trypanosoma* which is found in the blood and other tissues of vertebrates including livestock, wild life and people (Gupta et al., 2003; Blood and Radostits, 2007; Gupta et al., 2009; Sharma et al., 2012; Singla et al., 2015). Bovine trypanosomosis covering 10 millions of square kilometers of potentially productive land, results in drastic reduction of animal production and productivity in Ethiopia (Kitila et al., 2016). The species of trypanosomes are known to exist in Ethiopia, which are pathogenic to cattle are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*. Those species are distributed mainly in tsetse belt region of the country. However, *T. vivax* is also found in areas outside of the tsetse belt, where it can possibly be transmitted by mechanical vectors of biting flies (Getechew, 2005). In Ethiopia, trypanosomosis is wide spread in domestic livestock in the Western, South and Southwestern lowland regions and the associated river systems (that is, Abay, Ghibe Omo and Baro/Akobo). About 220,000 Km^2 of this region are infested with five species of tsetse flies namely *Glossina pallidipes*, *Glossina morsitans*, *Glossina fuscipes*, *Glossina tachinoides* and *Glossina longipennis* (NTTICC, 2004).

Besides Ethiopia trypanosomosis is a serious disease in domestic livestock that cause a significant negative impact in food production and economic growth in many parts of the world including Ethiopia (Kumar et al., 2012). African livestock producers are administering estimated 35 million US$ curative and prophylactic treatments annually (Holmes et al., 2004). The direct losses from trypanosomosis in livestock include mortality, morbidity, abortion, impaired fertility and the cost of implementing and maintaining trypanosomosis control operations (Juyal et al., 2005; Singh and Singla, 2013). Indirect losses stem from farmers responses to the perceived risk of the disease, including the reduction and in some cases, the exclusion of livestock from tsetse-infested grazing lands and reduced crop production due to insufficient animal draught power (Siyum et al., 2014). Tsetse transmitted animal trypanosomosis still remain as one of the largest cause of livestock production losses in Ethiopia (Kitila et al., 2017).

Trypanosomosis is one of the most important cattle problems in Jimma Horro District. This district is potential for cattle production but the district is infested with tsetse flies. As a result, the people suffer from low level of draught power and productivity that compromise the socio-economic and nutritional status of inhabitants. Hence, knowing the current status of bovine trypanosomosis and its associated risk factors is important to reducing economic losses by parasite. To effectively control such losses and realize benefit from cattle resource, it is crucially important to study prevalence of bovine trypanosomosis and factors contributing to its occurrence. Furthermore, science-based interventions could be made available for policy makers and animal health extension personnel. There is no any study conducted previously in Jimma Horro District. Therefore, objective of study was to determining the prevalence and associated risk factors of bovine trypanosomosis in the Jimma Horro District.

MATERIALS AND METHODS

Study areas

The study was conducted from October 2016 to October 2017 in four selected peasant associations (Nedi Gudina, Hambash, Gombo and Burka Gudina) of Jimma Horro District, Kellem Wollega Zone in Western Ethiopia. This district is bounded by Begi district in North, Gawo Kebe district in East, Yamalogi Wolel district in South and Gidami district in West. The area is located at about 665 km west of Addis Ababa. The area is located at an elevation of 1400-1830m above sea level. The Topography of this district is characterized by Forest of Wolel Mountain and Dati Wolel Park. The main river in this district is Supe, Burar and Kumbabe. The climatic condition alternates with long summer rain fall (June to September), short rainy season (March to May) and winter dry season (December to February). The minimum and maximum annual rain fall and daily temperature range from 800 to 1200 mm and 15 to 25°C, respectively. Jimma Horro District is characterized by Dega (19.7%), Woyna dega (48.5%) and Kola (31.8%). Livestock population in area is estimated to be about 68,500 heads of cattle, 5,761 mules, 8,786 donkeys, 233 Horses 19,952 sheep, 13,575 goats and 69,975 species of poultry. The farmers in the area practice mixed farming system (JHDAO, 2O16).

Study population

Study population were zebu cattle kept under extensive traditional husbandry condition in selected peasant associations of Jimma Horro District of Kellem Wollega Zone in western Ethiopia. The animals were managed by grazing the communally owned pasture land throughout the year under the same agro-ecology without any additional supplementary feedings.

Study design

Cross-sectional study was conducted from October 2016 to October 2017 to determine the prevalence of bovine trypanosomosis and associated risk factors of the disease.

Sampling method and sample size determination

The study district was selected purposively based on history of parasite reports. Simple random sampling technique was used to select the peasant associations. Four peasant associations were sampled from Jimma Horro District based on number of cattle population. Sampling frame of cattle was taken from respective

Table 1. Prevalence and distribution of *Trypanosoma* species in different peasant associations.

Peasant associations	Number of examined	Prevalence (95% CI)	Trypanosome species		
			T. congolence (%)	*T.vivax* (%)	*T. brucei* (%)
Nedi Gudina	99	1.1 (0.96-2.98)	0 (0.0)	1(7.1)	0 (0.0)
Hambash	96	3.1 (0.36-6.61)	2 (14.3)	1 (7.1)	0 (0.0)
Gombo	89	3.4 (0.38-7.12)	2 (14.3)	1 (7.1)	0 (0.0)
Burka Gudina	100	7.0 (2.0-12.0)	3 (21.4)	1 (7.1)	3 (21.4)
Total	384	3.7 (1.77-5.52)	7 (50)	4 (28.6)	3 (21.4)

peasant associations. During sampling age, sex, skin color, body condition of cattle and peasant association were recorded. Since there was no previous study done in the area, the sample size was determined based on the expected prevalence of 50% and absolute desired precision of 5% at confidence level of 95%. As a result a total of 384 animals were needed to be sampled according to formula given by Thrusfield (2005).

Sample collection and parasitological examination

Buffy coat technique

A little sample of blood was collected from an ear vein using heparinized microheamatocrit capillary tube. One end of the heamatocrit tube containing whole blood sample was sealed with hematocrit clay. The heamatocrit tube was centrifuged at 12000 rpm for 5 min. The capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of capillary tube was expressed on to slide, homogenized on to clean slide and covered with cover slip. The slide was examined under X40 objective and X10 eye piece for the movement of the parasites (Paris et al., 1982; Juyal and Singla, 2005).

Thin blood smear

Placed a drop of blood on clean slide and spread by using another clean slide at angle of 45°C air dried and fixed for 2 min in methyl alcohol, then immersed in Giemsa stain for 50 min (Cherenet et al., 2006). Drained and washed of excess stain using distilled water and allowed to dry by standing up right on the rack and was examined under microscope with oil emersion objective lens. In Giemsa stained smears the species were distinguished by their size, shape, position, location and size of the kinotoplast.

Measuring of packed cell volume

The capillary tubes containing blood samples were placed in microheamatocrit centrifuge with sealed end outer most. The samples were allowed to centrifuge at 12000 rpm for 5 min. Tubes were then placed in heamatocrit and readings were expressed as a percentage of packed cells to the total volume of whole blood. Animals with PCV <24% were considered to be anemic.

Data management and analysis

Data obtained from this study was recorded and stored in Microsoft® Excel for Windows 2010 and transferred to Statistical Package for the Social Sciences (SPSS) version 20.0. The prevalence of trypanosomosis in different variables (peasant association, body condition, skin color, sex and age) was analyzed by using logistic regression model. Student's t-test was employed to compare the mean PCV of the parasitaemic and aparasitaemic animals. Associations between outcome (trypanosomosis) and explanatory variables (risk factors) for all units of analysis were investigated by using logistic regression model. The strength of the association between outcome and explanatory variables was assessed using the crude and adjusted odds ratios (OR). The explanatory variables (P≤0.25) were further checked for multicollinearity using the variance inflation factor (VIF) and tolerance factor (TF) before multivariable logistic regression analysis. Variance inflation factor values of greater than 3 or tolerance less than 0.1 were considered the cut-off points (Apeanti, 2016) for the collinearity diagnostics. Variables were also tested for interaction effects using cross-product terms. For all the analyses, confidence level (CL) is at 95% and P≤0.05 were set for significance.

RESULTS

The overall prevalence of bovine trypanosomosis in the study areas was 3.7%. The prevalence in each peasant association was determined to be 1.1% in Nedi Gudina, 3.1% in Hambash, 3.4% in Gombo and 7.0% in Burka Gudina of Jimma Horro District. *Trypanosome congolence* was dominant species with proportion of 50%, followed by *T. vivax* (28.6%) and *T. brucei* (21.4%) in Table 1.

The mean PCV value for the parasitemic cattle was 23.29 +4.25 SD while the mean PCV value for the aparasitaemic cattle was 25.59+4.23 SD. There was statistically significant difference (P< 0.05) in mean PCV value between parasitaemic and aparasitaemic cattle (Table 2).

The highest (7.0%) and lowest (1.1%) prevalence of bovine trypanosomosis was recorded in Burka Gudina and Nedi Gudina peasant associations, respectively. However, there was no statistical significant difference (P>0.05) between prevalence of trypanosomosis and peasant associations. The prevalence of trypanosomosis was 5.18% in older age category than in adult age category (0.8%) cattle. The prevalence of trypanosomes infection between age group was not statistically significant difference (P>0.05). The prevalence of

Table 2. Mean PCV comparison parasitaemic and aparasitaemic cattle.

Condition	Number	Mean	SD	t-test	P-value
Parasitaemic	14	23.29	4.25	2.009	0.005
Aparasitaemic	370	25.59	4.23		

Table 3. Univariable logistic regression analysis of bovine trypanosomosis associated risk factors in the study areas.

Variables	Category	Total animals examined	Total animals positive (%)	OR (CI; 95%)	P-value
PA	Nedi Gudina	99	1 (1.10)	-	0.22
	Hambash	96	3 (3.13)	3.2 (0.32-3.9)	0.32
	Gombo	89	3 (3.37)	3.4 (0.34-3.5)	0.29
	Burka Gudina	100	7 (7.0)	7.4 (0.89-6.1)	0.06
Sex	Male	144	4 (2.78)	-	-
	Female	240	10 (4.17)	1.5 (0.47-4.94)	0.49
BCS	Good	193	6 (3.11)	-	0.029
	Medium	130	2 (1.54)	0.5 (0.97-2.41)	0.38
	Poor	61	6 (9.84)	3.4 (1.10-2.40)	0.41
Age	Young	8	0 (0.00)	-	208
	Adult	125	1 (0.80)	0.2 (0.01-3.30)	0.26
	Old	251	13 (5.18)	1.3 (0.16-1.23)	0.82
Skin color	White	157	1 (0.64)	-	0.17
	Red	122	7 (5.74)	1.3 (0.33-5.40)	0.69
	Black	40	3 (7.5)	0.1 (0.01-0.87)	0.04
	Mixed	65	3 (4.62)	0.7 (0.20-3.20)	0.75

OR: Odds Ratio; CI: Confidence Interval, Ref : Reference.

trypanosomosis was higher in female (4.17%) than male (2.78%) cattle, but there was no statistically significant difference (P>0.05). The highest prevalence of trypanosomosis was recorded in cattle with poor body condition cattle (9.84%). Moreover, variation in prevalence of trypanosomosis among the body condition was statistically significant (P<0.05). Poor body condition cattle being almost three times (OR=3.4) more likely to be infected with trypanosomes organisms compared to good body condition cattle. Highest prevalence of trypanosomosis was found in black skin color of cattle (7.5%), followed by red skin color (5.74%) and lowest in white skin color (0.64%) of cattle. However, there was no statistically significant difference (P>0.05) of skin color of cattle with prevalence of trypanosomosis (Table 3). Variables with a P-value less than 0.25 in the univariable analysis with no multicollinearity were entered into multivariable logistic regression model. No significant interactions between variables were detected. A Hosmer-Lemeshow goodness-of–fit value (P=0.98), indicated that the model fit the data. The final multivariable logistic regression model showed that body condition was independently associated with (P<0.05) bovine trypanosomosis (Table 4).

DISCUSSION

The present study showed that from a total of 384 randomly examined cattle, 14(3.7%) were positive for trypanosome parasite. Similar level of prevalence was reported by Teka et al. (2012), Dawit et al. (2015) and Fayisa et al. (2015), who reported that prevalence of 3.7% in Abaya district, 4.9% in Arbamich and 4.4% in Didesa District in Ethiopia, respectively. On the other hand, the prevalence of trypanosomosis reported in the current study is lower than the values reported by Olani and Bekele (2016) 7.8% in Lalo-Kile district; Fedesa et al. (2015) 7.1% in Darima District and Miruk et al. (2008) 20.4% in Wolyta and Dawero Zone of Southern Ethiopia;

Table 4. Multivariable logistic regression analysis of potential risk factor of bovine trypanosomosis in study areas.

Factor	Number of animals examined	Total animals positive (%)	Adjusted OR (95% CI)	P-value
Body condition				0.03
Good (Ref)	193	6 (3.11)	-	-
Medium	130	2 (1.54)	0.5 (0.10-2.90)	0.38
Poor	61	6 (9.84)	3.4 (1.10-2.93)	0.04

OR: Odds Ratio; CI: Confidence Interval, Ref :Reference

Siyum et al. (2014) 16.9% in Sayo District in Western Ethiopia; Yalew and Fantahun (2017) 21.5% in Bambasi woreda, Western Ethiopia and Kitila et al. (2017) 7.4% in Yayo District Iluababora zone of Western Ethiopia. This variation might be due to differences in environmental factors, breed and managemental system in study areas.

The present result shows that out of 14 positive cattle for trypanosomosis, *T. congolonse* (50%) was predominant species of trypanosome, followed by *T. vivax* (28.6%) and *T. brucei* (21.4%) in study area. This may be due to major cyclical vectors or *Glossina* species are more efficient to transmitters of *T. congolonse* than *T. vivax* and high number of serodems of *T. congolonse* as compared to *T. vivax* (Olani and Bekele, 2016). Moreover, *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolonse,* since *T. congolonse* is mainly confirmed in blood, while *T. vivax* and *T. brucei* invade tissue (Biyazen et al., 2014). This finding is consistent with some previous studies in different parts of Ethiopia (Begna et al., 2011; Biyazen et al., 2014; Kassaye, 2015; Tola et al., 2016; Kassaye and Tsegaye, 2016; Kitila et al., 2017). Similarly, *T. congolonse* was dominant species with a proportion of (69.7%) and followed by *T. vivax* (19.2%) and *T. brucei* (9.1) in Western Ethiopia also reported by Siyum et al. (2014) and Dawit et al. (2015).

The mean PCV value of trypanosome positive cattle was significantly lower (23.29 ± 4.25) than that of negative cattle (25.59 ± 4.23). The occurrence of positive animals with PCV of greater than 24% might be thought of as recent infection of the animals (Vanden and Rowlands, 2001). Low PCV value may not solely be due to trypanosomosis. However, these factors are likely risk for both parasitemic and aparasitemic cattle. Thus, the difference in mean PCV value between the parasitemic and aparasitemic cattle indicates that trypanosomosis is involved in reducing the PCV value in the infected cattle. This result was in line with Rowlands et al. (2001), who reported that the treatment resulted into an increase in PCV value of positive animals when PCV was less than 26%. Hence, the mean PCV was a good indicator for the health status of the herd in an endemic area. This result was also in agreement with previous report as anemia is the classical sign of the disease pathogenicity; the low PCV in parasitaemic animals could have contributed in reducing the mean PCV for cattle (Getachew et al., 2014; Efrem et al., 2013). Likewise, this result is in line with Mezene et al. (2014), who stated that parasitaemic animals had generally lower mean PCV value than aparasitaemic animals.

In the present study, body condition indicated that animals with poor body condition are three times more likely to be affected by trypanosomosis (OR= 3.4) than good body condition. This may be due to trypanosomosis results in progressive emaciation of the infected animals; never less, non-infected cattle under good condition have well developed immune status that can respond to any foreign protein better than those of non-infected cattle with poor body condition (Taylor et al., 2007). This finding is consistent with some previous studies in Ethiopia (Dawud and Molalegne, 2011; Girma et al., 2014; Getachew et al., 2014; Gona et al., 2016; Yalew and Fantahun, 2017) stated that prevalence of trypanosomosis was statistically significantly associated with body condition in cattle. This study finding is also in line with that of Bitew et al. (2011), Teka et al. (2012) and Fayisa et al. (2015), who reported that statistically significant association between prevalence of trypanosomosis and body condition in cattle. However, in contrary to this Abebayehu et al. (2011), Bekele and Nasir (2011), Tafese et al. (2012), Dawit et al. (2015) and Kitila et al. (2017) reported that body condition of cattle was not significantly associated with the prevalence of trypanosomosis in cattle.

In the present study, no statistically significant variation was observed in prevalence of bovine trypanosomosis among skin color of cattle. Comparison conducted between the different skin colors of cattle indicated that higher prevalence was observed in cattle's having black skin color (7.5%) followed by 5.7% red and 4.62% mixed skin color. Tsetse flies by nature are attracted toward a black color, so in animals having black skin color there is high prevalence of trypanosomosis recorded (Teka et al., 2012; Gona et al., 2016). The prevalence of bovine trypanosomosis was no statistical significant difference (P>0.05) among sex, age groups of cattle and peasant association. This might be because of an equal chance of exposure cattle to the parasite and even distribution of the disease in the district. This result is in line with the previous study (Abebayehu et al., 2011; Bekele and

Nasir, 2011; Tafese et al., 2012).

CONCLUSION AND RECOMMENDATIONS

Trypanosomosis is most important constraint for cattle production in Jimma Horro District. The present result showed that existence of *T. congolonse*, *T. vivax* and *T. brucei* were responsible for bovine trypanosomosis in study area. Body condition was statistically significance difference with prevalence of trypanosomosis in the district. However, age groups, sex, skin color and different peasant associations were not showed statistically significance difference. The mean PCV value of trypanosome cattle was significantly lower than negative cattle indicating the effect of trypanosomosis in lowering the PCV value. Thus awareness creation and appropriate control methods of trypanosomosis on its vectors and against the parasite should be designed and implemented.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

An observational and questionnaire based study on principles of herd health management on Jimma University dairy farms

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The study was in Jimma University dairy farm with objective to assess the dairy management aspects of Jimma University dairy farm and to identify the herd health problems of the dairy farm. Ethiopia has a large number of livestock populations and holds the 1st rank in Africa. Despite the huge number of cattle and their economic importance, the productivity is low due to constraints of disease, nutrition, poor management, lack of marketing facilities and opportunity, inadequate animal health services, uncoordinated development programs between various levels of government institutions. Both questionnaire and observational survey study was conducted on Jimma University dairy farm which have total of 45 animal populations (calf 16, heifer 15, bull 1, milking cow 12, and dry cow 1) found in Jimma University dairy farm. Observational study type conducted and the total animal populations in the dairy farm are observed by using census sampling method and the questionnaire developed for this purpose is filled with the information obtained from the close dairy attendants. Close observation considering animal welfare, house, management, feeding and watering and questionnaire survey was collected regarding health management, back ground history, productivity, and other related issues. After observation and questioner collection and the results from dairy farm obtained were compared with scientific standards of dairy herd health management and production. Dairy farm has been facing low prevalence of mastitis, lameness, calf diarrhea, bloating and external parasite density. The house has no separate rooms for different status of cows and calves and lacks proper waste disposal systems. In conclusion, the farm has many constraints that have to be improved for next.

Key words: Dairy farm, herd health, disease, management, university.

INTRODUCTION

Ethiopia has a large livestock population, a relatively favorable climate for improved, but the products and productivities were very low compared with their number. Considering such a potential, investing in development productivities were very low compared with their number. Considering such a potential, investing in development

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interventions to the dairy sector will contribute to poverty alleviation in the country by increasing the income of smallholder dairy producers and creating employment and transforming the existing largely subsistent type of milk production to commercial level (Zelalem et al., 2011).Industry has even greater potential for improving the living standards of people through improved nutrition arising from milk consumption and incomes raised from sales of milk and milk products (Njombe, 2011).

Despite the huge number of cattle and their economic importance, the productivity is low due to the constraints of disease, nutrition, poor management, lack of marketing facilities and opportunity, inadequate animal health services, and poor performance of indigenous breeds (DACA, 2006). The implementation of an integrative program of herd health and production management in dairy cows requires monitoring a large category of data, general status of the animals, clinical examination, para-clinical examinations (ultrasound, blood and urine biochemistry, bacteriological, virological, serological), reproduction parameters, housing conditions, nutrition, milk production and its quality, veterinary actions and their results (Solcan et al., 2005).

Among the major reproductive problems that have direct impact on reproductive performance of dairy cows are abortion, dystocia, retained fetal membrane, pyometra, metritis, prolapse (uterine and vaginal), anoestrus and repeat breeder. They are classified as before gestation (anoestrous and repeat breeding), during gestation (abortion, vagina prolapsed and dystocia) and after gestation (retained fetal membrane and uterine prolapsed) (Lobago et al., 2006; Shiferaw et al., 2005).

Infectious diseases can have a great impact on the economic performance of a farm and may also induce stress in the farmer and image of the dairy industry, including damage to public image, loss of market position and decreased slaughter value (Noordhuizen, 2012). Feed shortage is partly attributed to the shortage of land for forage development. The absence of or weak linkages among the different actors in the dairy value chain is considered to be another important factor that negatively affects the country's dairy development (Zelalem et al., 2011). Based on the above literature, Jimma University dairy farm is one of farm found in Jimma University that provide milk for college community, students for practice and for research purpose. To cross presence or absence of challenges in Jimma University dairy farm that are commonly facing dairy production in general, we come with the following objectives:

(i) To address the dairy management aspects of Jimma University dairy farm
(ii) To identify the herd health problems of the dairy farm and
(iii) To forward general recommendations based on observations

Characteristics of smallholder dairy cattle production systems

Smallholder dairy farming is an important part of farming throughout developing countries. Smallholder dairy farmers follow three main feeding systems for cattle rearing which are zero grazing (intensive), partial grazing (semi-intensive) and free range (extensive) (Msuya, 2002). Zero grazing is also used as a means to control communicable diseases by isolating crossbred and exotic cattle from the indigenous cattle (Reis and Combs, 2000). However, zero grazing can contribute to poor animal productivity due to a number of factors: failure to feed cattle during the night is often encountered in zero grazed animals, which is undesirable since stall-fed milking cows need night feeding like grazing animals (Phiri, 2001).

Smallholder farmers have the common characteristics of limited resources and income, their farming systems and culture differ widely from place to place. Farmers own mostly between one and five zero grazed dairy cattle (Kurwijila and Boki, 2003).

Generally, factors constraining performance of dairy industry include; inadequate feeding of dairy cattle for high production, inadequate control of epidemic diseases, unreliable supply of inputs and shortage of skilled labor for good management (Msechu et al., 1995). According to Mwatawala et al. (2003), problems of long calving interval (CI), short lactation length (LL) and long dry period (DP) could be reduced through improvement in management than manipulation of genetic constitution of the animals.

METHODOLOGY

Description of the study area

Observational and questionnaire survey study were conducted in Jimma University dairy farm, Southwestern part of Ethiopia. Jimma University is located in Oromia Regional state, 346 km Southwest of Addis Ababa at latitude of about 7013'-8056' N and longitude of about 35052'-37037' E, and at an elevation ranging from 880 to 3360 m above sea level. Observational study area receives a mean annual rainfall of about 1530 mm which comes from the long and short rainy seasons. The annual mean minimum and maximum temperature during the study period were 14.4 and 26.7°C respectively (JUDF, 2017/2018).

Study type and study population

Both questionnaire and observational survey was conducted on dairy farm from February to October 2017 at Jimma University farm (a total of 45 animal populations (calf 16, heifer 15, bull 1, milking cow 12, and dry cow 11)) found in Jimma University.

Sample size and sampling method

Observational study type conducted and the total animal populations in the dairy farm (45) are observed by using census sampling method and the questionnaire developed for this purpose is filled with the information obtained from the close dairy attendants.

Study methodology

Close observation considering animal welfare, house, management, feeding and watering and questionnaire survey was collected regarding health management, back ground history, productivity, and other related issues. After observation and questioner collection and the results from dairy farm obtained were compared with

scientific standards of dairy herd health management and production. No laboratory work was conducted to differentiate health problems in farm but questionnaire surveys for interviewees have tried to approve their occurrences.

RESULTS AND DISCUSSION

Observational study

Total of 45 animals (12 milking cows, 1 dry cow, 1 bull, 15 heifers, 16 calves) observed under observation and questionnaire survey.

Housing

The house of university dairy farm was constructed with ventilation, urinary disposal canal, feeding and watering canal, delivery pen, calf pen, group heifer pen, milk dispatch room, office, feed store and etc. This house construction with above component is good, even though it has its own constraints. Some of them are the followings:

(i) The house has no proper waste disposal system. Animal urine and feaces are storing on the floor and contaminate animals and their feeds.
(ii) There are no separate rooms for delivery, milking, calves, and there is no proper ventilation.
(iii) Two directions of the openings of house relative to west and east.
(iv) Feed is stored with other materials which are the source of contamination for dairy cows and there is no separate room for dairy and poultry feeds.

Herd management and sanitation

University dairy farm is currently well managed as compared to previous management system. The dairy cows and floor are washed with water and soap three times a week, each dairy cow udder has cleaned by separate towel, milking system is good in that if there is diseased cow with mastitis, the healthy cows are milked first, from diseased cow healthy udder milked first and followed by diseased udder last. The pregnant cow after eight month she separated to parturition pen. After parturition with in 24 h the calf was separated from mother and get in calf pen. The calf feed 2 L of milk twice a day. When the calf start feeding forage and supplementary feed, the milk allowed to calf is decreasing. Dairy cow feed twice a day concentrate feed (morning and afternoon during milking) and forage feed allowed 2-3 times a day. Concentrated feed and water are given to cows in feeding and watering trough. However, the management system of university dairy farm are facing the following challenges:
(i) The floor has no proper manure and urine disposal canals till; this contaminates feeds, animal udder and equipment
(ii) Forage feeding to animal directly come from the site which has some source of contamination and diseases.
(iii) Concentrated feed contaminated with wastes from animals and equipment
(iv) The concentrated feed allowed to cow is not measured enough
(v) There is no separate room for diseased animals.

Herd health management

In the dairy farm; the frequently recorded diseases and management problems include carbohydrate engorgement, lameness, mastitis, calf scour, diarrhea, decreased production etc. Lameness prevalence is lowered in its occurrence due to the change in sanitation of the house and general follow up of the dairy cattle for the diseases and sudden trauma. Carbohydrate engorgement is reduced to a manageable level because of the practice of animal feeding management to an acceptable level. The cows' udder washed with clean water and soap, and then dried with clean towel. Mastitis have been managed in a way of keeping good milking orders of health cows milked first followed by mastitis cow health udder and then finally the mastitis udder. Calf scour and production reduction are managed when faced the farm report to Jimma University for technical support. The school gives a sudden technical support immediately to the problem on the farm. In general, university dairy farm has the following weak sides to be considered:

(i) Concentrate feeds have no separate store from poultry feeds and equipments
(ii) Concentrate feed allowed to the dairy milking cow is not measured
(iii) Even though the floor is washed three times a week; there is a couple of contamination to the animal feed and water and equipment.
(iv) Whatever they care for the mastitis; there is no more attentions given to subclinical mastitis and environmental contamination to the udder and whole animal body. Feeding and watering troughs are not as hygienic as they required being.

Results of questionnaire survey

As it was indicated below that total number of 15 dairy farm laborers were interviewed from the total of 20 labors and 15 semi-structured questionnaires each has about 18 questions were distributed to get information concerning the diseases common to the dairy farm, the feeding and watering system, housing and feed storage system, the sanitations and general herd health management system. Then after the questionnaires were filled by the interviewers, they were picked and the responses to each question were evaluated and ranked according to Table 1

Table 1. Demographic characteristic of interviewers.

Questionnaires	Variables/responses	No. of respondents	Percentage
Sex of labor	Male	**9**	60
	Female	6	40
Marital status of labor	Single	4	26.7
	Married	11	73.3
Educational status of labor force	Illiteracy man	1	6.67
	Elementary	5	33.33
	Certificate	3	20
	Diploma	3	20
	First Degree	4	26.67

Table 2. Farm characteristics.

About farms	Variables	No. of respondents	Percentage
No. of dairy cows in farm	10-15	12	80
	16-21	1	6.7
	22-27	1	6.7
	28-33	1	6.7
	Above 34 cows	0	0
Total number of milking cows	10 – 14	12	80
	15 – 19	2	13.33
	20 - 24	1	6.67
No of dry cows in farm	1	0	73.33
	2	1	6.67
	3	3	20
	4	11	73.33

and compared to the results obtained by the observational study of the same period, study population and place. As it was indicated in Table 1, demographic characteristics of interviewers showed that labor worker of the farm was dominated by males 9(60%) and educational status of the worker was mostly by elementary 5(33.33%). The farm consisted of 12 (80%) dairy cows in the farm, about 12(80%) was milking cows and 11(73.33%) number of dry cows in the farm as the respondents responded as indicated in Table 2.

Feeding management

Most dairy cows were feed in zero grazing (10(66.67%) in the farm system (Charles et al., 2015). Tethering and stalling feed and grazing were also important types of feeding in the farm. Animal feed was mainly sourced on the farms from natural sources. The farmers employed a farm worker who collected feed from various sources including field and other communal lands (Table 3). In Table 4, house of the farm was constructed by modern systems (concreted floor) 10(66.67%) as responded by interviewee, but some of the respondents stated that house was constructed by semi-modern system 4(26.67%). This result agrees with the result of Charles et al. (2015) and MoLD (2007).

CONCLUSION AND RECOMMENDATIONS

Both observational and questionnaire survey study were carried out in Jimma University dairy farm which is found Southwestern part of Ethiopia. The study was conducted with objective of addressing herd health problems and dairy management aspects in Jimma University. Close

Table 3. Feeding systems.

Feed systems for farm	Variable	No. of Respondents	Percentage
Feeding system of the farm	Communal grazing land	1	6.67
	Tethering	2	13.33
	Private grazing land	0	0
	Stall feeding and grazing	2	13.33
	Zero grazing	10	66.67
Feeding source to the farm	Local/natural grass	8	53.33
	Silage	2	13.33
	Hay	3	20
	Concentrates	1	6.67
	Other	1	1.67
Source of concentrate feed for farm	Local area	1	6.67
	Market	11	73.33
	Manually prepared	3	20
Feeding related challenges	Feed shortage	9	60
	Food poisoning	2	13.33
	Food contamination	4	26.67

Table 4. Management activities and systems.

Management	Variables	No. of respondent	Percentage
Housing systems of dairy cow	Traditional	0	0
	Semi-traditional	1	6.67
	Semi-modern	4	26.66
	Modern/concreted floor	10	66.67
Frequency of cleaning of dairy cows and floor per week	Not at all	0	0
	Once a week	0	0
	Twice a week	1	6.67
	Three times a week	13	86.66
	Four times a week	1	6.67
Practical milking order	Yes	14	93.33
	No any order	0	0
	Sometimes	1	6.67
	Less important	0	0
	I do not know	0	0
Feed supply to dry and milking cows share equally	No	13	86.66
	Yes	1	6.67
	Not known	0	0
	Sometimes	0	0
	I do not know	1	6.67
House construction has waste disposal canal	Yes	2	13.33
	No	12	80
	I Do not Know	1	6.67

Table 4. Contd.

Diseased separated from health animals	**Yes**	**2**	**13.33**
	No	13	86.67
Common diseases in dairy farm	Carbohydrate engorgement	6	40
	Diarrhea	6	40
	Calf scour	1	6.67
	Mastitis	2	13.33

observation considering animal welfare, house, management, feeding and watering and questionnaire survey was collected regarding health management, back ground history, productivity, and other related issues. After observation and questioner collection and the results from dairy farm obtained were compared with scientific standards of dairy herd health management and production. The house of university dairy farm was constructed with ventilation, urinary disposal canal, feeding and watering canal, delivery pen, calf pen, group heifer pen, milk dispatch room, office and feed store. Known and very visible weakness of the farm was poor waste disposal system and lack of separate rooms for delivery, milking, calves.

The current status of dairy farm is well managed as compared to previous management system. That is after parturition within 24 h the calf was separated from mother and get in calf pen. Major management problems in the farm recorded was diseases (lameness) and other management problems or feeding problem (carbohydrate engorgement, lameness, mastitis, calf scour, diarrhea, decreased production). On the basis of the above conclusion; the followings are recommended:

(i) Improving the use of pasture through appropriate grazing land management systems
(ii) Lactating cows, dry cows and suckling calves are supplemented with concentrates with measurement
(iii) To improve the frequency of mastitis and lameness, more standard way of milking and special consideration should be given to environmental hygiene
(iv) The house of the dairy has to have proper waste disposal system that minimize contamination of environment and the dairy cows.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

The efficacy of Ovopet® in the treatment of hip dysplasia in dogs

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Hip dysplasia is a widespread condition that can affect dogs of all ages. Hip dysplasia is caused by a subluxation in the hip joint. This leads to the development of osteoarthritis that causes inflammation and pain. At this sight, the efficacy of a supplement with Ovopet®, eggshell membrane, was evaluated together with its tolerability and safety. Forty client-owned medium sized arthritic dogs were treated daily for a period of 40 days with placebo or Ovopet®. Every ten days, the dogs were evaluated for functional limitation and joint mobility (hip functional scale), muscular atrophy and mobility range (extension-flexion rating). Dogs were also examined for blood analysis (inflammatory markers), and sonographies of the hip joint space were taken before and at the end of the study. Performances in daily life activities and vitality assessed by the owners were also recorded. Based on these observations, significant ($p<0.05$) reduction in muscular atrophy and improvement of mobility range was noted in Ovopet® treated group. Parameters such as as starting lameness, walking lameness, running and playing resistance and limitation to little jumps also experienced a significant ($p<0.05$) improvement. The parameters for function and positive behaviour description and pain sensation assessed by the owners showed a significant improvement ($p<0.05$) since day 20 of treatment. Based on recorded data, Ovopet® (15 mgKg^{-1}dog) treatment provides a significant improvement, reducing the pain the dog has and therefore improving physical function.

Key words: Ovopet®, hip dysplasia, dogs, osteoarthritis, feed supplement, eggshell membrane.

INTRODUCTION

Osteoarthritis (OA) is a painful and progressive disease that involves the permanent, long term deterioration and destruction of the cartilage and components surrounding the joints, which results in chronic pain, inflammation and decreased mobility (Henrotin et al., 2014).

The hip is one of the most common areas of OA in the body. Hip dysplasia is highly prevalent in dogs, and affects primarily large and giant breeds of dogs, never the less this disease can also occur in medium-sized breeds and rarely in small breeds (Butler and Gambino, 2017). German Shepherds, Labrador Retrievers, Rottweiler's, Great Danes, Golden Retrievers and Saint Bernards appear to have a higher incidence, however these are all very popular breeds and may be over represented because of their popularity (Butler and Gambino, 2017). Dogs of all ages are subject to hip dysplasia and the

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resultant OA (Gail et al., 2001).

Hip dysplasia is associated with abnormal joint structure and a laxity of the muscles, connective tissue, and ligaments that would normally support the joint. As joint laxity develops, the articular surfaces of the two bones lose contact with each other (Kyriazis and Prassinos, 2016). This separation of the two bones within the joint is called a subluxation, and this causes a drastic change in the size and shape of the articular surfaces (Butler and Gambino, 2017). OA of the hip is the result of the degeneration of the joint due to a laxity caused by hip dysplasia (Butler and Gambino, 2017). When a dog has hip dysplasia, the joint wears out abnormally and the protective cartilage on the surface of the joint deteriorates and the resultant bone-to-bone contact creates pain (Butler and Gambino, 2017).

Moreover, the degenerative changes of OA appear frequently causing inflammation and pain (Henrotin et al., 2014). Pain is also related with the inflammation of the joint (synovitis) (Henrotin et al., 2014). This pain makes the dogs shift most of their body weight to the front end. As a result, dogs showing few clinical symptoms may develop increased shoulder musculature while hind limb musculature remains under-developed (Kyriazis and Prassinos, 2016). As damage to the joint progresses and secondary OA sets in, symptoms of stiffness and lameness may be present (Kyriazis and Prassinos, 2016).

Some forms of degenerative joint disease can be treated with surgery but drugs are the most common therapy for OA treatment. Nevertheless, pharmacological treatment is limited to clinical signs alleviation (Comblain et al., 2016). In this way, non-steroidal anti-inflammatory (NSAIDs) are commonly prescribed to address anti-inflammatory mechanisms. Unfortunately, the use of NSAIDs may be associated with detrimental effects, especially gastrointestinal side effects (Comblain et al., 2016). Some clinical studies have highlighted the beneficial effects of dietary supplement for the treatment of OA in dogs (Comblain et al., 2016).

Glucosamine and chondroitin are two compounds that have been widely used for the treatment of OA in animals. These aminosaccharides act as a preferred substrate for the biosynthesis of glycosaminoglycan(GAG) chains, and subsequently for the production of aggrecan, main proteoglycan used by chondrocytes to build the cartilage extracellular matrix (ECM). They also exert anti-inflammatory and anti-catabolic effects through the inhibition of nuclear factor κB (NF-κB) binding activity (Comblain et al., 2016).

Ovopet® is an innovative ingredient obtained from egg shell membranes at Eggnovo S.L. via a patented process. Eggshell membrane discovery as a natural source of glycosaminoglycan's, such as chondroitin sulfate and hyaluronic acid among others, has led to the consideration of this product as a potential approach for the treatment of OA as shown in a previous study (Blasco et al., 2016).

The goal of the present study is to assess the effectiveness of Ovopet® in the treatment of hip dysplasia and the consequent OA in dogs.

MATERIALS AND METHODS

Dog selection

A group of adults (63% female), privately owned arthritic dogs were used in this study. Their age was between 1 to 13 years old, with an average of 8±0.56 years. All the participants were medium-sized dogs. Their body mass ranged from 15 to 40 kg, with an average of 34±1.05kg. The body condition of the 66% of the dogs was ideal according to the body condition score published in the manufacturer's site (http://www.affinitypetcare.com/veterinary/obesity/obesity_dog/flash/bcs.html). The German Shepherd was the most common breed (40% of dogs) followed by Labrador and Golden (15% of dogs each). All dogs had radiographic evidence of hip dysplasia. They were recently diagnosed dogs in all cases, so they had not been previously treated with chondroprotective supplements or diets. Besides the afore mentioned stated inclusion criteria, the owner had to describe at least one of the following signs: difficulty to stand up, difficulty to jump, difficulty to climb stairs or clear lameness. The study protocol was performed in compliance with national guidelines for research onanimals. Throughout the study, dogs remained with their owners. The owner's consent was obtained at the beginning of the study.

Nutraceuticals

Ovopet® was obtained from Eggnovo S.L. (Navarra, Spain) in a sustainable and environmentally friendly manner without the use of chemicals. It consists of egg membranes separated from eggshells by a patented process. Compositional analysis of Ovopet® has identified a high content of protein (collagen types I-V-X, elastin, keratin) and moderate quantities of GAGs (chondroitin sulfate, HA) and glucosamine. Snacks were prepared by a Spanish pet food manufacturer.

Study protocol

The randomized and double-blind with placebo study was carried out in cooperation with Eudald Toralles and Sentmenat Veterinary Clinics (Barcelona). The veterinary monitoring was performed at days 0, 10, 20, 30 and 40. Veterinary Clinics were in charge of recruiting the dogs with hip dysplasia, getting the owners signed consent and performing the standard clinical tests according to the study protocol. If the inclusion criteria were met, the dogs could take part in the study. Firstly, the veterinary filled in the dogs' data (breed, age, body condition, sex and diet), did a clinical examination of the dog before starting the treatment and evaluated the OA grade with the Kellgren-Lawrence scale (Kohn et al., 2016). Based on the weight of the dog the veterinary established, the amount of fodder and the number of snacks that dogs had to intake during the study. This amount was revised in every veterinary visit to adjust the dosage of fodder. The performed protocol in each assessment day was as follows:

(1) To assess the muscle atrophy by measuring the perimeter of the rear legs
(2) To evaluate the extension-flexion range of the hip

(3) To fill in a hip functional scale questionnaire
(4) To fill in an evaluation questionnaire together with the owner
(5) To fill in the side effects questionnaire
(6) To draw blood for measuring inflammatory blood markers (at day 0 and 40)
(7) To make a sonography of the hip (at day 0 and 40)

Two groups of patients were established, one taking the chondroprotective supplement with Ovopet® (N=30) and the other group taking the placebo supplement (the same recipe but without Ovopet®) (N=10). The treatment lasted 40 days and the recommended daily dose of Ovopet® was 15 mg Kg^{-1} dog day^{-1}. The only authorised pharmacological treatment, when the lameness was intense or the quality of life decreased markedly was Metacam. The owners and the veterinary noted down if the dog needed rescue drugs.

Kellgren-Lawrence classification of OA degree

The OA degree (from 1 to 4) was classified using the Kellgren-Lawrence grading scale. The grades in the scale are described as follows:

Grade 1: Doubtful narrowing of joint space and possible osteophytic lipping.
Grade 2: Definite osteophytes, definite narrowing of joint space.
Grade 3: Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis and possible deformity of bone contour.
Grade 4: Large osteophytes, marked narrowing of joint space, severe sclerosis and definitedeformity of bone contour.

Assessment of muscular atrophy

The circumference of each thigh was measured at standard anatomical references based on the Bioarth assessment scale (Villaret al., 2016; Cuervo et al., 2014). The same investigator performed all measurements using a measure ribbon.

Assessment of flexion-extension range

To evaluate the hip mobility range, the veterinarian measured the flexion and extension degrees of the hip every ten days based on the Bioarth assessment scale (Villaret al., 2016; Cuervo et al., 2014). The range of movement was measured bilaterally for the hip using a goniometer. These measurements were taken for the maximum possible extensionand flexion values.

Hip functional scale

The hip functional scale is an adaptation of the Bioarth assessment scale (Villaret al., 2016) to quantify the degree of OA. This is the questionnaire that the supplement manufacturer R&D department trained personnel employs for researching. The questionnaire contains two different sections: functional limitation and joint mobility. The functional limitation assesses the lameness before starting to walk, the lameness during walk, the resistance to walk, the resistance to run and play, the difficulty to climb stairs and the difficulty in little jumps. The joint mobility assesses the pain at manual mobilisation of the hip, the pain during palpation and the pain during movement. These parameters were assessed by the veterinarian in the clinical visits every ten days. The scale for most of the questions goes from one (no functional limitation or no pain) to four (complete functional limitation orextreme pain). However, some items use a three-point scale, such as, difficulty in little jumps,pain during palpation and pain during movement.

Evaluation questionnaire with the owner

The evaluation questionnaire that the veterinarian completed with the owner is based partially on the Canine Brief Pain Inventory (Brown et al., 2018) and is divided in three sections: description of function, description of positive behaviour and visual scale.The description of the function compiles different questions in relation to the general activity of the dog, the ability to standing up from lying down, the ability to walk, the ability to run and the ability to climb stairs. The description of positive behaviour includes two questions: mood and feel like playing. The visual scale refers to the severity of the pain that the owner thinks that his/her dog suffers. Each section has different items and each item has a numerical rating scale from 0 to 10. Zero means no pain (or that pain does not interfere) and ten means extreme pain (or that pain interferes completely). Besides individual measurements for each parameter we obtained an average value for all the parameters included in the description of the function and another for the description of positive behaviour. The improvement with respect to the beginning of the study was calculated for each treatment (mean day 40 – mean day 0) x100 / mean day 0). We also calculated the difference in improvement between therapy and placebo (mean treatment – mean placebo).

Blood analysis

Blood was obtained from the cephalic vein for biochemical analyses of inflammation markers. It was collected under aseptic conditions in 5 ml tubes, and then centrifuged for 5 min at 2000 rpm to collect serum. Serum samples were analysed for Tumour necrosis factor-α (TNF-α) and nitric oxide (nitrite/nitrate) (NO). TNFα assay was performed using canine TNFα commercial ELISA set from R&D (Ref. CATA00, R&D systems, La Joya, CA), following the manufacturer's instructions. No assay was performed as previously described (Miranda, Espeyand Wink, 2001; García-Robledo, Corzo and Papaspyrou, 2014).

Sonography

Sonographic evaluations were performed under routine sedation in all dogs using the Vivid I (GE Healthcare, Wauwatosa, WI, USA) ultrasound system at day 0 and 40 after treatment. Linear transducer 12L-RS of high frequency (5-13 MHz) was used for the ultra sound examination. The animals were placed in dorsal decubit, with posterior members in neutralposition (between 10° flexion and 30° extension, 10° and 30° abduction and 0° and 10°external rotation), the knees forming an angle of 90° between the femur and the tibia/fibula. The images of the coxofemoral joints (both hips) were obtained in three sagittal views parallel to the longitudinal axis of the lateral side of the hip, dorsal to the greater trochanter of the femur. The measurement of the distance between the femur head and the acetabulum was taken for each view and the major value was considered for statistical analysis.

Statistical analysis

Data were analysed using the GraphPad (GraphPad Prism version 6.0 for Windows, GraphPad Software, Inc) program and the Epi Info TM (Epi Info version 7 for Windows, CDC). Data were assessed for normality with the D'Agostino and Pearson normality test, and for homoscedasticity with the Bartlett's test. Parametric analyses were performed with one-way ANOVA with repeated measures followed by Holm Sidak's multiple comparison post-test. Non-parametric

samples were compared with Friedman test for related samples.

RESULTS

All participants completed the study. The percentage of dogs taking part in the supplement study was similarly distributed among grade 1, grade 2, grade 3 and grade 4 of OA. Most of the dogs fulfilled two of the following inclusion criteria (48%): difficulty to stand up, difficulty to jump, difficulty to climb stairs or clear lameness, where the difficulty to stand up was the most common sign. Dogs were not administered rescue pain medication. The possible side effects related to the treatment such as: changes in appetite, vomits, diarrhoea and skin reactions were assessed in each veterinary visit. No significant side effects were seen during the treatment with Ovopet® and the veterinary did not relate the observed symptoms with Ovopet® as similar reactions were seen in the placebo group. Moreover, an initial test was carried out in 10 dogs giving them ten times the recommended daily dosage for 50 days to assess that Ovopet® was safe.

Assessment of muscular atrophy

A gradual improvement in the muscular perimeter in the group treated with Ovopet® was observed along the study reaching a significant improvement of 7.3% in the right ($p<0.0001$) and 8.9% in the left ($p<0.0001$) rear legs after 40 days of treatment with Ovopet® (Figure 1A and B). The placebo group showed only a slight increase (2.8%, $p=0.3155$ and 3.2%, $p=0.0011$) which differs from the continuous improvement showed in both legs of treated dogs since day 20 of treatment.

Assessment of flexion-extension range

To evaluate the hip mobility range, the veterinarian measured the flexion and extension degrees of the hip every ten days. Two patterns were observed depending on the treatment; the control group showed a significant increase in the flexion range (right rear leg, $p=0.0266$and left rear leg, $p=0.0243$) during the study while in the dogs treated with Ovopet® this parameter was significantly reduced ($p=0.0001$) in the right rear leg and it showed a tendencyto decrease in the left rear leg (Figure 1C and D). The opposite occurred with the extension angles, which augmented significantly only in the treated group (right rear leg, $p<0.0001$ and left rearleg, $p=0.0011$) (Figure 1E and F).

Hip functional scale

The functional limitation assesses the lameness before starting to walk, the lameness during walk, the resistance to walk, the resistance to run and play, the difficulty to climb stairsand the difficulty in little jumps (Figure 2A, B, C, D, E and F, respectively). In general, the pattern of each treatment was different. In the treated group of dogs, for the lameness before starting to walk ($p=0.005$), the lameness during walk ($p=0.0389$), the resistance to run and play ($p=0.0038$) and the difficulty in little jumps ($p=0.01$), there was a significant decrease while the placebo group remained stable or even augmented, although not significantly (Figure 2A, B, D and F). The assessment of the resistance to walk ($p=0.0015$) and the difficulty to climb stairs ($p=0.0024$) showed a progressive decrease after treatment which failed to reach statistical significance in the ANOVA post hoc tests. The opposite occurred with the placebo group, which showed a tendency to increase progressively (resistance to walk) or remained stable (difficulty to climb stairs) (Figure 2C and E).

The joint mobility assesses the pain at manual mobilisation of the hip, the pain during palpation and the pain during movement, parameters that the veterinarian measured in the clinical visits every ten days (Figure 3A, B and C respectively). In the group treated with Ovopet® there was a gradual decrease in pain since day 10 until the end of the study. There were statistically significant differences in the pain at manual mobilization of the hip ($p=0.0008$) and in the pain during palpation ($p=0.0193$) while the pain during movement only showed a tendency to decrease. Evaluation questionnaire with the owner compiles different questions in relation to the general activity of the dog (Figure 4A), the ability to stand up from lying down (Figure 4B), the ability to walk (Figure 4C), the ability to run (Figure 4D) and the ability to climb stairs (Figure 4E).

At the end of the study, there was a 5,6% of improvement in the description of the function between thetreated and the placebo group. Focusing on each item, the general activity of the dogs that took Ovopet® increased significantly ($p<0.0001$) since the beginning of the study while the general activity of the placebo group was maintained stable along the research. Similarly, in the rest of the items there was a progressive and significant improvement in the treated dogs compared to the placebo group: ability to rise from lying down ($p<0.0001$), ability to walk ($p<0.0001$), ability to run ($p<0.0001$) and ability to climb stairs ($p<0.0001$).

The description of positive behaviour includes two questions: mood (Figure 5A) and feel like playing (Figure 5B). In both items there was a significant ($p=0.0006$ for mood and $p<0.0001$ for desire to play) improvement between Ovopet® and placebo from day 20 on. This progress was 7,6% at day 40. The visual scale refers to the severity of the pain that the owner thinks that his/her dog suffers.In the placebo group the pain maintained stable along the study while in the group treated with Ovopet® there was a progressive and significant ($p<0.0001$) decrease in pain (Figure 5C). The significant

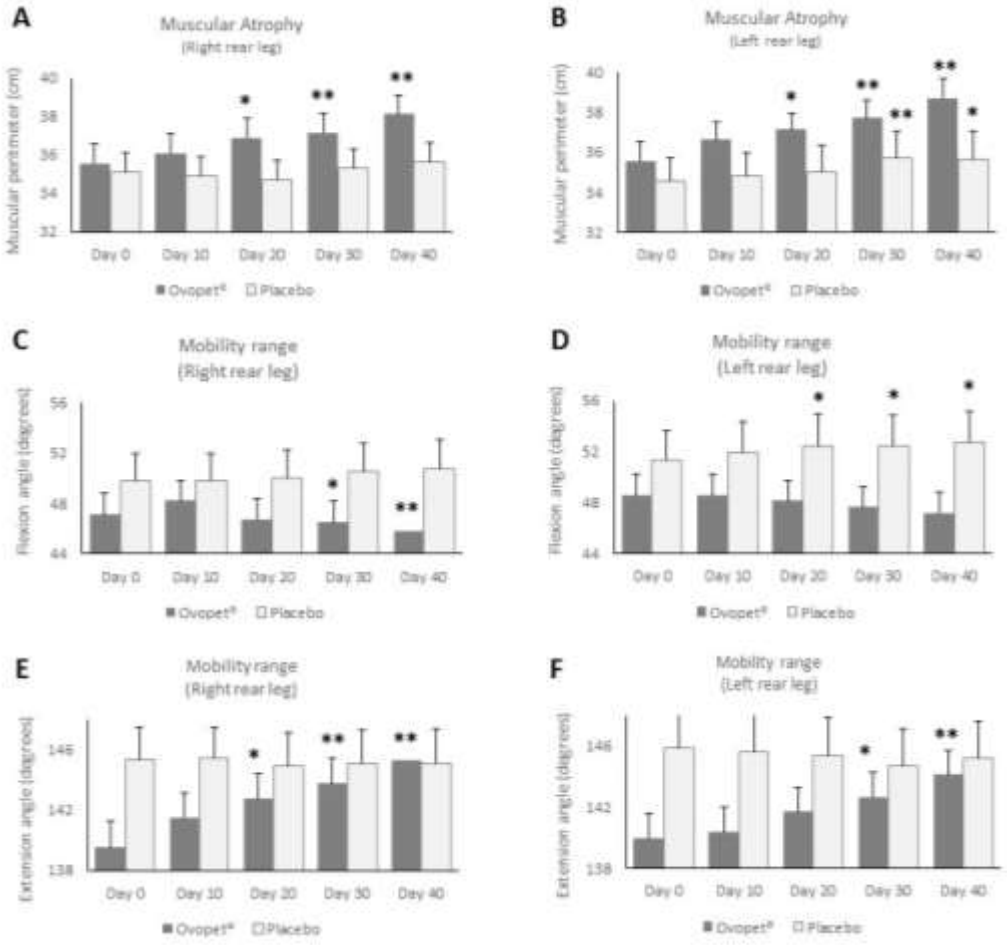

Figure 1. Evolution of the muscular perimeter and the mobility range (flexion and extension) during the treatment with Ovopet®. Muscular perimeter in the right (A) and in the left rear leg (B).Flexion of the right (C) and the left rear leg (D).Extension of the right (E) and the leftrear leg (F). Black asterisks indicate significant differences ($p \leq 0.05$) when compared to basal values in the post-test. Values are represented as mean ± SEM.

decline in pain in the treated group was 46,9% at the end of the treatment compared to day 0. Moreover, the difference in pain between the placebo and the Ovopet® group at day 40 was 37,9% less pain in the Ovopet® group.

Blood analysis

Anti-inflammatory blood markers, such as NO and TNF-α, were analysed before treatmentand at the end of the study. A change in NO levels from 19.4 to 35.6µmol/L reflects an 83.6% increase in NO in the placebo group ($p=0.0645$) while levels in the Ovopet® treated group remained stable (Figure 6). Serum levels of TNFα did not differ significantly between treatedand placebo group. Most of the values were below the detection levels (data not shown).

Sonography

The hip joint space from the legs was measured at day 0 and at day 40 using ultrasounds (Figure 7). The results showed a significant decrease in the joint space of both legs in the dogs treated with Ovopet® (Figure 7A and B) while in the placebo group the joint space maintained or increased. The improvement in synovitis at the end of the study was 19.6 and 24% in the right and left legs of Ovopet® treated dogs respectively (Figure 7C). The % of improvement in the Ovopet® group was statistically significant as compared with the placebo group in both legs ($p=0.0019$ and $p<0.0001$).

DISCUSSION

Medical treatment of hip dysplasia and OA has greatly improved in the last several years thanks to the

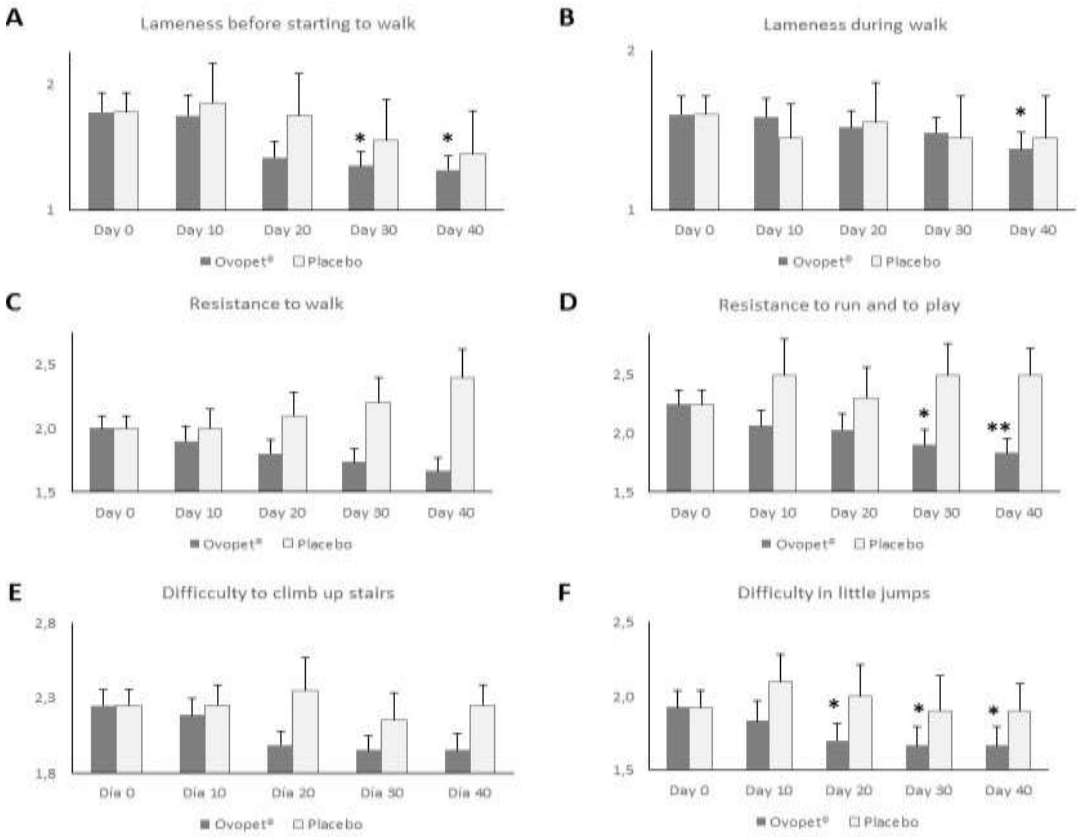

Figure 2. Evolution of the functional limitation parameters in the hip functional scale during the treatment with Ovopet®: lameness before starting to walk (A), lameness during walk (B), 0 resistance to walk (C), resistance to run and to play (D), difficulty to climb up stairs (E) and difficulty in little jumps (F). Black asterisks indicate significant differences ($p \leq 0.05$) when compared to basal values in the post-test. Values are represented as mean ± SEM.

introduction and approval of several new supplements and drugs (Scott et al., 2017). In the present study, we have tested the efficacy of a novel dietary supplement Ovopet®, obtained from eggshell membranes, for the treatment of OA in dogs with hip dysplasia. OA is a condition that causes pain, inflammation and stiffness in joints (Barrouin-Melo et al., 2016) that are associated with ligamentous laxity and muscle weakening (Arden and Nevitt, 2006).

Although there is a lack of consensus in the research community regarding the outcomes that should be assessed in canine OA, validated and objective methods are urged (Belshaw et al., 2016), for instance, the goniometer to measure the hip range of motion (Jaegger et al., 2002), imaging (de Sousa et al., 2017) or the use of validated questionnaires (Belshaw et al., 2016). The efficacy assessment Innvacion-Eggnovo performed in the present study aims to measure both, objective and semi-objective outcomes related to the known symptoms of OA and the physical limitations of the patients, to prove the efficacy of the natural and innovative eggshell-derived dietary supplement.

One of the symptoms associated to OA is the muscle weakening or muscular atrophy (Arden and Nevitt, 2006). In the present study, the muscular perimeter of rear legs was significantly improved in the Ovopet® group together with the range of motion (extension and flexion) of the hip. The decrease in the extension angle of dogs suffering hip dysplasia has been proven to worsen each year of a dog's life leading even to the inability of the dog to run, jump or climb steps (Greene et al., 2014; Dycus et al., 2017). Therapeutic exercises and diverse physical therapies have been employed to improve the hip range of motion and to decline OA symptoms such as muscle atrophy, pain, inflammation (Dycus et al., 2017). At the sight of the results, Ovopet® appears as an alternative treatment to improvethe mobility range of dogs suffering OA and hip dysplasia.

In this study, enhanced joint mobility and functionality were observed in treated dogs. The joint mobility parameter was based on a veterinary physical examination to evaluate pain. To our knowledge, although the assessment of pain has not been validated to be completed by veterinary surgeons, there is a need

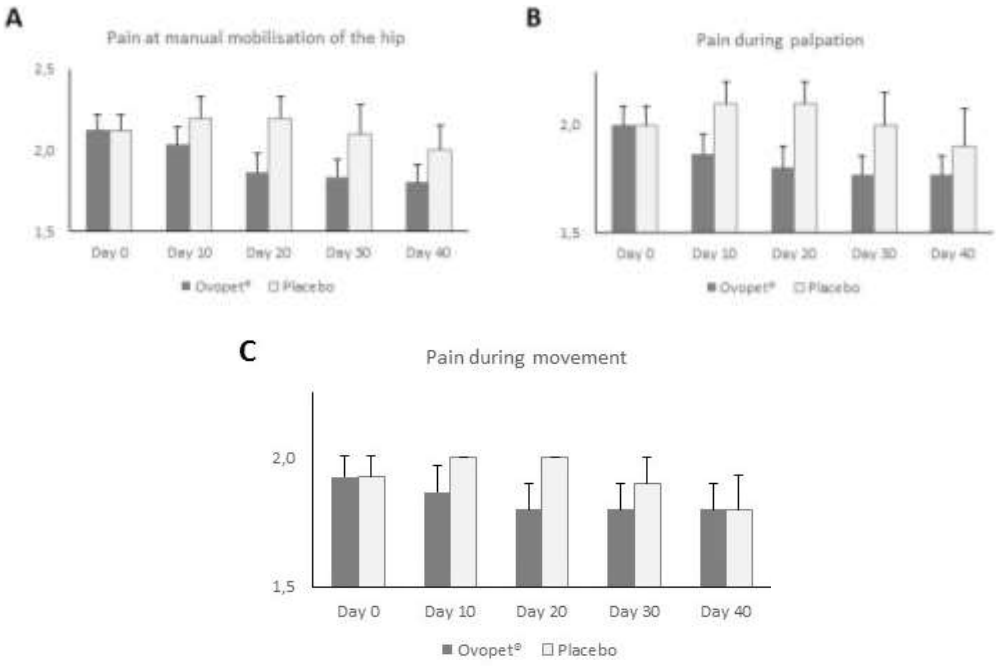

Figure 3. Evolution of the joint mobility parameters in the hip functional scale during the treatment with Ovopet®: pain at manual mobilisation of the hip (A), pain during palpation (B) and pain during movement (C). Black asterisks indicate significant differences ($p \leq 0.05$) when compared to basal values in the post-test. Values are represented as mean ± SEM.

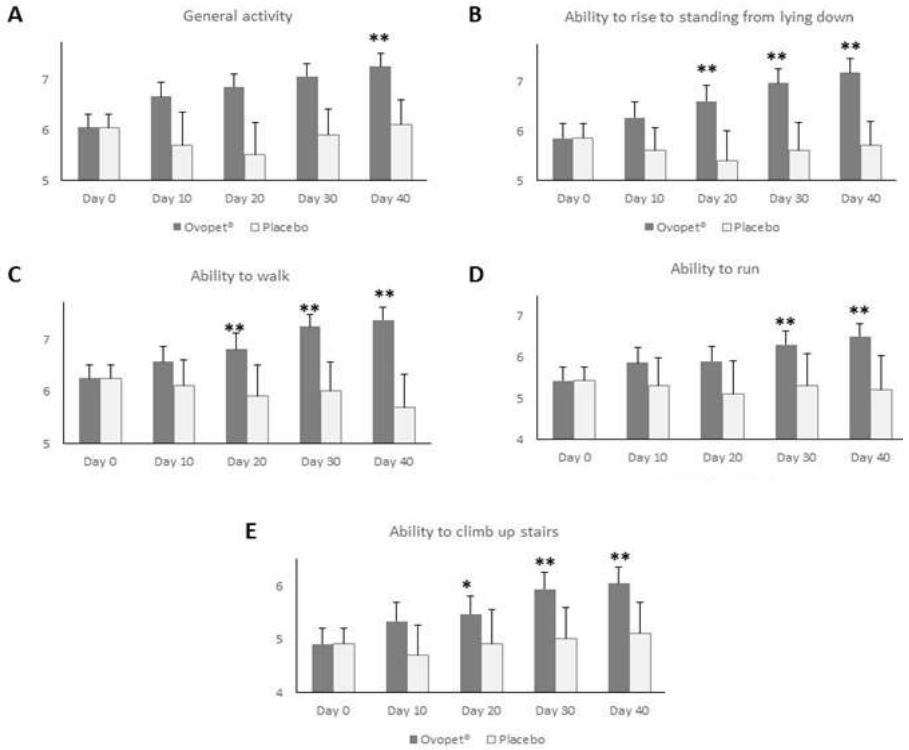

Figure 4. Evolution of the function description in the evaluation made by owners during the treatment with Ovopet®: general activity (A), ability to rise to standing from lying down (B), ability to walk (C), ability to run (D) and ability to climb up stairs (E). Black asterisks indicate significant differences ($p \leq 0.05$) when compared to basal values in the post-test.Values are represented as mean ± SEM.

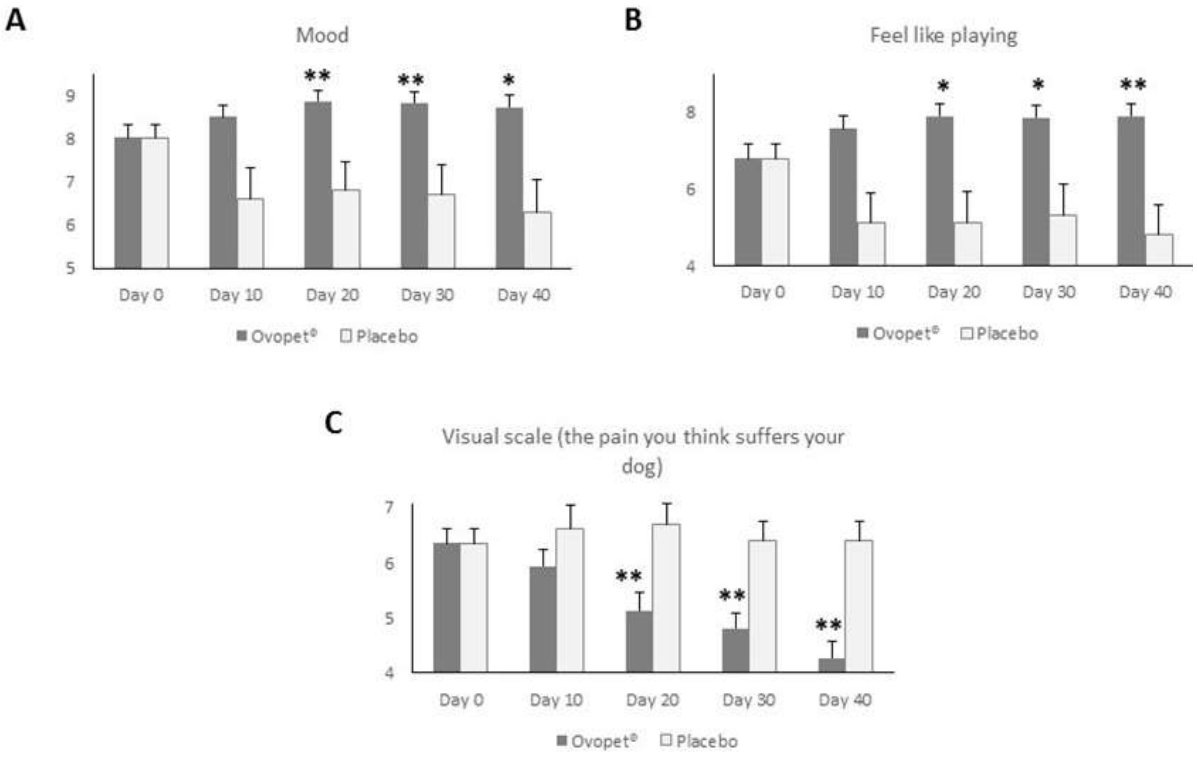

Figure 5. Evolution of the positive behaviour, mood (A) and feel like playing (B), and the pain that the owner thinks that suffers the dog (C) during the treatment with Ovopet®. Black asterisks indicate significant differences ($p \leq 0.05$) when compared to basal values in the post test.Values are represented as mean ± SEM.

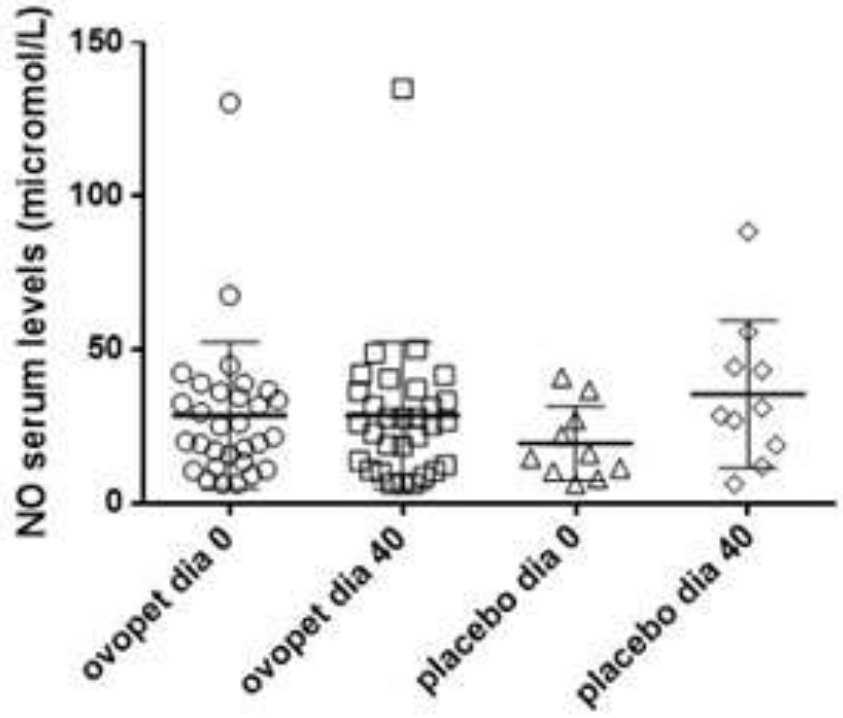

Figure 6. Serum levels of the inflammatory blood marker nitric oxide (NO) before and 40 days after treatment with placebo or Ovopet®. The horizontal line represents the mean ± SEM.

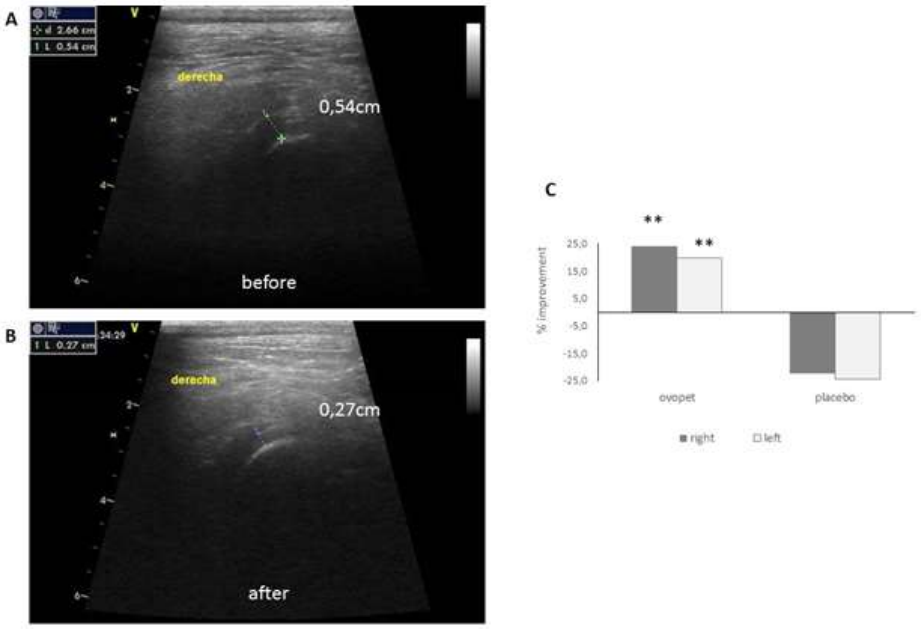

Figure 7. Sonographic evaluation of the evolution of the hip joint space after the treatment with Ovopet®. A: right hip of one treated dog before Ovopet® treatment. B: right hip of the same treated dog after treatment with Ovopet®. C: % of improvement of the hip joint space at the end of the study. (n = 30 for Ovopet®; n = 10 for placebo). Black asterisks indicate significant differences ($p \leq 0.05$) when compared to placebo.

to somehow measure pain by professionals as it is one of the most common outcomes when measuring canine OA (Belshaw et al., 2016). A tendency to decrease pain was measured in Ovopet® group that was not observed in the placebo group. The evaluation made by owners helped us to assess the function description and positive behaviour. In all the categories evaluated in the evolution of the function description there was significant improvement only in the Ovopet® treated group while in the dogs treated with placebo, all the parameters remained stable or even worsened. This is in contrast to what previously was observed in the evaluation of a patient response to treatment, where there was a care giver placebo effect (Scott et al., 2017).

Here, the placebo effect associated with the owners of the dogs regarding the function description was not observed in this study population which is in concordance with the statement of other researchers saying that when looking at the group average change in animal OA, there is no a placebo effect (Gagnon et al., 2017). Similarly, to assess canine behavioural changes affected by pain, an owner-reported instrument was employed. The detailed behaviour-based assessment performed by the ownershave been extensively employed (Belshaw et al., 2014; Essner et al., 2017) and offer the advantage of an extended assessment of dogs in their typical environmentand routine (Sharkey, 2013). These results are in good concordance with the data obtained in the hip functional scale for functional limitation that was assessed by the veterinarian. They are also in concordance with data of mobility range, which revealed an improvement of the flexion and extension angles, and with muscular atrophy, which showed an increase of the rear legs perimeter.

In OA and in synovitis there is an increase in the production of pro-inflammatory factors (Mobasheriet al., 2017; Bhattaram and Chandrasekharan, 2017). The anti-inflammatory properties attributed to eggshell membrane that are potentially responsible for reducing inflammatory cytokines IL-1β and TFN-α are shown in rats (Ruff and De Vore, 2014). There is a need to prove these effects in clinical studies with eggshell membrane products used as nutraceutical compounds for dogs. Our finding of decreased NO blood concentration in treated dogs, although failed to besignificantly different, may indicate that a similar anti-inflammatory mechanism was conferred by Ovopet®. Moreover, hip joint space was analysed by ultrasounds, revealing a significant narrowing of the hip joint space. The results obtained for blood measurements of NO and the sonographic evaluation of hip joint space indicates that Ovopet® was capable of reducing the inflammation.

The goal of dietary supplements intended to treat OA is to provide relief to the major clinical signs of OA. Ovopet® arises as an effective supplement to decline inflammatory pain, functional disability, lameness and

inflammation associated to hip dysplasia and OA, and therefore, to improve the quality of life of dogs. The data obtained in the present study support the use of this dietary supplement as an effective treatment for hip dysplasia and OA. Further research is needed to ascertain and better understand the mechanisms underlying the mode of action of eggshell membrane in osteoarthritic joints. For instance, the measurement of some biomarkers such as fragments of collagen type II (de Sousa et al., 2017) could be useful to gain insights in this complex mechanism.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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A study of Newcastle disease virus in poultry from live bird markets and backyard flocks in Kenya

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A countrywide cross-sectional study was conducted to determine the presence of Newcastle disease virus (NDV) in poultry in live bird markets (LBMs) and backyard poultry farms in Kenya. A total of nine hundred and twenty two (922) poultry in backyard flocks and four hundred and fifty four (454) poultry in LBMs were examined. Overall, NDV was detected in 10.1% (46/454) of the poultry sampled in live bird markets. In backyard flocks, NDV was detected in 3.6% (33/922) of the poultry sampled. Regional variations in NDV occurrence was observed in both live bird markets and poultry flocks. Markets in major towns and cities had significantly ($p<0.05$) higher NDV detection rates. Higher NDV detection rates were observed in backyard farms in Lake Victoria Basin than other regions. Chicken had the highest NDV detection compared to other poultry species. The study detected NDV in apparently healthy chicken and brought forward the probable high importance of carrier birds in the circulation and transmission of NDV and in causing outbreaks. The study also points to the usefulness of reverse transcription polymerase chain reaction (RT-PCR) in screening for NDV to prevent the outbreaks and control of ND in Kenya.

Key words: Backyard poultry, chicken, Kenya, live bird markets, NDV, RT-PCR.

INTRODUCTION

Poultry farming is an important socio-economic activity in the developing countries. It contributes to food security and livelihoods in poor rural households. In Kenya, poultry is particularly important to women, the youth and other vulnerable groups such as human immunodeficiency virus (HIV) affected households.

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Economically, the poultry industry contributes 30% to the agricultural sector and 7.8% of the Gross Domestic Product of Kenya (MoALF, 2015).

The poultry population in Kenya is estimated at 30 million of which 80% are extensively managed backyard poultry (MoALF, 2015). Despite the role poultry plays in the livelihoods of the rural communities and the entire economy of Kenya, poultry production is hampered by a wide range of constraints. Infectious diseases are significant constraints to poultry production around the globe including Kenya (Assam et al., 2011; Chaka et al., 2012; Eze et al., 2015; Olwande et al., 2016; Sandhu et al., 2009; Singla and Gupta, 2012; Sultana et al., 2014). Among various infectious diseases, Newcastle Disease (ND) is the main challenge for the backyard poultry in most households across Kenya (Olwande et al., 2016).

Newcastle disease is a highly contagious and fatal disease of poultry which is notifiable to the World Organization for Animal Health (OIE, 2018). It is caused by Newcastle disease virus (NDV), classified under the genus *Avulavirus* within the family *Paramyxoviridae* (Amarasinghe et al., 2017). In developing countries, the disease is endemic and causes periodic outbreaks that wipe out entire flocks with severe impact on production (ACIAR, 2014) and heavy economic losses annually (Ashraf and Shah, 2014; Hugo et al., 2017). ND is highly prevalent in both commercial and backyard poultry in most countries in Africa (Chaka et al., 2013; Jibril et al., 2014) and Asia (Khan et al., 2011; Sultana et al., 2014). However, in backyard flocks, ND is particularly difficult to control because biosecurity measures are most often entirely lacking (Rimi et al., 2017).

In Kenya, Newcastle disease outbreaks are documented to occur throughout the year in both the cold and dry periods with peaks in April, June to July and September to November (Kemboi et al., 2013). Various management factors on backyard flocks such as keeping of mixed poultry species, mode of disposal of poultry waste and restocking with birds from markets are associated with the occurrence of ND outbreaks in Kenya (Njagi et al., 2010b).

Vaccination of poultry with ND vaccine boosts their immunity and reduces the occurrence of ND (ACIAR, 2014). However, vaccination of backyard poultry remains a challenge due to the complex production dynamics of these flocks (Alexander et al., 2013). Kenya is no exception; millions of backyard poultry remain unprotected and thus vulnerable to ND infection during outbreaks. Losses of up to 100% are reported in unvaccinated poultry population. ND control is therefore of importance in enhancing the productivity of backyard poultry.

A major step in the control of ND is understanding the dynamics of maintenance and spread of the viral agent in between major outbreaks. The virus is thought to be maintained in healthy birds which act as a source of infection to susceptible poultry in a flock. Human activity and increased turnover in live bird markets has been thought to aid in maintenance and transmission dynamics of NDV leading to outbreaks (Abdisa and Tagesu, 2017).

In order to enhance epidemiological knowledge on maintenance and transmission of ND, and develop sustainable and appropriate ND control strategies in Kenya, a study was conducted to examine poultry for the presence of NDV in apparently healthy backyard poultry flocks and live bird markets.

MATERIALS AND METHODS

Study area and design

A cross-sectional study was conducted between November 2015 and March 2016 in backyard flocks and live bird markets in Kenya. Semi-structured questionnaire interviews with poultry keepers and live bird market sellers were conducted to establish the management and trade practices and dynamics as well as health and disease status of birds. At the same time, selected poultry were sampled to establish their NDV status.

Backyard poultry flocks were sampled in three agro-ecological zones; Western Highlands, Lake Victoria basin and Coastal zones were purposively selected based on high population density of backyard poultry in these Zones (KNBS, 2016). Western Highlands is an agricultural hub of Kenya. It is a vast zone and stretches from Kisii and Bomet in the South to Tranzoia, Uasin Gishu, and Mt Elgon area of Bungoma County through to Nandi, Kakamega and Vihiga. This zone lies above 1500 m above level and experiences heavy rains averaging 1500 mm most part of the year and temperatures of between 11 to 20°C. The zone holds half of the country's backyard poultry population (KNBS, 2016).

The Lake Victoria basin is a zone located in the Southwest part of Kenya around Lake Victoria which includes Busia, Siaya, Kisumu, Migori and Homabay counties. This zone is hot and humid and experiences average rainfall of 500 to 1000 mm that occurs twice per year. Population of backyard poultry in the area is estimated at 6 million (KNBS, 2016). On the other hand, the Coast region lies within the Coastal lowlands, which may extend from sub-humid to arid zones.

The population is approximately 3.3 million within an area of 79,686 km^2. Average annual rainfall is poorly distributed and unreliable and ranges from 500 to 750 mm. The mean annual temperature is higher than 24°C, and mean maximum temperature is lower than 33°C.

To sample live bird markets; major markets were selected from five zones namely; Western Highlands, Lake Victoria Basin, Coastal strip, Eastern and Nairobi metropolitan. The sampled markets included; Western Highlands (Chwele, Bungoma, Kitale, Kericho, Kakamega and Bomet); Lake Victoria Basin (Bumala, Kisumu, Homabay, Migori); Coast (Majengo, Marikiti, Kilifi); Eastern (Meru, Makueni) and Nairobi metropolitan (Burma, Kibera, Kawangware, Machakos and Kitengela). The LBMs were categorized into three market types; City, major town and trading centre markets. LBMs selected from Nairobi, Kisumu and Mombasa accounted for the city markets. Figure 1 shows the location of sampled villages and live bird markets.

Sample size and selection of study birds

A total of 922 poultry were sampled from 225 backyard flocks. The

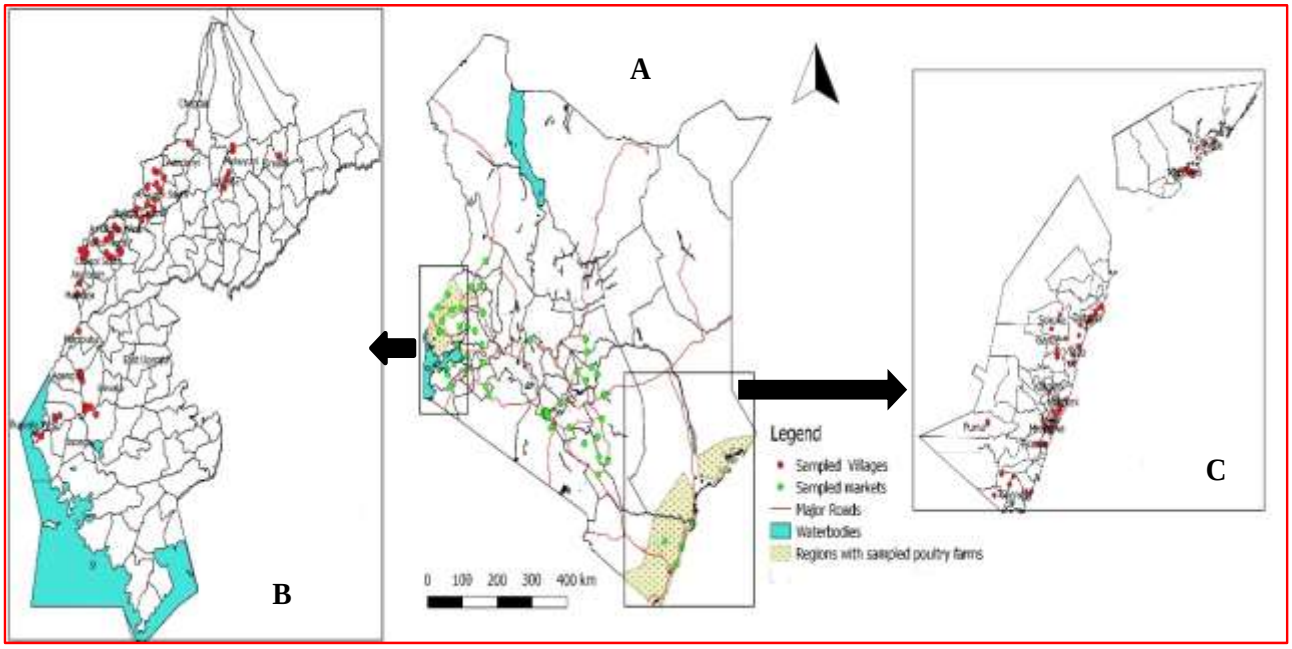

Figure 1. Geographical location of sampled backyard poultry flocks and live bird markets in Kenya, November 2014 to March 2016; (A) A map of Kenya showing the location of sampled live bird markets and poultry flocks (B) Geographical representation of sampled backyard poultry flocks in Western Kenya (C) Geographical representation of sampled backyard poultry flocks in Coastal region of Kenya.

sample size for each of the three zones was calculated based on the formula (Charan and Kantharia, 2013).

$$n=1.962\ p\ (1\text{-}86\ p)/L2$$

Where:

n= required sample size, p= prevalence of ND and L= precision.

Prevalence of ND was estimated to be 30% from a previous study (Njagi et al., 2010a; Olwande et al., 2016), a confidence level of 95% and precision of 5% was used. A multistage sampling criterion was used to select poultry to be sampled. Sub-locations were listed from the three selected zones (Western Highlands, Lake Victoria Basin and Coastal strip) and 5 were randomly selected from each zone. From each sub-location backyard poultry keepers were listed with the help of the field extension staff. Fifteen (15) poultry keepers were selected randomly from each sub location. From each farm, 4 adult birds were sampled. For farms with mixed species of birds; 2 birds of each of the other species were also sampled.

In live bird markets, a total of 454 birds were sampled from 124 traders. The number of birds sampled in each market was 20, assuming a market size of 50 to 100 birds, a minimum expected prevalence of 10% and confidence interval of 95%. Twenty (20) live bird markets (LBM) were selected from the five zones. In the LBMs, five sellers were selected randomly and four birds of were randomly selected per seller for sampling. However, in 7 live bird markets that had high bird turnover, we sampled 7 traders each. This included Kisumu, Kericho, Majengo, Burma, Meru and Chwele markets. Table 1 shows the number of birds sampled in poultry farms and LBMs. To sample birds, we collected tracheal and cloacal swabs in 1000µl of RNAlater®. We transported samples in a cool box and stored at −80°C until processing.

Newcastle disease virus screening

Screening for the presence of NDV in swab samples collected from birds was done using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) (Ganar et al., 2014). This involved extraction of Ribonucleic acid (RNA) from samples using Trizol LS Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Complementary DNA was synthesized from RNA using Superscript® III (M-MLV) reverse transcriptase of the First-strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). Briefly, for each reaction; 5 µl of sample total RNA was combined with 3 µl of 50 ng/µl of random hexamer, 1 µl of 10 mM dNTP mix, 2 µl of DEPC treated water and heated at 65°C for 5 min. We placed the mixture on ice immediately for 1 min to anneal the primers to the 3' terminal sequences of the RNA. The SuperScript™ III reverse transcription mix was then prepared according to the manufacturer's instructions. Each reaction containing; 1µl of 50 U of Superscript™ III Reverse Transcriptase, 1µl of 40 U of RNaseOUT™ Recombinant Ribonuclease Inhibitor, 2µl of 0.1 M DTT, 2µl of 10X First Strand Buffer and 4µl of 25mM $MgCl_2$ were annealed at 25°C for 10 min, extended at 42°C for 50 min. Thereafter, we incubated at 70°C for 15 min to inactivate the reverse transcription enzyme, and later chilled at 4°C. Amplification of NDV was done by conventional PCR using primers previously published by Liu et al. (2008) which target a 535bp region of the virus. The PCR was performed using Taq DNA polymerase and 5 µl of cDNA with the cycling parameters starting with a denaturation step of 95°C for 3 min; followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 3 min, followed by 72°C for 10 min. PCR products were visualized by agarose gel (1.5% w/v)

Table 1. Number of birds sampled in markets and poultry farms in different regions of Kenya between 2014 and 2016.

Regions	Areas with sampled households	Sampled markets	Number of birds sampled	
			Households	Markets
Lake Victoria basin	Aktes, Aturet, Aterait, Budalang'i; Amukura	Bumala, Kisumu, Homabay, Migori	288	58
Western Highlands	Kimilili, Cheptais, Kaptama,Kabuchai, Chwele	Chwele, Bungoma, Kitale, Kericho, Kakamega and Bomet	324	135
Coastal	Dabaso, Gede,Matsangoni, Mkomani, Shella	Majengo, Marikiti, Kilifi	310	71
Eastern	-	Meru, Makueni	-	64
Nairobi metropolitan	-	Burma, Kibera, Kawangware, Machakos and Kitengela	-	126
Total	-	-	922	454

electrophoresis and UV illumination after staining with GelRed™ (Biotium).

Data analysis

Data on management and trade dynamics captured through the questionnaires were entered in a spreadsheet (Microsoft Excel) and linked with the results from NDV screening. Descriptive analysis on management and market practices and NDV positivity was carried out using R version 3.2.3 (R CRAN). NDV detection with 95% confidence intervals (CI) was calculated as the proportion of the number of NDV positive birds on RT-PCR to the total number of samples tested. Frequencies (percentages) for categorical variables were calculated and Chi-square test was used for comparison. Statistically significant difference was accepted at a probability (p) of $p<0.05$.

RESULTS

General Information of sampled backyard poultry farms and live bird markets

Two market types were encountered; open air (64.7%) and enclosed (35.3%). In the open-air markets, birds were placed outdoors either in cages, traditional baskets or tethered to a pole. Enclosures were mainly permanent or temporary structures and birds were kept in stalls or cages. Majority (64.7%) of the sampled markets had between 100 and 200 birds. LBMs were categorized as city, major town or trading centre markets. Two-thirds of the sampled live bird markets routinely offered slaughter services, that is, traded both, live and slaughtered birds. However, only 17.7% of these had designated slaughter facilities. Chicken was the predominant poultry species kept by 98.2% (221/225) of sampled backyard farms, two thirds (65%) of the households also kept other poultry species including ducks, guinea fowls, turkeys, pigeons and geese. Similarly, sampled live bird sellers predominantly sold chicken (98.4%:122/124). While all sampled backyard farms kept only local chicken ecotypes, almost half of market sellers (46%) sold both local chicken ecotypes and exotic chicken (Table 2).

Occurrence of NDV in sampled birds

Figure 2 is a gel image showing the NDV negative and positive samples. Overall, NDV was detected in 3.6% (33/922; range 2.0 to 7.4) of the sampled backyard poultry in farms. Of the positive birds, 32 were chicken and 1 duck. There were regional variations in household NDV detection; Lake Victoria basin had significantly higher NDV detection ($p<0.05$) compared to the other zones (Coast and Western Highlands) (Table 3). Aterait, Aturet and Aktes had significantly higher proportion of NDV positive birds. NDV was not detected in any of the birds sampled in Gede, Amukura and Shella sub-locations. Overall, NDV was detected in 10.1% (46/454) of the sampled birds. NDV detection was significantly higher ($p<0.05$) in open air markets (21.2%) compared with enclosed markets (8.1%). Similarly, there were significant ($p<0.05$) differences in NDV detection across the five sub-regions with Nairobi having the highest detection rate and Coast region having the lowest detection rate (Table 4). In addition, LBMs in the cities and major towns presented higher detection rates than those from trading centres ($p<0.05$). Overall, 19 out of 22 LBM (86.3%) had at least one bird that tested positive for NDV. Amongst the LBMs, Kibera (58.3%, OR = 7.7, $p<0.04$), Homabay (45.5%, OR = 4.6, $p = 0.12$), Kitengela (38.5%, OR = 3.4, $p = 0.197$), Bungoma (40.0%, OR = 3.7, $p = 0.16$) and Burma (33.3%, OR =2.8, $p = 0.303$) had the highest NDV detection rates in that order. NDV was not detected in birds sampled from LBMs of Chwele, Kisumu and Marikiti (Table 4).

DISCUSSION

This study utilized the RT-PCR to detect the presence of

Table 2. Characteristics of sampled live bird markets and poultry farms.

Characteristics	No. households (N=225)	Proportion sampled	SE
Farm type			
Chicken only	113	50.2 (37.3-61.8)	0.04
[1]Mixed species	112	49.8 (38.2-62.7)	0.04
Chicken flock size			
Less than 10	136	60.4 (45.9-72.3)	0.03
11-30	62	27.6 (18.8-34.1)	0.04
>30	23	10.2 (7.1-24.8)	0.04
Type of treatment			
Conventional	72	32.0 (19.7-41.2)	0.04
Herbal	58	25.8 (13.4-32.0)	0.04
None	95	42.2 (23.1-55.5)	0.04
Vaccination			
Yes	53	23.6 (16.6-31.8)	0.04
No	172	76.4 (68.2-83.5)	0.04
Market characteristics	**No of markets (N=20)**	**-**	-
Market enclosure			
Open air	12	60.0 (46.6-71.8)	0.11
Enclosed	8	40.0 (38.2-53.5)	0.11
Bird population			
<100	5	25.0 (16.8-32.7)	0.12
100-200	7	35.0 (22.6-41.9)	0.11
>200	8	40 (28-53.1)	0.11
Slaughter services			
No	8	40.0 (35.2-52.5)	0.11
Yes	12	60.0 (48.2-69.7)	0.11
Category of market			
Trading centre	4	20 (15.5-27.4)	0.13
Major town	9	45.0 (37.3-57.9)	0.11
City market	7	35.0 (29.0-41.8)	0.11

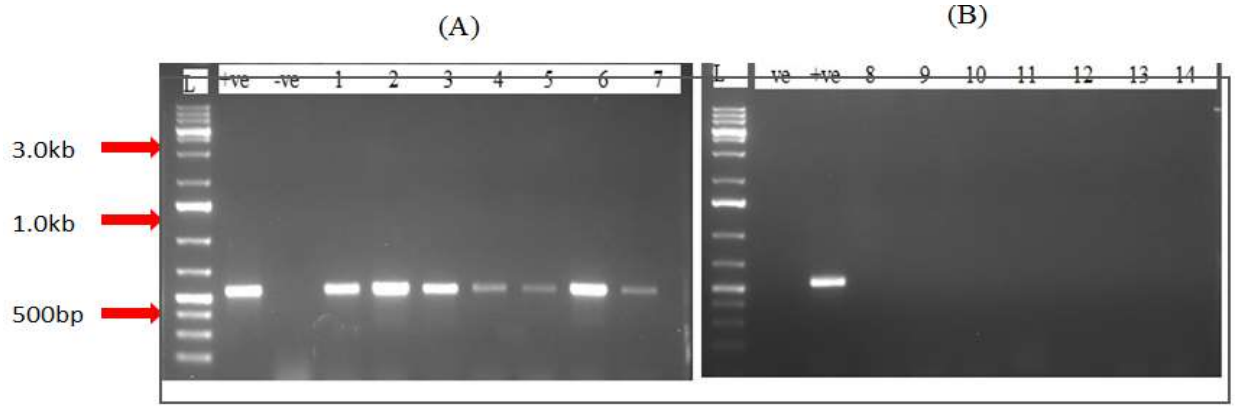

Figure 2. Gel images of amplified products showing (A) Positive samples (1-7) with band size of 535bp (B) Negative samples (8-14); L-represents the marker/ladder used (1KB plus) to estimate the product sizes as indicated by the red arrows; +ve - Positive control; -ve - Negative control.

Table 3. Number and proportion of birds (n=922) that tested NDV positive by geographical location.

Factor/variable	No of sampled birds(N)	No. NDV positive birds (n)	Proportion positive birds (%)	SE	p-value
Region					
Coastal strip	310	4	1.3 (0.5-3.5)	-	0.01**
Western Highlands	324	8	2.5 (1.5-4.9)	-	0.03**
Lake Victoria basin	288	21	7.3 (5.3-9.1)	-	Reference
Sub-location					
Aktes	53	4	7.6 (4.9-10.3)	-	0.01**
Ateriat	57	10	17.5 (12.7-22.9)	-	0.001**
Amukura	58	0	0	-	
Aturet	59	6	10.2 (4.5-14.8)	-	0.001**
Budalangi	60	1	1.7 (0.06-2.9)	-	
Kimilili township	60	3	5.0 (3.5-8.7)	-	0.02**
Cheptais	69	2	3.3 (2.9-7.1)	-	0.03**
Kaptama	62	1	1.6 (0.4-2.2)	-	0.06
Kabuchai	62	1	1.6 (0.4-2.2)	-	0.06
Chwele	75	1	1.3 (0.2-2.6)	-	0.1
Gede	60	0	0	-	
Dabaso	60	1	1.7 (0.06-2.9)	-	0.062
Matsangoni	65	2	3.1 (1.9-4.7)	-	0.03**
Mkomani	67	1	1.5 (0.06-3.6)	-	0.07
Shella	58	0	0	-	Reference

**Proportion with asterisks are different compared to others ($p<0.05$); Parentheses represent 95% confidence intervals.

NDV in poultry in live bird markets and poultry farms in various regions in Kenya in order to establish the NDV status of backyard poultry in between outbreaks. We detected NDV in approximately 3.6% of the sampled poultry in backyard flocks.

Olwande et al. (2016) and Njagi et al. (2010a) reported higher prevalence of ND in backyard poultry flocks in Western and Eastern Kenya. The difference could be due to the difference in the assays used. While Njagi et al. (2010a) and Olwande et al. (2016) used an antibody assay to estimate prevalence, our study utilized a PCR assay. Antibody assays test for long term immunoglobulins and are therefore bound to detect more positives than antigen based tests like PCR (Chaka et al., 2013). However, the antibody tests detect exposure to infection rather than presence of the viral agent as detected by PCR (OIE 2015). This study detected NDV in 10% of sampled poultry in live bird markets. Similar NDV prevalence has been reported in LBMs in other countries in the Eastern Africa region (Byarugaba et al., 2014; Chaka et al., 2013; Mulisa et al., 2014).

The presence of NDV in LBMs has also been reported in other regions of Africa (Jibril et al., 2014; Omony et al., 2016; Solomon et al., 2012) and Asia (Barman et al., 2016). LBMs are reported to contribute to the persistence and spread of NDV and serves as a source of infection to backyard poultry flocks (Jibril et al., 2014). This finding therefore indicates the important role played by LBMs in the epidemiology of NDV in Kenya.

The study detected NDV in apparently healthy poultry in both LBMs and backyard flocks. This suggests the possibility of presence of poultry that are carriers of NDV (Munir et al., 2012). This has grave epidemiological implications because apparently healthy poultry mix with other birds. These NDV positive but apparently healthy birds may be incubating the virus with no obvious clinical signs and may transmit the virus to other birds (Ashraf, and Shah, 2014).

Variation in NDV detection in both LBMs and poultry flocks were observed on the basis of the geographical location. For instance, LBMs in major towns and city markets tended to have a higher frequency of poultry testing positive to NDV than those from trading centre. It is highly likely that this is associated with the volume and diversities of poultry trading and patterns that favour the maintenance and circulation of NDV. For instance, Nairobi metropolitan is a large urban area that attracts poultry centripetally from most parts of the country and as far as from Uganda. Poultry with unknown disease status are mixed, and transported over long distances from various sub-regions and arrive at Nairobi city stressed and immunocompromised with increase susceptibility to

Table 4. Number and proportion of birds (n=454) that tested NDV positive in different zones and markets.

Variable	No. of birds sampled	No. of NDV positive birds	Proportion (%) positive birds	p-value
Sampled regions				
Nairobi	104	24	23.1 (16.8-34.1)	Reference
Western Highlands	153	16	10.5 (7.3-15.6)	0.023**
Lake Victoria Basin	82	6	7.3 (3.6-9.1)	0.021**
Eastern	41	4	9.8 (4.5-11.7	0.032**
Coast	74	4	5.4 (3.6-8.3)	0.013**
Sampled markets				
Kitengela	26	5	19.2 (11.7-22.8)	Reference
Bungoma	17	6	35.3 (19.7-57.6)	0.163
Chwele	28	0	-	-
Kitale	27	0	-	-
Kericho	41	7	17.1 (10.9-29.3)	0.947
Kakamega	22	1	4.6 (3.1-9.6)	0.003**
Bomet	18	2	11.1 (8.1-14.4)	0.146
Bumala	20	0	-	-
Kisumu	28	2	7.1 (5.9-15.6)	0.01**
Migori	16	1	6.3 3.8-13.5)	0.005**
Homabay	18	3	16.7 (10.1-23.7)	0.406
Majengo	31	1	3.2 (0.8-4.8)	0.004**
Marikiti	26	0	-	-
Kilifi	17	3	17.7 (11.3-27.3)	0.746
Meru	17	0	-	-
Makueni	24	4	16.7 (9.9-28.6)	0.654
Burma	25	3	12 (6.8-17.4)	0.303
Kibera	25	8	32.0 (15.5-40.3)	0.035**
Kawangware	28	2	7.1 (5.5-12.2)	0.02**
Machakos	23	6	26.1 (13.0-37.9)	0.045**
Market type				
Open air	237	29	12.2 (6.2-17.9)	Reference
Enclosed	240	25	10.4 (5.2-14.9)	0.976
Bird population				
<100	112	17	15.2 (8.9-18.2)	Reference
100-200	177	14	7.9 (5.2-12.9)	0.079
>200	212	27	12.7 (8.4-17.9)	0.139
Slaughter services				
No	133	16	12.0 (7.3-19.8)	Reference
Yes	321	38	11.8 (9.7-13.1)	0.967
Category of market				
Trading centre	82	4	4.9 (2.0-13.5)	Reference
Major town	206	31	15.5 (12.3-19.4)	0.003**
City market	189	19	10.1 (6.6-17.2)	0.032**

**Proportion with asterisks are different compared to others ($p<0.05$); Parentheses represent 95% confidence intervals.

infectious pathogens (McCarron et al., 2015). Geographical variation as seen in this study could also indicate the locality variation in epidemiology and ecology of NDV, which results in some areas experiencing the inter-epidemic period with low virus activity while others experience high viral activity in early infection (Roy, 2012).

The study revealed low awareness and use of ND vaccination among poultry keeping households in the study area. This is in agreement with Ndegwa et al. (2015) who reported low adoption of ND vaccination in village poultry flocks in Kenya. Vaccination is the most effective control method for ND (ACIAR, 2014), however various socioeconomic factors are thought to limit its uptake in village poultry flocks (Copland and Alders, 2013). This highlights the need for intervention to improve uptake and sustainability of ND vaccination in village poultry.

In this study, reverse transcription-polymerase chain reaction (RT-PCR) allowed rapid detection of NDV directly from diagnostic tracheal and cloacal swabs without the need to first isolate the virus in embryonated eggs. Although virus isolation in embryonated chicken eggs remains the “gold standard” method of NDV identification, it is time-consuming (5 to 10 days) and requires additional period to determine the isolate’s pathogenicity (OIE, 2015). Using RT-PCR, we detected NDV within 24 h. This suggests that the approach could significantly reduce the time required to respond to the introduction of Newcastle disease outbreaks (ND) and impact on the spread of the disease from farms or across regions (Abdisa and Tagesu, 2017). It can also become a powerful tool where targeted control of ND is needed.

Ethical considerations

All interviewed persons gave their informed consent prior to their inclusion in the study. All procedures performed in studies involving handling and slaughter of birds was in accordance with the ethical standards of the animal welfare committee of the Veterinary Research Institute-Kenya Agricultural and Livestock Research Organization.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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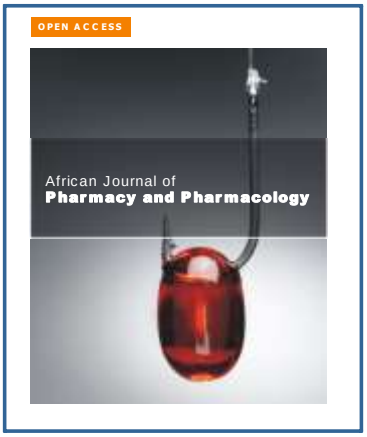

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