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Veterinary Science & Veterinary Medicine

Highlights

- Xanthium Strumarium Leaf
- Study on Prevalence and Monetary
- Quantitative Real Time RT-PCR
- Haemoparasites and Haematological

Discovering Thoughts, Inventing Future

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By Hussein Kafeero Mukasa, Frank Norbert Mwiine, David Kalenzi Atuhaire, Sylvester Ochwo & Ann Nanteza

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Abstract- Foot-and-mouth disease (FMD) is a viral disease of Ungulates; both Artiodactyla and Perissodactyla. The mortality rates are low in adult animals but it affects milk yield and international trade. In endemic countries, diagnosis can be based on clinical signs. But these are shared by other vesicular diseases, so a laboratory is needed to confirm the disease. In Uganda the commonly used assays for the laboratory diagnosis of FMD are NSP ELISA and RT-PCR. Serology using ELISA techniques may fail to distinguish between vaccinated and new infection so compromising its sensitivity. The gel passed PCR is involves a lot of advance sample treatment increasing errors due to carry over which also compromises its sensitivity. This work reports comparative the detection of foot-and-mouth virus by NSP ELISA and RT-PCR with real time PCR which was taken as the gold standard. The assays were compared in terms of sensitivity, specificity and disease prevalence and likelihood ratios. A total of 176 cattle were used from which samples that included epithelial tissues (17.05%) and oral swabs (84.09%) were collected from outbreak cases in Eastern Districts of Mbale and Budaka.

Keywords: NSP-ELISA, RT-PCR, sensitivity, specificity, real time PCR, focal screening.

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Comparative Detection of Foot-and-Mouth Disease Virus by the two Commonly used Assays of NSP ELISA and RT-PCR in Uganda with Quantitative Real Time RT-PCR on Field Samples

Hussein Kafeero Mukasa [α], Frank Norbert Mwiine [σ], David Kalenzi Atuhaire [ρ], Sylvester Ochwo [ω] & Ann Nanteza [¥]

Abstract- Foot-and-mouth disease (FMD) is a viral disease of Ungulates; both Artiodactyla and Perissodactyla. The mortality rates are low in adult animals but it affects milk yield and international trade. In endemic countries, diagnosis can be based on clinical signs. But these are shared by other vesicular diseases, so a laboratory is needed to confirm the disease. In Uganda the commonly used assays for the laboratory diagnosis of FMD are NSP ELISA and RT-PCR. Serology using ELISA techniques may fail to distinguish between vaccinated and new infection so compromising its sensitivity. The gel passed PCR is involves a lot of advance sample treatment increasing errors due to carry over which also compromises its sensitivity. This work reports comparative the detection of foot-and-mouth virus by NSP ELISA and RT-PCR with real time PCR which was taken as the gold standard. The assays were compared in terms of sensitivity, specificity and disease prevalence and likelihood ratios. A total of 176 cattle were used from which samples that included epithelial tissues (17.05%) and oral swabs (84.09%) were collected from outbreak cases in Eastern Districts of Mbale and Budaka. These were used for molecular assays of real time PCR and Conventional PCR using primers and probes targeting the 3D ^{pol} gene. The corresponding sera from all the 176 cattle (100%) were used for NSP ELISA using the Prio CHECK®FMDV NSELISA kit. The sensitivities and specificities of conventional PCR and NSP ELISA were compared with real-time PCR taken as the gold standard. The RT PCR and NSP ELISA had sensitivities of 100.00% (95% CI=86.77% - 100.00%) and 37.50% (95% CI=29.92% - 49.04%) respectively. However, NSP ELISA was more specific than with a RT PCR with sensitivities of 95.83% (95% CI= 89.67% - 98.85%) and 94.67% (95%CI=89.76% - 97.67%) respectively. The kappa value for diagnostic agreement between real time PCR and RT PCR was 0.84 (95% CI = 0.733 – 0.947) at a standard error (SE) of 0.055 showing a very good agreement while that for the agreement between real time PCR and NSP ELISA was 0.35 (95% CI=0.231 – 0.496%) at astandard error (SE) of 0.061 showing a fair agreement. The RT-PCR assay was more sensitive than NSP-ELISA and can be recommended for genotyping and confirmation of FMD in national reference laboratories while NSP ELISA be used for routine screening.

Keywords: NSP-ELISA, RT-PCR, sensitivity, specificity, real time PCR, focal screening.

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I. Introduction

Foot-and-mouth disease (FMD) is a devastating viral disease effecting cloven hoofed animals including cattle, pigs, sheep, and goats. The burden of the disease is manifested through reduced productivity and limitation of international trade in live animals and their product causing serious economic losses (Syed & Graham, 2013). It is a highly contagious, trans-boundary, acute, vesicular disease of cloven-hoofed animals including those in the wild (Alexandersen & Mowat, 2005) which act as reservoirs of the virus for transmission to the domestic animals (Anderson, Anderson, Doughty, & Drevmo, 1975). The causal agent of FMD is called foot-and-mouth disease virus (FMDV). It is a small, non-enveloped, single stranded RNA virus 8.5 kb long with a positive polarity surrounded with icosahedral capsid symmetry belonging to the genus *Aphthovirus* of the *Picornaviridae* family (Boothroyd *et al.,* 1981). It has seven serotypes A, O, C, Asia 1 and the Southern African territories (SAT) 1-3 of which all have occurred in most East African countries (Vosloo, Bastos, Sangare, Hargreaves, & Thomson, 2002) except Asia 1 (Rweyemamu, 1982). Studies have shown that the predominant FMDV serotypes in Uganda are O and SAT-2 (Balinda *et al.,* 2010). Other serotypes reported include SAT-1 and SAT-3 (Vosloo *et al.,* 2002), serotype C was last recorded in early 1971 (Vosloo *et al.,* 2002).

The disease is characterized by short lasting fever, epithelial lesions on the tongue, dental pad and inner mouth area leading to excessive salivation and drooling and lesions on the feet causing lameness

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(Margo, E Chase-Topping Handel et al., 2013). The initial virus multiplication takes place in the pharynx epithelium producing vesicles and lesions and later vesicles appear on the feet (Burrows *et al.,*1981) making the tissues in these areas preferred specimens for diagnosis (Sutmoller, 1992).

In Africa the epidemiology of FMD in Africa is not well understood (Ayebazibwe *et al.,* 2010). The widespread movement of animals, the wide host range of the virus involving wild and domestic animal reservoirs and the presence of multiple strains and sub-strains complicating the epidemiology of the disease.

In Uganda the assays commonly used assays for detection of FMD include conventional reverse transcription polymerase chain reaction (Kasambula, Belsham, Siegismund, H.R Muwanika1, & C, 2012) and antibody ELISA (Mwiine et al., 2010). A recent study by Namatovu et al., 2013 showed that the exclusively collected sample in East African countries in general and Uganda in particular is serum. So in East Africa nearly all the national referral laboratories use antibody ELISA (Namatovu et al., 2013) because it is cheap and can be used to test large volume of samples (OIE, 2009) and does not depend on virus isolation (Paixao et al., 2008) or the expensive molecular techniques such as real time RT-PCR and conventional RT-PCR (Kafeero et al., 2016). In the same study by Namatovu et al. 2013, national reference laboratories are understaffed yet most molecular methods rely on services of well trained staff. This makes antibody ELISA the major assay used in diagnosis of foot-and-mouth disease. In the study by Kafeero et al. 2016, foot-and-mouth disease virus reverse transcription loop mediated assay has been evaluated. It was found to have a comparable sensitivity as the foot-and-mouth disease virus real time RT-PCR giving hope for FMD diagnosis even in the field with high sensitivity. None the less despite its high popularity due to the high sensitivity, specificity, rapidity, cost-effectiveness, field applicability, colorimetric detections (Notomi et al. 2000, Mori et al. 2001, Nagamine & Hase, T Notomi 2002, Matovu et al. 2010, Hopkins et al. 2013, Atuhaire et al. 2014, Kafeero et al. 2016), it has not received a lot of attention.

In this study we report the diagnostic challenges of foot-and-mouth disease virus in Uganda by comparing the results from the two commonly used assays of NSP ELISA and conventional PCR in national and research laboratories in Uganda. The results from the two assays were compared with real time quantitative PCR as the gold standard (OIE 2008).

II. Methods and Materials

a) Study sites

The study was carried out between July 2014 to July 2015 on samples collected from Bungokho county Mbale district and Kamonkoli County in Budaka district during the foot-and-mouth disease 2014/2015 outbreak in our country.

b) Study design

A cross-sectional study was carried out following reports of foot-and-mouth disease outbreaks in Mbale district, Bungokho County and in Budaka district, Kamonkoli County as described in our previous study (Kafeero et al., 2016). Purposive sampling was done based on animals having clinical symptoms like oral lesions, history of infection but having healing lesions and any other asymptomatic cattle in the same farm/kraal or grazing with the symptomatic cattle as reported by the Sub-count Veterinary Officer and or the farmers. The inclusion criteria were cattle with clinical symptoms and the asymptomatic ones in the same farm while exclusion criteria were cattle in farms without any clinical signs or history of clinical signs. All farmers in the villages where sampling was done keep few cattle on average 3-4 animals per house hold and on zero grazing basis, transmission of the virus was assumed to be low between kraals/farms.

c) Sample size determination

The desired confidence interval for sensitivity estimates was 95% (width of 0.05). The specificity of NSP ELISA in previous studies by Diego, Brocchi, Mackay, & De Simone, 1997 was in the range 99%. This was consistent with the studies by Minga et al., 2015 which gave a diagnostic specificity of 99.4% and a diagnostic sensitivity of 64.00%. Sample size at the required absolute precision level for sensitivity was calculated by applying Buderer's formula (Buderer, 1996).For sample size calculation, an estimate of specificity of 95% and a precision of 5% within the 95% confidence level was considered. In addition, a prevalence of 50% as recommended in outbreak cases was used (Buderer, 1996). From this a total of 176 cattle were used from which 176 sera were obtained for NSP ELISA test. 176 tissues/ swabs were obtained for nucleic acid tests of real time RT-PCR as the gold standard (Office International des Epizooties (OIE), 2008) and gel based PCR. The sensitivity, specificity, likelihood ratios and disease prevalence values of the two assays relative to the real time PCR as the OIE recommended gold standard(Office International des Epizooties (OIE), 2008) were established.

d) Sample collection

Samples were collected from Mbale and Budaka Districts of Eastern Uganda during the 2014-2015 foot-and-mouth disease outbreak in Uganda as previously described in our study (Kafeero et al., 2016). Briefly, samples were collected from cattle with clinical signs, those which had healing lesions in the mouth, dental pad or on the feet and the asymptomatic animals in same kraals/ from the same farmer. Three types of samples were collected from animals; epithelial tissues

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(ETs), oral swabs (OSs) and blood. The ETs were obtained from animals with vesicles in the mouth, feet or teats. The OSs were obtained from animals with no clinical signs but sharing the same kraal with those having clinical signs. Blood was obtained from all the study animals from which serum (S) sample was also obtained. Exclusion criterion involved cattle from kraals with no any animal having clinical signs. These were taken as the non-cases.

After the identification of the animal as a case, it was restrained and blood was collected from either the caudal vein or the jugular vein into red top vacutainers by a trained technician using disposable vacutainer needles and given a field identification number. Blood was left to stand at the ambient temperature for serum to separate out and the red blood cells to sediment to the bottom of the tube and later separated in the evening of each day and aliquoted into crayon vials then kept on ice. Epithelial tissues and swabs were collected in the crayon vials containing virus transport medium PBS/Glycerol, given a field identification number and kept in liquid nitrogen. The date of sample collection, district, county, sub-county, parish, GPS number, type of sample collected as well as the presence of clinical signs were all recorded in the field book. All samples were transported to the virology laboratory, College of Veterinary Medicine Animal Resources and Bio security, Makerere University. The tissues/ swabs were kept at -80°C while the serum was kept at -20°C pending further use.

A total of 176 cattle were used in this study. From all animals (n=176), blood to be used for obtaining serum (100%) was obtained. From 30 animals (n=30) epithelial tissues (17.05%) were obtained. From 148 animals (n=146) oral swabs (82.95%) were obtained (Table 1). Serum was used for serological test using the NSP ELISA while swabs and epithelial tissues were used for molecular assays of real-time PCR and conventional PCR.

Table 1 : Total number of samples and sample type collected.

Sample type	Number of Sample (%)
Serum	176 (50%)
Epithelial Tissues	30 (8.5%)
Oral Swabs	146 (41.5%)
Total	352 (100%)

All the epithelial tissue, ET (n=30) and oral swabs from the dental pads, OS (n=146) were used for molecular diagnosis while all the sera samples (n=176) were used for serological tests using the NSP ELISA.

e) The RNA extraction

Total RNA was extracted from 140 µl original epithelial tissue/ swab suspension using Qiagen RNA extraction kit following the manufactures instructions as described in our previous study (Kafeero et al., 2016). Briefly, 140 µl of original epithelial tissue/ swab suspension was added to 560µl Buffer AVL- carrier RNA in the micro centrifuge tube, vortexed for 15 sec to mix and then incubated at room temperature (25°C) for 10 minutes. The tube was briefly centrifuged to remove drops from the inside of the lid, then 560µl of ethanol (96%) was added to the sample and mixed by pulse-vortexing for 15 seconds followed by brief centrifuging to remove drops from the inside lid. Then 630µl of the solution were applied to the QiAmp Mini column in a 2ml collection tube and centrifuged at 6000xg (8000rpm) for 1minute and the filtrate discarded. This procedure was performed twice. Then 500µl of Buffer AW1 was added and centrifuged again at 6000x (8000 rpm) for 1 minute. The filtrate was discarded and the column was placed in a fresh 2ml collection tube. Then 500µl of buffer AW2 were added to the column then centrifuged at 20,000 X g (14,000 rpm) for 3 min and the filtrate was discarded. Then 65 µl of Buffer AVE was added to the column, equilibrated at room temperature for 1 minute then centrifuged at 6000 X g (8000 rpm) for 1 min. The RNA samples were stored at -80°C until required for RT-LAMP and conventional RT-PCR.

f) The cDNA synthesis

This was synthesized using the Invitrogen superscript First-Strand cDNA synthesis kit following the manufacturer's instructions as described in our previous study (Kafeero et al., 2016). Briefly 2µl of 10X RNA primer mix, 0.8µl of 25X dNTPs, 2 µl of 10X RT buffer, 1µl of RNase inhibitor, 3.2µl of RNase free water and 1 µl of Supperscript III Reverse Transcriptase to a 0.5 ml microcentrifuge tube to a total volume of 10 µl. The mixture was vortexed briefly to mix then placed on ice. Then 10µl of RNA sample were dispensed to the reaction tube to make up the total reaction volume of 20µl. The mixture was incubated in a thermal cycler at 42°C for 2 hours followed by termination of the reaction at 80°C for 15minutes. The mixture was chilled at 4°C for 30 minutes then transferred to ice and 1 µl of RNase H added followed by incubation at 37°C for 20minutes to degrade the RNA template leaving only a single stranded DNA product. The cDNA was stored at -80°C until required for PCR and LAMP (Kafeero et al., 2016).

g) Real time RT-PCR reaction

In this study, the primers and probe previously described by Callahan *et.al* (2002) that detect the 3D RNA polymerase encoding gene were used as described in our earlier study (Kafeero et al., 2016). Forward Primer: 5'-ACTGGGTTTTACAAA CCT GTGA-3' Reverse Primer: 5'-GCG AGT CCT GCCACGGA-3' 3D

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Probe: (5'-FAM-TCC TTT GCA CGC CGT GGG AC-TAMRA-3'). This probe labeled with 6- (FAM) at the 5' end and the quencher tetramethylrhodamine (TAMRA) at the 3'end in Real-time RT-PCR reaction detects the $3D^{pol}$ gene sequence in all the FMDV serotypes.

The rRT-PCR reaction was based on one-step procedure combined with reverse transcription and Real-time assay. Therefore Real-time assay was carried out by Superscript III/Platinum Taq one-step rRT-PCR kit (Invitrogen). The composition of the 25 µl reaction/ Master Mix for the One-Step rRT-PCR included the following: 12.5 µl 2x- reaction buffer, 2.0 µl (10 pmol/µl) of each of the forward and reverse primer, 1.5 µl (1.5 µl) of the probe, 5.0 µl extracted RNA, 0.5 µl Superscript 111 RT/Platinum Taq mix, 1.5 µl of molecular grade H_2O. The amplification was done at the following temperature cycle: Reverse transcription (one cycle), 48 °C for 30 minutes, the initial denaturing (one cycle), 95 °C for 10 minutes; then 40 cycles consisting of 95°C for 15 seconds and 60°C for 1 minute and 72°C for 30 seconds. Negative and positive controls were included in each run. PCR amplification was carried out in the thermal cycler Rotor- Gene Q (Qiagen, German)

h) The PCR reaction

The PCR was carried out as previously described by (Moniwa, Clavijo, Li, Collignon, 2007) using primers designed to target the 3D polymerase encoding gene; forward primer: 5′ CACTTCCACATGGA TTATGGAACTG-3′ and the reverse primer: 5′ -ACATCT GAGGGATTATGCGTCAC-3′ ; Gene bank accession number JF749843 that amplified the 260 bp fragment of the highly conserved RNA polymerase (3D) gene of FMDV. Briefly, the 25 µl reaction mixture composed of 12.5 µl 2X TaqMan Universal Master Mix, 1 µl of each of the forward primers and reverse primers , 5.5 µl of PCR grade water and 5 µl of cDNA template. Negative control (nuclease free water) and positive control (field isolate) were included in each run. The reactions were carried out in an HBA Cycler machine (Mj Research Inc. USA). The following conditions: 95°C for 10 min for Taq man polymerase activation, 95°C for 15 sec for denaturation, 58°C for 30 sec annealing , 72°C extension. These three steps were repeated for 35 cycles and a subsequent hold temperature of 12°C was used.

i) NSP ELISA assay

All sera were screened for antibodies against FMDV nonstructural proteins using Prio CHECK®FMDV NS kit (PriomicsLelystad B.V, The Netherlands). The Prio CHECK®FMDV NS kit is a blocking ELISA that detects antibodies against the non-structural 3ABC protein of FMDV of all the seven serotypes. The test plates are coated with 3ABC specific monoclonal antibody (mAb) followed by incubation with antigen (3ABC protein). Hence test plates of the kit contain FMDV NS antigen captured by the coated mAb. The Prio CHECK®FMDV NS kit detects FMDV infected animals independent of the serotype that has caused the infection and independent of the fact that the animal is vaccinated or not.

Standard protocols and procedures were followed according to manufacturer's instructions. Briefly, 80 µl of ELISA buffer were dispensed to all wells, 20µl of Negative Control to wells A1 and B1, 20µ l of Weak Positive Control to wells C1 and D1, 20µl of Positive Control to wells E1 and F1 and 20µl of test samples to the remaining wells. Test Plate was sealed using the enclosed plate sealers and shaken gently then incubated overnight (16hours) at room temperature (25°C).The Test Plate were emptied after the incubation period and washed 6 times with 250µl washing solution (200x) made to a working solution (1x) with de-mineralized water using a micro plate washer (Mrc scientific, Marty Enterprises ltd, Nairobi, Kenya). 100 µl of diluted conjugate was dispensed to all wells and incubated at room temperature for 60minutes at room temperature (25°C).The Test Plates were emptied after the incubation period and washed 6 times with 250µl washing solution using the plate washer as previously described. Then100 µl of Chromogen; tetra methyl benzidine (TMB) Substrate were dispensed to each of the wells and incubated for 20 minutes at room temperature (25°C) .Then 100µl of Stop Solution was dispensed to each of the all wells.

j) Measurement of the optical density (OD) of the samples

The optical densities (OD) of the wells at 450nm were measured within 15minutes after colour development stopped using Multiskan Ascent spectrophotometer (Thermo lab systems OY UK).

The mean OD_{450} value of wells A1 and B1 (OD450 max) for negative control was calculated as;

$$\left(\frac{ODA\ 1 \times ODB\ 1}{2}\right) = OD_{450}max$$

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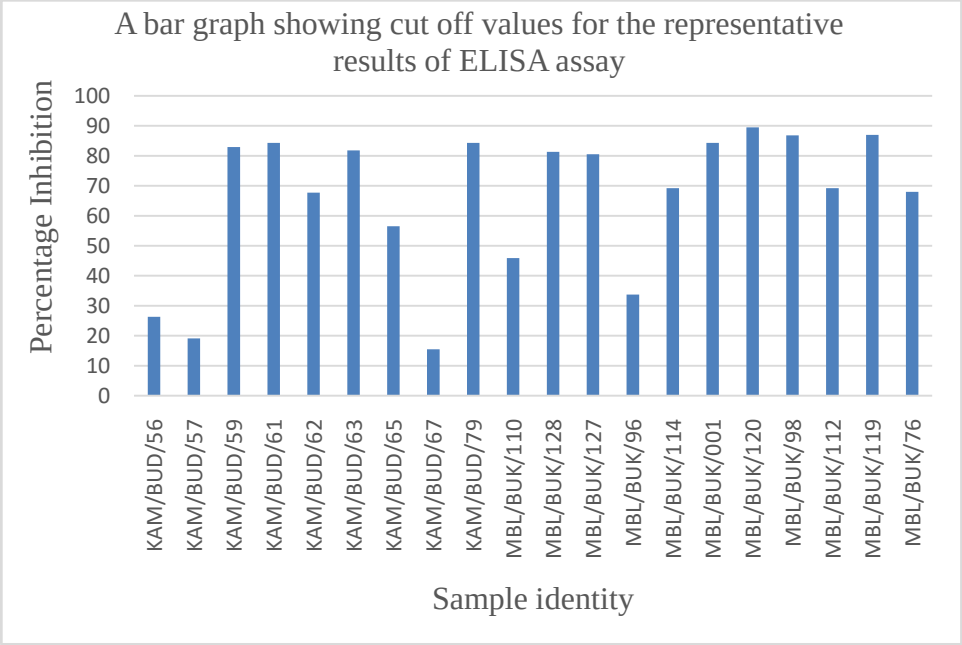

Figure 1 : Representative NSP 3ABC FMDV ELISA results: Positive samples represented by PI ≥50% while negative samples by PI < 50%

The OD 450 values of all samples were expressed as percentage inhibition (PI) relative to the OD450 max.

$$PI = 100 - \left(\frac{OD450\ test\ sample}{OD450\ max}\right) \times 100$$

PI< 50% was interpreted as negative while PI≥ 50% was positive.

k) Detection of amplification products

i. *Real time reverse transcription polymerase chain reaction (rRT-PCR)*

The PCR amplification was carried out in the thermal cycler Rotor- Gene Q (Qiagen, Germany). The successfully amplified target gave an amplification curve and the cycle threshold, Ct at which the target amplicon was initially detected above the background fluorescent levels as determined by the instrument software noted. Each rRT-PCR was perfomed minimally in duplicate and the mean Ct value with standard deviation reported.

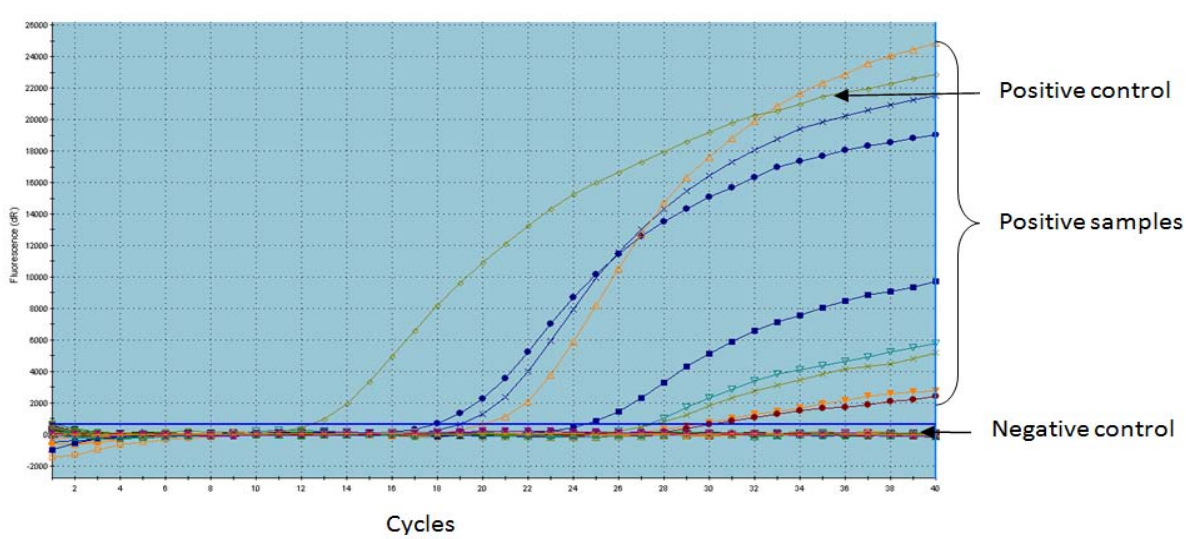

Figure 2 : Real time representative results showing amplification curves: The positive control (field isolate), negative control (Molecular grade water) and positive sample are indicated

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The Ct values in the range ≥40.0 indicated a negative sample and Ct values < 40 indicated positive sample (Figs 2 & 3). In all cases, the positive control gave the minimum Ct value and the negative control gave no Ct.

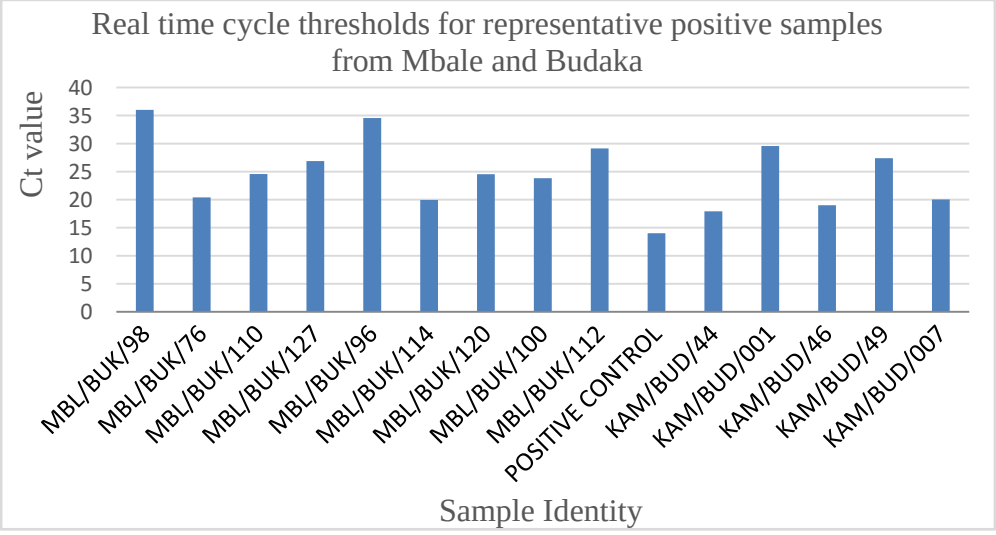

Figure 3 : Representative real time results with Ct values ≤ 40 showing positive animals for FMDV

ii. *Reverse transcription polymerase chain reaction (RT-PCR)*

The 2 µl of the reaction mixture was electrophoresed on a 2% agarose gel electrophoresis after ethidium bromide staining under UV light using a ΦX174 marker (Amersham Biosciences, UK) to determine the size of the PCR product.

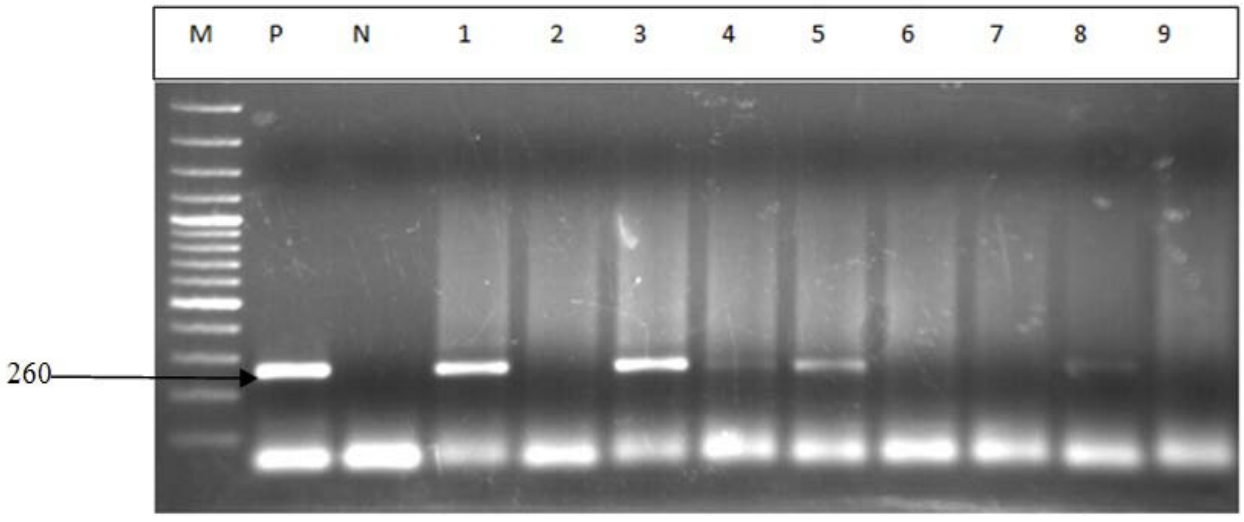

Figure 4 : Conventional PCR representative gel for FMD virus detection

A 2% agarose gel electrophoresis of PCR products: Lane M, DNA 2000bp marker (Invitrogen), lane P is for positive control (field isolate), lane N is negative control (nuclease free water), lanes 1-9 FMDV samples. Lanes 1, 3, 4,5and 8, are positive samples while lanes 2, 6, 7 and 9are negative samples

Positive samples and the positive control gave bands corresponding to the 260bp (Fig. 4) as determined from the marker since it is the size of the 3D pol gene. Negative samples gave no bands.

l) Data analysis

Every sample was tested twice by each of the methods and in case of a disagreement; the test was repeated for all the three assays to come up with the final result. Sensitivity and specificity of each test was then determined as percentages with 95% confidence intervals (CIs). The two tests were then each compared to the reference test/gold standard (rRT-PCR) using Fisher's exact test. The sensitivities and specificities of each test compared to the gold standard were determined. Kappa values to assess the level of test agreement were also determined. All analyses were done at 95% CI.

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III. Results

Table 2 : Summarizes results of two molecular assays of conventional PCR and real time PCR and, the NSP ELISA assay using the Prio CHECK®FMDV NS kit for all the 176 cattle samples.

NSP ELISA	Conventional PCR	Real Time PCR	Number of Cows
Positive	Positive	Positive	24
Positive	Positive	Negative	00
Positive	Negative	Negative	50
Negative	Negative	Negative	92
Positive	Negative	Positive	06
Negative	Positive	Negative	00
Negative	Negative	Positive	02
Negative	Positive	Positive	02

A total of 24 of the 176 cattle tested positive by all the three assays of conventional PCR, real time quantitative PCR and NSP ELISA. A total of 92 cattle tested negative for all the three assays. Real time quantitative PCR identified 34 animals as being positive with FMDV RNA.

The NSP ELISA assay identified 80 out of the 176 animals as positive of which only 30 animals were also positive by the gold standard and 50 negative by the gold standard (Tables 2,3 and Figs 1, 2, 3) giving a diagnostic sensitivity of 37.50% (95% CI=26.92% - 49.04%) and a specificity of 95.83% (95% CI= 89.67% - 98.88%). The RT-PCR assay also identified 24 animals as positive out of the 34 animals identified as positive by real time PCR and missed out 8 animals (Tables 2,3 and Fig. 4) giving a diagnostic sensitivity of 100% (95% CI = 86.77% - 100.00%) and a specifiity of 94.67% (95% CI = 89.76% - 97.67%). These results for both assays NSP ELISA and RT-PCR were statistically significant (P< 0.0001) when analyzed by Fisher's exact test.

Table 3 : Sensitivity and specificity of conventional PCR and one NSP ELISA assays for identification of foot-and-mouth disease virus in serum and swab/ tissue samples from cattle in Budaka and Mbale districts of Eastern Uganda.

Diagnostic Assay	Medium Sensitivity	95% Confidence internal		Medium Specificity	95% Confidence interval	
		Lower	Upper		Lower	Upper
NSP ELISA	37.50%	26.92%	49.04%	95.83%	89.67%	98.85%
RT-PCR	100.00%	86.77%	100.00%	94.67%	89.76%	97.67%

The study cattle FMDV prevalence (Table. 4) was estimated at 45.45% (95%CI=37.95% - 53.12%) by NSP ELISA and 14.77% (95%CI=9.88%-20.89%). The corresponding medium Positive likelihood ration (Table. 4) was 9.00 with a 95% credible interval of 3.31 to 30 for NSP ELISA and 18.75 for RT-PCR at a 95% credible interval of 9.55 to 36.80. Both likelihood ratios show that the test result is associated with the disease with RT-PCR showing a twice chance of post test probability of the disease (Table.4). The kappa value for agreement between RT-PCR and gold standard, test real time PCR was 0.84 (95% CI=0.733 – 0.947) at a standard error (SE) of kappa of 0. 055 showing a very good agreement between the two assays. On the other hand the kappa value for agreement between NSP ELISA and real time PCR assay was 0.35 (95% CI = 0.231 – 0.469) at a standard error (SE) of kappa of 0.061 showing a fair agreement.

Table 4 : Disease prevalence and positive likelihood ratio of conventional PCR and NSP ELISA assays for foot-and-mouth disease virus in cattle from Budaka and Mbale districts of Eastern Uganda

Diagnostic Assay	Medium Disease Prevalence	95% Confidence interval		Medium Positive likelihood ratio	95% Confidence interval	
		Lower	Upper		Lower	Upper
NSP ELISA	45.45%	37.95%	53.12%	9.00	3.31	24.47
RT-PCR	14.77%	9.88%	20.89	18.75	9.55	36.80

IV. Discussion

The aim of this study was to compare the sensitivity and the specificity of the NSP ELISA and conventional PCR which are the commonly used assays in the detection of FMD virus in Uganda (Mwiine *et al.,* 2010, Kasambula, 2011) using real time PCR as the gold standard (Office International des Epizooties (OIE), 2008). Previous studies by Saliki, 2000 have shown that

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disease recognition is essential for any disease control program. This is again paramount in the control of FMD due to the several serotypes and topotypes causing clinically indistinguishable disease (Vosloo et al., 2002).

In the present study, the results of RT- PCR and NSP ELISA were compared with real time PCR as the gold standard. The ELISA results indicated more infected animals than all the three assays on samples from the same animals. It is noted that 24 (13.64%) of the 176 cattle examined were positive on all the three techniques. However, ELISA positive were 80 (45.46%) and ELISA negative were 96 (54.54%) (Table 2, Fig.1) whereas the RT-PCR positive 26 (14.77%) and RT-PCR negative were 150 cattle (85.23%) (Table 2, Fig.3). This gave FMD virus NSP ELISA sensitivity of 37.50% and specificity of 95.83% as well as the FMD virus RT-PCR sensitivity of 100% and a specificity of 94.67%. The FMD virus NSP ELISA sensitivity in the current study was lower than the sensitivity in the earlier study by Minga et al., (2015) which gave a sensitivity of 64.00%. However the specificity in our study was almost consistent with that identified by Minga et al., (2015) of 99.40%. On the other hand, the FMD virus RT-PCR gave a specificity and a sensitivity of 100.00% and 94.67% respectively consistent with the earlier findings by Moniwa M, Clavijo A, Li M, Collignon B, (2007).

The high ELISA positive in this study is not surprising since it has been explained in earlier studies by Alexandersen *et al.,* (2003). Initial virus multiplication occurs in the vesicular epithelium and mucosal swabs in the five days after infection. Later the antibodies remain in plasma for several weeks, or months sampling could have been done in this time when the antibodies have remained in the plasma. Secondly, the high false positives by antigen ELISA assay been explained in earlier studies by Ma *et al.,* (2011). According to their work on overview of ELISA techniques for FMD diagnosis," no single ELISA technique can differentiate infected from vaccinated animals with confidence. This is aggravated by the use of non-purified vaccines in Eastern Africa which elicit antibodies against NSPs increasing chances of false positive (Ayebazibwe, Mwiine, Balinda, Jornehoj, & Alexandersen, 2012). In addition antibodies agaist NSPs do not appear until 8-9 days after infection (Lu et al., 2007) increasing chances of false negative. Consequently to be effective, NSP ELISA should be used for sera sampled in late sub-acute or even under chronic or persistent FMDV infection. Fortunately or un fortunately the antibodies against NSP persist for long post infection and therefore NSP ELISA cannot be used with absolute confidence to differentiate new and previous infection (Sørensen et al., 1998). This is consistent with the findings of the current study. This posits a challenge for FMD diagnosis in our country where NSP ELISA is the most commonly used assay for routine detection of FMD in cattle and other domestic ungulates (Namatovu et al., 2013) due to its simplicity. Conventional PCR though it has demonstrated higher sensitivity and specificity compared to NSP FMD virus ELISA both in earlier studies by Moniwa M, Clavijo A, Li M, Collignon B, (2007) and in our study. However in our country, the RT-PCR for foot-and-mouth disease is restricted to research institutions but in national reference laboratories NSP ELISA is the most commonly used as underlined in the previous study by Namatovu et al., (2013)

V. Conclusions and Recommendations

Our study compared the sensitivity and specificity of the two commonly used assays of NSP ELISA and gel based PCR for the detection of FMD in our country using real time PCR as the gold standard. The NSP ELISA assay has demonstrated a high false positive rate compared to gel based PCR using real time PCR which is recommended as the gold standard in countries whose biosafety levels do not permit them to perform virus isolation including Uganda. The conventional PCR demonstrated a higher sensitivity and specificity as compared to NSP ELISA but it uses sophisticated equipment and requires special training of the laboratory staff, its use for routine screening is not practical. So in Uganda, focal screening of FMD is based on NSP ELISA nearly in all regional and national reference labs due to its simplicity and its ability to screen large volumes of samples. This puts FMD diagnosis in our country in an empirical dilemma yet FMD is a highly contagious disease and its management is contingent upon accurate and timely diagnosis. The high frequency of the misclassification of cattle when using NSP ELISA suggest that FMD prevalence estimates based on NSP ELISA may be inflated, therefore confirmation by nucleic acid techniques should be the priority in national referral laboratories. We recommend the use of RT-PCR in the national reference laboratories for foot-and-mouth disease virus for confirmation, genotyping and to justify fresh infection, otherwise the NSP ELISA can be used for routine screening. We further recommend that more studies be done using large samples to improve on the accuracy of the findings. The scope of the sample types can also be extended to oral pharyngeal fluids in asymptomatic animals. Finally we recommend that vaccine strains should be matched with field strains and purified vaccines should be used to reduce on the false positive rates and hence more reliable results.

Conflict of interest

We declare that we have no competing interests in regards to the authorship of this article or its publication.

VI. Acknowledgement

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Haemoparasites and Haematological Parameters of the One Humped Camel (*Camelus Dromedarius*) Slaughtered in Maiduguri Abattoir, Nigeria

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Abstract- Haemoparasitic diseases account for substantial losses in terms of decreased working capacity, growth and productivity of camels. A survey of the one humped camel (*Camelus dromedarius*) slaughtered in Maiduguri was conducted from January to June, 2016 to determine the prevalence of haemoparasites and their effects on some haematological parameters. Blood samples were randomly collected from 209 camels at the point of slaughter and subjected to standard haematological procedures to determine the white blood cell count (WBC), packed cell volume (PCV), heamoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC). Blood films and Giemsa stained thin smears were prepared on clean glass slides and examined for the presence of haemoparasites. Haemoparasites were identified microscopically to generic level based on morphological features. A total prevalence of 12.6% was recorded for Anaplasma (37.7%), Trypanosoma (33.3%) and Babesia (22.2%), in addition to microfilariae of *Dipetalonema* species (7.5%).

Keywords: camels, haemoparasites, haematological parameters, maiduguri, prevalence.

GJMR-G Classification : NLMC Code: WA 360

HAEMOPARASITESANDHAEMATOLOGICALPARAMETERSOFTHEONEHUMPEDCAMELCAMELUSDROMEDARIUSSLAUGHTEREDINMAIDUGURIABATTOIRNIGERIA

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Haemoparasites and Haematological Parameters of the One Humped Camel (*Camelus Dromedarius*) Slaughtered in Maiduguri Abattoir, Nigeria

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Abstract- Haemoparasitic diseases account for substantial losses in terms of decreased working capacity, growth and productivity of camels. A survey of the one humped camel (*Camelus dromedarius*) slaughtered in Maiduguri was conducted from January to June, 2016 to determine the prevalence of haemoparasites and their effects on some haematological parameters. Blood samples were randomly collected from 209 camels at the point of slaughter and subjected to standard haematological procedures to determine the white blood cell count (WBC), packed cell volume (PCV), heamoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC). Blood films and Giemsa stained thin smears were prepared on clean glass slides and examined for the presence of haemoparasites. Haemoparasites were identified microscopically to generic level based on morphological features. A total prevalence of 12.6% was recorded for Anaplasma (37.7%), Trypanosoma (33.3%) and Babesia (22.2%), in addition to microfilariae of Dipetalonema species (7.5%). The haematological parameters of infected and uninfected camels were within normal range but, there was a significant difference ($p<0.05$) in Mean±SE RBC counts of infected and uninfected camels, while thee Mean±SE PCV, Hb, WBC, MCV, MCB and MCHC were comparable ($p>0.05$). We report the first occurrence of *Dipetalonema* species in one humped camel in Maiduguri. The role of camels as carriers or reservoir of haemoparasites for other species of domestic animals in Maiduguri is suspected. Routine screening, treatment including preventive chemoprophylaxis and vector control is recommended. There is also need for molecular studies to identify various species of haemoparasite circulating in trade camels in Maiduguri.

Keywords: camels, haemoparasites, haematological parameters, maiduguri, prevalence.

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I. Introduction

Nigeria has an estimated 87,000 camels of which 30.9% are found in Borno state (FDLPCS). Camels are highly adapted to the semi-arid environments and are confined to the northern borders of Sokoto and Borno states in Nigeria (Schwartz and Dioli, 1992; Blench, 1999). They contribute significantly to the food security of the nomadic pastoral households (El-Naya and Barghash, 2016) and economy of northern Nigeria (FDLPCS, 1992), in addition to their work ability, environmental conservation and the provision of meat and milk (Chafe *et al.*, 2003). Despite their role as a member of the food producing family of livestock, camels have for a long time remained the most neglected animal in the field of scientific research. Furthermore, camels are hardy animals that have a strong adaptation to the harsh weather conditions of arid regions because of their unique physiological characteristics (Karimi *et al.*, 2014).

Camels are known to suffer from various types of parasitic diseases which are major constraint in improvement of camel health (Parsani *et al.*, 2008). Haemoparasitic diseases such as Anasplasmosis, Babesiosis, Trypanosomosis, Theileriosis and Dipetalonemiasis have adverse effects on the health, growth, productivity and working capacity of camels (Ahmad *et al.*, 2004). Various species of haemo-parasites have been reported in camels in Nigeria (Egbe-Nwiyi and Chaudhry, 1994; Bamaiyi *et al.*, 2011) and elsewhere (Abdelrahim *et al.*, 2009; Swelum *et al.*, 2014; Faham *et al.*, 2015; El-Naya and Barghash, 2016). Among haemoparasitic diseases of camels, trypanosomosis also known as "sura" is one of the important and serious disease caused by *Trypanosoma evansi* (Soulsby, 1982). It is mechanically transmitted non-cyclically by haematophagous flies such as *Tabanus*, *Stomoxys* and *Hippoboscid*, which are common in Africa, Nigeria inclusive (Agbede, 2013; Eyob and Matios, 2013). Trypanosomosis in camels usually occurs in chronic form but may be acute when the animal is under stress (Parsani *et al.*, 2008). In the acute form, clinically affected camels show fever, anorexia, marked

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generalized oedema, deteriorate rapidly and die while in the chronic form, there is intermittent high fever, progressive loss of body weight, marked generalized muscular atrophy and occasionally abdominal oedema (Eyob and Matios, 2013). Piroplasmosis due to tick-borne Anaplasma, Babesia and Theileria species have also been reported in Camels in Nigeria (Bamaiyi *et al.*, 2011) and elsewhere (Swelum *et al.*, 2014; El-Naya and Barghash, 2016).

Extra intestinal filarid nematodes like Onchocerca, which produces microfilaria have been reported in camels (Parsani *et al.*, 2008). *Onchocerca fasciata* produces subcutaneous nodules on the head and neck regions while *Dipetalonema evansi* occurs in blood vessels in the spermatic cord, pulmonary arterial tree, right auricle, lymph nodes and mesentery. The microfilaria is sheathed and found in the blood circulation. Basic diagnosis of haemoparasitism relies on clinical symptoms, haematological evaluations and microscopic examinations of blood film or blood smear (Soulsby, 1982).

There has been a steady increase in the number of camels slaughtered for human consumption, as an alternative to goat, sheep and cattle meat in Maiduguri. The increased demand on camel meat is also accompanied by a corresponding rise in prevalence of haemoparasites among them (Egbe-Nwiyi and Chaudhry, 1994; Bamaiyi *et al.*, 2011). It is against this background that this study was conducted to ascertain the prevalence rate of haemoparasites in slaughtered camels so as to design a better preventive and chemotherapeutic approach that could fit into policy formulation in the region.

II. Materials and Methods

a) Study Area and Population

Maiduguri is located in the North east arid zone of Nigeria between Latitude 11° N and Longitude 13° E, and shares international boundaries with Republics of Niger and Chad in the north and Cameroon in the east. It is characterized by a long period of dry season which lasts from October to May and a short period of rainfall from June to September (Hess *et al.*, 1995). The State derives great economic activity from its rich livestock and fishery products (NPC, 2006). Camels are important trade livestock in Maiduguri and also used for meat and milk in addition to their use as portage animals in rural localities. The camels used for this study were trade stock presented for slaughter at the Maiduguri abattoir. The sex were differentiated based on appearance of external genitals while aging was based on rostral dentition as described by Bello *et al.* (2013). Thus, camels <5 years were categorized as young while older (>5years) ones were regarded as adults.

b) Study Design and Sample Collection

A cross-sectional study was conducted from January to June, 2016, to investigate the occurrence of haemoparasites and associated changes in some haematological parameters of slaughtered camels. A total of 209 camels were randomly selected at the point of slaughter, and the age and sex of each sampled animal were observed and recorded appropriately. 10ml of blood was collected into two labelled bottles containing sodium EDTA, by jugular venipuncture at the point of slaughter. The samples were submitted to the Veterinary Parasitology and Pathology Laboratories for parasitological and haematological examinations, respectively.

c) Parasitological Examinations

Blood smears were prepared from fresh whole blood on microscope glass slides (75mm by 25mm), air dried, fixed in methanol and stained with Giemsa's stain while blood films were prepared to examine trypanosomes and microfilaria according to Soulsby (1982). Haemoparasites were identified by direct microscopic examination using X40 and X100 oil immersion objectives of a compound microscope (Olympus, USA), based on morphologic keys described by Soulsby (1982).

d) Haematological Examinations

The blood samples were analyzed for hemoglobin (Hb) by acid hematin (Sahli's) method, packed cell volume (PCV) by microhaematocrit, and total red blood cell (RBC) and total white blood cell (WBC) counts by Neubauer hemocytometer (Brar *et al.*, 2000). The erythrocyte indices (mean corpuscular volume, MCV; Mean Corpuscular Hemoglobin, MCH; and Mean Corpuscular Hemoglobin Concentration, MCHC) were calculated using standard formula (Jain, 1998).

e) Statistical Analysis

Prevalence was calculated as P (%) = d/n where P= prevalence, d= number infected and n= number examined (Thrusfield, 2005), and the 95% confidence intervals on prevalence was calculated using Vassar Stats® statistical computation web site. The student t-test was used to compare the haematological parameters of infected and uninfected camels and $p<0.05$ was considered significant.

III. Results

Overall prevalence of haemoparasites and their 95% confidence intervals (CI) in the one humped camel (*Camelus dromedarius*) slaughtered in Maiduguri is presented in Table 1. Out of 209 blood films and smears examined, 27 (12.4%) were positive for various types of haemoparasites. Young (19.4%) and male (20.8%) camels had insignificantly higher ($p>0.05$) prevalence than the adult (11.8%) and female (11.9%) counterparts.

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The 4 types of haemoparasites identified in blood films and stained blood smears of the one humped camels (*Camelus dromedarius*) slaughtered in Maiduguri is shown in Table 2. A total of 3 genera of haemoprotozoa including *Anaplasma* (37.7%), *Babesia* (22.2%) and *Trypanosoma* (33.3%), in addition to *microfilariae* of nematode, *Dipetalonema* (7.5%) were detected.

Mean values of haematological parameters of the infected and uninfected one humped camel (*Camelus dromedarius*) slaughtered in Maiduguri is shown in Figure 1. All the haematological parameters of the infected and uninfected camels examined in this study were within range of normal values. However, the mean values of RBC in the infected and uninfected camels examined in this study were significantly different ($p<0.05$) but the mean values of PCV, Hb, WBC, MCV, MCH and MCHC of infected and uninfected slaughtered camels were comparable ($p>0.05$).

IV. Discussion

The prevalence of haemoparasites in slaughtered trade camels in Maiduguri has progressively increased in the last two decades. Egbe-Nwiyi and Chaudhry (1994) reported 2.5% prevalence, Bamaiyi *et al.* (2011) reported 5.7% prevalence while the current study recorded an overall prevalence of 12.9%. The observed increase in prevalence of haemoparasites in this locality could be attributed to preponderance of arthropod vectors due to favorable micro climatic conditions in the region (Biu and Konto, 2011). Also, previous reports on prevalence of haemoparasites in other species of domestic animals in different parts of the country suggests that haemoparasitism is endemic in Nigeria. Biu *et al.* (2005) reported an overall prevalence of 17.3% in cattle from Maiduguri. Ameen *et al.* (2008) reported a total prevalence of 4.1% in ruminants from Oyo state. Okeiyeto *et al.* (2008) reported a total prevalence of 13% for various species of haemoparasites in pastoral sheep from Kaduna state. Shamaki *et al.* (2009) reported a prevalence of 9.1% for *Trypanosoma* species in cattle from Gombe state. Furthermore, Ademola and Onyiche (2013) reported a prevalence of 5% in ruminants from Ibadan. These reports further validate our findings and suggest that the various species of haemoparasites constantly circulates among different species of domesticated and semi-domesticated animals in Nigeria, with some semi-domesticated species probably serving as permanent reservoir of infection. The role of arthropod vectors in transmission of haemoparasites has been described (Soulsby, 1982; Urquhart *et al.*, 1996), and the transhumant conditions under which camels are traditionally raised in the tropics exposes them to the arthropod vectors of haemoparasites.

The higher prevalence of haemoparasites recorded in younger camels in this study agrees with previous report by El-Naga and Barghesh (2016). Similarly, Ademola and Onyiche (2013) also reported an inverse age related decrease in prevalence of haemoparasites in slaughtered animals in Nigeria. The higher prevalence of haemoparasites recorded in male than female camels in this study is in agreement with Ahmed and Bringa (2014) but disagrees with El-Naga and Barghash (2016) who reported a higher prevalence of haemoparasites in female than male camels. Also, Shamaki *et al.* (2009) reported a higher prevalence of haemoparasites in female donkeys, sheep and cattle than their male counterparts. Generally, male animals under the extensive system of management in which camels are traditionally raised have high natural tendencies of acquiring diseases than the females because they tend to move about in search of mates for courtship and breeding purposes.

All the 3 genera of haemoprotozoa identified in this study were previously reported in camels (Egbe-Nwiyi and Chaudhry, 1994; Bamaiyi *et al.* 2011) and domestic animals in Nigeria (Abenga *et al.*, 2004; Biu *et al.*, 2005; Kamani *et al.*, 2010; Ademola and Onyiche, 2013; Okorafor and Nzeako, 2014; Qadeer *et al.*, 2015) and elsewhere in the world (Soulsby, 1982; Alonso *et al.* 1992). The high frequency of *Anaplasma* species in this study may be due to the abundance of suitable environmental conditions that favours multiplication and survival of the arthropod vectors (Soulsby, 1982; Shah-Fischer and Say, 1989). Similarly, the high prevalence of *Trypanosomes* in the present study may be linked to the abundance of biting flies such as *Stomoxys*, *Tabanus* and Hippoboschids in the region (Agbede, 2013), and the transhumant conditions under which camels are reared may increase their exposure to the arthropod vectors. Previously, few cases of trypanosomosishave been reported in camels from Maiduguri (Egbe-Nwiyi and Chaudhry, 1994). These occurrences were linked to the movement of camels through tsetse infested to tsetse free zone as they travel down towards the northern limit of tsetse distribution in Borno state. Moreover, mechanical vectors such as biting flies which have been incriminated in transmission of trypanosomosis in tsetse free zones (Soulsby, 1982) are abundant in Maiduguri and environs, and could play a significant role in transmission. The occurrence of *Dipetalonema* species Microfilaria in camels in this study never reported in Maiduguri and the low prevalence rate indicate that filariid nematodes are erratic in the geographical region due to unavailability of suitable ecological conditions for propagation of *Simulium* species which serve as their natural vector (Soulsby, 1982). Moreover, Mosquitoes are known to play a significant role in transmission of microfilaria (Soulsby, 1982).

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The mean values of RBC, PCV, Hb, WBC, MCV and MCH were within normal range of values in desert camels (Farooq *et al.*, 2011) but the MCHC was below normal range. Moreover, mean values of most haematological parameters of infected and uninfected slaughtered camels examined in this study were comparable ($p>0.05$). The absence of anemia, which is a reliable indicator of severity in haemoparasitic infections (Adejinmi *et al.*, 2004) may be due to the fact that infected camels were probably carriers with latent infection. In the presence of favourable immunity and good nutrition, there may be adequate compensatory haematopoietic response in the course of most haemoparasitic infections, which could mask the initial anemia, hence the observed normal heamogram in this study. The significantly ($p<0.05$) higher mean RBC counts observed in infected camels than uninfected ones may be explained on the basis of active haematological response to the presence of haemoparasites, which usually occurs in the course of natural infections (Soulsby, 1982).

V. Conclusion

This study reports endemic proportion of haemoparasites and the first occurrence of microfilaria of *Dipetalonema* species in one humped camel in Maiduguri. The results obtained from this study also indicate that camels in Maiduguri may harbor subclinical infections involving various genera of haemoparasites. The role of camels as carriers and or reservoirs for other species of domestic animals is suspected since infection is not associated with significant changes in haematological parameters.

VI. Recommendation

We recommended the need for further studies using molecular methods to elucidate the various species of haemoparasites circulating in camels within the region. Also trade camels coming to Maiduguri for slaughter or other purposes should be screened for and be treated against haemoparasites. There is an immediate need to educate camel herders in this locality on preventive chemoprophylaxis and vector control using effective insecticides, acaricides and environmental management as well as chemotherapeutic control measures.

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Conflict of Interest

The authors did not declare any conflict of interest concerning this work.

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Table 1 : Overall Prevalence of Haemoparasites and their 95% CI in the one Humped Camel (*Camelus dromedarius*) Slaughtered in Maiduguri Abattoir.

Variables	No. Examined	No. Positive (%)	95% CI	
			L	U
Age				
Young	31	6 (19.4)	0.09	0.36
Adult	178	21 (11.8)	0.08	0.17
Sex				
Male	24	5 (20.8)	0.09	0.21
Female	185	22 (11.9)	0.08	0.17
Total	209	27 (12.9)	0.09	0.18

CI= 95% confidence interval on prevalence, L= lower limit, U= upper limit

Table 2 : Types of Haemoparasites identified in the one Humped Camel (*Camelus dromedarius*) Slaughtered in Maiduguri Abattoir.

Haemoparasites	No. Positive (%)
Anaplasma	10 (37.0)
Babesia	6 (22.2)
Microfilaria	2 (7.4)
Trypanosoma	9 (33.3)
Total	27 (12.9)

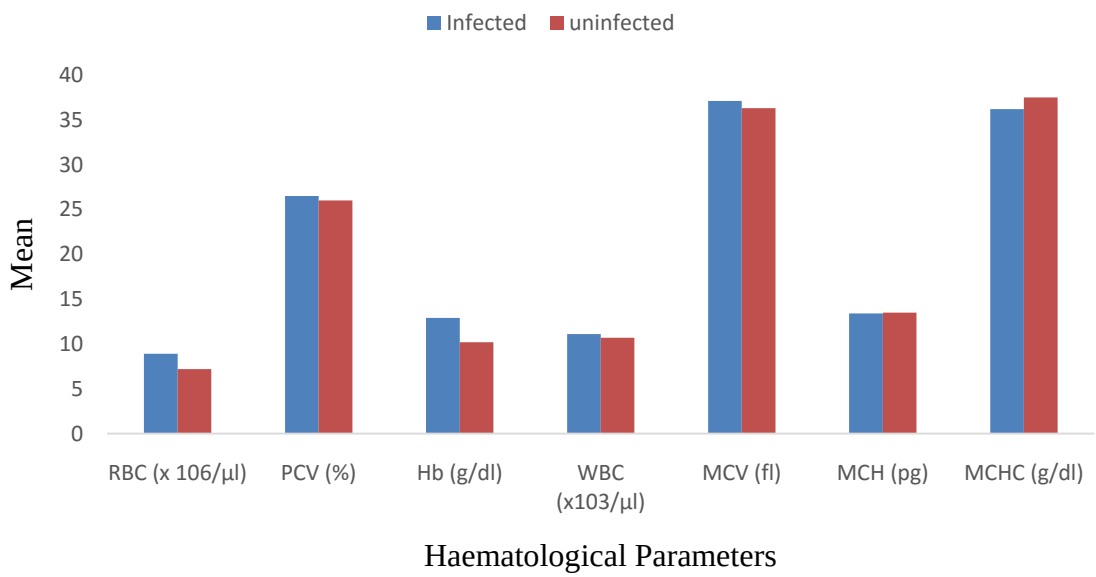

Figure 1 : Mean values of Some Haematological Parameters of Infected and Uninfected one Humped Camel (*Camelus dromedarius*) Slaughtered in Maiduguri Abattoir.

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Study on Prevalence and Monetary Loss Attributed to Hydatidosis in Cattle Slaughtered at Jimma Municipal Abattoir, Southwestern Ethiopia

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Abstract- A cross-sectional study was conducted on bovine hydatidosis from November 2015 to June 2016 with the objectives of investigating its prevalence and Monetary loss in cattle slaughtered in Jimma municipality abattoir. Routine ante mortem and post-mortem inspection was performed on a total of 400 selected slaughtered cattle. Infection organs 223 cattle positive, 200 (89.7%) had cysts more in lungs, 20 (8.9%) in liver, 1 (0.45%) in kidney, 1(0.45%) in spleen, whereas, the rest of 1(0.45%) in heart infections involved organs. A significant association was observed ($P<0.05$) between the disease positivity and age groups, body condition. It was concluded that these zonotic cestodes deserve due attention to safeguard public health and that further studies are needed on epidemiology and public health importance of Echinococcus granulosus in the study area.

Keywords: *bovine, hydatidosis, prevalence, monetary loss jimma, abattoir.*

GJMR-G Classification : *NLMC Code: WC 900*

STUDYONPREVALENCEANDMONETARYLOSSATTRIBUTEDTOHYDATIDOSISINCATTLESLAUGHTEREDATJIMMAMUNICIPALABATTOIRSOUTHWESTERNETHIOPIA

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Study on Prevalence and Monetary Loss Attributed to Hydatidosis in Cattle Slaughtered at Jimma Municipal Abattoir, Southwestern Ethiopia

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Abstract- A cross-sectional study was conducted on bovine hydatidosis from November 2015 to June 2016 with the objectives of investigating its prevalence and Monetary loss in cattle slaughtered in Jimma municipality abattoir. Routine ante mortem and post-mortem inspection was performed on a total of 400 selected slaughtered cattle. Infection organs 223 cattle positive, 200 (89.7%) had cysts more in lungs, 20 (8.9%) in liver, 1 (0.45%) in kidney, 1(0.45%) in spleen, whereas, the rest of 1(0.45%) in heart infections involved organs. A significant association was observed (P<0.05) between the disease positivity and age groups, body condition. It was concluded that these zonotic cestodes deserve due attention to safeguard public health and that further studies are needed on epidemiology and public health importance of Echinococcus granulosus in the study area.

Keywords: bovine, hydatidosis, prevalence, monetary loss jimma, abattoir.

I. Introduction

Hydatidosis caused by the larval stage (metacestode) of Echinococcus granulosus is the most widespread parasitic zoonoses (Ibrahim, 2010; Getaw et al., 2010). Dogs are the usual definitive hosts while a large number of mammalian species are intermediate hosts, including domestic ungulates and man. It is a familiar with many different countries (cosmopolitan) zoonotic infection (Azlaf and Dakkak, 2006).

Despite the large efforts that have been put into the research and control of echinococcosis, it still remains a disease of worldwide significance. In some areas of the world, Cystic echinococcosis caused by E. granulosus is a re-emerging disease in places where it was previously at low levels (Urquhart *et al.,* 1996; Kebede *et al.,* 2009a).

Echinococcus granulosus infection is endemic in East and South Africa, Central and South America, South Eastern and Central Europe, Middle East, Russia and China. The highest incidence is reported mainly from sheep and cattle rearing areas (Arene, 1995). The disease is most important in livestock production which is based mainly on extensive grazing system. Several reports from different parts of Ethiopia indicate that hydatid cyst is prevalent in livestock population of the country (Jobre *et al.,* 1996; Kebede *et al.,* 2010).

According to Abebe and Yilma (2011) a prevalence of 72.4%, 37.72%, 33.78% and 13.7% in cattle slaughtered in Asella, Adama, Gonder, and Dire Dawa was documented respectively indicating its importance in the livestock industry. Its distribution is higher in developing countries especially in rural communities where there is close contact between dogs (definitive host) and various domestic animals intermediate hosts (Eckert and Deplazes, 2004). By affecting many animal species, intermediate animal hosts and humans, hydatid cyst causes tremendous economic losses worldwide and specially in those areas where the parasite is endemic (Urquhart *et al.,* 1996).

Knowledge about the prevalence of the diseases together with associated risk factors as part of the epidemiology of the disease is crucial for any attempt of prevention and control of the disease in question. Moreover, determination of the economic significance of the disease is important for decision making, planning, and implementation of local control strategies. The present study were, therefore, conducted in the area with objective of determining the prevalence of Hydatidosis, its associated risk factor in cattle slaughtered at Jimma municipal abattoir and to estimate the economic significance of the disease in cattle.

II. Material and Method

a) Study area

The study was conducted in Jimma town which is located at about 352km south west of Addis Ababa. The area receives a bimodal rain fall with an average annual rain fall of 1530mm. The long rainy season occur during the months of June to September while the short rainy season occurs during the months of March to May. The climatic condition of the town is "Weynadega" and the town is located at an altitude of 1915masl. The annual maximum and minimum temperature ranges from 24-30C◦ and 7-14C◦ respectively. According to the statistical data obtained (CSA, 2009), Jimma district has a livestock population of 2,016,823 cattle, 288,411 goats, 942,908 sheep and 74574 horses, 49,489 donkey, 28,371 mules and 1,139,735 poultry.

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b) Study animals

The study animals were local breeds of cattle coming from different wereda of the Jimma zone to Jimma municipal abattoir. Only male cattle and sheep were slaughtered, but majority of animals slaughtered in the abattoir were male cattle. The majority of slaughter animals came from seven weredas, this are Agaro, Asandabo, Bilida, Dedo, Jimma, Sarbo and seka. The body condition score was classified into poor, medium and good (fat), (Nicholson and Butter worth, 1986). The age was determined by dentition formula according to the method described by De Lahunta and Habel (1986), and animals categorized into three age groups (< or = 5, 5 – 8, and > 8 years).

c) Sample size and sampling method

The study animals were selected from the slaughter line using simple random sampling technique. The required sample size was determined based on prevalence of 61% (Koskei, 1998) using the formula given by (Thrusfield, 2005). The study considered 95% confidence interval and 5% precision level. Accordingly a total of 384 animals were calculated, but to increase precision, the number of examine animals were reached to 400. For this study sex, age, origin and body condition of animals were considered as risk factors.

d) Study Design

A cross sectional study was conducted from November 2015 to June, 2016 by collecting data on events associated with hydatidosis in cattle slaughtered in Jimma municipal abattoir. This study was conducted to determine update information on the prevalence and economic impact on bovine hydatidosis at Jimma municipal abattoir. (Two slaughtering days per week) visits were made to abattoir.

e) Study Methodology

i. *Ante mortem inspections*

Pre-slaughter examinations of cattle were conducted in the lairage in order to determine the sex, age, body condition and origin of animals. Identification number was given for each animal to examine after evisceration. During ante-mortem examination, animals were clinically examined for any sign of illness while standing and moving according to (Urquhart *et al.,* 1996). And following the judgments passed by (FAO, 1994).

ii. *Post mortem inspection*

During post mortem examination organs especially liver, lung, spleen, kidney and heart as a whole were systematically inspected for the presence of hyatid cyst by applying the routine meat inspection procedure of primary examination followed by secondary examination. The primary examination involves visualizations and palpation of organs, were as secondary examination involves further systemic incision of each organs into pieces and whenever evidence of hydatid cyst was found, it was classified as live or calcified and the cyst distribution into organs was recorded.

iii. *Examination of cysts for fertility and viability*

Based on the presence or absence of brood capsules containing protoscolices in hydatid fluid, cysts were identified and classified as fertile and infertile according to the method described by Macpherson (1985). Individual hydatid cysts were carefully incised and examined for protoscolices, which resembled white dots on the germinal epithelium; such cysts were characterized as fertile cysts.

Fertile cysts were subjected to viability test. A drop of the sediment containing the protoscolices were placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movements with 40x objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices in hydatid fluid on microscope slide with the principle that viable protoscolices should completely or partially exude the dye while the dead ones absorb it (Macpherson *et al.,* 1985). Furthermore, infertile cysts were further classified as sterile or calcified. Sterile hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in their content. Typical calcified cysts produce a gritty-sound heard at incision (Soulsby, 1982).

iv. *Determination of Monetary losses due to hydatid cyst*

An attempt was made to estimate the annual economic losses from hydatidosis in cattle taking into account losses from cost of organ condemnation and from carcass weight.The retail market price of average size offal (lung, liver, kidney, heart and spleen) and the cost of one kg beef were obtained from information gathered from local butchers. Annual economic loss due to organ condemnation was determined by considering annual slaughter rate of cattle and prevalence of hydatidosis per organ and an estimated 5% carcass weight loss (Getaw et al., 2010) was considered. Average carcass weight of Ethiopian local breed cattle is estimated as 108 kg (Negassa et al., 2010). The total economic loss was calculated as the summation of cost of offal condemned plus the cost of carcass weight losses (Kebede et al., 2009a; Getaw et al., 2010).

$$LOC = (NAS \times ph \times plu \times cplu) + (NAS \times ph \times phr \times cphr) + (NAS \times ph \times pli \times cpli) + (NAS \times ph \times psp \times cpsp) + (NAS \times ph \times pkid \times cpkid);$$

Where NAS –Average number of cattle slaughtered annually

Loc-loss of organs condemned

Ph-prevalence rate of hydatidosis

Plu-percent involvement of lung

Cplu-current mean retail price of lung

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Phr- percent involvement of heart

Cphr- current mean retail price of heart

Pli- percent involvement of liver

Cpli - current mean retail price of liver

Psp- percent involvement of spleen

Cpsp - current mean retail price of spleen.

Pkid- percent involvement of kidney

Cpkid - current mean retail price of kidney

N: B-All prices are determined from the price at Jimma town.

v. *Total Monetary Loss Estimation*

Total economic loss was evaluated by considering both loss from organ condemnation and loss from carcass weight loss. Total loss = direct loss (loss from organ condemnation) + indirect loss (loss from carcass weight loss).

f) Data Analysis and Management

The data obtained was coded in Microsoft excel sheet 2007 and subjected to descriptive statistics and chi-square in order to assess the magnitude of the difference of comparable variables using SPSS version 20.0 software. Statistically significant association between variables is considered to exist if the p-value is less than 0.05.

III. Results

a) Prevalence and Risk Factors

i. *Age group*

Out of the total 400 heads of cattle slaughtered and examined, 218 (54.5%) were infected with hydatid cyst, more cysts involving different visceral organs (lung and liver). Rate of infection in different age groups (<5 and, 5-8 and >8 years) was assessed and described in (Table 1). Prevalence in age groups have shown as statistically highly significant variation ($P<0.05$, $\chi2 = 16.615$) with young group having higher infections.

ii. *Body condition score*

Prevalence was also assessed in terms of body condition score (Table 2). It was found that cattle having poor body condition had the highest prevalence (74%) followed by medium (46.6%) and good (52.5%). There was highly significant difference revealed between body condition scores ($P<0.05$, $\chi2=28.332$) with poor animals groups having higher infections.

iii. *Origin of animals*

Prevalence of Hydatidosis in cattle slaughtered at Jimma Municipal abattoir in origin of animals at Bilida (61%) was higher infected but, at Sarbo 43.5% was less infected (Table 3).

b) Cyst Distribution

Overall distribution of cysts in different organs of cattle slaughtered at Jimma Municipal abattoir was described (Table 4). Of the 223 cattle positive, 200 (89.7%) had cysts merely in lungs, 20 (8.9%) in liver, 1 (0.45%) in kidney, 1(0.45%) in spleen, whereas, the rest of 1(0.45%) in heart infections involved organs.

c) Characters of hydatid cyst in different organs

Out of 98 organ infected by cysts to tested for fertility, 50(17.3%) cysts of lung, 45(54.87%) cyst of liver, 1(100%) cysts of kidney,1(100%) cysts of spleen, and 1(100%)cysts of heart origins had protoscolices detected and hence, fertile. Out of the total cyst counts, 98(26.2%) cyst counts are fertile, 216(57.8%) are sterile and 60(16%) calcified. Fertility status of cysts from different organs has shown, but the cysts of lung origin being highly fertile (Table 5).

d) Estimated Monetary loss incurred by hydatidosis

Due to aesthetic value and to break the life cycle of the Echinococcus parasites infected organs are condemned. A total of lung, liver, kidney, spleen and heart were condemned due to hydatidosis with an economic loss of 89249.2ETB, 22312.3 ETB, 676.89 ETB, 225.63ETB and 676.89 ETB respectively. The direct and indirect economic loss was about 133140.91 ETB and 3249072 ETB respectively. The total annual financial loss due to bovine hydatidosis was estimated to be 3362212.9 ETB, (Table 6).

Table 1 : Prevalence of hydatidosis in different age groups of cattle slaughtered at Jimma Municipal abattoir

Age group (years)	Number of cattle examined	Infected	Infected Prevalence
Group 1 (< 5years (young)),	38	23	62.2%
Group 2 (5-8 years (adult))	303	159	52.3%
Group3 (>8years (old))	39	36	61%
Total	400	218	54.5%

$\chi2 = 16.615$, $P = 0.034$

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Table 2 : Prevalence of Hydatidosis in cattle slaughtered at Jimma Municipal abattoir on body condition basis

Body condition score	Animals		Animals
	Examined	Infected	Prevalence
Poor	77	57	74%
Medium	146	68	46.6%
Good	177	93	52.5%
Total	400	218	54.5%

$\chi 2 = 28.332$, $P = 0.00$

Table 3 : Prevalence of Hydatidosis in cattle slaughtered at Jimma Municipal abattoir on origin of animals

Origin of animals	Number of examined	Number of infected	Total % of infected
Agaro	48	28	58%
Asandabo	45	26	57%
Bilida	94	58	61%
Dedo	59	30	50%
Jimma	48	25	52%
Sarbo	79	35	43.5%
Seka	28	16	56.5%
Total	400	218	54.5%

$X^2 = 64.742$, $p = 0.000$

Table 4 : Distribution of Hydatid cysts in different organs of positive cattle at Jimma Municipal abattoir

Organs affected		
	Number of cases	Percentage
Lung only	200	89.7%
Liver only	20	8.9%
Kidney only	1	0.45%
Spleen	1	0.45%
Heart	1	0.45%
Total	223	100%

Table 5 : Fertility, sterility and viable of cysts collected from different organs of cattle slaughtered at Jimma Municipal abattoir

Organ	Fertile cyst (%)	Sterile cyst (%)	Calcified (%)	Total cyst counts%
Lung	50(17.3)	180(62.28)	59 (20.4)	289(77.27)
Liver	45(54.87)	36(43.9)	1(1.2)	82(21.9)
Kidney	1(100)	-	-	1(0.26)
Spleen	1(100)	-	-	1(0.26)
Heart	1(100)	-	-	1(0.26)
Total	98(20)	216(57.75)	60(16)	374(100)

Table 6 : Direct economic losses associated with CE in infected cattle in Jimma municipal abattoir. organs condemned and their price in ETB during study period

Organs	No.of organs condemned	% of condemned	price per organs	Total price in ETB
Lung	200	89	20	4000
Liver	20	8.9	50	1000
Kidney	1	0.45	30	30
Spleen	1	0.45	10	10
Heart	1	0.45	30	30
Total	223	100	140	5070

Direct economic loss from loss of organs condemned=NAS X ph [(plu x cplu) +(pli x cpli) +(psp x cpsp) +(phr x cphr) + (pkid x cpkid).

NAS=Number of animals slaughtered annually in Jimma municipal abattoir were=9600

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Loc=9600 x 0.545[(0.89 x20) + (0.089 x 50) + (0.0045 x 30) + (0.0045 x 10) +(0.0045 x30)] = 5014[(17.8) +(4.45) + (0.135) + (0.045) + (0.135)

=5014[(22.565)] =113140.91 ETB

*1USD=20 ETB

=2262818.2 USD

In direct Monetary loss

Loss from carcass wegheit loss= NAS X ph X CPB X 5% X 108Kg

1 Kilo gram of beef meat in Jimma town is 130ETB

LCWL=9200 X 0.545 X 120 X 0.05 X 108

=3249072 ETB
=64981440 USD

Total Monetary loss=direct loss + in direct loss

= 113140.91 + 3249072
= 3362212.9ETB
= 67244258 USD

IV. Discussion

In the present study the prevalence of Bovine hydatidosis in Jimma Municipal abattoir was found to be 54.5% which was comparable with the results of other works conducted, this study was much higher compared to the prevalence reported at Jimma 31.44% (Tolossa *et al,.* 2009) and 22.4% (Moges, 2003), Konso 22.57% (Fikre, 1994), Adigrat 20.3% (Kebede, *et al.,* 2009b) and Nekemte 31.19% (Feyissa, 1987). Much lower prevalence was also reported by Kebede, 2009b (7.5%) in Shire and Tsehaye, 1995 (7.2%) in Debre Birhan and also high in Asella 61.0% (Koskei, 1998), 62.96% around Bale Robe (Woubet, 1988), and 59.9% Bahirdar (Nebiyou, 1990).

The present prevalence rate was high (54.5%). This might be due to the abundance and frequent contact between the intermediate and infected final hosts. It could also be associated to slaughtering of aged cattle which have had considerable chance of exposure to the parasitic ova, backyard slaughtering of small ruminants and provision of infected offal's to pet animals around homesteads. Moreover, poor public awareness about the disease and presence of few slaughter houses could have contributed to such a higher prevalence rate.

Generally, variation among the prevalence of hydatidosis at different geographical location could be associated to the strain difference of *Echinococcus granulosus* that exist in different geographical locations (McManus, 2006). Additionally variation could be with age factors of the animals and other factors like difference in culture, socio-economical activities and attitudes to dogs and their population. Similar to the present finding, it was reported that cystic Echinococcosis infection was higher for older animals (Azlaff & Dakkak, 2006; Fayesa *et al.,* 2010). Animals with more than eight years of age were found to be highly infected that stastically significant (P value < 0.05). This could be mainly due to the fact that aged animals have longer exposure time to *Echinococcus granulosus* eggs. In addition, older animals might have weaker immunity to combat against infection (Himonas, 1987). This finding is similar to the finding of Fikre Lobago (1994), Hagos Yihdego (1997), Umur (2003), Azlaf and Dakkak (2006) and (Esatgil and Tuzer, 2007).

The prevalence of hydatidosis by origin of slaughtered cattle was assessed and statistically significant difference (P value < 0.05) was found indicating that geographical regions play an important role in distribution of the cysts. This could be due to the difference in the socio-economic status and animal husbandry practices of community in all areas from where animals were brought for slaughter and frequent contact of animals with infected definite host.

The prevalence of hydatidosis among different organs involved in harboring of the cyst showed that lung was found to be the most commonly affected organ (50%) followed by liver (43%) and this was equivalent with Bizuwork (2013), 50.5% for lung and 40.6% for liver and also similar result of 54.5% and 43.5% was reported by Debas and Ibrahim (2013) on lung and liver respectively. This finding was higher than finding of Abunna *et al.,* (2012) who reports 12.5% and 4.25% prevalence for lung and liver respectively while 92.7% in lung and 53.2% in liver which is higher than this study was also reported by Abera *et al.,* (2013).

In this study number of cysts collected from lung 200(89.7%) was greater than that collected from liver 61(8.9%) and that of spleen, heart and kidney in which 1(0.45%) was recorded. Comparable results were reported by (Alemu and Yitagele, 2013), 47.04% and 44.2% for lung and liver respectively and 9.41% for spleen, heart and kidney. This might be due to the fact that cattle are slaughtered at older age, during the time the liver capillaries are dilated and most oncospheres directly pass to the lung; additionally, it is possible for the hexacanth embryo to enter the lymphatic circulation

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and be carried via the thoracic duct to the heart and lungs in such a way that the lungs may be infected before or instead of liver (Arene, 1985).

Additionally, the lung and liver which are most commonly infected organs, this could be due to the fact that lungs and livers posses the first great capillaries of sites encountered by migrating Echinocooccus onchosphere (hexacanth embryo) which adopt the portal vein route the first large capillaries encountered by migrating blood borne onchospheres and primarily negotiate pulmonary and hepatic filtering system sequentially before any other organ is involved. However, development of hydatid cysts occur occasionally in other organs like spleen, kidney and heart and other organs and tissues when onchosphers escaped into general systemic circulation (Urquhart *et al.,* 1996).

Lung harbored highest number of calcified cysts (20.4%) followed by liver (1.2%). This finding is comparable with finding of Mesele *et al. (*2013) 60.2% ,22.6% and 4.3% respectively for lung, liver and spleen and higher than the report of Bizuwork *et al.(*2013) with the prevalence's of 36.8% for lung,14.6% for liver and 0% for spleen. This can be due to the host defence mechanisms of killing more efficiently the parasitic larvae at the early stage of development (Himonas, 1987).

The percentage of fertile cysts in this study was 26.2%. This is higher compared to the fertility rate of 26.9%, 24.4% and 19.3% reported by Fayesa *et al.* (2010), Solomon (2011) and Zelalem (2008) respectively from different parts of Ethiopia. But the present study was quite lower compared to the 96.9% reported from South Africa (Arene, 1985). Yet much lower fertility record such as 1.76%, 9.85% and 6.2% were reported in cattle from Wolayita Soddo (Nigatu, *et al.,* 2009), Nekemt (Bersissa, 1994) and Bahir Dar (Nebiyou,1990) respectively. The variation in fertility rates among different species and in different geographical Zones could be due to difference in strain of *Echinococcus granulosus* (McManus, 2006). Strain of the parasite and the host can modify the infective pattern of the parasite (Gemmel *et al.,* 2002).

Comparison of fertile cyst from different organs was found to be lower for lung (17.3%) than liver (54.87%). This finding was agreement with finding of (Debele *et al.,* 2014) 66.7 and 40.7% for lung and liver respectively and the present finding was higher than Debas and Ibrahim(2013) 25% and 7.5% for lung and liver respectively.

Out of a total 400 cattle carcasses, 218(54.5%) were infected with hydatid cysts. Of these cysts 98 (20%) fertile, 216(57.75%) sterile and 60(16%) were calci fied. Higher infected when compare with (Tolossa *et al,.* 2009) in Jimma out of a total 512 cattle, 161(31.44%) were infected with hydatid cysts. a total of 1171 hydatid cysts being collected from the infected animals. Of these cysts, 223(19.4%) were fertile, 505(43.13%) were sterile and 349(29.80%) calcified. These indicate that cattle are an important intermediate host for the perpetuation of the life cycle of the parasite in Jimma and its surroundings.

The annual economic loss incurred by hydatidosis was calculated to be 3362212.9 ETB. The result was relatively comparable with the report of (Zelalem, 2008) 5,544,591.74 ETB in Addis Ababa abattoir and lower than that of the Terefe *et al.* (2012) 19,847,704.5ETB at Addis Ababa abattoir enterprise and higher than that of Belina *et al.* (2015) and Zewdu *et al.* (2010) with annual economic loss of 841,419.3 and 160,032.23 respectively. The economic losses was different from the reports of others studies in the country which may be due to the variation in prevalence of the disease and mean annual number of cattle slaughtered in different Abattoirs and variation in retail market price of organs (Polydorus, 1981).

As described above Hydatid disease is generally considered to be a rural disease because of its way of transmission cycle, which involves domestic herbivorous animals (cattle, sheep, pigs and so on) and dogs. However, it is possible that urban residents may have been in contact with *Echinococcus granulosus* eggs, in this matter backyard slaughtering and inappropriate disposal affected organs plays major role for the continuity of parasite life cycle.

V. Conculusion

The overall prevalence observed in the study indicated relatively high and an important zoonotic disease in the area and this could be due to several factors of which keeping dogs in close association with cattle. Hydatidosis also causes substantial visible and invisible economic losses in cattle of the study area as a result of condemnation of edible offal and carcass weight loss. The most preferred predilection sites of hydatid cyst in cattle like liver, kidney, heart and lungs and condemnations of these important organs having a single or multiple hydatid foci is really a huge loss. From the result obtained in the present study and considering the reality in Jimma municipal abattoir and its surrounding, it is mandatory for launching a control program proper disposal of affected offal's freely for dogs and wild canids (the usual practice in the community) should be stopped and all the condemned organs should be either buried or incinerated. Moreover, further studies are needed on genotyping, epidemiology and public health importance of *Echinococcus granulosus* in the study area

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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