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Full Length Research Paper

Prevalence and possible risk factors for caprine paratuberculosis in intensive dairy production units in Guanajuato, Mexico

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In order to determine the prevalence of caprine paratuberculosis and the possible risk factors associated a cross-sectional epidemiological study (February to June, 2009) was carried out in dairy goat intensive production units in Guanajuto, Mexico. Blood (n=821) and fecal (n=240) were sampled from goats (older than one year of age) were randomly selected from thirteen dairy goat intensive production units. Serum samples were analyzed using immunodiffusion serological test (AGIT) and fecal samples were used for confirmatory diagnosis by IS 900 Nested PCR. Analysis of results was carried out with STATA 7® software. Paratuberculosis prevalence in the studied population was 9.87% (range 1 to 32%), females had 10.25% and male 6.24%; for body condition were 14.02% emaciated, 7.94% thin and 8.7% good. By Nested PCR analysis detected 64 (26.67%) fecal samples as positive. Risk factor analysis indicated that alpine breed had a OR=2.1 (95%, CI 0.76 to 7.65), females in theirs second parturition showed OR= 2.94 (95%,CI 1.04 to 8.3), from three to fifth parturition had OR= 5.88 (95%, CI 4.3 to14.7) and with more than six parturitions had an OR=7 (95%, CI 2.79 to 18.21)., Animals that presented pasty to liquid feces had OR=2.10 (95%, CI 0.84 to5.25). The results in this study suggest that there is an increased risk for paratuberculosis as the number of parturitions increases and is also related to the consistency and handling of feces in the flocks.

Key words: Paratuberculosis, caprine, epidemiology, nested, polymerase chain reaction (PCR), immunodiffusion, intensive production.

INTRODUCTION

Paratuberculosis is a chronic infectious disease caused by the facultative intracellular bacteria *Mycobacterium avium* subspecies *paratuberculosis* (Map) that affects the gastrointestinal tract causing granulomatous enteritis of a large variety of animals, among which are domestic ruminants such as bovines, sheep and goats (Kennedy

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and Benedictus, 2001; Amoldi et al., 1983; Ramírez et al., 1983).

The main elimination pathway of Map is through feces which contaminate water and feed. Animals are infected in the first weeks of life through water, colostrum or milk that has been contaminated with feces of the animals infected with paratuberculosis. The animals that are most susceptible to be infected are newborns all the way up to 6 months of age (Gilardoni et al., 2012; Martínez et al., 2012). Feces of goats and sheep infected with paratuberculosis lose their normal consistency and become pasty with intermittent diarrhea accompanied with progressive loss of body condition and death (Gumber et al., 2006). It is thought that stressing conditions such as nutritional deficiencies, concurrent diseases, crowding, parturition and high production pressure contribute to the start of clinical signs (Merkal et al., 1975). Unlike cattle, background on ovine and caprine paratuberculosis prevalence data are scarce and much of case is known, It assumes that is similar to cattle, paratuberculosis has a worldwide distribution, the prevalence in some countries is high as 45%, as is the case for the United States of America (Pithua and Kollias, 2012), similar rates are reported in Canada (Bauman et al., 2016, Sorensen et al., 2003). In cattle, it is endemic in The Netherlands, Austria, and Belgium, where the prevalence is 54, 7 and 41%, respectively (Singh et al., 2008). In Australia, infection rates fluctuate between 9 and 22% respectively. In Argentina reports prevalence rates is 18.8% in dairy farms and 6.8% in beef farms (Gilardoni et al., 2012). In Rio de Janeiro, Brazil, the prevalence is 33%. In Venezuela, 72% of the herds is infected (Sánchez-Villalobos et al., 2009), in Chile report prevalence of 9.3% (Kruze et al., 2007; Pinto et al., 2002).

Paratuberculosis in Mexico is widely distributed and studies indicate that the prevalence rates between 5 and 30% mainly in cattle, goats, sheep and fighting bulls (Córdova et al., 2010). The main economic losses incurred by infection with paratuberculosis are due to lower milk production, premature culling and/or seizure of carcasses at slaughterhouse (Harris and Barletta; 2011).

Diagnosis of paratuberculosis can be done based on clinical and epidemiological aspects, through the study of lesions present, direct identification of Map in clinical samples and indirectly by measuring the immune response of the animal against the infection. Serological diagnosis of paratuberculosis in sheep and goat can be done using agar-gel immunodiffusion test (AGIT) which has a sensitivity between 70 and 80% and 100% specificity (Ferreira et al., 2003). Confirmatory diagnosis of paratuberculosis is done by bacteriological culture although the limiting factor is that the incubation period for a Map culture is between 8 and 16 weeks. A further diagnosis option is to use a PCR test (polymerase chain reaction), with primers designed to detect the *IS900* insertion sequence that is specific to Map, using ADN from bacterial cultures, tissue samples from granulomatous lesions (mesenteric lymph nodes and small intestine), feces and milk, which has a sensitivity and specificity above 90% and the result is obtained in one to two weeks (Erume et al., 2011; Garrido et al., 2000). Paratuberculosis causes important economic losses to livestock production and as such it is important to determine the possible pathways by which production units and herds become infected and develop the clinical phase of the disease.

The purpose of this study was to determine the prevalence and possible risk factors associated to paratuberculosis in dairy goat intensive production units.

MATERIALS AND METHODS

Selection of sample size and study population

A cross-sectional epidemiological study was carried out on the dairy goat population from thirteen intensive dairy goat production units located in the State of Guanajuato, Mexico. The study was carried out between February and June 2009. Sample size was determined using a livestock census of 3,500 heads which was the total present in the livestock production units (LPU), goat farmers that were invited to participate; they signed an informed consent letter. In order to determine the sample size, the proportions formula (Levy and Lemeshow, 1980) was used with an estimated prevalence of 0.03 and a 0.1 estimated error; 10% was added to the calculated “n” to account for losses in field and/or in the laboratory. Animals older than one year were randomly selected from each production unit taking into account the existing animals present at the time of visit following the sample size calculation formula (Cannon and Roe, 1982), which provides the probability of detecting at least one sick animal considering the expected prevalence. Information was collected using questionnaires for each LPU and individual charts for each animal selected.

Epidemiological information

All information collected was blind-coded in order to ensure the confidentiality of the participants. Questionnaires of each LPU included location, type of handling, feeding variables and time as goat farmer. The individual charts consisted of age, gender, physiological status, breed, body condition, and type of animal, place of origin, treatment of diarrhea and physical characteristics of the feces sample. All information, together with the corresponding diagnostic test results, was transferred into two electronic databases (one for the LPU data and one with the chart data) for their epidemiological analysis using the STATA 7® software, descriptive analysis and odds ratios using logistic regression models was calculated.

Blood and fecal samples

Blood samples were collected from the jugular vein in sterile tubes in order to obtain sera, and fecal samples were collected directly from the rectum using a polyethylene bag. Samples were individually tagged for their latter analysis in the laboratory.

Serological diagnosis

Sera samples were evaluated by duplicate throughout the agar-gel

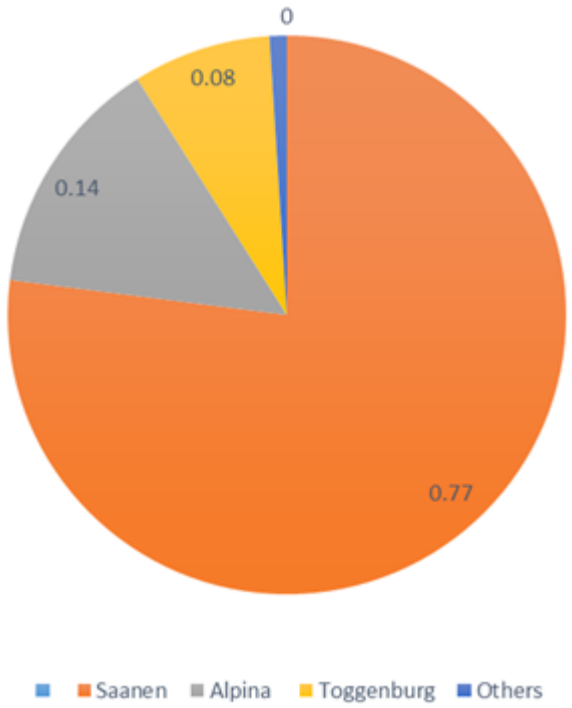

Figure 1. Distribution of the study population by breeds.

immunodiffusion test (AGIT) which was done following the method described in Hernandez (2007). The antigen used in the test was a protoplasmic antigen of the 3065 strain of *Mycobacterium avium* subspecies *paratuberculosis*.

Nested polymerase chain reaction test (Nested PCR)

For the nested PCR diagnostic test, selected samples that were positive to the AGIT test and also randomly negative samples from each LPU were selected, up to a total of 240 samples. DNA was extracted following the methods described in Jaimes et al. (2008) and Garrido et al. (2000).

Nested PCR was carried out following the methods described in Erume et al. (2001), using the following primers for *IS 900*. The first PCR: ptb1 (5' TGA TCT GGA CAA TGA CGG TTA CGG A 3') and ptb4 (5'CGC GGC ACG GCT CTT GTT 3'). The amplification product was to 563 base pairs (bp) and used for the second reaction using the primers: ptb2 (5' GCC GCG CTG CTG GAG TTA A 3') and ptb3 (5' AGC GTC TTT GGC GTC GGT CTT G 3'), amplification product was 210 bp. Amplification products were visualized in 2% agarose gels stained with ethidium bromide.

RESULTS

Serological diagnosis

Serological diagnosis for caprine paratuberculosis was done by the agar-gel immunodiffusion tests (AGIT) to determine the presence of antibodies against Map in this study in which 81 of 821 of evaluated animals were AGIT positive.

IS900 Map nested PCR analysis

A 210 bp amplification product of the *IS900* Map was observed in 64 (26.67%) of 240 samples evaluated and at least one animal of each farm was positive nested PCR test.

Descriptive analysis

Goat farmers had between 4 and 38 years of being involved in goat production with a mean of 20 years. Of the LPUs, 69.2% (9 of 13) had animals born within the same LPU, while the remaining 30.7% (4 of 13) had animals that were purchased from outside the LPU.

All of the LPUs had an animal health plan that encompassed cleaning of pens, feces handling. Regarding cleaning of pens and feces handling, each farm had their own activities schedule with 45.15% (6 of 13) doing them daily, 7.69% (1 of 13) every month and 15.38% (2 of 13) every six months. Each LPU had their own feces handling procedures of which 69.23% (9 of 13) incorporated feces into soil as organic fertilizer, 15.28% (2 of 13) donate them to brick manufacturing entities, 7.69% (1 of 13) used them for compost and the remaining LPU did not provide specific information.

The total sample size was 821 goats composed of 89.16% (732) females and 10.84 % (89) males . The predominant breed was Saanen (77.1%), followed by Alpine (14%) and Toggenburg (8.2%). The remaining goats were grouped into a category that included Lamancha, Nubian and Boer breeds (Figure 1).

Caprine paratuberculosis prevalence

The general prevalence of caprine paratuberculosis was 9.87% (range 1to 32 %). Females had 10.25% and male 6.24%; the prevalence estimated by municipality was for Guanajuato 31.7%, Juventino Rosas 10.53%, Apaseo el grande 9.2% and Celaya 1.75%. For each LPU the prevalence varied, and the rates between 1.59 and 31.71%.

For dairy breads alpine, saanen and togenburg prevalence obtained was 10.64, 10.00 and 5.97%, respectively.

For body condition the prevalence in emaciated animals had 14.2%, 7.94% in thin animals and 8.7% for animals with good body condition. The animal purchased within the same municipality had 12.5%, while the animals purchase in other municipality had 6.25% of the prevalence (Table 1).

Risk factors analysis

In order to identify risk factors associated with prevalence, a logistic regression analysis was performed. Risk factor analysis for breed, gender, place of origin and

Table 1. Prevalence of caprine paratuberculosis in dairy goat intensive production unit.

Variable	n	Prevalence %	Range %	p
Municipality			1.75 - 31.71	0.000
Apaseo El Grande	685	9.2		
Celaya	57	1.75		
Guanajuato	41	31.71		
Juventino Rosas	38	10.53		
Livestock Production Unit (LPU)			1.59 – 31.71	0.000
Farm 1	38	10.53		
Farm 2	67	1.59		
Farm 3	73	4.11		
Farm 4	86	2.33		
Farm 5	57	1.75		
Farm 6	131	3.85		
Farm 7	40	10		
Farm 8	57	14.04		
Farm 9	30	23.33		
Farm 10	59	5.08		
Farm 11	41	31.71		
Farm 12	49	28.57		
Farm 13	98	16.33		
Breed			0 - 10.64	0.384
Saanen	630	10		
Toggenburg	67	5.97		
Alpina	94	10.64		
Other	6	0		
Gender			6.74 - 10.25	0.295
Male	89	6.74		
Female	732	10.25		
Body condition			7.94 -14.02	0.043
Emaciated	214	14.02		
Thin	441	7.94		
Regular-Good	138	8.7		
Place of origin			6.1 -10.84	0.198
Born within the LPU	686	10.84		
Externally purchased	82	6.1		
Type of animal			0 – 16.8	0.000
First year female	13	0		
Billy goat	89	6.74		
First parturition	116	0		
Second parturition	130	7.69		
Third to fifth parturition	252	14.29		
More than six parturitions	125	16.8		
Place of purchase			0 – 12.5	0.344
Within the community	11	0		
Within the same municipality	16	12.5		
Other municipality	16	6.25		
Another State	10	0		
Another country	17	0		
Feces			5.01 - 100	0.000
Normal	359	5.01		
Pasty	69	8.7		
Liquid	1	100		

Table 2. Risk factors associated with the prevalence of caprine paratuberuculosis, in dairy goat intensive producton unit, in Guanajuato, México.

Variable	Odds ratio	CI 95%	
	O R	Lower	Upper
Muncipality			
Celaya	1.00	-	-
Apaseo el Grande	5.67	0.77	41.67
Guanajuato	**26.00**	3.24	208.93
Juventino Rosas	6.58	0.71	61.41
Livestock Production Unit (LPU)			
Farms 2,4,5	1.00	-	-
Farm 1	**5.94**	1.42	24.89
Farm 3	2.16	0.42	9.91
Farm 6	2.02	0.53	7.67
Farm 7	**5.61**	1.34	23.46
Farm 8	**8.24**	2.39	28.50
Farm 9	**15.37**	4.18	56.51
Farm 10	2.71	0.59	12.44
Farm 11	**23.45**	7.14	76.94
Gender			
Female	1.00	-	-
Male	0.63	0.27	1.50
Place of origin			
Purchased	1.00		
Born within the LPU	1.83	0.71	4.68
Breed			
Toggenburg. Boer, Nubian, Lamancha	1.00	-	-
Alpine	**2.42**	**0.76**	**7.65**
Saanen	1.92	0.68	5.43
Body condition			
Good, fat	1.00	-	-
Emaciated	1.71	0.84	3.47
Thin	0.91	0.56	1.80
Type of animal			
Fist year female, Billy goat, first parturition	1.00	-	-
Second parturition	**2.94**	1.04	8.30
Third to fifth parturition	**5.88**	4.43	14.27
More than six parturitions	**7.13**	2.79	18.21
Consistency of feces			
Normal	1.00	-	-
From pasty to liquid	**2.10**	**0.84**	**5.25**

body condition did not have statistical significance, but alpine breed had an OR=2.1 (95%, CI 0.76 to 7.65). For animal type, females in theirs second parturition showed OR=2.94 (95%, CI 1.04 to 8.3) from three to fifth parturition had OR=5.88 (95%, CI 4.3 to 14.7) and with more than six parturitions had an OR=7 (95%, CI 2.79 to 18.21). For municipality Guanajuato had OR=26 (95%, CI 3.24 to 208.93), Juventino Rosas OR=6.58 (95%, CI 071 to 61.41) and Apaseo el Grande OR=5.67 (95%, CI 0.77 to 41.67). Animals that presented pasty to liquid feces had OR=2.10 (95%, CI 0.84 to 5.25) (Table 2).

DISCUSSION

The prevalence obtained in this study was 9.87%; results agree with those obtained from studies in caprine production units in several states of the Mexican Republic as Puebla 28% (Gallaga, 2001), Coahuila 18.36% (Toledo et al., 2010) and Guerrero 3.4% (Villalobos, 2011) indicating that the prevalence of the disease had wide

distribution between caprine production units in Mexico.

In Chile 2500 sera samples were evaluated for detection of antibodies anti-Map by ELISA (Pinto et al., 2002), the prevalence showed in the intensive production system was 9.3%, and It could be because of the animals stay for long time in the flocks. In México, it is very common for goat farmers to have for long periods time their animals and the average life span of a goat in this system is about six or more years. Serological diagnosis of paratuberculosis in sheep and goat is done using the AGIT methodology with sensitivities ranging from 70 to 80% and 100% specificity. This methodology is simple and quick for testing whole herds and in this study it was considered an efficient test to determine the presence of antibodies against Map. The main disadvantage of this test is that it detects the presence of the disease once the clinical phase has been initiated (Hernandez, 2007; Gumber et al., 2006; Ferreira et al., 2003).

Kruze et al. (2007) evaluated 383 caprine fecal samples for bacteriological culture and *IS900* Map PCR, in which was found 50% of flocks and 9.1% of the animals tested were positive from all flocks with intensive management and presence of imported breeds. This study focused on intensive production systems and indeed all thirteen production units had at least one animal positive to paratuberculosis diagnosed by Nested PCR. The Nested PCR detected 26.67% of animals as positive confirming that the disease is present in the flocks. The used of the Nested PCR as confirmatory test, allowed the validation of the results obtained by AGIT screening test. The Nested PCR has the advantage of detecting animals that are sheding in feces low quantities of bacilli and are at an early infection stage of the disease which has a low humoral immune response. It is recommended that for integral diagnosis of paratuberculosis both a serological test and a confirmatory test such as nested PCR are carried out (Erume et al., 2011; Jaimes et al., 2008).

As risk factors, gender, breed and place of origin, there were no statistical differences but with respect to place of origin; the introduction of infected animals with Map, is the main route of spread diseases among flocks. This practice was also common for all infected flocks, since it was found that farmers, directly or indirectly, had imported animals selected for dairy genetic fitness of countries whose paratuberculosis infection status is widely recognized (Kennedy and Benedictus, 2001).

In this study 30.7% of the farmers goat have a common practice to buy or trade animals in the same region, generally the animal`s health status is unknown, so there is a risk of introducing infected goats in the flocks, so the health status should be emphasize in relation to paratuberculosis at the time of goats acquisition. Body condition is considered as an indicator of the presence of the disease, and even though the design of this study did not allow the determination of causality or the start of the disease due to the temporal structure of the data, the "emaciated" body condition category had higher seroprevalence of the disease; these animals are considered to be in the clinical phase of the disease and therefore are the main infection source for the rest of the flock.

The main dairy goat breeds evaluated in this study were alpina, toggenburg , and saanen, had a high prevelence, possible causes of these may be due to various management measures each farm. Dairy goats are subjected to stress conditions of productive, reproductive rate, and genetic predisposition, makes them more susceptible and predispose the animals infected with Map (Pithua and Kollias, 2012).

In Korea the apparent regional prevalence of caprine paratuberculosis was estimated at 21.1, 18.2 and 38.2% for Northern, central and southern regions respectively (Lee et al., 2006). They conclude that possible risk factor for caprine paratuberculosis were all associated with intensive management. In addition a flock size more than 100 heads and presence of foreign specialized dairy goat breeds are associated with prevalence. Guanajuato in Mexico is one of the states with the largest goat production, and high prevalence observed by municipalities, can be due to the fact that ten years ago, the introduction of specialized goat breeds is increased, without knowing their paratuberculosis health status and the mobilization of animals between farms was carried out without restrictions.

Handling of feces constituted a risk factor with an OR = 2.10, which agrees with Villalobos (2012); that reported that when pen cleaning and feces removal was not carried out regularly this had an OR= 3.6 for the presence of the disease into the flocks. These management practices should be considered as an important part of the measures disease control.

Results in this study showed that the dairy goat production units had a 9.87% prevalence of the disease (considering the AGIT results) and, since paratuberculosis is a flock problem and the detection of a single animal can implicate that up to 25% of the herd could be infected, there could be many more infected animals. These results point to handling practices within and between production units that are obligatory but are nevertheless dependent upon the degree of cooperation and expressed interest for goat farmers, in reducing the risk of infection. Such practices include slaughter of positive animals, and the limitation of animal movement between production units, especially towards the LPUs that were negative to paratuberculosis. Also, it must be considered that the diagnosis of this disease should be done before a recently purchased animal enters the herd, and also a regular monitoring program should be in place.

Conclusions

Paratuberculosis is a chronic infection that affects dairy

goat herds that are under intensive production systems and further studies should be carried out in order to determine the epidemiological distribution of the disease and quantify its economic impact on production.

Conflict of Interests

The authors have not declared any conflict of interests.

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Major causes of lung and liver condemnation and associated risk factors in cattle slaughtered at Hawassa Municipal abattoir, Southern Ethiopia

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The study was conducted from November, 2013 to March, 2014 at Hawassa municipal abattoir, Southern Ethiopia with the aim of identifying the major causes of liver and lung condemnation and assessing possible risk factors associated with organ condemnation. Standard ante-mortem and post-mortem procedures were followed throughout the study and abnormalities were recorded. A total of 399 cattle were considered for ante mortem and post-mortem examination by systematic random sampling technique. In ante mortem inspection the most commonly encountered abnormalities were 3 (0.75%) lameness, 4 (1%) nasal discharge, 2 (0.5%) skin lesions and 4 (1%) rough hair coat. During post mortem inspection, 224 (56.1%) of lungs were condemned due to hydatidosis, calcification, emphysema and marbling and 207 (51.8%) of livers were condemned due to fasciolosis, hydatidosis, calcification, hardening and haemorrhage. The condemnation rate of lung with respect to each risk factor was found to be 35.3, 0.78, 9.7 and 10% for hydatidosis, calcification, emphysema and marbling, respectively. The condemnation rate of liver with respect to each risk factor was also found to be 20.5, 17.2, 4.2, 10.2 and 0.5% for fasciolosis, hydatidosis, hardening, calcification and hemorrhage, respectively. The study indicated that hydatidosis (35.5%) for lung and fasciolosis (20.7%) for liver were the major cause of condemnation, respectively. Many risk factors such as age, body condition, origin and breed of animal determined liver and lung condemnation rate. But among these only body condition showed statistically significant difference (x^2= 11.524, P=0.003) with condemnation rate of each organ. The results of this study showed that condemnation of liver and lungs at the abattoir is very high, thereby preventive measure need to be designed to overcome further loss by applying appropriate treatment control and preventive measures of slaughter animals.

Key words: Abattoir, cattle, condemnation, Hawassa, liver, lungs, municipal.

INTRODUCTION

Ethiopia has the largest live-stock population in Africa with an estimated population of 44,318,877 cattle, 23,619,720 sheep and 23,325,113 of goats, 6 million equines, 2.3 million camels and 43 million poultry (CSA, 2008). But this great live-stock potential is not properly used due to different factors such as traditional

management system, limited genetic potential, lack of appropriate veterinary services. Each year a significant loss results from death of animals, inferior weight gain, condemnation of edible organs and carcass at slaughter (Gryseal, 1988).

In developing countries, abattoir plays a major role in providing and serving source of information and reference center for disease prevalence. Meat inspection is conducted in the abattoir for the purpose of screening animal products with abnormal pathological lesions that are unsafe for human consumption. These efforts help in reduction of food borne parasitic zoonoses (Chhabra and Singla, 2009). It assists to detect certain diseases of live-stock and prevent the distribution of infected meat that could give rise to diseases in animal and human being and to ensure competitiveness of products in the local market (Hinton and Green, 1993). Animals showing signs of abnormality during ante mortem inspection should not be allowed to enter the abattoir for slaughter. After animals entered into slaughter, routine post mortem inspection of carcass and organs should be carried out as soon as possible (Teka,1997).

Although various investigations have been conducted through abattoir survey to determine the cause, prevalence and economic losses resulting from organ condemnation in Ethiopia, there is shortage of study especially about the major causes and possible factors of organ condemnation in Hawassa municipal abattoir. Therefore this study was aimed to fill this gap. Generally the objective of this study was: (i) to identify the major causes of organ condemnation in cattle slaughtered at Hawassa Municipal abattoir and (2) to assess the possible risk factors associated with organ condemnation.

MATERIALS AND METHODS

Study area

The study was conducted at Hawassa municipal abattoir, which is found in Hawassa town, the Capital city of South Nations Nationalities and Peoples Regional State, at a distance of 273 km south of Addis Ababa. Geographically, the area lies 1680 m above sea level and has an average annual rain fall and temperature of 953 mm and 25°C, respectively (Community Supported Agriculture (CSA), 2008).

Study population

The study animals were cattle (local, cross and exotic) breeds presented to Hawassa municipal abattoir for slaughter. These breeds were brought from different source areas: Harar, Nazereth, Arsi Negele, Shashemene and Hawassa Zuria as informed by responsible individuals. The study animals were transported to the abattoir using vehicles or on foot depending on the site of collection. Antemortem and post mortem examinations were carried out for every animal. Data were recorded and animals were grouped into adult (2 TO 8 years) and old (above 8 years) age categories by using eruption of incisor teeth according to Pace and Wakeman (2003).

Sample size and sampling methods

Systematic random sampling technique was employed for selection of individual animal (local, cross and exotic) breeds. The sample size was determined by the formula given by Thrusfield (2007) considering expected prevalence being 50% with the desired precision level of 5%. Adding a few more sample to increase accuracy, a total of 399 cattle (local, cross and exotic) breeds were considered in the study.

Study design and study methodology

A cross sectional study design was followed and the study was conducted from November, 2013 to March, 2014 at Hawassa municipal abattoir to determine major causes of liver and lung condemnation.

Antemortem inspection

Before slaughter, examination of cattle was conducted at lairage with various risk factors: age, body condition score, breed and origin of each study animal being recorded simultaneously on specially designed data recording sheet. Animals were inspected looking for their gait, structure, conformation and any disease abnormality detected by physical examination and the findings were registered according to the standard of ante mortem inspection procedure (Gracey et al., 1999). Accordingly, judgments were passed based on Food and Agricultural Organization (FAO) (2007).

Post mortem inspection

All animals that had been examined by ante mortem inspection were subjected to post mortem examination. The post mortem examination was performed by visual inspection, palpation and systematic incisions for the presence of cysts, adult parasites and other abnormalities. The results were recorded and decisions were classified as fit for human consumption and then approved, totally condemned as unfit for human consumption or partially condemned as fit for human consumption after processing of each which is determined by looking at the percentage of lesions existing on the organ (FAO, 2007).

Data management and analysis

The raw data collected during inspection were entered into excel spread sheet (Microsoft Excel 2000) and filtered. Descriptive

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Table 1. Abnormal conditions encountered during ant mortem inspection.

Abnormality	Adult (274)	Old (125)	Total (399)(%)
Lameness	1	2	3 (0.75%)
Nasal discharge	2	2	4 (1%)
Rough hair coat	1	3	4 (1%)
Skin lesion	1	1	2 (0.5)
Total	5	8	13 (3.25%)

Table 2. Major causes of liver condemnation and frequency of lesions.

Causes of condemnation	Frequency of lesions and % of condemned organs	
	Frequency	Percent (%)
Fasciolosis	82	20.5
Hydatidosis	69	17.2
Hardening	17	4.2
Calcification	41	10.2
Hemorrhage	2	0.5
Total	211	52.6

statistics was used to determine organ condemnation rates and defined as proportion of condemned organs to the total number of organs examined. The variability between condemnation rates of specific organs by risk factors of age, origin, body condition and breed were evaluated by Pearson's chi square (x^2) and differences were regarded statistically significant if p-value was less than 0.05 using statistical package for social sciences (SPSS) version 20.0 for windows.

RESULTS

Antemortem examination

Out of the total of 399 cattle examined during ante mortem inspection, 13 (3.25%) were found to have abnormalities of lameness, mouth discharge, rough hair coat and skin lesions (Table 1).

Post mortem examination

All cattle that had been examined in ante mortem inspection were subjected to post mortem examination. From the total organs examined, 211 (55.5%) livers (Table 2) and 223 (56.4%) lungs (Table 3) were totally condemned from local market due to various gross pathological abnormalities such as fasciolosis, hydatidosis, calcification, marbling, emphysema, hardening and hemorrhage. Association among many risk factors such as age, body condition, origin and breed of animals was computed agianst liver and lung condemnation rate. But among these only body condition showed statistically significant difference (x^2= 11.524, P=0.003) with condemnation rate of each organ (Table 4). The result indicated that rate of liver and lung condemnation has no statistically significant difference in animals coming from different origin (Table 5).

DISCUSSION

In this study, routine ante mortem and post mortem inspection was carried out to detect abnormalities encountered in Hawassa Municipal Abattoir. The most commonly encountered abnormalities during ante mortem inspection were 3 (0.75%) lameness, 4 (1%) nasal discharge, 2 (0.5%) skin lesions and 4 (1%) rough hair coat. Both the nasal discharge and rough hair coat were the highest encountered ante mortem problems followed by lameness and skin lesion. All of these ante mortem findings may be associated with stress following transport of animals from the source areas and also lameness and skin lesions may be partly contributed by traumatic effect of transport vehicles.

Out of 399 cattle slaughtered and examined in the abattoir, 224 (56.1%) lungs and 207 (51.8%) liver were rejected from local market due to their gross abnormalities (Table 6). The current study showed very high rejection rates of liver at post mortem as compared to previous studies conducted by Asmare et al. (2012) and Yifat et al. (2011) who reported rejection rates of 23.7 and 31.1% of liver at Bahir Dar and Gondar, respectively.

Table 3. Major causes of lung condemnation and frequency of lesions.

Cause of Condemnation	Frequency of lesions and % condemned	
	Frequency	Percent
Hydatidosis	141	35.3
Calcification	3	0.78
Emphysema	39	9.7
Marbling	40	10
Total	**223**	**55.78**

Table 4. Condemnation rate of liver and lung based on age and body condition score.

Variable		Frequency of lesion and % of condemned organ		
		Total No. examined	Lung condemnation (%)	Liver condemnation (%)
Age	Adult	274	150(54.7)	138(50.3)
	Old	125	74(59.2)	69(55.2)
Body condition score	Good	269	144(53.5)	124(46.1)
	Medium	108	67(62)	71(65.7)
	Poor	22	13(59.1)	12(54.5)

x^2=0.445, p=0.514, and x^2=11.524, p=0.003.

Table 5. Condemnation rate of liver and lung based on origin of cattle in this study.

Variable	Municipal	Frequency of lesion and % of condemned organ		
		Total No. examined	Lung condemned (%)	Liver condemned (%)
Origin	Tula	308	170 (50.7)	165 (49.2)
	Hawassa	45	23 (54.7)	19 (45.2)
	Harar	5	3 (60)	2 (40)
	Negele	22	20 (57.1)	15 (42.8)
	Shashemene	12	7 (46.6)	5 (33.3)
	Nazereth	7	1 (50)	1 (50)

x^2=0.586, p=0.44.

Lung rejection rate in this study (56.1%) was also increased as compared to Amene et al. (2012), Yifat et al. (2011) and Asmare et al. (2012) who reported prevalence of 46.22, 29.4 and 25.78% in Jimma Municipal, Gonder ELFORA and Bahir Dar Municipal abattoirs, respectively. This difference may be due to difference in agro-ecological conditions from where the slaughter animals are originated and prevalence of diseases in different areas. The post mortem examination of the study also showed fasciolosis (20.5%), hydatidosis (17.2%) and calcification (10.2%) as major and hardening (4.2%) and hemorrhage (0.5%) as minor causes of liver condemnation; whereas hydatidosis (35.3%) as major and calcification (0.78%), marbling (10%) and emphysema (9.7%) as minor causes of lung condemnation. Such variation in the degree of different factors as a cause for organ condemnation may be related with difference in the prevalence of these conditions from different geographic areas and in different animals in animals from the same geographic area.

In this study both fasciolosis and hydatidosis accounted for greater proportion of organ condemnation. This agrees with what was reported by different reports from studies by different researchers even though variation is still visible. The rejection rate of liver due to fasciolosis (20.5%) was high when compared with the rejection rate of 12.7 and 8.6% by Fufa et al. (2010) at WolaitaSodo and Mellau et al (2011) at Tanzania, respectively. On the

Table 6. Condemnation rates of organs in association with breed of cattle.

Organ Examined	N (% condemned)	Breed of cattle		
		Local (%)	Exotic (%)	Cross (%)
Lung	224 (56.1)	216 (51.6)	3 (75)	5 (55.5)
Liver	207 (51.8)	202 (48.3)	1 (25)	4 (44.4)
Total	431	418	4	9

other hand it was low when compared to 24.32% in Mekelle (Geberetsadik et al., 2009) and 68.7% in kombolcha (Andualem, 2007). This could be due to differences in environments and ecological conditions of the study areas from where the study animals are originated.

In this study the prevalence of hydatidosis in lungs (35.5%) was higher than that found in the liver (17.2%). This was in line with the findings of Asmare et al. (2012) and Fitsum (2009) who reported a prevalence of 35.7 and 38.8%, respectively; but is greater than that reported by Shegaw (2008), Andualem (2007), Marta (2010) who reported a prevalence of 25.2, 26.7, 32.8, 22.7%, respectively. Such variation may be attributed to the difference in the geographic origin and agro ecological conditions of slaughter animals.

The findings of the study depending on the age, origin and breed of the slaughter animals did not show a significant difference ($P > 0.05$) indicating that cattle in each category in all variable types are prone for factors causing organ condemnation and this was in line with the findings of the study by Asmare et al. (2012) who reported the presence of no significant difference in these risk factors.

However, body condition of animals showed a statistically significant difference ($p < 0.05$) for the rate of liver and lung condemnation. This finding agreed with the result of Asmare et al. (2012) who reported the presence of a statistically significant difference in the rate of organ condemnation in different body condition of animals. This indicated that disease conditions that cause organ condemnation are highly associated with body condition score of animals.

Conclusion

The study showed that fasciolosis and hydatidosis are the major causes of liver and lung condemnation, respectively. The age, breed and origin of the animals did not show a significant difference whereas the body condition score of the animal did show a significant difference on the rejection rate of organs. Therefore, this study avails information to take appropriate actions to reduce the factors that contribute for greater condemnation rate of organs.

Conflict of Interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of knowledge, attitude and practices of agro-pastoralists on tsetse fly(*Glossina* sp.) in Western Serengeti Tanzania

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A cross-section study was conducted to evaluate knowledge, attitude and practices about tsetse (Glossina) and trypanosomiasis among the agro-pastoralists communities around the Ikorongo/Grumeti game reserves in the Western Serengeti. Structured questionnaire were administered to 80 respondents and out of these 95% had adequate knowledge about tsetse flies and 79% knew the local names of the fly. The study also revealed that 25.5% of respondents recognized the common hideouts of tsetse to be the grazing areas near the reserves 29% along rivers and 20.4% in bushes and forests. Twenty four percent of respondents knew the clinical signs of African Animal Trypanosomiasis (AAT) which included rough coat, emaciation (21%), diarrhea (13.3%) and loss of appetite (11.9%). Other mentioned disease symptoms which had small proportions included miscarriages, coughing, reduced milk yield and break tail. Ninety six percent of respondents ranked Animal Trypanosomiasis the first among other existing animal diseases in the area including Contagious Bovine Pleuropneumonia (CBPP), Foot and Mouth Disease (FMD), East Coast Fever (ECF) and Anaplasmosis. Chemotherapy was the most practiced method to control trypanosomiasis in the western Serengeti. Cattle owners used isometamedium chloride (samorin) and dimenazine aceturate (berenil) to treat sick animals. Economic losses caused by the disease in their animals were identified to be deaths, reduced milk production and income. Lack of enough resources to contribute to the maintenance of cattle dips and the grazing of animals inside the reserves especially during the dry season when there was shortage of pastures outside the reserves were the main constraints associated with cattle productivity in the area.

Key words: Agro-pastoralists, animal trypanosomiasis, Glossina,

INTRODUCTION

Tsetse-transmitted trypanosomiasis in man and livestock poses a serious threat to the lives and livelihoods of people in African countries. Tsetse (Glossina) infestation in Africa covers over 10 million km^2 representing 37% of

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the continent (Jordan, 1986). There are 23 identified species and 8 subspecies of *Glossina* (Moloo, 1993) and all are potential vectors of Animal African Trypanosomiasis (AAT) and Human African Trypanosomiasis (HAT). African animal Trypanosomiasis remains to be an obstacle to sustainable development of livestock production in the tsetse infested areas of sub-Saharan Africa (PATTEC, 2001). An estimated annual losses in meat and milk yield as well as cost of the disease control amounts to about US $ 1.2 billion (Kuzoe and Schofield, 2004). It is estimated that the removal of trypanosomiasis would lead to as much as a threefold increase in cattle numbers in affected countries and to economic benefits of billions of dollars per year (FAO-PAATIS, 2006). Surveys by Daffa et al. (2013) in Tanzania had shown that about 33% of the land is tsetse infested by 7 *Glossina* species and all play a significant role in the transmission of AAT and HAT. The losses suffered annually in cattle due to the disease in terms of mortality, morbidity and reduced milk yield have been estimated at $7.98 million (MOAC, 1995). About 4 million people are at risk of acquiring HAT and about 400 cases are reported annually (Komba et al., 1997; Sindato et al., 2008). The presence of tsetse excludes livestock from large area of considerable agricultural potential by virtue of the severity of the disease caused by tsetse and transmitted trypanosomes (Msangi et al., 1999). Western Serengeti provides a significant grazing ground for livestock in villages surrounding the Ikorongo/Grumeti Game Reserves. The reserves and the National Parks are homes of tsetse flies and the game animals that provide blood meals to tsetse and at the same time acting as reservoirs of trypanosomes. The interaction between humans, livestock, game animals and the tsetse flies greatly influences disease transmission in western Serengeti. The privatization of veterinary services in Africa including Tanzania had led a situation where diagnosis, treatment and prevention of the AAT has remained almost entirely in the hands of cattle owners or extension workers who are unskilled in differential diagnosis and also lack knowledge on appropriate drug to be used for various diseases (Doran and van den Bossche, 1997). In western Serengeti trypanocidal drugs have been widely used by livestock owners due to lack of large scale tsetse control at national level. At farmer level trypanocides provides a way for individual to take actions to control the disease and very often only sick animals are treated because they constitute the problem perceived by the farmer. The extensive use of trypanocidal drugs and insecticides for trypanosomiasis treatment and tsetse control or the use of other drugs for tick control and other insects of economic importance could cause changes in the tsetse populations. Little was known on the situation in the western Serengeti therefore such information was of vital importance in the determination of disease epidemiology.

This study was conducted in order to determine the level of knowledge, altitude and practice towards tsetse management and control in the course of prevention of devastating trypanosomiasis in the study area. The results obtained from this study could be used in the assessment of the impact of drug use on Glossina populations in western Serengeti. Similarly the results can be used in the formulation of extension material in disease prevention.

MATERIALS AND METHODS

The study area is located in Serengeti district which lies between latitude 2° 00 S and longitude 34 50 E. The four villages (Makundusi, Natta-Mbiso, Rwamchanga and Bunchugu) were involved in the study. The ethnic groups found in this area include the Ikoma, Sukuma, Ikizu, Natta and Taturu. The Sukuma, Taturu and Ikizu are mainly agro-pastoralists keeping large herds of cattle some people having up to 4,000. The climate is savannah and the area experiences bimodal rainfall pattern. The area is covered by a range of vegetation types including grasslands, open woodlands, closed woodlands and riverine vegetation found along existing rivers.

Selection Criteria of Agro-pastoralists to be interviewed based on the number of cattle the person is having, readiness to be interviewed, the time one has spent in the area and those practicing communal grazing near Ikorongo/Grumeti game reserves.

Administration of questionnaires

Interviews were conducted in Kiswahili (the national language) to respondents from the selected villages (Figure 1). Pretest interviews were conducted with twenty copies to ensure that questions were comprehensive and acceptable. The questionnaires were administered by the researchers, veterinary officers and drug vendors. The three parameters assessed were Knowledge, Attitude and Practice (KAP). Under knowledge respondents were assessed on the knowledge of tsetse fly causes of HAT and AAT, clinical signs of the disease. On attitude the respondents were asked to rank the problem of AAT as compared to other animal diseases found in the study area. The practice section dwelt with the methods of control, trypanocidal drugs used, where do they obtain them, frequency of drug use, prices and dose rates. Eighty questionnaires out of 100 were retrieved from the respondents.

Analysis of data

All data collected through the questionnaires were entered into Microsoft excel sheet and the SPSS I6 and STATA II statistical tools were used to analyze and interpret the data. Descriptive statistics (frequency and percentage) were used to analyse qualitative data.

RESULTS

Participants' characteristics

The results (Table 1) shows that majority of respondents 34 (42.5%) fall within the age group of 45 to 60 years and 28.8% were within the age group of 36 to 45. Other 11 respondents (13.8%) were in the age group of 21 to 35 years while 10 respondents (12.5%) were above 61 years. The total numbers of respondents was 80 and out

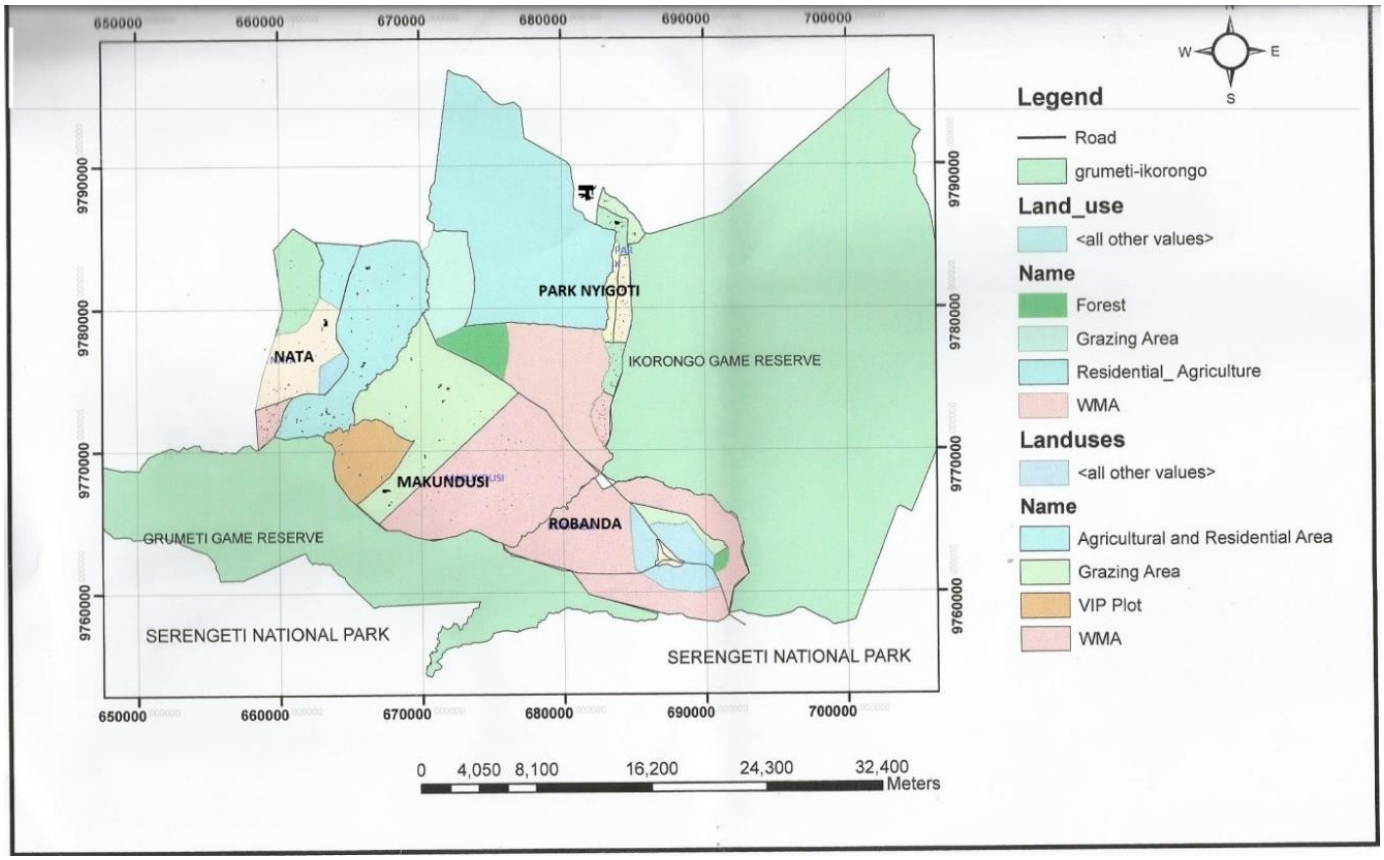

Figure 1. Villages in western Serengeti where interviews were conducted.

Table 1. Age of respondents in years.

Age category	Frequency	Percent
Below 20 years	2	2.5
21 to 35 years	11	13.8
36 to 45 years	23	28.8
46 to 60 years	34	42.5
61 to 90 years	10	12.5
Total	80	100
Sex	Frequency	Percent
Male	69	86.2
Female	21	13.8
Total	80	100

of these males were 69 (86.2%) and females were 11 (13.8%).

From the results in Table 2, it was revealed that 76 (95.0%) of respondents knew about tsetse flies and 54 (67.5%) of them were able to give its local name. Few respondents 3 (3.8%) did not know tsetse flies. From the same table most respondents (69%) knew that tsetse flies cause the disease to animals and humans.

Table 3 indicates that, 54 (29.0%) respondents know that tsetse flies hides along the rivers, 47 (25.3%) in grazing areas, 47 (25.3%) near Serengeti National Park and 38 (20.4%) in bushes and forest.

Table 5 indicates that 72 (82.8%) respondents ranked tsetse fly to be the first cause of animal trypanosomiasis while 11(12.6%) believed that the disease was caused by other flies and ticks. 4(4.6%) respondents associated the

Table 2. Evaluation of knowledge of tsetse fly in Serengeti District.

Knowledge N= 80	Frequency	Percent
Do you know tsetse fly	76	95
If yes give the local name		
Ndorobo	11	20.4
Endorobo	20	37
Andorobo	12	22.2
Other six names	11	21.4
Do not know	3	3.8
Do tsetse transmit disease to animals and human beings		
Yes	69	86.2
No	10	12.5
Do you know the disease		
Yes	76	95
No	3	3.8

Table 3. Evaluation of the common hideouts of tsetse in the community (Effect N = 80).

Hideouts	Frequency	Percent
Grazing areas	47	25.3
Along rivers	54	29
Near Serengeti National Park	47	25.3
Bush and forest	38	20.4

disease with the presence of wild animals. Table 6 indicates that the respondents ranked trypanosomiasis (ndorobo) first important among disease existing in the area. Other animal diseases are CBBP, FMD, ECF, anaplasmosis, dizziness and LCD.

Table 7 shows that respondents were aware of the effect of tsetse on livestock productivity. Animal deaths ranked first with 72 (37.3%) respondents, loss of income 52 (26.9%) and11 (5.7%); respondents mentioned other consequences like reduced draft power and animals not being marketable. From the same table other respondents 18 (9.3%) sited miscarriage as another effect of the disease and failure of oxen to plough were 5 (2.6%).

Table 8 shows that majority of respondents were using different methods to control AAT in the study area. Sixty two (35.0%) treating their sick animals, 69(39.0%) are using sprays and 46(26%) use vaccines.

Table 9 indicates that respondents are using drugs in the treatment of animal trypanosomiasis and other diseases. Sixty one (21.4%) respondents Samorin, novidium 42(21.4%), dimenazine 37(18.9%) and are the most used drugs. Other drugs used for treatment include: Berenil 12(6.1%) and veriben 9(4.6%). For the treatment of other diseases they use OTC, DIP and TRIDOX.

Table 10 shows that drugs are obtained from different sources: 65 (56.0%) of respondents obtained from private and animal chemical stores, 5(4.3%) government subsides, 43 (37.1%) livestock officers and 3(2.6) from neighbors.

DISCUSSION

The findings indicate that most respondents were men 69(86.2%) aged between 45 to 60 years and also there was a good number of active reproductive age 36 to 45 it is easier for these groups of people to acquire and share new skills on disease prevention. It was also discovered that majority of the respondents had knowledge 76(95%) of tsetse fly and knew the local name of the flies. Respondents also knew that tsetse fly bite cause the disease to human and livestock. Similar observation was made Nigeria by Njoku et al. (2003) and Gumel et al. (2012) were farmers new the local names for tsetse flies. Respondents have identified riverside, forest and grazing areas as the most risky places for fly and trypanosomes exposure. This is in line with scientific description about the biology and ecology of the flies (Hargrove, 2004: Leak, 1999). Similar observation in a study done in Nigeria (Gumel et al., 2012) showed that farmers believe livestock contact tsetse in forest and riverine areas. Majority of respondents were aware of the disease symptoms in their animals which are used as

Table 4. Respondents views on animal trypanosomiasis symptoms (Effect N= 80).

Symptoms	Frequency	Percent
Rough coat	52	23.9
Loss of appetite	26	11.9
Abortion	10	4.6
Diarrhea	29	13.3
Coughing	5	2.3
Hump licking	22	10.1
Reduced milk yield	14	6.4
Emaciation	47	21.6
Break tail	13	6

(11.9%) loss of appetite, 22 (10.1%) and hump licking were the main symptoms of the disease. Others symptoms mentioned included; abortion, coughing and reduced milk production.

Table 5. Evaluation on the main causes of Trypanosomiasis (Effect N = 80).

Cause	Frequency	Percent
Mbungo (tsetse)	72	82.8
Flies	5	5.7
Ticks	6	6.9
Wild animals	4	4.6

Table 6. Important diseases/conditions perceived by respondents (Effect N= 80).

Disease	Frequency	Percent
Ndorobo (trypanosomiasis)	74	31.0
CBPP	26	10.9
FMD	18	7.5
Worms	30	12.6
ECF	22	9.2
Anaplasmosis	29	12.1
Dizziness	13	5.4
LCD	19	7.9

Table 7. Evaluation of respondents on the effect of tsetse fly on livestock (Effect N = 80).

Consequences	Frequency	Percent
Animal deaths	72	37.3
Not marketable	40	20.7
Miscarriage	18	9.3
Animals fetch low price	6	3.1
Loss of income	52	26.9
Oxen fail to plough	5	2.6

basis for treatment. It was observed that agro-pastoralists base on clinical signs to diagnose sick animals this could lead to the wrong treatment of animals and hence drug misuse. A similar observation was made in the study by Magwisha et al. (2013).in Southern Tanzania where the diagnosis of disease based entirely on clinical signs. It

Table 8. Evaluation of respondents on the methods of control (Effect N = 80).

Control method	Frequency	Percent
Treatment	62	35.5
Spraying	69	39
Vaccination	46	26

Table 9. Evaluation of respondents on drug use (Effect N= 80).

Drug	Frequency	Percent
Novidium	42	21.4
Samorin	61	31.1
Diminasan	37	18.9
Veriben	9	4.6
Berinil	12	6.1
Saulinin	1	0.5
OTC	9	4.6
Alben	7	3.6
DIP	3	1.5
TRIDOX	15	7.7

Table 10. Source of drugs as per respondents (Effect N = 80).

Source	Frequency	Percent
Private animal chemical stores	65	56
Government subsides	5	4.3
Livestock officers	43	37.1
Neighbors	3	2.6
Total	116	100

was revealed that the majority of respondents understands the causes of animal trypanosomiasis to be the tsetse fly, while other respondents related the disease with presence of wild animals. This result coincides with tsetse fly ecology that their availability is influenced by the presence of host animals (Leak, 1999). Of the reported diseases African animal trypanosomiasis has been perceived as the number one obstacle to cattle production in the study area. These findings were consistent with a study done in Southwestern Ethiopia (Seyoum et al., 2013) and Kenya (Ohaga et al., 2007) where trypanosomiasis was perceived as the number one among other diseases inaffecting cattle production. From this study respondents have emphasized that if cattle was suspected of trypanosomiasis; noticeable reductions could be observed on milk production, body condition, oxen working ability, growth rate and price of animal and increased mortality in untreated cases. This indicates average level of awareness of the respondents on the role played by tsetse in the disease transmission to their cattle. Similar observation was made in the study by Onyiah (1997) and Gumel et al. (2012) where most respondents knew that tsetse bite has an effect on cattle. This study revealed that trypanocidal drugs are mostly used by respondents for treatment and control of animal trypanosomiasis. This indicates that the respondents have a good knowledge on the effects caused by tsetse fly on their livestock which influenced them to use drugs to control the disease. Similar findings were reported by Magwisha et al. (2013) in Southern Tanzania and Gumel et al. (2012) in Nigeria they found that livestock keepers had a good knowledge on tsetse and the disease are causing to their animals. On practices for disease control the study revealed that majority of respondents were using different methods to control the AAT which included treating sick animals, using drugs and vaccines. Most of these drugs were obtained from private stores and livestock officers while few were from the government subside. This indicates that majority of the agro-pastoralists had knowledge and were aware of the

consequences of the disease to their livestock.

Conclusion

The findings from this study indicate that respondents have a high knowledge of tsetse fly and the effects it causes on cattle productivity. Their attitudes and practices towards control of tsetse fly and the disease are good though knowledge and sensitization is needed for livestock keepers to contribute towards dip maintenance and guidance on the proper administration of drugs. In this area the tsetse burden could be successful reduced by involving the agro-pastoralists and the management of Ikorongo/Grumeti reserve in government supported control campaign using insecticide impregnated targets.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Hematology and serum biochemistry of farmed bullfrog, *Lithobates catesbeianus* during the active and hibernating periods

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The Bullfrog *Lithobates catesbeianus* is a species of economic important edible frog and often chosen as experimental animal model. Although, some studies in the literatures have reported the parameters of hematology and serum biochemistry in several Rana species, a comprehensive profile of hematology and biochemistry in farmed bullfrogs was very limited, especially during the hibernating period. 140 apparently healthy farmed bullfrogs (70 males and 70 females) were used during the active and hibernating periods to determine the hematology and serum biochemistry parameters including morphometry of erythrocytes, PCV, HGB, MCV, MCH, MCHC, RBC count, WBC count, differential leukocyte count, glucose (Glu), triglycerides (TG), cholesterol (Cho), blood urea nitrogen (Bun), uric acid (UA), creatine (Cre), total protein (TP), albumin (Alb), globulin (Glo), γ-glutam (GGT), total bilirubin (TB), alkaline phosphatase (Alp), a-amylase (Amy), CK, AST, ALT, LDH, K, Na, Cl and nCa (that is, the ionized calcium level when pH=7.4). Differences between sexes showed that male bullfrogs possessed a statistically higher LDH activity level, and statistically lower levels of Cre, Na and Ca concentrations. Additionally, it was noted that bullfrogs during the active period had significantly lower values for HGB, PCV, MCV, ALT, Glo, Bun, Na, Cl and surface areas and volumes of RBCs and their nuclei, and significantly higher values for WBCs counts and Cre than the hibernating ones. These baseline data could be used for health monitoring and disease diagnostics in bullfrogs artificial farming and serve as general reference values for future studies on the physiology of this species.

Key words: Bullfrogs, hibernation, hematology, serum biochemistry.

INTRODUCTION

Hematology and serum biochemistry parameters, as measured in peripheral blood, are useful in assessing the health and nutritional status of humans and animals (Artacho et al., 2007; Allender and Fry, 2008). The combination of many parameters is required to identify anemia, inflammatory diseases, parasitemia,

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hematopoietic disorders and homeostatic alterations (Campbel et al., 2007; Vasaruchapong et al., 2013). The measurements of erythrocyte dimension can be used for studies on environmental, seasonal, or altitudinal acclimatization and relationship between exchange of gas with tissues and metabolic rate (Ruiz et al., 1989; Pagés et al., 1992). The number of erythrocytes, HGB and PCV reflect the efficiency of oxygen carrying capacity and nutritional status. Leukocytes are involved in the defense of the organism under several conditions, such as stress (Silveira-Coffigni et al., 2004), inflammation (Martins et al., 2006) and parasitism (Azevedo et al., 2006), the number of which could be used as an indicator for infectious diseases (Pessier, 2007; Jamalzadeh et al., 2009). The serum biochemical analyses provide information on internal organs, proteins, electrolytes, and nutritional and metabolic parameters (Newman et al., 1997).

So far, most studies on hematology and serum biochemistry focused on fishes, reptiles and mammals when compared with amphibians. Some limited previous studies have reported on different hematology and serum biochemistry parameters of several amphibian species, including *Rana esculenta* (Sinha, 1983), rhacophorid frog (Mahapatra et al., 2012) and dubois's Tree Frog *Polypedates teraiensis* (Das and Mahapatra, 2014). Hematology and serum biochemistry parameters vary under different pathological, physiological, ecological and environmental conditions in animal population (Llacuna et al., 1996; Sarasola et al., 2004; Seaman et al., 2005). Amphibians are considered to be sensitive animals and show physiological variables to acute environmental changes (Carey, 2005). Two characteristic phases can be distinguished in the annual life cycle, that is, periods of activity and hibernation. Up to date, few documents were found on hematology and serum biochemistry parameters in amphibians at hibernating state.

Bullfrogs, *Lithobates catesbeianus*, being widely farmed over the world, is a species of economic important edible frog for farmers and often chosen as experimental animal model. Cathers et al. (1997) reported on hematology and serum biochemistry parameters of anesthetized American bullfrogs, *Rana catesbeiana*. However, no studies have been published in the literature on the hematology and serum biochemistry reference ranges during the active and hibernating periods for farmed bullfrogs. A relevant knowledge of bullfrog blood physiology is getting increasingly imperative due to diagnostic demand with the increase of breeding industry. It would also help to ensure food safety for eaters.

The aim of this study was to establish the hematology and serum biochemistry reference ranges of farmed bullfrogs during the active period (in May) and the hibernating period (in January). These baseline data could be used for health monitoring and disease diagnostics in bullfrogs artificial farming and serve as general reference values for future studies on the physiology of this species and other anuran species.

MATERIALS AND METHODS

Animals

140 farmed bullfrogs (35 males and 35 females each period) with mean weight 0.39±0.10 kg, ranging from 0.28 to 0.55 kg, were purchased from local raising households in Wuhu City, Anhui Province, China in January and May, respectively. Wuhu is in the southern region of China, with subtropical monsoon climate. These local bullfrogs would turn into the state of hibernation, that is, they neither eat nor move and breathe mainly through the skin when environmental temperature continuously fall below 10°C in January.

Sample collection

The blood samples of 140 bullfrogs were obtained using cardiocentesis. Prior to blood collection, the hibernating bullfrogs were housed in the terrarium for a 20-day continuous hibernation to ensure that they are still in the hibernating state, and the blood samples of active bullfrogs were collected in the morning over 12 h after a last feeding in May. Tubes coated with K_2-EDTA were used to collect samples for hematologic analysis and serum separation tubes without anticoagulant were used to collect samples for serum biochemistry and electrolyte parameters. All experiments were performed in compliance with national and provincial guidelines and in accordance with the Guide for the Care and Use of Laboratory Animals.

Analysis of hematology and biochemistry parameters

The serum biochemistry parameters including activities of CK, ALT, AST, GGT, LDH, Alp, Amy and concentrations of Cre, UA, Bun, TP, Alb, Glo, GLU, TB, Cho and TG were measured on serum samples by using an autoanalyzer (KHB 450, Shanghai, China). And the concentrations of K, Na, Cl and Ca in serum were determined using an electrolyte analyzer (IMS-972，HORRON, Shenzhen, China).

Total number of erythrocytes (RBC) and leukocytes (WBC) were analyzed in Neubauer Chamber, with dilution being performed by standard Hayem's solution for RBCs and Turk's solution for WBCs (Parida et al., 2011). The HGB concentration was determined by using an automated hematology analyzer (BC-3000plus, Mindray, Shenzhen, China). The PVC was examined by the microhematocrit method (Parida et al., 2011). The tubes were spun in a microhematocrit centrifuge (TDL-50B, Anke, Anting Scientific Instrument, Shanghai, China) for 5 min at 12000 rpm and the PVC was calculated with the total blood level divided by the blood cell level. The MCH, MCV and MCHC were calculated according to standard formulas (Campbell and Ellis, 2007).

In the laboratory, blood smears were stained with Wright Stain Solution (Tianda Diagnostic reagents co., LTD. Hefei, China) for the differential leukocyte count and then examined under a microscope (BM2000, Jiangnan Yongxin Co., LTD. Nanjing, China). The percentages of different leukocytes were determined after counting a total of 100 white blood cells.

The sizes of RBCs and their nuclei were acquired by measuring their long and short axes (major and minor axes) under a light microscope with an ocular micrometer (ERMA, Japan). Twenty to thirty cells from each blood slide of frogs (n=72) were selected for measurement of the major and minor diameters of erythrocytes and their nuclei (Benfey et al., 1984). The cell surface area, nuclear area and their volume were calculated using the below formula suggested by Lemoine and Smith (1980):

$S=a\times b\times \pi/4$ and $V= (a/2) \times (b/2)\ 2\pi\times 4/3$

Where a is the major and b is the minor axis of the cell or nucleus.

Statistical analysis

Hematology and serum biochemistry data resulting from our study were presented as means and standard deviation (SD) via the software SPASS19 for windows. The results were compared among periods in each sex and among sexes within each period. Significant differences were determined using an independent sample t test model. Results were considered significant at $p<0.05$.

RESULTS

In this study, no parasites were observed, no hemolysis occurred and no chyle blood serum appeared. Values obtained are expressed as mean±SD (ranges). Morphometry parameters of RBCs and their nuclei, hematology and biochemistry values of bullfrogs during the active and hibernating periods are summarized in Tables 1, 2 and 3, respectively.

Morphometry parameters of erythrocytes in bullfrogs during the active and hibernating periods

The morphometry parameters of erythrocytes are shown in Table 1, major and minor axes of erythrocytes and their nuclei were measured values, while surface area and volume of erythrocytes and their nuclei were calculated values. No significant differences were found in erythrocyte morphometry parameters between the two sexes. But the male bullfrogs tended to have lower values for cell minor axis, cell surface area, cell volume, nuclear minor axis, nuclear major axis, nuclear surface area and nuclear volume, than the female ones. The bullfrogs had significantly lower values for morphometry parameters during the active period than those during the hibernating period, particularly for surface area and volume of red blood cells and their nuclei, which were significantly increased during the hibernating period ($p<0.05$).

Hematology parameters of bullfrogs during the active and hibernating periods

The hematology parameters are shown in Table 2. The male bullfrogs had higher values of RBC count, neutrophil percentage and eosinophil percentage, but lower values of PCV and MCV than the females without a statistical significance. The total number of WBCs were found to be significantly higher during the active period than those during the hibernating period ($p<0.05$). However, the values of hemoglobin concentration, MCV and PCV percentage were statistically lower during the active period than those during the hibernating period ($p<0.05$). In addition, the values of MCH and RBC during the active period were lower than those during the hibernating period, but no statistical significance.

Morphologically, leucocytes of bullfrogs could be classified into the following five types: monocytes, lymphocytes, neutrophils, basophils, eosinophils, the first two were agranulocytes while the rest were granulocytes. The percentage of lymphocyte was observed to be the highest in bullfrogs, and the percentage of basophil was the lowest. The percentage of monocyte was close to that of neutrophil. It was found that males possessed slightly higher percentage of eosinophil than females. The percentage of the neutrophil and the N/L ratio during the active period were lower than those during the hibernating period, but not statistically significant.

Serum biochemistry parameters of bullfrogs during the active and hibernating periods

The serum biochemistry parameters are shown in Table 3. The male bullfrogs had significantly higher levels of LDH activity, and significantly lower levels of Cre, Na and Ca concentrations than the females ($p<0.05$). Additionally, the males tended to have high activity levels of AST, ALP, ALT, Amy, TB, but low GLU, TG, Cho and K concentration levels versus those for females ($p > 0.05$). The values of ALT, Glo, Bun, Na and Cl were significantly lower in both sexes during the active period than those during the hibernating period ($p<0.05$). Conversely, the Cre value of bullfrogs was statistically higher during the active period than those during the hibernating period in two sexes ($p<0.05$). In addition, the values of Amy, TP and Glu were found to tend to decrease during the active period ($p>0.05$). However, the values of GGT and Cho were found to increase during the active period versus those during the hibernating period ($p>0.05$).

DISCUSSION

Hematology and serum biochemistry are important tools for assessing health status in human and animals. It is well known that many hematology and serum parameters vary with sex, age, season, and physiological state (Boily et al., 2006). This study may be the first to present hematology and biochemistry reference ranges for farmed bullfrog and comparison between the active period (in May) and the hibernating period (in January).

Non-mammalian erythrocyte such as in fishes, amphibians, reptiles and birds is nucleated, flattened and ellipsoidal (Rowley and Ratcliffe, 1988). This study showed that the shape of RBCs was elongated or oval or elliptical in bullfrogs. The measurement of erythrocyte dimensions is often an important component of standard hematologic survey in amphibians (Hartman and Lessler, 1963). There was a relationship between cell size and metabolic rate. The cells with the smaller surface have lower metabolic cost per unit of cell mass (Olmo et al., 1989). In this study, the measurements of erythrocytes and their nuclei in bullfrogs during the active period were lower than those during the hibernating period, which were in agreement with the results observed for *Rana Esculenta* (Sinha, 1983), it was inferred that decrease in size of erythrocytes during the active period might be a physiological adaptation required for quicker blood circulation due to increased physical activity. Prosser (1973) reported that a higher MCH value is due to larger size of the RBCs. It was found that bullfrogs had higher values of MCH, RBCs counts, PCV percentage and HGB concentration during hibernation. In our opinion, an increase in MCH, RBCs count, PCV percentage, hemoglobin concentration and erythrocyte dimensions during hibernation may be explained by the “respiratory compensation” mechanism. According to Guijarro et al. (2003), respiratory compensation is necessary for fish to keep high oxygen availability to tissues in low oxygen condition. Likewise, this theory is applied to frog species. As known, the bullfrogs during hibernation breathed oxygen by low efficient way, that is, through the skin. Therefore, bullfrogs were trying to supply a more demand

Table 1. Morphometry parameters of erythrocytes in bullfrogs during the active and hibernating periods.

Parameters	Males		Females	
	Active period	Hibernation	Active period	Hibernation
Cell minor axis (um)	15.2±1.02(14.1-16.7)	16.3±1.36(15.0-17.9)	15.7±1.87(13.6-17.4)	17.0±2.27(16.3-19.9)
Cell major axis (um)	25.7±2.67(22.8-28.4)	26.0±4.91(23.4-30.1)	25.6±2.73(22.9-28.4)	26.2±2.90(22.5-28.6)
Cell surface area (um^2) [a]	306.7±34.85(256.3-330.9)	332.9±69.72(300.6-416.1)	315.5±39.83(272.9-355.5)	349.6±47.50(309.1-404.2)
Cell volume (um^3) [a]	817.7±49.73(762.4-869.0)	887.8±260.41(701.6-1199.7)	841.4±47.15(790.2-891.4)	932.3±126.90(824.3-1077.6)
Nuclear minor axis (um)	4.2±0.97(3.4-5.2)	5.9±1.02(4.8-7.0)	4.3±0.93(3.3-5.6)	6.8±1.18(5.1-7.9)
Nuclear major axis (um)	8.3±0.16(7.4-8.8)	9.4±1.60(8.1-10.7)	8.7±1.24(7.3-9.9)	10.0±2.06(9.1-13.3)
Nuclear surface area (um^2) [a]	27.4±8.76(19.0-35.3)	43.5±7.01(38.2-51.6)	29.4±9.88(20.0-38.7)	53.4±9.80(46.2-63.8)
Nuclear volume (um^3) [a]	72.9±2.10(69.3-74.1)	116.1±18.40(100.2-133.6)	78.3±2.42(67.9-80.4)	142.3±18.11(120.2-163.7)

[a] Significant difference($P < 0.05$) according to different periods.

Table 2. Hematology parameters of bullfrogs during the active and hibernating periods.

Hematology Parameters	Males		Females	
	Active period	Hibernation	Active period	Hibernation
RBC($\times 10^{12}$/L)	0.25±0.09(0.19-0.36)	0.34±0.14(0.16-0.51)	0.24±0.05(0.18-0.33)	0.32±0.07(0.26-0.40)
WBC($\times 10^9$/L) [a]	9.93±0.81(6.25-12.40)	2.41±0.79(1.71-3.50)	9.03±3.06(6.87-13.10)	2.43±1.03(2.00-3.63)
HGB(g/L) [a]	73.0±7.55(65.0-81.0)	105.0±13.1(93.0-119.0)	60.7±21.76(38.0-88.0)	106.0±14.7(95.0-123.0)
PCV(%)[a]	20.24±2.00(18.22-23.41)	29.78±6.40(22.90-38.13)	20.32±5.81(16.24-25.11)	29.91±2.40(26.02-35.56)
MCV($\times 10^3$fl)[a]	809.60±88.91(732.14-848.77)	875.88±67.32(832.31-957.07)	846.66±41.33(807.21-889.76)	934.68±93.82(874.20-1029.35)
MCH(pg)	290.00±74.10(226.02-335.12)	310.82±36.94(189.22-377.14)	252.92±81.78(193.30-309.28)	331.25±45.50(290.00-377.43)
MCHC(g/L)	360.67±40.64(318.7-407.8)	352.78±44.94(310.2-387.4)	300.72±51.43(253.7-320.2)	354.40±37.68(311.3-400.1)
Neutrophil(%)	23.09±10.19(10.72-31.10)	29.33±18.4(17.50-55.21)	20.63±6.72(12.93-25.08)	29.21±6.50(24.26-36.59)
Lymphocyte (%)	41.86±14.46(27.50-56.41)	39.66±9.10(29.52-62.11)	52.87±9.23(44.61-62.50)	39.43±5.20(36.58-45.83)
Monocyte(%)	25.31±8.18(17.86-35.00)	25.07±12.40(17.31-40.50)	22.28±4.39(18.00-26.79)	25.33±5.12(20.83-30.77)
Eosinophil(%)	6.43±3.90(2.36-10.17)	4.61±1.13(1.19-5.43)	4.59±0.69(4.00-5.36)	3.88±2.11(2.44-6.41)
Basophil(%)	3.30±1.52(1.56-4.36)	1.17±0.18(0-2.93)	2.73±1.45(1.79-4.41)	2.23±0.91(0-3.17)
N/L ratio	0.53±0.41(0.24-1.18)	0.74±0.09(0.59-0.89)	0.39±0.17(0.22-0.63)	0.74±0.04(0.67-0.81)

N/L ratio: Neutrophil/lymphocyte ratio. [a] Significant ($P < 0.05$) difference according to different periods.

for tissue oxygen content through this mechanism. Leukocytes are cells that are directly associated with specific and unspecific immunological responses (Iwama and Nakanishi, 1996). In this study, leukocyte count was significantly more during the active period than those during the

Table 3. Serum biochemistry parameters of bullfrogs during the active and hibernating periods.

Biochemistry parameters	Males		Females	
	Active period	Hibernation	Active period	Hibernation
CK(U/L)	584.0±176.17(215.0-801.0)	414.3±135.21(257.5-499.1)	403.5±144.64(243.0-592.0)	508.5±161.23(183.3-702.2)
Amy(U/L)	907.2±314.30(612.7-1359.0)	1099.7±177.50(902.1-1407.0)	901.8±296.41(577.8-1278.3)	1057.3±432.31(462.7-1523.2)
LDH(U/L) [b]	205.8±80.72(101.4-312.6)	281.5±62.32(117.4-677.1)	161.5±76.27(71.9-291.0)	152.6±24.70(92.4-177.6)
ALT(U/L) [a]	14.5±9.11(5.7-26.3)	41.3±9.13(11.1-52.8)	14.1±8.22(3.6-29.4)	38.9±11.01(27.6-49.1)
AST(U/L)	111.5±70.00(30.3-191.7)	118.3±20.13(33.7-217.0)	101.2±66.50(29.0-181.3)	100.0±42.16(64.7-155.2)
GGT(U/L)	5.09±2.16(3.01-8.43)	4.09±2.60(2.10-7.43)	5.25±2.87(2.04-9.42)	4.01±0.51(2.90-5.91)
ALP(U/L)	18.40±8.79(8.93-31.00)	18.23±6.80(13.0-26.30)	17.75±10.09(3.87-28.41)	17.70±5.11(8.40-20.42)
TP(g/L)	29.21±6.36(19.32-37.53)	34.42±5.40(30.11-40.60)	29.07±9.65(19.31-38.70)	36.06±8.56(27.50-43.03)
Alb(g/L)	19.82±4.40(14.70-25.31)	16.83±2.33(15.12-19.53)	17.73±8.61(10.40-17.73)	17.96±4.01(13.30-20.60)
Glo(g/L) [a]	9.38±0.27(9.10-9.69)	17.62±2.99(14.90-21.11)	9.34±0.31(8.96-9.69)	18.10±3.98(15.21-24.42)
A/G	1.99±0.60(1.04-2.16)	0.93±0.06(0.88-1.10)	1.90±0.28(1.50-2.25)	0.96±0.04(0.80-1.06)
TB(umol/L)	0.93±0.31(0.70-1.99)	0.99±0.25(0.79-2.06)	0.92±0.39(0.66-1.97)	0.90±0.30(0.63-1.62)
Bun(mmol/L) [a]	4.14±0.55(2.70-4.74)	7.48±1.99(5.30-9.21)	4.82±2.21(3.02-7.03)	7.08±2.01(5.13-9.94)
Cre(umol/L) [a b]	18.56±8.96(10.01-30.14)	9.66±2.21(3.80-12.17)	27.51±12.89(12.02-40.17)	15.76±3.27(4.32-24.03)
UA(umol/L)	NO	7.33±3.32(4.00-12.03)	NO	7.23±3.05(4.06-19.84)
GLU(mmol/L)	1.99±0.18(1.12-2.20)	2.21±0.92(1.41-2.97)	2.00±0.21(1.19-2.55)	2.23±1.00(1.67-2.99)
TG(mmol/L)	0.14±0.06(0.05-0.20)	0.13±0.04(0.09-0.19)	0.15±0.06(0.11-0.24)	0.16±0.05(0.10-0.29)
Cho(mmol/L)	1.25±0.15(1.06-1.39)	1.16±0.28(0.77-1.38)	1.36±0.13(1.00-1.74)	1.20±0.87(0.98-1.55)
K(mmol/L)	6.13±0.34(5.17-6.58)	6.01±0.41(4.77-6.39)	6.43±0.52(5.91-7.12)	6.37±1.90(4.18-7.56)
Na(mmol/L) [a b]	108.7±3.80(102.0-110.6)	121.4±17.66(109.5-140.7)	116.8±8.24(108.5-121.9)	136.0±30.52(105.2-181.0)
Cl(mmol/L) [a]	54.6±6.43(41.6-57.8)	75.1±22.84(61.2-101.4)	52.3±6.27(42.7-58.3)	80.9±27.01(49.9-99.7)
nCa(mmol/L) [b]	0.89±0.09(0.86-0.99)	0.87±0.17(0.71-1.05)	0.99±0.08(0.89-1.07)	0.96±0.23(0.81-1.35)
Pb(ug/L)	18.0±4.08(12.03-21.10)	17.1±4.12(14.97-20.78)	17.8±4.00(15.38-20.01)	17.6±3.98(16.11-20.54)

A/G: Albumin/globulin ratio. NO: not detectable. [a] Significant ($P < 0.05$) difference according to different periods. [b] Significant ($P < 0.05$) difference according to sex.

hibernating period, and was less than those reported earlier in wild-caught *Xenopus laevis* by Wilson et al. (2011). The levels of leukocyte in the whole blood vary depending on environmental quality (Lea Master et al., 1990), nutritional state (Barros et al., 2002), the presence of infectious agents (Martins et al., 2008) and parasitism (Martins et al., 2004). This study showed that lymphocytes in bullfrogs were the most predominant cells as compared to the other types of leukocytes. This result was consistent with data presented for Wild Caught Dubois's Tree Frog (Das and Mahapatra, 2014) and for anesthetized American bullfrogs (Tama Cathers et al., 1997), suggesting that lymphocytes are the major cells involved in the immunological responses in frogs. In contrast, the percentage of basophil was the lowest in the present study on bullfrog, which was consistent with dubois's Tree Frog (Das and Mahapatra, 2014). The percentage of basophil depends on species and possibly on season, geographic region and age of the animal or may be associated with blood parasites or viral infection (Vasaruchapong et al., 2013).

As far as we know, no information is available on the serum biochemistry parameters of frogs in winter. Serum hepatic enzyme tests in animals are used to indicate hepatocellular injury or repair (Kasamatsu et al., 2012). ALT was produced by the liver, being more liver-specific in human and animals. In some animals with hepatic disease, the level of serum ALT activity would get higher than those in normal conditions. This study showed that serum ALT activity level during hibernation was significantly increased, which was consistent with the report for captive finless porpoises by Kasamatsu et al. (2012). This change probably suggested that there was hepatocellular injury in bullfrogs during hibernation. As known, Glo was also often used as an index of hepatic disorder. Serum Glo concentration of bullfrogs was strikingly increased during hibernation, which was likely due to promoting immune defense under the hibernating state.

Cre, being a break-down product in muscle, is usually produced at a very constant rate depending on muscle mass (Di Wu et al., 2014). There was a slow catabolism of Cre at a rate directly proportional to muscle mass (Kasamatsu et al., 2012). In the present study, the value of Cre concentration was found to be markedly increased during the active period, which was in general agreement with previous report for captive finless porpoises (Kasamatsu et al., 2012). Additionally, male bullfrogs had a statistically higher value of Cre in comparison with female bullfrogs, which may be attributed to variations in physical activity intensity, and increases in renal blood flow and glomerular filtration rate during the active period. The Cre concentration level in bullfrogs was higher than those in dubois's tree frog (Das and Mahapatra, 2014), which might be due to species specificity and variations in the muscle mass.

Besides Cre, Bun was also an important indicator of kidney disease. The Bun concentration level reported here for bullfrogs during the active period was notably lower than those during the hibernating period, but higher than those in wild-caught *Xenopus laevis* (Sabrina Wilson et al., 2011). The significant difference in Bun concentration level between bullfrogs and *X. laevis* was not in agreement with the conclusion drawn previously by Wilson et al. (2011), who observed no statistical difference in Bun concentration level between bullfrogs and *X. laevis.*

LDH is a cytosolic enzyme, which is originally present in all the tissues involved in glycolysis, especially in cardiac tissue. Consequently, detection of raised concentration of this enzyme released into the blood stream from the damaged tissue has become a definitive diagnostic and prognostic criterion for various disorders and diseases (Saravanan et al., 2012). In this study, the value of serum LDH activity was notably lower when compared with those of wild-caught *Xenopus laevis* by Wilson et al. (2011). The level of LDH activity in male bullfrogs was statistically higher than those in females, which was in accordance with those previously obtained for captive finless porpoises by Kasamatsu et al. (2012). So far, the mechanism of serum LDH activity change between sexes was not illustrated in amphibians in the literatures.

Serum electrolyte values were important basic information for clinicians and researchers to assess the health status of animals. In this study, male bullfrogs had lower values of serum Na and Cl concentrations than those of female bullfrogs, which coincided with the results obtained earlier for wild-caught *X. laevis* (Wilson et al., 2011), and for anesthetized American bullfrogs (Cathers et al., 1997). In addition, it was found that serum Na and Cl concentrations significantly increased in bullfrogs during the hibernating period.

In summary, hematology and serum biochemistry parameters are effective at identifying the changes of body function prior to a sign of clinical abnormality. As far as we know, this study provided the first set of reference ranges of hematology and serum biochemistry for healthy farmed bullfrogs according to their sexes and two different periods. These baseline data could be used for future studies on its physiology and assessments for health monitoring and disease diagnostics. It is believed that these baseline data could also serve as general reference values for future investigations involving this species and other anuran species.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations:

RBC, Red blood cell; **WBC,** white blood cell; **HGB,** hemoglobin; **PCV,** packed cell volume; **MCV,** mean corpuscular volume; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **ALP,** alkaline phosphatase; **Amy,** a-amylase; **ALT,** alanine aminotransferase; **AST,** aspartate aminotransferase; **Cho,** cholesterol; **Cre,** creatine; **Glu,** glucose; **TG,** triglycerides; **TB,** total bilirubin; **UA,** uric acid; **Bun,** blood urea nitrogen; **GGT,** γ-glutam; **LDH,** lactate dehydrogenase; **CK,** creatine kinase; **TP,** total protein; **Alb,** albumin; **Glo,** globulin.

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Assessment of public knowledge, attitude and practices towards rabies in Debark Woreda, North Gondar, Ethiopia

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The study was conducted from November 2015 to April 2016 to assess the knowledge, attitudes and practices (KAP) on rabies and associated risk factors among the community of Debark district, North Gonder, Amhara regional state, Ethiopia. For this cross-sectional study, a simple random sampling procedure was employed to select kebeles. From the list of kebeles, six were randomly selected using lottery method. Then, 70 households were selected and interviewed from each kebeles using systematic random sampling method. A structured questionnaire was used to collect the data through face-to-face interviews among 422 respondents. Then, the data was analyzed using SPSS statistical software version 20. The frequency distribution of both dependent and independent variables were worked out by using descriptive statistics technique (Frequencies, mean, SD and percentage). Association between independent variables and KAP scores on rabies was calculated using Pearson's Chi square. Out of 416 respondents interviewed, 240 (57.7 %%) of them were males and 176 (42.3%) females. 151 (36.3%) of the respondents were between 15 to 29, 153(36.8%) were between 30 to 45 and 112 (26.9%) were >45 years old. The majority of the respondents 391 (94.0%) were Orthodox Christians. Almost all of the respondents indicated that they had previously heard about rabies. Out of this 251 (60.3%) had good level of KAP on rabies. There was strong association between KAP scores and educational level; occupation and sex ($p<0.05$). Generally, these findings indicate that the Debark Woreda community has good knowledge about rabies. However, a need for further awareness creation on the attitude and practice for appropriate prevention and treatment measure. Therefore, Veterinarians and health professionals should prepare and deliver continuous and strategic community awareness programs on prevention and control of rabies in the study area.

Key words: Attitude, debark district, Ethiopia, knowledge, practice, rabies.

INTRODUCTION

Rabies is characterized by an acute encephalitis illness caused by rabies virus genus *Lyssavirus* in the family of *Rhabdoviridae* that affects virtually all mammals. Infected species invariably die from the disease once clinical signs are manifested (Jackson and Wunner, 2007). It is the most widely recognized example of salivary transmission

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of viruses. Inoculation of infected saliva through the bite of a rabid animal appears to be the predominant mode of rabies. Contamination of broken skin and mucous membrane such as mouth, nasal cavity or eyes by fresh saliva or neurological tissues may also result in infection (OIE, 2013). Rabies virus (RABV) is a highly neurotropic pathogen that typically leads to mortality of infected animals and humans. It is estimated that nearly 55,000 human fatalities occur each year due to RABV (Andrea and Jesse, 2012). Although actual number is likely much higher due to unreported exposures or failure of diagnosis. In the 21st century, rabies remains as one of the most feared and important threats to public health. As a neglected zoonotic disease, rabies is present throughout the world, with many deaths in human beings occurring in Africa and Asia in children younger than 15 years. Rabies is regarded as under-reported in many regions (Fooks, 2005). Dogs are the principal vector for human rabies, and are responsible for more than 99% of human cases. Hence, controlling rabies in dogs, and especially free-roaming (stray) dogs, is the first priority for prevention of human rabies.

The disease causes a severe and long-lasting societal and economic burden and the implications are especially apparent in poverty-stricken developing countries. Shortage of resources and a limited public health infrastructure in many rabies-endemic countries precludes data collection and analysis. Rabies has been successfully controlled in dog population in the America. In both dog and terrestrial wildlife populations, rabies has been successfully eliminated from Western Europe. Thus, rabies can be controlled with sufficient resources (Nottidge, 2005). The demographic characteristics of dogs biting humans and livestock have not been fully elucidated. Besides, the rabies status of dogs biting humans has not been known. It has been a common practice to provide post-exposure vaccines to humans bitten by dogs irrespective of their rabies status. In Ethiopia, rabies is an endemic disease with a the incidence rate of 73% (Eshetu et al., 2012). Unfortunately, individuals who are exposed to rabies virus often see traditional healers for the diagnosis and treatment of the disease. These widespread traditional practices of handling rabies cases are believed to interfere with timely seeking of post exposure prophylaxis (PEP). Rabies victims specially, from rural areas seek PEP treatment after exhausting the traditional medicinal intervention and usually after a loss of life from family members (Deressa et al., 2010).

Community awareness about rabies is very crucial in rabies prevention and control. For efficiently increasing awareness, the knowledge gap among the community should be identified and targeted. Community awareness of all aspects of rabies is generally lacking or limited, such as first aid or management of animal bites, pre- and post-exposure prophylaxis, responsible pet dog ownership, dog population management. Regarding the immediate measures to be carried out after a bite exposure, there is inadequate knowledge of the crucial need to wash wounds with soap and running water and apply antiseptics and where vaccine is available. People may also contact local traditional healers for treatment, thus losing precious time and increasing the danger of infection and death (WHO, 2004). In Debark town elimination of stray dogs and prophylactic vaccination was practiced in October 2015 but was limited to the three kebeles (the smallest administrative unit) of the town. There is lack of accurate quantitative information on rabies both in humans and in animals and little is known about the awareness of the people about the disease to apply effective control measures in Ethiopia. Even if there were reports of death of humans and animals in the study area, no prior studies were undertaken about the prevalence and public awareness towards rabies. Thus, the objective of this study was to assess the level of knowledge, attitudes and practices regarding rabies and associated risk factors among the communities of Debark District.

MATERIALS AND METHODS

Study area

The study was conducted from November 2015 to April 2016 to assess the level of knowledge, attitudes and practices towards rabies in Debark district, North Gondar, Amhara regional state, Ethiopia. Debark district has a total of 30 kebeles which are located 830km far from Addis Ababa, the capital city of Ethiopia. The district has latitude of about 13.133^{o}N and longitude of about 37.900°E and an elevation ranging from 2712 to 3122 m above sea level (m.a.s.l). The area receives an annual rainfall of ranging from 900 to 1400 mm, which comes from long and short rainy seasons. The average minimum and maximum annual temperature ranges between 6.2^{o}C and 20.7°C, respectively with humidity of about 25 to 83.5%. The total human population of the district is estimated about 169835, from which 85594 are male and 84242 are female population. Debark has a livestock population of cattle (380403), equine (27449), shoat (185922), poultry (159612) and dogs (15000) (CSA, 2009).

Study design and study population

A cross-sectional study design employed to assess the knowledge, attitudes and practices (KAP) on rabies and associated risk factors among the community of Debark district. The study population was household heads or their spouses who had lived in randomly selected kebeles of Debark district (Mikara, Debir, Miligebsa, Kino, Kebele 01 and 02) as permanent residents for more than six months.

Sample size determination and sampling techniques

The required sample size for this study was estimated by considering 50% of the population knowing about rabies since earlier there is no awareness study on rabies had been conducted in the study area. Thus, the sample size was calculated according to Thrusfield formula by using 95% confidence interval and 0.05 absolute precision (Thrusfield, 2005) as follow:

$$N = \frac{1.96^2 P_{exp}\,(1-P_{exp})}{d^2}$$

Where N = required sample size; Pexp = Expected proportion of population knowing about rabies are 50%; d^2 = Desired absolute precision (0.05).

As a result, 384 respondents were selected as study population by adding 10% non-response rate; thus, the total sample size was 422 subjects. A simple random sampling procedure was employed to select kebeles for this study. From the entire primary sampling unite that is, 30 kebeles (lowest administrative structure), six were randomly selected using lottery method. Then, 70 households were selected and interviewed from each kebeles using systematic random sampling method, as there was no significant difference in number of household's. Whenever the selected household was found locked, the next household (on the right side) was substituted automatically for interview. A pretested structured questionnaire consisting of closed ended questions was used for this study. The data were collected via face-to-face interview. The questionnaire was first developed in English and then translated in to Amharic language (native language) for appropriateness and easiness in approaching the study participants.

Inclusion and exclusion criteria

Household who live more than 6 months as a permanent resident in the study area were included in this study and household who live less than six months and respondents in the household who cannot communicate and under 15 year were excluded from this study.

Data management and analysis

After collecting, the data were cleaned and checked for its completeness. Those incomplete and inconsistent were corrected when possible and removed otherwise. After complete check-up, the data were coded and entered to Microsoft Excel and transport to SPSS version 20.0 statistical packages for windows and analysis made. The frequency distribution of both dependent and independent variables were worked out by using descriptive statistics technique (Frequencies, mean, Standard deviation (SD) and percentage). Association between independent variables and KAP scores on rabies was calculated using Pearson's Chi square. All p values less than 0.05 was considered as statistical significance.

Ethical clearance

The study protocol was reviewed and approved by Institutional Review Board of Jimma University Research and Community Service Office. Oral informed consents were obtained from each participant after informing them about the purpose of the study as well as the risks, benefit and rights of the study participants. Only voluntary participants were involved in the study. All the information obtained from the study participants was kept confidential.

RESULTS

Socio-demographic characteristics

Four hundred twenty two heads of household were interviewed during the study period of this research. Of these, the data collected from six respondents were found to be incomplete and excluded from the analysis. Only data from 416 households were considered for the analysis. The majority of the respondents were male 240 (57.7%) and were above 15 years of age, of which 36.3 and 36.8% were between 15 to 29 and 30 to 45 years old, respectively, with a response rate of 99.0%.The majority of the respondents 391(94.0%) were Orthodox followed by Muslim 25 (6%). Concerning educational status, 182 (43.8%) of the participants were illiterate, 46 (11.1%) had profession with diploma and above and 113 (27.2%) were in high school and preparatory program (Table 1).

Community KAP about rabies in study area

Twenty-two questions were asked from each respondent regarding cause, sources, mode of transmissions, clinical signs and prevention practices and treatment measures of rabies. The questions were with multiple choices. Respondents who answered the questions correctly had got one mark and those who selected wrong answers had zero marks. The number of questions for which respondents give correct answer was counted and scored. Then, the scores were pooled together and the mean score was computed to determine the overall KAP of respondents. The respondents who score greater than or equal to the mean value (Mean=13.75, SD=3.15) grouped to good KAP and less than the mean value were grouped as Poor KAP level. As table 2 indicates, out of 416 respondents, 251 (60.3%) and 210 (40.7%) were found to have good and poor KAP towards to rabies.

Knowledge of participants related to cause and host range

All of the respondents (100%) were familiar with the rabies. It is called as*'Yebed wusha beshata'* and '*lekift*' locally, which mean madness. Out of 416 respondents 143(34.4%) were got the knowledge about rabies through formal way such as radio and television. However 146 (35.1%) and 127 (30.5%) of the respondents had the awareness through informal (such as traditional healers neighbors, friends and relatives) and both (formal and informal) ways, respectively. Sixty-seven (16.7%) of the respondents knew as virus is the cause of rabies. 404 (97.1%) knew that rabies is transmitted from animal to human, 231(55.5%) knew as all mammal can be affected by the disease and 363 (87.3%) were aware of the fact that dog is the most common source of rabies (Table 2).

Knowledge of participants related to mode of transmission, sign and symptom and treatment

Biting, scratching and saliva contact with open wound

Table 1. Socio-demographic information of the study participants in Debark woreda, North Gondar, Ethiopia, during 2015 to 2016.

Variables	Category	Frequency	Percent
Sex	Male	240	57.7
	Female	176	42.3
Age (in years)	15-29	151	36.3
	30-45	153	36.8
	>45	112	26.9
Household size	1-3	158	38.0
	4-6	230	55.3
	>6	28	6.7
Educational status	Illiterate	182	43.8
	Primary school	75	18.0
	Secondary school	113	27.2
	Diploma and above	46	11.1
Occupation	Government employee	43	10.3
	Merchant	76	18.3
	Farmer	236	56.7
	Unemployed	7	1.7
	Other	54	13.0
Religion	Orthodox	391	94.0
	Muslim	25	6.0

Table 2. Knowledge of participants related to cause and host range in Debark Woreda, North Gondar, Ethiopia, during 2015 to 2016.

Variables	Category	Frequency	Percent
Have you heard rabies before	Yes	416	100
	No	0	0
Source of information	Formal	143	34.4
	Informal	146	35.1
	Mixed	127	30.5
Can dog get rabies	Yes	416	100
Cause of rabies	No	0	0
	Psychological problem	1	.2
	Associated with sprit	31	7.5
	Virus	67	16.7
	Starvation & thirst	264	63.5
	I don't know	52	12.5
Spp. Affected by rabies	Dog	6	1.4
	Human & dog	44	10.6
	Human, dog, cattle, equine & shoat	135	32.5
	Wild animals	-	-
	All	231	55.5

Table 3. Knowledge of participants related to mode of transmissions, sign and symptom and treatment of rabies in Debark Woreda, North Gondar, Ethiopia, during 2015 to 2016.

Variables	Category	Frequency	Percent
Transmit from animal to human	Yes	404	97.1
	No	12	2.9
Mode of transmission	Biting	99	23.8
	Scratching	10	2.4
	Saliva contact with open wound	5	1.2
	All	302	72.6
Common source of rabies	Dog	363	87.3
	Dog & cat	3	.7
	Dog &wild candies	50	12.0
Sign and symptom	Stops eating and drinking	17	4.1
	Biting and change in behavior	68	16.3
	Salivation	15	3.6
	All	316	76.0
Incubation period	Immediately	7	1.7
	-40 day	147	35.3
	-90 day	208	50.0
	I don't know	54	13.0
Is rabies fatal	Yes	412	99.0
	No	2	.5
	I don't know	2	.5
Easily treatable after onset of clinical signs	Yes	1	.2
	No	380	91.3
	I don't know	35	8.4

were mentioned by 302(72.6%) of the respondents as a mode of transmission. Regarding to sign and symptom of the disease 316 (76.0%) stated that rabid animals stop eating and drinking, further there is notable change in behavior, hydrophobia; paralysis and salivation are common in rabid animals. 380 (91.3%) of the respondents knew that the disease could not easily treatable once clinical signs are manifested and 412 (99.0%) were awarded fatal nature of the disease (Table 3). Of all the respondents 144 (34.6%) had dog; among them 102 (70.8%) vaccinated their dog and 11 (7.6%) castrated their dog. Regarding to castration 254 (61.1%) have knowledge as castration decrease incidence of rabies.

Practices and attitudes to prevent rabies after suspected animal/dog bite in Debark district

Concerning to immediate action taken after bite, 318 (76.4%) of the participants washed with water and soap and 228 (54.8%) used traditional healer after first aid and 175(42.1%) contacted health center. The attitude on anti-rabies vaccine was positive by 279 (67.1%) of the respondents. Killing of rabid animal was the first choice by 350 (84.1%) of the participants. Killing was also the first option of 235 (56.5%) of the participants to control stray dog. 270 (64.9%) of the respondents revealed that meat of rabid domestic animals will not cause disease during consumption by human (Table 4).

Factors associated with community KAP on rabies in Debark Woreda

Association between independent variables and KAP scores on rabies was calculated using Pearson's Chi square (Table 5). There was significant association between KAP scores and sex ($p < 0.05$).

The good KAP scores were recorded higher in males 155 (64.6%) than females 96 (54.5%). Educational status had strong significant associated with KAP scores ($p = 0.00$). All respondents with diploma and above education levels had good KAP of rabies. As Table 5

Table 4. Practices and attitudes to prevent rabies after suspected animal/dog bite in Debark woreda, North Gondar, Ethiopia, during 2015 to 2016.

Variables	Category	Frequency	Percent
Do you have dogs	Yes	144	34.6
	No	272	65.4
Do you vaccinated your dog	Yes	102	70.8
	No	42	29.2
Was your dog Castrated/spayed	Yes	10	6.9
	No	134	93.1
Does castration decrease incidence	Yes	254	61.1
	No	75	18.0
	I don't know	87	20.9
Family exposure	Yes	8	1.9
	No	408	98.1
Immediate action after bite	Tie the wound with cloth	76	18.3
	Wash with water and soap	318	76.4
	Apply herbal extract	16	3.8
	I don't know	6	1.4
After 1st aid	Health center	175	42.1
	Traditional healer	228	54.8
	Holly water	9	2.1
attitude to vaccine	Positive	279	67.1
	Negative	137	32.9
Actions taken for rabid animals	Let free	28	6.7
	Tie	38	9.1
	Killing	350	84.1
Measures to control stray dogs	Killing	235	56.5
	Animal birth control	10	2.4
	Aware the owner	171	41.1
Is safe consumption rabid food animal	Yes	270	64.9
	No	100	24.0
	I don't know	46	11.1

indicated, there was no significant association ($p>0.05$) between KAP score and age and religion of the respondents. Occupation had statistical significant association with knowledge levels ($p=0.001$); in which government employee has good KAP level (90.7%) than unemployed (57.1%) respondents.

DISCUSSION

The findings of this study indicated that, all respondents (100%) were aware of rabies and dog are the common source of rabies. In line with current report, Digafe et al. (2015) and Singh and Choudhary (2005) in Gondar Zuria District and rural community of Gujarat, India, indicated that 99.3 and 98.6% of people have heard about rabies before, respectively have previously heard about rabies. Jemberu et al. (2013) also reported high level of awareness (98%) about rabies in Gondar Zone, Ethiopia. However, the current investigation is higher than other reports from Addis Ababa, Ethiopia and India that

Table 5. Factors Associated with Community KAP on Rabies in Debark woreda, North Gondar, Ethiopia, during 2015 to 2016.

Variables		Good	Poor	x^2	P-value
Sex	Male	155(64.6%)	85(35.4%)	4.275	0.039
	Female	96(54.5%)	80(45.5%)		
Age(in year)	15-29	82(54.3%)	69(45.7%)	4.429	0.109
	30-45	94(61.4%)	59(38.6%)		
	>45	75(67.0%)	37(33.0%)		
House hold size	1-3	94(59.9%)	63(40.1%)	1.719	0.633
	4-6	139(60.4%)	91(39.6%		
	>6	18(64.3%	10(35.7%)		
Educational status	Illiterate	89(48.9%)	93(51.1%)	32.534	0.000
	Primary school	42(46.0%)	33(44.0%)		
	Secondary school	78(69.0%)	35(31.0%)		
	Diploma and above	42(91.3%)	4(8.7%)		
Occupation	Government employee	39(90.7%)	4(9.3%)	42.023	0.000
	Merchant	60(78.9%)	16(21.1%)		
	Farmer	114(48.3%)	122(51.7%)		
	Unemployed	4(57.1%)	3(42.9%)		
	Other	34(63.0%)	20(37.0%)		
Religion	Orthodox	235(60.1%)	156(39.9%)	0.149	0.699
	Muslim	16(64.0%)	9(36.0%)		

reported 83 and 68.7%, respectively (Ali et al., 2013, Ichhupujani et al*.,* 2006). The reason could be due to real difference in incidence of rabies in the areas of study and living status of the community as stated by Digafe et al*.* (2015) as rural community has better communication and information about what is happening in their residential area, including animal disease situations, which may contribute to their high level of awareness. The case of KAP level of the community about 60.3% of the respondents had good level of knowledge, attitude and practices about rabies. Relatively similar result was reported by Tadesse et al*.* (2014) about 64.1% among the community of Bahir Dar town. In contrast to this finding higher KAP level was reported from Sri Lanka (Gino et al*.,* 2009). This difference probably explained by sample size difference and lack of health education programs about rabies in Ethiopia. The source of information for the majority of the participants (34.4%) were formal and (35.1%) was through mixed (both formal and informal) which was higher compared to the report from Bahir Dar town (10.7%) by Tadesse et al*.* (2014) and from Addis Ababa (21.5%) by Ali et al. (2013). This difference may be due to the presence of community based radio station in the study area and radio is the major source of information in the rural area of Ethiopia. Of those respondents, 63.5% had misunderstanding on the cause of rabies; they believed that the disease in dog is caused by starvation and thirst. It was higher when compared with the result obtained from study conducted in and around Dessie town, Ethiopia which was 49.6% (Gebeyaw and Teshome, 2016) and in Bahir Dar town, 39.9% (Tadesse et al*.,* 2014). In addition, current study was lower than the findings of Jemberu et al*.* (2013) which was 86%, from Gondar and Dabat, Ethiopia. This could be due to sample size difference, study area and community awareness difference. Beside this, the higher misunderstanding may arise from the notion of asymptomatic rabies carrier dogs in which stressors like starvation and thirst might induce development of clinical rabies in carrier dogs. However, the notion of asymptomatic rabies carrier dogs by itself is a contentious issue (Zhang et al*.,* 2008; Wilde et al*.,* 2009). Human rabies caused by the classical rabies virus continues to be almost 100% fatal, with no specific treatment available anywhere in the world (WHO, 2013). In the present study, KAP analysis revealed that 99.0% of respondents recognize rabies as danger and a fatal disease. The current finding was in line with the study conducted in Bahir Dar town (94.5%) by Tadesse et al*.* (2014) and New York, USA (94.1%) by Eidson et al.

(2004). However, this result was disagreed with the study reported (30.97%) from Addis Ababa (Ali et al., 2013). This could be due to the high rate of incidence and frequent death of the affected host in the study area.

In the study area, 55.5% respondents knew that the disease could affect all mammals of domestic and wild animals. In contrast to this study, Tadesse et al. (2014) reported a lower result (21.4%) from Bahir Dar. This difference may be due to the availability of different host range in the rural district of our study site. Higher result (71.9%) was also reported in the city of New York, USA (Eidson et al., 2004) which could be due to the educational status and/or awareness of the community. Regarding to public health importance, about 97.1% of the respondents claimed that the disease is transmitted from animal to human, 72.6% of the respondents knew that the mode of transmission of rabies was through by biting, scratching and open wound contact with saliva. This finding was supported by report from Addis Ababa, 75.5% of respondents knew that rabies to be transmitted through animal bite (Ali et al., 2013). A higher result was reported from North Gondar, Ethiopia by Jemberu et al. (2013) about 84% of the participant stated any type of contact (irrespective of the skin condition) with saliva of affected individual could transmit the disease. A few respondents mentioned that rabid equine and donkey can transmits the disease but ruminant could not transmit the disease to human through biting or through their milk and meat during consumption. Inoculation of infected saliva through the bite of a rabid animal appears to be the predominant mode of rabies transmission (Radostits et al., 2007). Contact of infected saliva with broken skin or mucous membrane can transmit the disease (WHO, 2014). About 76.0% of the respondents were aware of common clinical signs of rabies in animals. This finding was in line with Tadesse et al. (2014) and Asabe et al. (2012) reported from Bahirdar, Ethiopia (76.8%) and Nigeria, respectively. In the present study, dog vaccination was practiced by 24.5% of the respondents. In line with this report, dog vaccination practice was generally very low and very nonexistent in rural district of Dabat and was good in Gondar town (Jemberu et al., 2013). In contrast, higher result (36%) was reported in and around Dessie city by Gebeyaw and Teshome (2016). This low level of report for vaccination in study area was claimed by the respondents' due to lack of access and low awareness towards rabies vaccines. Raising awareness about dog vaccination and improving access and affordability of the vaccine should be considered in control of the disease, as dogs are the main reservoir of the disease. Two point six percent of the participant had been castrating their dog and most of them used to castrate through traditional method by removing the testicle. Most of the respondents (61.1%) had awareness as castration decrease the incidence of the disease. Castration (sterilization) is another option for canine population management of male dogs, which has been used in Mexico, Brazil and other countries (Oliveira et al., 2012, Soto et al., 2009). However, sterilization efforts should not focus only on males, as females are also critical target for effective population management (Jackman and Rowan, 2010). More often, however, rabies control programs have attempted to cull dog populations, even though this approach has been shown to be ineffective (Dalla et al., 2010; Morters et al., 2013). Such lethal management strategies require the elimination of 50 to 80% of dogs a year, which is neither financially possible nor ethically acceptable (Rupprecht et al., 2002). Among the immediate action taken after bite, wash with water and soap was reported by most of the respondents (76.4%) in this study. This result is higher compared to the study conducted in Gondar zuria district (30.7%) (Digafe et al., 2015) and in a Rural Community of Gujarat, India (31.1%) (Singh and Choudhary, 2005). The variation may be due to the study area and awareness level of the community. Washing of rabies-infected wounds with soap and water can increase survival by 50% (Radostits et al., 2007). This treatment is cheap, readily available and feasible for all to apply. All the participant 279 (67.1%) had positive attitude for anti-rabies vaccine. This result is higher compared to a study conducted in Bahirdar (42.8%) (Tadesse et al., 2014). This difference could be due to skill and awareness of the community for vaccination.

Low level (42.1%) of preference for health center (for PEP) was observed in this study. Most respondents choose other options like traditional healer (54.8). Similarly, studies conducted in Gondar zuria district, Ethiopia, reported about 62.2% of the study participants had strong beliefs in traditional medicine (Digafe et al., 2015). In Satkhira, Bangladesh, 59% of the dog bite victims first seek treatment from traditional healers instead of visiting the hospitals (Ghosh et al., 2016). A higher (84%) reliance of respondents on traditional treatment was reported from Dabat and Gondar (Jemberu et al., 2013). In contrast to these report, almost all respondents agreed to consult health professional in case of animal bite was reported in Addis Ababa (Ali et al., 2013). The preference for traditional practices might be arise from many factors including easy access to traditional medicine, lack of awareness, long duration of treatment. Reliance on traditional medicines with unproven efficacy is very risky and nothing can be done to save one's life after the first symptoms of the disease occur. After suspected or proven exposure to rabies virus, immediate use of efficient anti-rabies vaccine with proper wound management and simultaneous administration of rabies immunoglobulin is almost invariably effective in preventing rabies (WHO, 2005). Most of the respondents (64.9%) in our study claimed that meat of rabid food animal is safe for consumption. These results were consistent with that reported in Gondar Zuria district by Digafe et al. (2015) which revealed consumption of cooked or boiled meat from

rabid animals was considered as safe by 67.0% of the respondents and about 19% replied even raw meat is safe for human consumption. According to WHO (2014), consumption or preparation of meat from rabid animals is a risk. The consumption of raw meat from an infected animal requires PEP. Cooked meat does not transmit rabies; however, it is not advisable to consume meat from an infected animal (WHO, 2013). Even though the extent of transmission varies, all possible modes of transmission including bite, contact with saliva, and consumption of animal products from infected animal should be avoided. During analysis of KAP with independent variables, the good scores were higher in males (64.6%) than females (54.5%). The same proportion of statistical difference on KAP score of male (53.4%) and female (10.75%) was reported in Bahirdar town by Tadesse et al. (2014) and comparable result was reported from Addis Ababa in male (77.09% moderate and 10.55% good) and female (73.62% moderate and 5.08% good) (Ali et al., 2013). The statistical significant difference (P<0.001) in KAP score between males and females might be due to increased activity of males in their daily life compared with females and better chance of acquiring correct information about rabies. The other factor that identified to be significantly associated with knowledge on rabies was educational status. Statistically significant association (P<0.001) was observed between KAP score and educational levels where by higher levels of educations were associated with higher knowledge scores. All respondents with diploma and above education levels had good KAP of rabies. Numerically, this finding show (51.1%) for illiterates, (69.0%) for secondary school preparatory students, (91.3%) for Diploma and above education level. This finding was also supported by a study conducted in Bahir Dar (Tadesse et al., 2014), from Addis Ababa (Ali et al., 2013) and the studies conducted in Flagstaff, Arizona, USA (Andrea and Jesse, 2012). The possible explanation could be educated person would have better information access and can easily understand the disease. Occupation also another risk factor that has high statistical association with KAP score (p<0.001). High score was recorded in government employee (90.7%) and low score in farmers (48.3%). In line with this finding, a statistically significant association ($\times^2$ =40.971, p<0.001) with the highest knowledge level (9.89%) in employed/professionals and low level (4.94%) among unemployed/ housewife was reported from Addis Abeba by Ali et al. (2013). This result conveys that employment has a direct relation with source of knowledge. The type of occupation could also determine the source of information that relates government employee to formal source of information, which increases their level of knowledge.

CONCLUSION AND RECOMMENDATIONS

This study showed that rabies was a well-known disease in the study area. The KAP level towards to rabies of the community of Debark woreda found to be good. But still there are some gaps in the community concerning with cause and mode of transmission, host range of the disease, clinical signs of rabies, prevention methods after suspected animal bite and attitude to anti-rabies vaccine. In addition, the respondent said consumption of meat from rabies-infected animal has a less risk of zoonosis. On the other hand, there is a lack of knowledge about what to do after exposure, like immediate visits to health facilities, and use of anti-rabies post exposure prophylaxis, which might be due to lack of awareness creation. Moreover, sex, educational status and occupation of the respondents found to have a significant association with KAP score. Good KAP score has found related to educational status and employment that implies most of the illiterate of the rural area and farmers, spend their life with a variety species of animals, are prone to the disease easily. Therefore, based on the aforementioned conclusion the following recommendations are forwarded:

1. Veterinarians and health professionals should prepare and deliver continuous and strategic community awareness programs on prevention and control of rabies in the study area.
2 Governmental organizations like Federal Ministry of Health, Federal Ministry of Livestock and fishery resource and University of Gondar should work in cooperation with information sources like radio and television programs to give an information which will enhance the awareness level of the community.
3. The Amhara Regional Health Bureau should also design accurate and urgent Community based rabies education program with emphasis on mode of transmission, clinical signs and immediate benefits of wound management and need for Anti-rabies vaccine following dog bite.
4. The Amhara livestock and fishery resource Bureau should register the dog population of the region and prepare a legislation that will enforce the owners to vaccinate their animals.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sero-prevalence of Newcastle disease virus antibodies in local and exotic chickens in Gwagwalada, Nigeria

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This present study was conducted to determine the sero-prevalence of Newcastle disease (ND) antibodies in both local and exotic chickens in Gwagwalada, Nigeria. Two hundred sera were randomly collected post slaughter from chickens and analysed by Haemagglutination (HI) and Haemagglutination Inhibition Test (HIT). Fifty structured questionnaires were distributed among poultry keepers, workers and other stakeholders to evaluate their level of awareness and impact of avian ND in the study area. Results revealed an overall sero-prevalence of 63.5%. Breed sero-prevalence was 54% and 73% for local and exotic chickens respectively. However, 46% local chickens and 27% of exotic chickens had no detectable antibodies. Statistical analysis of the avian ND antibody titres showed association between breeds of chickens [$P<0.05$; ($\chi^2=7.79$); df= 1)]. The questionnaire study further revealed high level of ND awareness and the adverse effects of ND virus in poultry. Local breeds were rarely vaccinated against ND virus as indicated by the respondents and low antibody titres detected. This study provides preliminary information on ND prevalence in exotic and local chickens in Gwagwalada. Hence, the need to conduct further researches on ND in the study area using molecular diagnostic techniques in order to affirm disease burden and impact especially amongst local chickens for the purpose of control.

Key words: Newcastle disease antibodies, sero-prevalence, haemagglutination, questionnaires.

INTRODUCTION

Newcastle disease (ND) is one of the most devastating poultry diseases in Nigeria. The disease has synonyms such as avian paramyxovirus infection, Pneumo encephalitis, Pseudo fowl pest, Ranikhet disease or Pseudoplague of fowl disease (CIDRAP, 2003). ND is an infectious disease of birds caused by the avian paramyxovirus serotype 1 virus (APMV-1) (OIE, 2000) which has four pathotypes namely, velogenic (highly virulent), mesogenic (moderately virulent), lentogenic (low virulence) and avirulent (Spradbrow, 1987). ND has been reported consistently from all continents worldwide (Munir et al., 2012). The epizootics of ND in poultry continue to occur in Asia, Africa, Central and South America while in Europe, sporadic epizootics occur (Naveon et al., 2013). The negative impact of the disease in both commercial and village rearing poultry production

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systems is of great significance especially in Africa (Nwanta et al., 2006; Mohamed et al., 2011; Rezaeianzadeh et al., 2011).

In Nigeria, poultry production is estimated at 137.6 million, with backyard poultry production constituting 84% (115.6 million) (FMA, 2006). This poultry production provides quality protein and financial income for rural families and the nation as a whole (Abubakar et al., 2008). ND has been associated with mild conjunctivitis and influenza-like symptoms with slight public health significance in human beings (Nelson et al., 1955). Annual economic losses in millions of dollars have been associated with the disease course (Susta et al., 2011) and heavy mortality patterns in poultry (Waheed et al., 2013). However, these continuous outbreaks have been poorly reported and demonstrated with un-robust laboratory diagnostic investigations in Gwagwalada Area Council. The paucity of information on ND status both in local and exotic chickens within this Guinea savanna zone premised this survey. This study aimed at establishing the sero-prevalence of avian ND virus antibodies in this study area with the view of quantifying the infection or disease burden and providing sustainable control and possible eradication measures.

MATERIALS AND METHODS

Study area

The study was conducted in Gwagwalada metropolis, Gwagwalada Area Council of the Federal Capital Territory, FCT Abuja. Gwagwalada is one of the six Area Councils of the Federal Capital territory, Abuja; alongside Abaji, Kuje, Bwari, Kwali and Abuja Municipal Area Council within this Guinea savanna zone. Gwagwalada covers an estimated land mass of 1043 km^2 and a population of 157,770 during the 2006 census (Anon, 2009), where the University of Abuja is located. Gwagwalada is located on geographical coordinates of 8°56′29″ North, 7°5′31″ East (3D Google Earth). The area is characterized by two seasons consisting of the raining season and the dry season. The dry season lasts between May and October with a uni-modal peak of rainfall in August. Gwagwalada Area Council is extremely hot in terms of temperature with a mean daily temperature of 31°C (Awowole and Francis, 2007). The high temperature has been suggested as a predisposing factor of Newcastle disease outbreaks (Njagi et al., 2010). This hot weather in Gwagwalada also premised the choice of this location for the survey (Figure 1).

Sample collection and processing

A total of 200 blood samples were collected post slaughter from both local chickens (N=100) and exotic chickens (N=100) (consists mainly of broilers) at the Gwagwalada market for ND virus antibody detection. Blood samples were collected in plane sample bottles that contain no anti-coagulant during slaughtering on a weekly basis between February and March, 2015. The samples were preserved at 45°C slants for sera separation at room temperature for two hours (OIE, 2012). The sera were then transferred into cryovials, labelled and stored at 4°C.The sera were later transported in ice parked flask for analysis at the Avian Influenza laboratory, Virology Unit, National Veterinary Research Institute (NVRI), Vom, Nigeria.

Laboratory analysis

Haemagglutination test

This test was conducted in accordance with the procedures outlined by OIE (2012). 0.025 ml of Phosphate buffered saline (PBS) was dispensed into each well of a plastic V-bottomed microtitre plate. 0.025 ml of the virus suspension (infective or inactivated allantoic fluid) was placed in the first well. For accurate determination of the Haemagglutination Assay (HA) content, this was done from a close range of an initial series of dilutions, 1/3, 1/5, 1/7…..1/20. Two fold dilutions of 0.025 ml volumes of the virus suspension were made across the plate. A further 0.025 ml of PBS was dispensed to each well. 0.025 ml of 1% (v/v) chicken Red Blood Cells (RBCs) was dispensed to each well. The solution was mixed by tapping the plate gently. The RBCs were allowed to settle to a distinct button for about 40 min at room temperature (20°C). Plate titling and observation for the presence or absence of tear shaped RBCs, streaming was used for determination of HA. The titration was read against the highest dilution giving complete HA (no streaming) which represents one HA unit (HAU) and was calculated accurately from the initial range of dilutions.

Haemagglutination inhibition test

This was also conducted based on the OIE Terrestrial Manual (2012). About 0.025 ml PBS was dispensed into each well of a plastic V-bottomed microtitre plate and 0.025 ml of serum was later placed into the first well of the plate. Two fold dilutions of 0.025 ml volumes of the sera were made across the plate. Four HAU virus/antigen in 0.025 ml was added to each well and the plate was left for a minimum of 30 min at room temperature (20°C). Later, 0.025 ml of 1% (v/v) chicken RBCs was added to each well and mixed gently, the RBCs were allowed to settle to a distinct button for about 40 min at room temperature (20°C). The Haemagglutination Inhibition (HI) titre was read from the highest dilution of serum causing complete inhibition of four HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as the control wells (positive serum, virus/antigen and PBS controls) were considered to show inhibition. The validity of this result was assessed against a negative control. Serum titre greater than or equal to 1/4 (2^2 or $\log_2 2$) when expressed as reciprocal was considered as positive.

Questionnaire survey

Fifty questionnaires were distributed amongst poultry farmers, farm workers and undergraduate students in Faculties of Veterinary Medicine and Agriculture in Gwagwalada, F.C.T-Abuja. This was to establish the level of awareness of ND amongst stakeholders as well as the attitude and practice of poultry farmers during ND management. The indices assessed include ability to suspect the disease, clinical signs usually noticed, age mostly affected, season of outbreaks, mortality patterns, and farmers' attitude towards ethno-veterinary measures, routine treatment attempts employed by farmers, other veterinary interventions and vaccination protocols employed.

Statistical analysis

Statistical Package for the Social Science (SPSS) was used for the

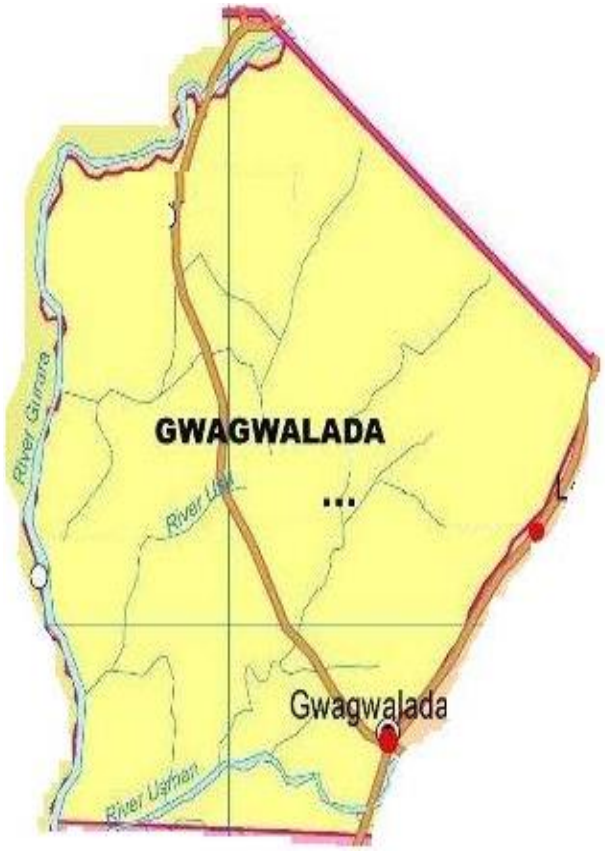

Figure 1. Map of Gwagwalada showing the location of sample collection at the Chicken Slaughter Market.

Table 1. Sero-prevalence of Newcastle Disease antibodies in local and exotic chickens in Gwagwalada.

Category of chickens	Local chicken	(%)	Exotic chicken	(%)
Number of positive	54	54	73	73
Number of negative	46	46	27	27
Total number tested	100	100	100	100

data analysis as adopted by Njagi et al. (2010). The numbers of positive antibody titers were expressed using descriptive statistics including frequencies and percentages. Data was also extrapolated as bar charts while Chi-square (χ^2) was used to compare the level of association between sero-prevalence of ND antibodies and the breeds of chickens. $P<0.05$ was significant.

RESULTS

Antibody titer

Out of 200 sera samples collected from 100 local and100 exotic chickens and analyzed using haemagglutination test and haemagglutination inhibition test, one hundred and twenty seven sera showed detectable antibody. The overall sero-prevalence of ND antibody revealed in this study was 63.5%. Breed distribution, showed sero-prevalence of 54% in local and 73% in exotic chickens. The results also revealed that 46% local chickens and 27% of exotic chickens showed no detectable antibodies for ND as indicated in Table 1. Statistical analysis of the avian ND antibody titres showed association between breeds of chickens [$P<0.05$; ($\chi^2=7.79$); df= 1)]. Figure 2 showed HI titer of 1.2 ($\log_2$ 2) as the most frequently found titer in the two breeds of chickens examined.

Questionnaire survey response

Questionnaire survey revealed the knowledge, attitude

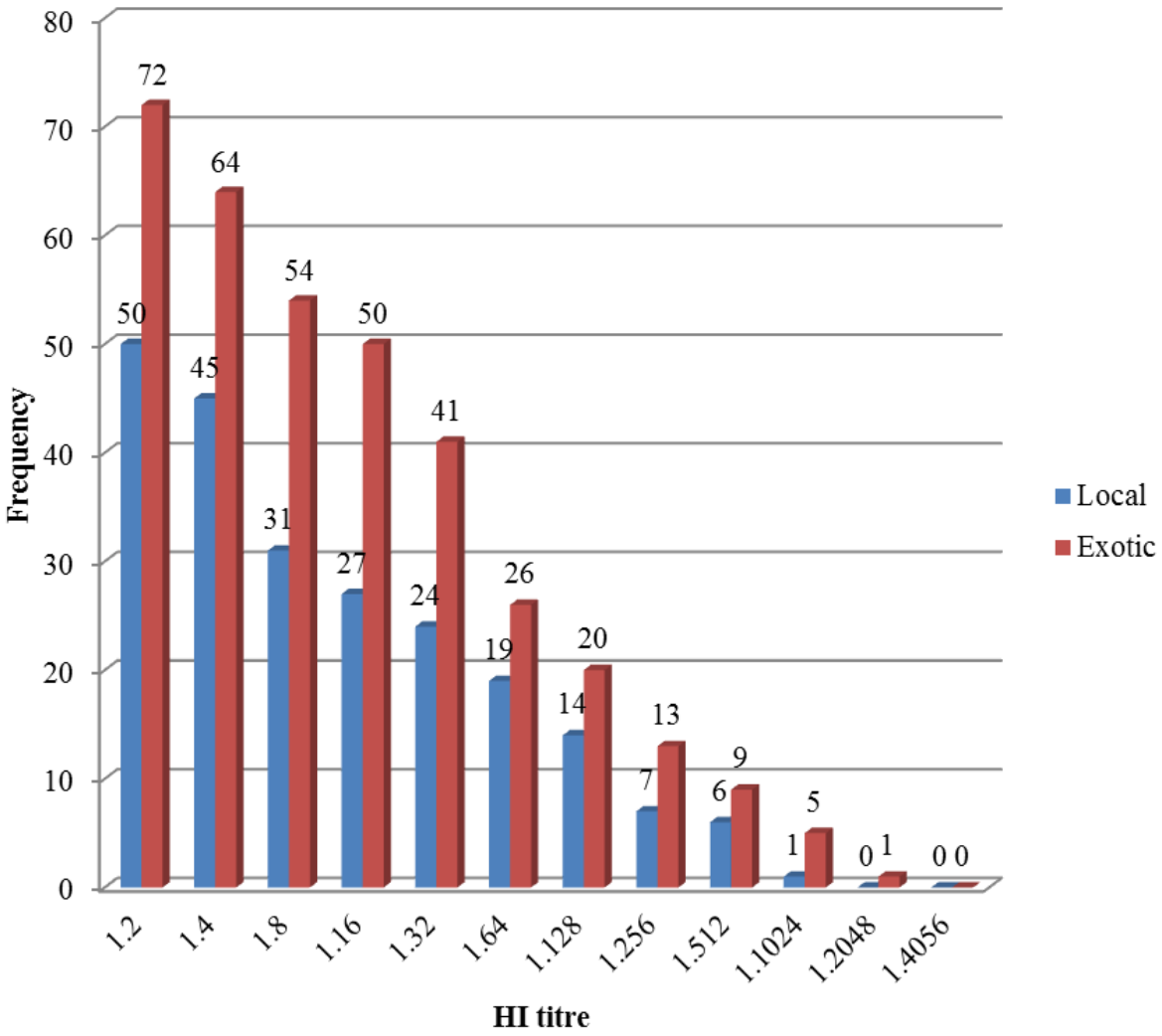

Figure 2. A bar chart showing ND antibody titres of both local and exotic chickens assayed in Gwagwalada.

and practice of farmers in the phase of ND outbreaks as well as the general awareness amongst Veterinary Medical Students. The questionnaire responses obtained is as shown in Table 2. This indicated 84% of respondents can suspect ND, and 68% of birds within the ages of 2-4 months were mostly affected. It also showed that exotic chickens (70%) are more susceptible to ND than local chickens (30%). Respondents (70%) reported ND outbreaks occur more during dry season than in rainy seasons. Most respondents (94%) had no idea on possible veterinary interventions for ND during outbreaks while 6% of respondents acknowledged that they used antibiotics as attempts for treatment of secondary bacterial infection. This questionnaire survey also revealed that 100% of the exotic breeds were vaccinated against ND while only 4% of the local chickens were exposed to ND vaccines. However, the knowledge on ND zoonotic importance in humans indicated low awareness response of 14%.

DISCUSSION

The results of this study conducted in Gwagwalada area council reveals the occurrence of ND antibodies in both local and exotic chickens. The finding in this study is in-line with previous report (Alders and Spradbrow, 2001). The overall ND sero-prevalence of 63.5% encountered in this study is lower than 97% recorded by Saidu et al. (2004) in Zaria, Nigeria. This variation may be attributed to physical factors including vaccination, vaccine failure or infection rates (Spradbrow, 1994; Anebo et al., 2014). Although, comprehensive history taking and laboratory analysis to differentiate ND antibodies associated with vaccination, vaccine failure or infection was not conducted in this study. The high prevalence of ND antibodies detected may therefore suggest the burden and endemicity of ND in the study area.

Sero-prevalence of 73% of ND antibodies recorded for exotic chickens in this study is higher than 22% ND

Table 2. Response of Newcastle Disease questionnaire survey administered to farmers and veterinary students in Gwagwalada.

Parameter	Response	Number of respondent	(%)
Ability to suspect ND	yes	42	84
	No	8	16
Age mostly affected	0-2months	12	24
	2-4months	34	68
	4-6months	4	8
Breed susceptibility	Exotic	35	70
	Local	15	30
Season of Outbreaks	Dry season	35	70
	Rainy season	15	30
Veterinary intervention	No idea	47	94
	Antibiotic therapy	3	6
Routine vaccination			
Local breed	Yes	2	4
	No	48	96
Exotic	Yes	50	100
	No	0	0
Zoonotic effects	Conjunctivitis	7	14
	No idea	43	86

antibody titres demonstrated in unvaccinated exotic birds by Oranusi and Onyekaba (1986) suggesting that the study birds were either previously vaccinated or were exposed to recent ND booster vaccinations prior to slaughter (Chandrasekar et al., 1988; Aldous and Alexander, 2001). This may have accounted for the high levels of protective antibody titres observed in these exotic chickens. Consequently, this observed prevalence may not reflect the true indication of ND in the study area. This would require further evaluation using other diagnostic methods.

The seroprevalence of 54% recorded in the local birds is in conformity with the findings of Adu et al. (1986) in Ibadan but higher than the 23.6% reported by Abraham et al. (2014) in Udu, Delta state using Haemagglution Inhibition Test. However, it was lower than the 73.3% prevalence reported by Ohore et al. (2002) using Enzyme Linked Immunosorbent Assay (ELISA) in Ibadan. The lower ND seroprevalence recorded in this study can be attributed to the low sensitivity of haemagglution test as compared with the more sensitive ELISA (Solano et al., 1986; Snyder, 1986). In addition, low ND antibody detection observed in some local birds could be attributed to absence of antibodies despite ND virus infection or presence of low un-detectable serum antibodies by haemagglutination test as previously reported by Adu et al. (1986). Although previous reports indicates uncommon vaccination practice in free ranged management system of local birds in Nigeria (Abdu et al., 1987), the sero-prevalence of 54% recorded amongst local birds in this study suggests exposure to ND infection in unvaccinated chickens as previously reported (Alexander, 1991). This none vaccination practice amongst local bird keepers could further enhance ND carrier status and possibility of re-infection (Adene et al., 1985) as well as disease spread to other in contact susceptible poultry especially the exotic chickens, ducks, geese (Ibrahim et al., 2005), domestic and wild scavenging birds such as pigeons, bats, doves and canaries (Bisalla et al., 2005). The questionnaire result indicates that most respondents were aware and could suspect ND to affect younger birds within the ages of 2 – 4 months. Most respondent also indicates ND outbreaks occur more frequently during dry season than in the rainy season. Vaccination history further reveals total and effective compliance with vaccination practice for exotic chickens as against the occasional use of vaccines in local chickens. However, ND zoonotic importance is not known as indicated by the responses.

In conclusion, this finding provides baseline data on the occurrence of Newcastle disease antibodies using HI and HIT tests in both local and exotic chickens in Gwagwalada, Abuja, Nigeria. The research also presents the low level of ND awareness in this study area.

Therefore, there is a need for continuous public awareness campaign programs especially amongst poultry farmers on effective ND control measures. Periodic antibody monitoring and disease evaluation in poultry flocks within the study area using molecular techniques is thus recommended.

Conflict of Interests

All authors re-affirm no conflict of interest during and after the conduct of this study.

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Authors wish to thank all poultry stakeholders in the study area for their support and cooperation during the questionnaires survey and sample collection. Efforts of the Staff of Virology diagnostic division, National Veterinary Research Institute (NVRI) Vom, Nigeria is also appreciated, for their assistance during laboratory sample analysis.

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Full Length Research Paper

Assessment of cadmium (Cd) residues in organs and muscles of slaughtered pigs at Nsukka and environs in Enugu State, Nigeria

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Pork is a major source of protein to man and widely consumed in Enugu State. The present study was undertaken to ascertain the presence and levels of cadmium in muscles and organs of slaughtered pigs at Nsukka agricultural zone. From a total of 160 slaughtered pigs, liver, kidney and muscle samples of each pig were collected and processed for the detection of cadmium residue using atomic absorption spectrophotometer. A prevalence rate of 76.25% was recorded for cadmium residue in Nsukka agricultural zone. There is a strong association (P<0.0001) between occurrence of cadmium residue and the source of samples. The mean concentrations of cadmium in liver (0.041, 0.035 and 0.056 mg/kg) and kidney (0.041, 0.035 and 0.056 mg/kg) from different sources (Nsukka, Orba and Obollo-Afor, respectively) were significantly higher (p<0.05) than what was obtained in muscles and also significantly higher (p<0.0001) than their specific maximum permissible limits (MPL). The levels of cadmium in few samples that exceeded the maximum permissible levels may pose human health threat to pork consumers in the study area.

Key words: Cadmium, kidney, liver, muscle, pig, slaughter house.

INTRODUCTION

Meat and meat products form an important part of human diet. In many African countries, as well as in Nigeria, internal organs (liver, kidneys, heart, and lungs) are sold and consumed as a cherished food source. The risk of

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heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations (Santhi et al., 2008). Heavy metals such as cadmium are naturally occurring elements in the earth's crust, and thus direct or indirect exposure to them from natural sources is inevitable especially for animals that are not intensively reared. Animals reared on contaminated pasture or fed with contaminated feed become a source of heavy metal residues in edible animal products (meat, fish, egg, milk). Indiscriminate dumping of waste materials on land and water bodies, illegal mining of ores, painting of animals' houses, and methods of processing slaughtered pigs have been incriminated in habitual contamination of animals and their products with heavy metals (Bala et al., 2012). Water bodies located near the abattoir often get contaminated with these hazardous substances through bad abattoir practices, improper management and supervision of abattoir activities. Pigs may drink water from ponds, streams, rivers and other possible contaminated water sources.

Cadmium is the most abundant, naturally occurring element; it was discovered in early 19th century and widely distributed in air, soil, water and plants (Bernard, 2008). Some other sources include cigarette smoking, industrial and agricultural chemicals and contaminated food products. Cereals and vegetables are said to be the major sources in food because of their high consumption rate, followed by meat and offals. It is found in the environment mainly associated with zinc and to a lesser extent with lead and copper. Exposure to cadmium has been associated with hepatic injury, lung damage, hypertension nephrotoxicity, osteoporosis, neurotoxicity, genotoxicity, teratogenicity, and it has been classified as a human carcinogen Group 1 (Huff et al., 2007; Gallagher et al., 2008).

The polluted meats from the edible animal products exposed to heavy metals in the environment are sold in the market for human consumption. It is therefore imperative that this study be carried out with the major aim to investigate the possible presence and prevalence of cadmium residues in organs and muscles of slaughtered pigs in the study area and also, to determine its level (concentration) in the tissues.

MATERIALS AND METHODS

Study area

The study was done in Nsukka agricultural zone of Enugu State, South East Nigeria. Nsukka agricultural zone has three major slaughter houses located at: Nsukka urban with map coordinates of 6°51′24″N and 7°23′45″E; Orba with a map coordinates of 6°51′0″N and 7°27′0″E and Obollo-Afor with coordinates of 6°N and 7°E (Figure 1). Nsukka has a total land area of about 17.5 sq mi (45.38 km^2), and has an elevation of 1,810ft (522 m) with a population of 309,633 (National Population Census (NPC), 2006).

Study design

The research work was a four month cross sectional survey and laboratory analysis of post slaughter matrix samples from slaughtered pigs, to determine the presence and concentration of Cadmium.

Sampling technique and sample collection

One (Nsukka) out of the three agricultural zones in Enugu State was randomly selected. The three major slaughter houses (Nsukka urban, Orba and Obollo Afor) were purposively selected. Stratified random sampling was used to select pigs from each slaughter house assigning them into female and male sex strata and systematic random sampling was used to select 1 in 3 pigs slaughtered from each group, twice a week for four months. A total of 480 fresh samples of liver, kidney and muscle from 160 slaughtered pigs were collected between the months of June, 2014 and September, 2014. Eighty (80) pigs were sampled from Nsukka urban since it has a higher slaughter capacity than the other two. Forty (40) pigs each were sampled from Orba and Obollo-afor slaughter houses since they have the same slaughter capacity. Age was determined using teeth eruption and wearing. About 50g each of liver and muscle samples and a whole kidney of each selected slaughter pig was packed in sterile polythene bags, labeled and sent to Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka for freezing pending analysis. The frozen samples were transported in a cold chain to Springboard Research laboratory, Awka Anambra State, Nigeria, for chemical analysis. Information on the method of processing and the type of materials used was collected by observation and pictures were taken.

Sample processing

Digestion of sample (Dry digestion)

Liver, kidney and muscle samples were dried in the oven at 45°C. After drying, individual sample was crushed into fine powder using mortar and pestle, and 1.0 g of the fine powdered sample was weighed into porcelain crucible and ignited in a muffle furnace at 500°C for 6 to 8 h. The samples were then removed from the furnace and allowed to cool in desiccators, and weighed again. 5 cm cube of 1 M Trioxonitrate (V) acid (HNO_3) solution was added to the left-over ash and evaporated to dryness on a hot plate and returned to the furnace for re-heating at 400°C for 15 to 20 min until perfect grayish-white ash was obtained. The samples were then allowed to cool in desiccators. 15 ml (cm^3) hydrochloric acid (HCL) was then added to the ash to dissolve it and the solution was filtered into 100 cm^3 volumetric flask. The volume was made to 100cm^3 with distilled water.

Analysis

Cadmium (cd) residues were tested for in the digested liver, kidney and muscle under specified condition using Atomic Absorption Spectrometer (AAS). The procedure was done according to the

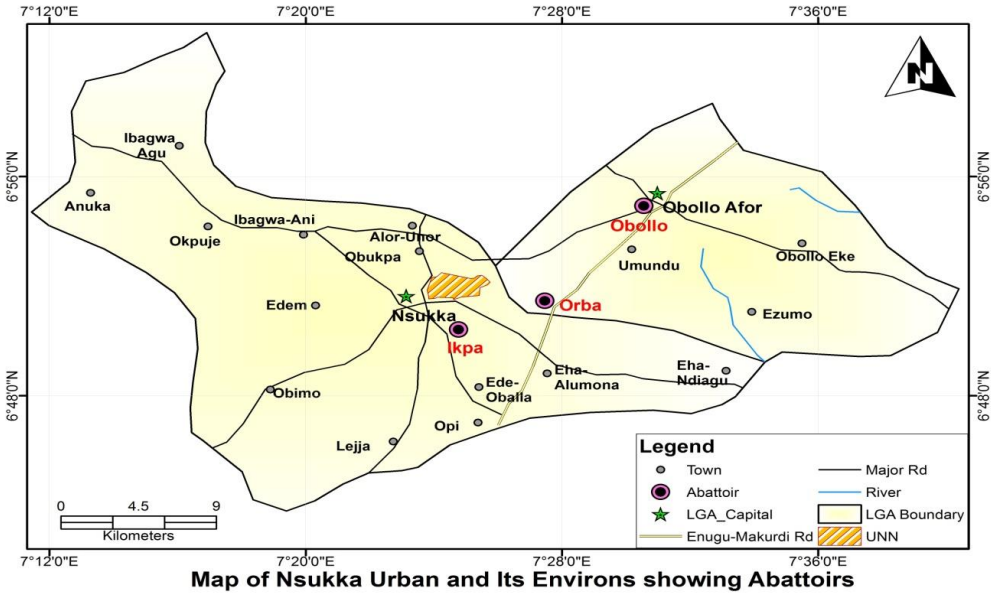

Figure 1. Map of Nsukka agricultural zone showing major slaughter houses.

manufacturer (AA-6800, Shimadzu Atomic Absorption Spectrophotometer) (Szkoda and Zmudzki, 2005).

Stock standard solution: Cadmium**,** 100 mg/L. Dissolve 1,000 g of cadmium metal in a minimum volume of (1+1) HCl, Dilute to 1 liter with 1% (v/v) HCL.

Light sources: Hollow cathode lamps were used for cadmium.

Data analysis and presentation

The data generated from the study were statistically analyzed using both SPSS version 17 and GraphPad Prism Statistical software version 5.02 (www.graphpad.com). Gaussian distribution of data sets was tested for, using D'agostino Omnibus Normality test before choosing the most appropriate statistical tests. Chi square analysis was used to determine if there is an association between the occurrence of cadmium residue and the source of samples and type of organ. Analysis of variance and post hoc test were performed to determine if there is significance difference in the mean concentrations of cadmium among various age groups. One–sample t test was used to determine if there is a significant difference in the mean concentration of cadmium in the organs and their specific maximum Permissible Limit (MPL). The alpha value of significance was set at the probability level of < 0.05.

RESULTS

Prevalence of cadmium residue in slaughtered pigs in Nsukka agricultural zone

Out of a total of 160 pigs sampled, 122 (76.25%) were positive while 38 (23.25) were negative for cadmium residue and from 480 organs sampled (160 each of liver, kidney and muscle from the 160 pigs), 262 (54.58%) were positive for cadmium residue (Table 1).

Distribution of cadmium in slaughtered pigs in the three slaughter houses

Forty (50%) out of the 80 pigs sampled from Nsukka slaughter house were positive for cadmium residue, 34 (85%) of 40 pigs in Orba and 38 (95%) of 40 In Obollo Afor slaughter houses were positive for cadmium residue (Figure 2). There was a strong association ($p<0.0001$) between occurrence of cadmium and the source of samples.

Organ distribution of cadmium in slaughtered pigs

In Figure 3, the presence of cadmium was recorded in 86 (53.75%) of liver, 96 (60%) of kidney and 80 (50%) of muscle samples. There is no association ($p=0.1926$) between occurrence of cadmium and the organ type.

Comparison of the number of positive samples and the mean concentrations of cadmium in the different organs from different sources with their specific MPLs

Table 2 shows that out of 160 samples of each organ

Table 1. Prevalence of cadmium residue in slaughtered pigs in Nsukka agricultural zone.

Status	No of Pigs (%)	No of Organ types (%)			Total
		Liver	Kidney	Muscle	
Positive	122 (76.25)	86 (53.75)	96 (60)	80 (50)	262 (54.58)
Negative	38 (23.75)	74 (46.25)	64 (40)	80 (50)	218 (45.42)
Total	160	160	160	160	480

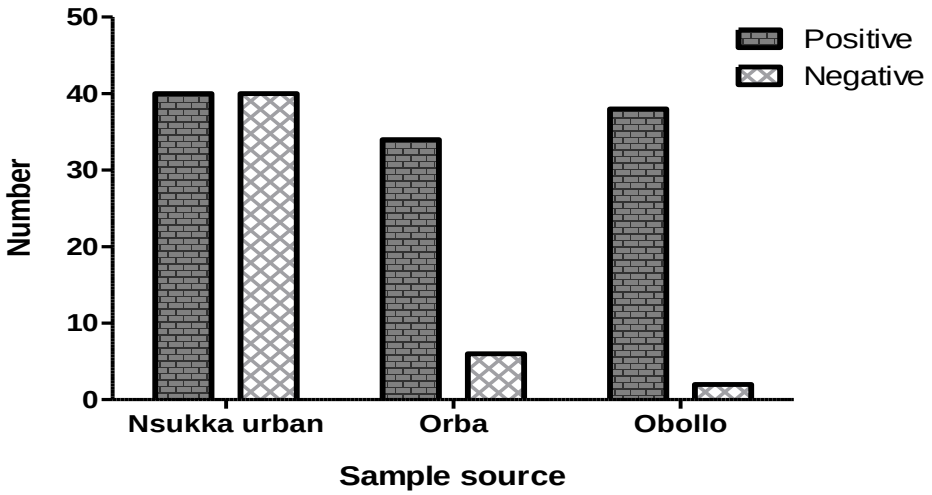

Figure 2. Source/Location distribution of cadmium in slaughtered pigs.

sample, 4 (2.5%) of kidney, 3.75% of the liver and 21.25% of the muscle samples were above the MPL of 1.0, 0.5 and 0.05 mg/kg, respectively. Mean concentrations of 0.1371, 0.052 and 0.13 mg/kg were recorded for kidney samples in Nsukka, orba and Obollo slaughter houses, respectively, there is a significant ($p<0.0001$) difference between the mean concentrations of the cadmium in the kidney samples and its MPL. The mean concentrations of 0.041, 0.035 and 0.06 mg/kg were recorded for liver and mean concentrations of 0.024, 0.02 and 0.05 mg/kg in muscle samples were recorded in Nsukka, Orba and Obollo slaughter houses, respectively. There are significant differences ($P<0.0001$) in the mean concentrations of cadmium in the liver and muscle samples with their MPLs in all the locations except the mean concentration of muscle samples from Obollo which has no significant ($p=0.80$) difference (Figure 4).

Age distribution of Cadmium concentrations in Nsukka, Orba and Obollo slaughter houses

The mean cadmium concentrations in age range of slaughter pigs, 0 to 1 year were 0.23, 0.091, and 0.068 mg/kg in kidney, liver and muscle samples, respectively (Table 3). In age range 2 to 3 years, the cadmium concentrations of 0.142, 0.035 and 0.009 mg/kg were recorded in kidney, liver and muscle, respectively. The mean cadmium concentrations in age range 4 to 5 years were 0.017, 0.008 and 0.009 mg/kg in kidney, liver and muscle, respectively. The mean cadmium concentrations for the age range ≥ 6 years were 0.013, 0.030 and 0.009 mg/kg in kidney, liver and muscle, respectively. However, mean values for cadmium in all the organs and muscle samples were below the MPL except in the muscle of age range 0 to 1 year (0.0679 mg/kg) which was slightly higher but not statistically significant ($p<0.001$).

Processing of slaughter pigs in some of the slaughter houses

Figures 5a to c show different ways slaughter pigs are processed in the slaughter houses. In Figure 5a, old tyre is used to light fire for singeing the pigs. Figure 5b shows the direct use of petrol on the skin of the slaughtered pigs for faster burning and singeing at Orba and Obollo

Table 2. The number (%) of organs of slaughter pigs with mean concentration above MPL.

Sample type	No sampled	Maximum Permissible Limit (MPL)	
		No (%) below	No (%) above
Kidney	160	156 (97.5)	4 (2.5)
Liver	160	154 (96.25)	6 (3.75)
Muscle	160	126 (78.75)	34 (21.25)
Total	**480**	**436 (90.83)**	**44 (9.17)**

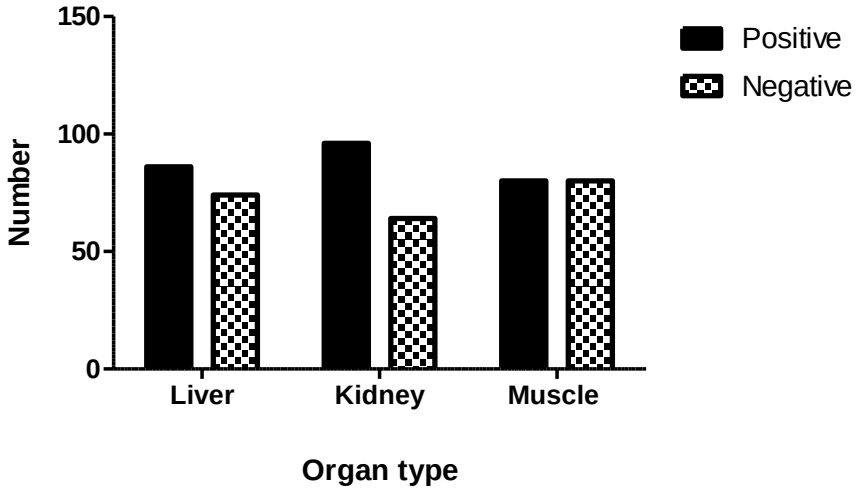

Figure 3. Organ distributions of cadmium.

Table 3. Mean and SEM organ concentration of cadmium in slaughtered pigs according to Age range.

Age range of pigs	Organ mean and standard error of the mean (SEM) conc.entration (mg/kg)		
	Kidney	Liver	Muscle
0-1	0.2301±0.0916[a]	0.0907±0.0320[a]	0.0679±0.0175[a]
2-3	0.1419±0.0896[b]	0.0353±0.0072[b]	0.0187±0.0059 [b]
4-5	0.0174±0.0080[c]	0.0079±0.0040[c]	0.0094±0.0058[c]
≥6	0.0131±0.0068[d]	0.0304±0.0252[d]	0.0093±0.0061[d]

Values within same column with different superscripts are statistically different at $P < 0.05$. SEM: Standard Error of mean.

slaughter houses and Figure 5c shows the use of old plastic bottle to aid the fire for singeing at Orba slaughter house.

DISCUSSION

The 76.25% prevalence of cadmium in the study area seems significant and indicative of high exposure of pig consumers to cadmium residue. The prevalence is at par with 65% prevalence recorded by Oladipo and Okareh (2015) in slaughter goats at Ibadan central abattoir but slightly differs from 100% prevalence recorded by Bala et al. (2012) at Sokoto. The parity between the prevalence of cadmium residue in pigs from Ibadan central abattoir and Nsukka urban abattoir in this study which recorded 50% prevalence could be likened to the fact that both are located in urban areas and students are taken to those abattoirs for meat inspection and teaching indirectly creating awareness, because of their proximity to the

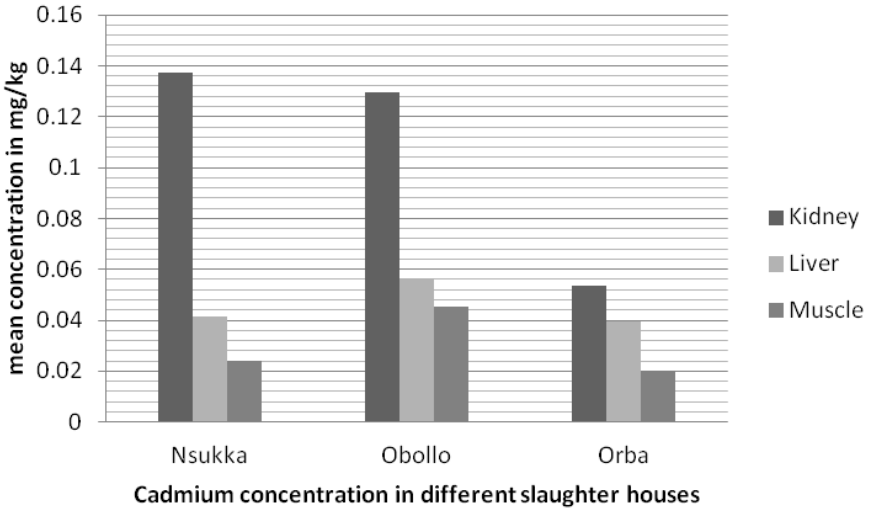

Figure 4. Levels of cadmium concentrations in kidney, liver and muscles of slaughtered pigs in Nsukka, Orba and Obollo slaughter houses, Enugu State.

University. Orba and Obollo Afor slaughter houses are interiorly located and so are exposed to more mundane singeing practices and higher rate of exposure to cadmium due to the processing materials or methods (fueling of wood with kerosene, fuel, plastic and tyre) used in singeing the slaughtered pigs as also reported by Ekenma et al. (2014). Also, awareness of the dangers of such practices is low as such exposure to cadmium due to such practices may be due to ignorance on the part of the butchers. Hence, there is a strong association between the occurrence of cadmium residue and location/source of samples.

Although it has been reported that cadmium residue accumulates more in kidney and liver (Nwude et al., 2010; EI-Salam et al., 2013) as also detected in this work owing to the fact that kidney and liver are organs of biotransformation and detoxification, but no association was found between the type of tissue and the occurrence of cadmium residues. The non association could be likened to the singeing practices which makes accumulation of cadmium in the muscle almost as high as in the internal organs (kidney and liver). Accumulation in internal organs (kidney and liver) occur due to oral exposure (consumption of cadmium contaminated food and water). Animals, especially free range pigs are exposed to heavy metals in our local environment through scavenging in open waste or refuse dumps, and polluted drinking water (Obiri-Danso et al., 2008; Okoye and Ugwu 2010). Okoye and Ugwu (2010) also reported high levels cadmium in soils from Enugu State which they claimed could serve as a source of heavy metals in animals grazing in such areas of the State. Although the rate of occurrence of cadmium residue is more for the more interiorly located sites, Nsukka recorded a higher range value and mean concentration of cadmium than the other locations (Orba and Obollo). This may be attributed to the higher level of industrial activities (mechanics and automobiles engines) observed in Nsukka than Obollo Afor and Orba.

The higher levels of cadmium recorded in organs in the present study is similar to what was reported by Bala et al., 2012 in pigs slaughtered at Nasarawa State. The number of positive samples (2.5 and 3.75%) out of 160 samples of each organ, with concentrations higher than their respective MPL as recommended by the EC (2011) for kidney (1.0 mg/kg) and liver (0.5 mg/kg) are small and the mean concentrations of the two organs in the different locations are significantly lower than their respective MPLs. The number of muscle samples with concentrations higher than its MPL is high at 21.25% but the mean concentration for both Nsukka and Orba slaughter houses are significantly lower than the MPL. There is no significant difference in mean concentration of cadmium in muscle samples from Obollo and its MPL; again, this could be due to the processing method recorded in this study of the use of petrol, rubber and plastic to singe the pigs as shown in the pictures. The results from this study implies that Cd accumulates more in kidney compared to other organs and is in agreement with some other studies (Iwegbue, 2008; Rahimi and Rokni, 2008; Bala et al., 2012).

The result shows that the mean concentrations of cadmium decreased as the age increased, mean cadmium concentrations in age range 0 to 1in kidney

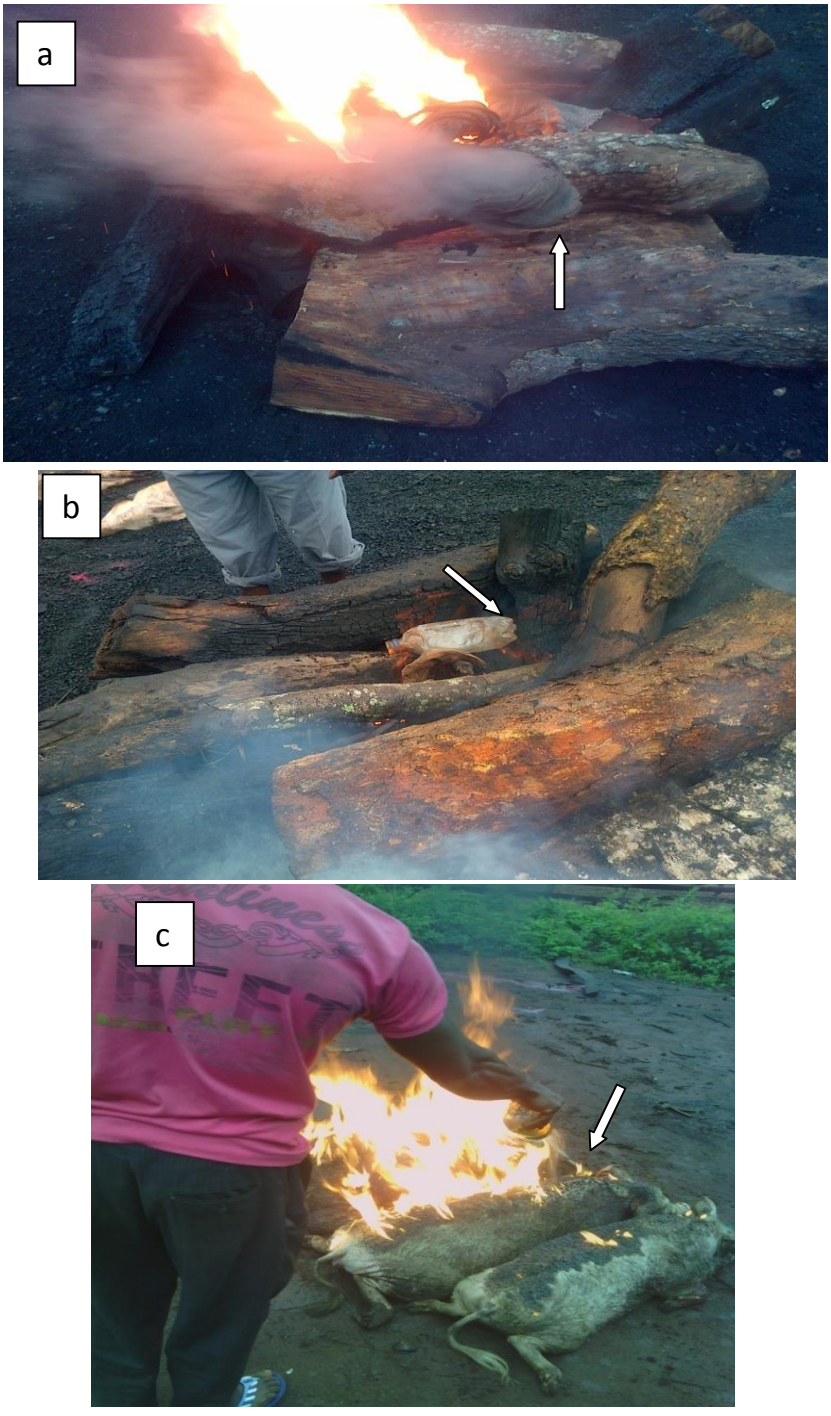

Figure 5a-c. Indicate arrows showing the direct use of tyre, plastic and petrol in scorching the skin of slaughtered pigs respectively.

(0.2301 mg/kg), liver (0.0907 mg/kg) and muscle (0.0679 mg/kg) was significantly higher than the age range 2 years and above. The reason for the variation in age is because younger pigs have immature metabolic rate, innate curiosity and active calcium absorption mechanism Generally, mean values for cadmium in all the organs and

muscles of pigs slaughtered in the study area were below the European commission and WHO recommended maximum permissible level except in the muscle of age range 0 to 1 year, which had higher mean concentration than its MPL, but the difference is not statistically significant. The fact that the mean concentration of the muscle tissue is higher than its MPL, although not significant, it may still pose serious public health threat to consumers considering the high rate of exposure recorded in this study. Consumption of cadmium has also been reported to have a known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic (Nazir et al., 2015).

Conclusion

It is clear from this study, that heavy metals bioaccumulate in different concentrations in organs and muscles of pigs. The level of cadmium in pigs slaughtered at Nsukka, Orba and Obollo Afor varied with majority of the samples falling below the MPL. It could also be concluded that the cadmium residue accumulates more in kidney and liver than the muscle although higher mean concentration was seen in muscle tissue. Younger pigs are more prone to cadmium residue accumulation than the older ones. The potential risk posed by cadmium bioaccumulation and toxicity may continue to increase unless adequate environmental control measures are put in place and enforced by public health authorities. Therefore, to protect public health and ensure food safety; routine monitoring measures for heavy metal residues should be put in place and enforced by the government to guarantee food safety for consumers.

Conflict of Interests

The authors have not declared any conflict of interests.

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