Journal of Veterinary Medicine and Animal Health

Volume 10 Number 2 February 2018
ISSN 2141-2529

Academic Journals

ABOUT JVMAH

The **Journal of Veterinary Medicine and Animal Health (JVMAH)** is published monthly (one volume per year) by Academic Journals.

The **Journal of Veterinary Medicine and Animal Health (JVMAH)** is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject like the application of medical, surgical, public health, dental, diagnostic and therapeutic principles to non-human animals.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JVMAH are peer-reviewed.

Contact Us

Editorial Office: jvmah@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/JVMAH

Submit manuscript online http://ms.academicjournals.me/.

Editors

Dr. Lachhman Das Singla
Department of Veterinary Parasitology
College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences
University
Ludhiana-141004
Punjab
India

Dr. Viktor Jurkovich
Szent István University,
Faculty of Veterinary Science,
István utca 2. H-1078 Budapest
Hungary

Editorial Board Members

Journal of Veterinary Medicine and Animal Health

Table of Contents: Volume 10 Number 2 February 2018

ARTICLES

academicJournals

Vol. 10(2), pp. 45-54, February 2018
DOI: 10.5897/JVMAH2017.0602
Article Number: 1021EAD55557
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Assessment of dairy farmers' hygienic milking practices and awareness on cattle milk-borne zoonoses in Bishoftu, Ethiopia

Lencho Getechew Kebede* and Seblewongel Ayichew Megerrsa

School of Veterinary Medicine, Wolaita Sodo University, Ethiopia.

Received 8 June, 2017; Accepted 12 September, 2017

A cross-sectional study was conducted in Bishoftu town, Ethiopia, from November, 2016 to April, 2017, to assess smallholder urban dairy farmers' milk hygiene practices and awareness on cattle milk-borne zoonoses. Data were collected from a total of 100 randomly selected dairy farmers using structured questionnaire. The results of the study showed that all respondents practiced hand milking, with twice (90%), once (8%) and thrice (2%) milking frequency per day. Most of the respondents (86%) cleaned their barn before milking and 98% used treated pipe water supply for farm activities. Plastic containers were commonly used for storage and transportation of milk. About 26 and 28% of the farmers used individual and common towel for wiping udder after washing, respectively. Most of the farmers (98%) did not practice post-milking dipping of teats. In all the farmers interviewed, respondents' awareness levels of milk-borne zoonoses were 38.89, 33.33, 19.84, 6.35 and 1.6% for tuberculosis, mastitis, anthrax, brucellosis and salmonellosis, respectively. Based on the findings of this study, farmers' awareness level on cattle milk-born zoonoses was low except for tuberculosis and mastitis. In conclusion, there was little awareness about milk borne diseases and some farmers adhered to some dairy hygiene practices. Therefore, it is imperative to strengthen farmers' awareness, extension services and training programs for smallholders in dairy industry on milking hygiene practices and post-harvest handling of milk, to minimize the likely losses due to rejection of spoiled milk and milk-borne dangers which may occur due to consumption of contaminated milk.

Key words: Bishoftu, farmers' awareness, milk-borne zoonoses, milking hygiene.

INTRODUCTION

Ethiopia has the largest livestock population in Africa. The total cattle population of the country in 2013 was estimated to be about 55.03 million. Out of this total cattle population, the female cattle constitute about 55.38% and the remaining 44.62% are male cattle. From the total cattle population of the country, 98.71% are local breeds. The remaining are cross and exotic breeds that accounted for about 1.15 and 0.14%, respectively (CSA, 2014). Despite its huge population, the livestock subsector in the country is less productive in general, and

*Corresponding author. E-mail: lenchogetachew29@gmail.com. Tell: 0920413611.

and as compared to its potential, the direct contribution to the national economy is limited (Kedija et al., 2008; Sintayehu et al., 2008). Consequently, the national milk production and overall milk consumption in Ethiopia are very low, when compared with other African countries with lowest livestock population (Zelalem, 2003).

Milk is universally recognized as a complete diet due to its essential components (Benta and Abtamu, 2011). Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microorganisms may contaminate milk at various stages of milking, processing and distribution. The ill health of the cow and its environment, improperly cleaned and sanitized milk handling equipment, and unhygienic workers who milk the cow, and come in contact with milk due to a number of reasons could serve as sources of contamination for the milk. Lack of refrigeration facilities at farm and household level in developing countries of tropical regions, with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Godefay and Molla, 2000). Once they enter into milk, microorganisms can multiply and cause changes to its quality. If pathogenic microorganisms are involved, they can cause harm to consumers by causing human illnesses and diseases (Barros et al., 2011). Therefore, milk and milk product handling need special care to reduce spoilage and food borne illness (Ashenafi and Beyene, 1994; Degraaf et al., 1997).

According to Bertu et al. (2010) humans may be infected with milk-borne pathogens through consumption of infected raw or unpasteurized milk and milk products. Although, milk and milk products are minor constituents in most diets, contaminated milk are responsible for up to 90% of all dairy related diseases of humans (De Buyser et al., 2001).

Infections that are naturally transmissible from vertebrate animals to humans and vice-versa are classified as zoonoses (WHO, 2009). It has been estimated that about 61% of human infections are zoonotic (Taylor et al., 2000). In the dairy sector, zoonotic pathogens are normally present in dairy animals, raw milk, milk products, meat and the farm environment but are often difficult to diagnose. These zoonoses can be transmitted to humans in several ways that include consumption of infected raw milk (mostly) and contact with infected dairy animals and products, and infected farm environments (Zinsstag et al., 2007).

Milk produced at smallholder farms in Ethiopia is marketed without any form of pasteurization or quality control measures. According to former reports in Ethiopia, on the total milk production, it is reported that 71 to 97% of milk is consumed through an informal market that is basically characterized by selling of low quality milk and milk products (Stanly, 2012). This implies the need for training in dairy production and processing in the country particularly at smallholders level to enhance the hygienic quality of the dairy products (Godefay and Molla, 2000).

Currently, a large number of smallholder urban dairy productions are operating in the present study area using improved dairy breeds. However, information on milking hygiene practices and farmers' awareness on cattle milk-borne zoonoses remains scarce. Thus, lack of information could result in public health risks and economic losses affecting the livelihoods of smallholder dairy producers. Hence, an understanding of farmers' knowledge on milking hygiene and cattle milk-borne zoonoses is very important to reduce risk of cattle milk-borne zoonoses transmission.

Therefore the aim of this study was to assess hygienic milking practices and the general handling practices of milk and to evaluate farmers' awareness on cattle milk-borne zoonoses in smallholder urban dairy producers in Bishoftu, Ethiopia.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Bishoftu town which is located at a distance of 45 km South East of Addis Ababa, Ethiopia. The town is located in east Showa zone of Oromia region and it lies 9° North latitude and 40° East longitude at an altitude of 1850 m above sea level in the central high land of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September) and the remaining in the short rainy season extending from March to May. The dry season extends from October to February. The mean annual maximum minimum temperatures of the area are 26 and 14°C respectively, with mean relative humidity of 61.3%. Mixed farming system followed in the area, crop and livestock production are an intensive type of production. Cattle, small ruminant, poultry and equines are the major livestock species kept with fast growing smallholder dairy production (IPMS, 2005).

Study population

The study was conducted in smallholder dairy farmers in Bishoftu, Oromia Regional State, Ethiopia. The majority of dairy producers in Bishoftu town were market oriented smallholder dairy farmers with average herd size of three cows which are organized under one dairy cooperative called, Ada'a milk and milk products marketing cooperative share company.

Study design

Cross-sectional questionnaire-based study design was used from November 2016 to April 2017 across the smallholder dairy farms in the study area and data collection questionnaire format was developed and used.

Sampling procedure

A random sampling technique was used to select the households for the purpose of this study and a random survey of 100 smallholder urban dairy farmers who were actively involved in dairy production was conducted. A list of households owning dairy farms was obtained from records maintained by Ada'a milk and milk products marketing cooperative share company.

The sample size for collecting the questionnaire data was determined by using formula as indicated by Bartlett et al. (2001). A list of 162 dairy farmers was considered as the sampling frame (N).

$$n = \frac{N}{1+ (N (e)^2)}$$

Where, n = the sample size of the research; N = total number of smallholder in each kebele; e = maximum variability or margin of error 5% (0.05); 1 = the probability of the event occurring.

Therefore, a total of 115 farms were selected at 5% standard error with 95% confidence interval. But depending on willingness and availability of dairy farmers, 100 dairy farms were interviewed in this study.

Data collection

A single-visit-multiple-subject formal survey technique (ILCA, 1990) was used to collect data through face-to-face interviews using a structured and pretested questionnaire using local language. Data obtained from respondents were on demographic characteristics, housing management, sources of farm water, milking system, milking frequency, milking hygienic practices (washing of milkers' hand, milk utensils and udder before milking), and farmers' awareness on cattle milk-borne zoonoses.

Statistical analysis

Microsoft Excel was used for data management and entry. All the collected data were coded and entered into the computer with Excel. The Statistical Package for Social Sciences (SPSS) software version 20 computer program was used for data analysis. Descriptive statistics such as frequencies, distribution and percentages were used to summarize the data. The association of demographic characteristics of the respondents and their milk hygienic practice was analyzed using Chi-square.

RESULTS

Socio demographic characteristics of the respondents

A total of 100 smallholder dairy farmers were interviewed in this cross sectional study in the nine selected kebeles of the Bishoftu town. Females comprised 61% of the respondents while the remaining 39% were males of different age and educational levels. Most of the respondents, 51% (51) belong to the age group of 36-50 years, this indicates that majority of the respondents were in potential productive age. Regarding the educational level, 35 (35%) were illiterate, 31 (31%) attended primary education, 24 (24%) had attended secondary education and 10% had college or university courses. In this study, 93% of the respondents managed their cows intensively and 7% managed their cows semi-intensively. Most of the respondents (86%) rear exotic breed, 13% rear cross breed and the other 1% rear both cross and exotic breeds (Table 1).

Dairy cattle housing characteristics

In the study area, all the respondents (100%) use separate house for keeping the animals and most of the cows (93%) were housed in concrete type floor barn and 6% were in muddy soil floor and only 1% are in wooden floor. Regarding barn cleaning, most of the respondents (41%) clean the barn twice a day, 27% clean once a day, 20% clean thrice a day and 12% clean more than thrice a day (Table 2).

Milking hygienic practices

Results of this study showed that milking is done by hand (100%), with milking frequency of twice (90%), once (8%) and thrice (2%) a day. All respondents milk their animal in barn, most of the respondents (86%) clean their barn before milking, while 14% do not clean their barn before milking, and 98% of the dairy farmers had access to pipe water supply and 2% use ground water.

In this study, most of the farmers (76%) reported that they washed their hands with water only and it was noted that only 24% used water and soap for washing their hands. Most of respondents (49%) use warm water for udder and teat washing, 46% uses cold water and 4% cooled water and detergent and only 1% wash udder and teats with warm water and soap. About 26% of the respondent's use individual towels and 28% use common towels for wiping udder after washing, whereas, the rest 44% do not use towels for drying. Most of the respondents (61%) do not use teat lubricant and it was noted that only 39% use it. 98% farmers did not practice teat dipping, only 2% practice teat dipping (Table 3).

Milking equipment and milk handling practice

In this study, most of the respondents (95%) use plastic containers for collecting milk and only 5% use stainless steel for collecting and transporting milk. All respondents clean milk handling containers; however, 39% wash containers with cold water, 40% wash containers with soap and cold water and only 21% wash containers with hot water and soap. Majority of the respondents (58%) do not remove foremilk during milking and 41% of the respondents remove foremilk. Concerning milk filtering to storage containers, most of the respondents (75%) do not practice milk filtering, only 25% practice milk filtering into containers. Concerning milk storage, 96% of the respondents store milk as milked, only 4% store their milk in refrigerator. Most of the respondents (88%) deliver milk to other users immediately after milking and 12% of respondent's delivery milk within one hour after milking (Table 4).

Farmers' awareness of cattle milk-borne zoonoses

Almost all of the respondents (99%) consume milk and

Table 1. Socio demographic characteristics of the respondents.

Parameter	Category	Frequency	Percentage
Sex	Female	61	61
	Male	39	39
Age	18-35 yrs	17	17
	36-50 yrs	51	51
	>50 yrs	32	32
Occupation	Self –employ	21	21
	Farmer	71	71
	Government	2	2
Education	Illiterate	35	35
	Primary education	31	31
	Secondary education	24	24
	Diploma and above	10	10
Farm type	Intensive	93	93
	Semi intensive	7	7
Breed	Cross breed	13	13
	Exotic	86	86
	Cross and exotic	1	1

Table 2. Housing characteristics of the farms.

Parameter	Category	Frequency	Percentage
Housing	Separate house	100	100
Floor type	Concrete	93	93
	Wooden	1	1
	Muddy soil	6	6
Barn cleaning	Once a day	27	27
	Twice a day	41	41
	Thrice a day	20	20
	> thrice a day	12	12

only 1% do not. Most of the respondents (57.5%) consume milk after boiling it, 23.4% consume raw milk as milked and 19.1% consume milk after processing (yogurt). Most of the respondents (62%) discards milk of sick animals, 27.6% gave milk sick animals to their pets, 7.8% use the milk of sick animals after processing it and 2.6% gave milk of sick animals to their calves. Most of the respondents (64.9%) discards milk of drug treated animals, 26.32% gave milk of drug treated animals to their pets, 5.3% use the milk of drug treated animals after processing it and 3.5% gave milk of drug treated animals to their calves.

With regards to farmers' knowledge on milk-borne zoonoses, they were aware of tuberculosis (38.89%), mastitis (33.33%), anthrax (19.84%), brucellosis (6.35%) and salmonellosis (1.6%). Most of the respondents (91%) have not suffered from any milk borne illness before, whereas 9% of the respondents have suffered from milk borne illness in the past. Most respondents (93%) reported that disease from human being are not transmitted to animals, only 7% stated that human disease can be transmitted to animals (Table 5).

Prevention practice of the farmers

In this study, most of respondent (39%) boil milk before

Table 3. Milking methods and hygienic milking practices followed by farmers.

Parameter	Category	Frequency	Percentage
Milking area	In barn	100	100
Cleaning milking area	Yes	86	86
	No	14	14
Milking frequency	Once a day	8	8
	Twice a day	90	90
	Thrice a day	2	2
Source water	Pipe water	98	98
	Well water	2	2
Hand washing before Milking	Wash with water	76	76
	Wash with water and Soap	24	24
Washing udder and teats	Cold water	46	46
	Warm water	49	49
	Cold water with soap	4	4
	Warm water with soap	1	1
Use of towel	Individual towel	26	26
	Common towel	28	28
	No use of towel	46	46
Using of lubricant	Yes	39	39
	No	61	61
Teat dipping	Yes	2	2
	No	98	98

use as disease prevention method, 25% stated that keeping hygiene prevent disease transmission, 24% had no idea on disease prevention methods, 19% reported treating of sick animals, 15% stated vaccination of animals and the other 2% stated the use of artificial insemination prevent disease transmission. The study showed that there was no practice of medical examination of farm workers, particularly milkers for prevention of contamination of milk by diseases carried by man.

In this study, half of the respondents (50%) were trained only on hygienic milking and all (100%) did not acquire training on cattle milk borne zoonoses. In this study, most of the respondent (67%) got veterinary professionals service at farm on phone call, while the rest 33% do not have veterinary professionals that follow their animal health (Table 6).

Association of age with hygienic milking practice

Among hygienic milking practices use of PPE, source of water used, washing udder, removing foremilk and milking equipment were significantly ($p<0.05$) associated with age of the respondents (Table 7).

Association of sex with hygienic milking practice

Among hygienic milking practices, washing animals, drying udder, cleaning milking utensils, removing foremilk and use of towel were significantly ($p<0.05$) associated with sex of the respondents (Table 8).

Association of education with hygienic milking practice

Among hygienic milking practices, washing animals, source of water, cleaning milking utensils, milking equipments and milk storage were significantly ($p<0.05$) associated with educations of the respondents (Table 9).

DISCUSSION

This study aimed to assess the hygienic milking practices

Table 4. Milking equipment and milk handling practice.

Parameter	Category	Frequency	Percentage
Milk containers	Plastic	95	95
	Stainless steel	5	5
Milk utensils cleaning	Cooled water	39	39
	Soap and cold water	40	40
	Soap and hot water	21	21
Removing foremilk	Yes	42	42
	No	58	58
Filtering milk	Yes	25	25
	No	75	75
Milk storage	In refrigerator	4	4
	As milked	96	96
Time to reach collectors	Immediately after milking	88	88
	within one hour	12	12

and awareness of milk-borne zoonoses among smallholder dairy farmers. The results of the present study showed that majority of the respondents (69%) in the study area who were engaged in milk production were females than males which is similar to Bereda et al. (2012) report in Ezha district of the Gurage zone, that dairying offers more opportunities for females to be closely involved in the daily management than males. In contrast with the present findings, Azage (2004) and Yitaye et al. (2008) reported that in Addis Ababa and northwest Ethiopia, there were more male-headed households. The present study showed that majority of the participants handling milk were females, it may be because men work in the field and attitude of the society towards dairy farms. The sex of the respondents had significant level of variation with hygienic milking practice ($P<0.05$).

The present study indicated that most of the respondent's educational levels were found between illiterate and primary school. This is in agreement with report from Illu Aba Bora Zone, Southwest Ethiopia (Bereda et al., 2014), where the educational level attained by majority of the household heads falls between illiterate and primary school. In this study, the educational level of the respondents had significant level of variation ($P<0.05$) with hygienic milking practice. This indicates that more intervention is needed to make farmers to be aware, in order to improve their hygienic dairy production and husbandry practices.

In this study, most of the respondents (51%) were in the productive ages which agreed with Teshager et al. (2013) report in Ilu Aba Bora Zone. In this study, the age of the respondents had significant level of variation of hygienic milking practice ($P<0.05$).

The survey result showed that, all the respondents (100%) use separate house for keeping the animals and most of the cows (93%) were housed in concrete type floor barn. In agreement with the present findings, Bruktawit (2016) reported that in Addis Ababa, majority of the respondents used barn floor made of concrete. As observed in the current study, 98% of the respondents used pipe water as main water sources for cleaning the udder or teats, wash their hands and milking equipment, and the other 2% use well water source for cleaning and washing purpose. Similarly, Bruktawit (2016) reported that in Addis Ababa, 98.9% of the respondents use pipe water and the other 1.1% use well water. According to Zelalem (2009), when water from non-tape sources is used for cleaning purpose, it is important that producers should at least filter and heat treat it before use because the quality of water determines the amount of bacterial counts.

Results of this study showed that milking is done by hand (100%), with milking frequency of twice (90%), once (8%) and thrice (2%) a day. In agreement with these findings, Milligo et al. (2008) reported that all smallholder farmers in peri urban areas in Burkina Faso practiced hand milking. The findings of Zelalem (1999) showed that in Holetta, Selale and Debre Zeit, 83.3, 93.3 and 96.7% of crossbred cows are milked twice a day, respectively. Yitaye et al. (2007) reported that 83.8% of the farmers in northern Ethiopia milked their cows twice a day. Once and thrice per day milking frequency was also reported by Sintayehu et al. (2008) in other urban dairy farms in

Table 5. Farmers' awareness of cattle milk-borne zoonoses at urban dairy farms.

Parameter	Category	Frequency	Percentage
Milk consumption	Yes	99	99
	No	1	1
Milk consumption ways	Raw	39	23.35
	Boiled	96	57.485
	Processing	32	19.16
Milk of sick animals	Discarded	72	62
	Given to pet	32	27.6
	Given to calves	3	2.6
	Using it after Processing	9	7.8
Milk of drug treated animals	Discarded	74	64.9
	Given to pet	30	26.32
	Given to calves	4	3.5
	Using it after Processing	6	5.3
Disease transmit from milk	Yes	20	20
	No	80	80
Named milk-borne zoonoses	TB	49	38.89
	Anthrax	25	19.84
	Mastitis	42	33.33
	Salmonellosis	2	1.59
	Brucellosis	8	6.349
Human disease transmit animals	Yes	7	7
	No	93	93

Table 6. Prevention practice of the farmers

Parameter	Category	Frequency	Percentage
Prevention methods	No idea	24	24
	Boiling milk	39	39
	Keeping hygiene	25	25
	Treating sick Animals	19	19
	Vaccination	15	15
	Using AI	2	2
Vaccination	Yes	96	96
	No	4	4
Training on hygiene	Yes	50	50
	No	50	50
Vet. Professionals	Yes	67	67
	No	33	33

Ethiopia.

The production of milk of good hygienic quality for consumers requires good hygienic practices, such as clean milking utensils, washing of milker's hands, cleaning udder and use of individual towels during milking and handling, before delivery to consumers or processors (Getachew, 2003). In this study, most of respondents (76%) washed their hands with water only and 24% of them used water and soap for washing their hands. Most of the respondents (49%) use warm water for udder and teat washing, 46% use cold water and 4% cooled water and detergent and only 1% wash udder and teats with

Table 6. Association of age with hygienic milking practice.

Parameter	Category	Age			X^2	p-Value
		18-35 years	36-50 years	>50 years		
Use of PPE	Yes	16	22	13	15.287	0.000
	No	1	29	19		
Source of water	Pipe water	15	51	32	9.964	0.007
	Well water	2	0	0		
Washing udder	Cold water	6	21	19	14.424	0.025
	Warm water	8	29	12		
	Cold water with soap	3	0	1		
	Warm water with soap	0	1	0		
Removing foremilk	Yes	13	19	10	10.282	0.006
	No	4	32	22		
Milking equipment	Plastic	14	49	32	7.534	0.023
	Stainless steel	3	2	0		

Table 7. Association of sex with hygienic milking practice

Parameter	Category	Sex		X^2	p-value
		Female	Male		
Washing animals	Yes	11	15	5.16	0.023
	No	50	24		
Drying udder	Yes	23	27	9.458	0.002
	No	38	12		
Removing foremilk	Yes	19	23	7.562	0.006
	No	42	16		
Use of towel	Individual towel	12	14	8.234	0.016
	Common towel	14	14		
	No use of towel	35	11		

warm water and soap. Consistent with this study, Duguma and Geert (2015) reported that majority (96.3%) of the farmers in Jimma practiced hygienic milking, such as washing of hand, milk containers and udder before milking.

In this study, 26 and 28% of the farmers used individual and common towels for wiping udder after washing, respectively. This is in agreement with the findings of Zelalem and Faye (2006) who reported that in the central highlands of Ethiopia, small and large scale dairy producers used common towel for drying udder. Duguma and Geert (2015) reported that only 13% of the farmers in Jimma town, southwestern Ethiopia, used individual towel and this is lower than the present findings (26%). The use of common towel may result in transmission of diseases, particularly mastitis. The high percent of using individual towel might be due to more awareness and modern dairy farms being in this study area.

As shown in this survey, most respondents (61%) do not use teat lubricant and it was noted that only 39% used it. In this study, 98% farmers did not practice teat dipping, only 2% practice teat dipping. In contrast to the present findings, Benta and Abtamu (2011) reported that 10% of the farmers in Wolayta Sodo used teat dip solutions after milking and this is higher than the present findings (2%). This might be due to the fact that farmers in the study area lack awareness on teat dipping practices.

In this study, most of the respondents (95%) use plastic containers for collecting milk and only 5% use stainless steel for collecting and transporting milk. In agreement with this study, Duguma and Geert (2015) reported that about 92.6 and 3.7% of the farmers in Jimma collected milk using plastic buckets and stainless steel cans,

Table 8. Association of education with hygienic milking practice.

Parameter	Category	Education				X^2	p- value
		Illiterate	Primary education	secondary education	Diploma and above		
Washing animals	Yes	4	10	6	6	10.514	0.015
	No	31	21	18	4		
Source water	Pipe water	35	31	24	8	18.367	0.000
	Well water	0	0	0	2		
Cleaning milking utensils	Cooled water	19	9	6	5	13.578	0.035
	Cold water and Soap	11	17	8	4		
	Hot and water soap	5	5	10	1		
Milking equipment	Plastic	34	31	23	7	15.163	0.002
	Stainless steel	1	0	1	3		
Milk storage	In Refrigerator	0	1	1	2	8.175	0.043
	As milked	35	30	23	8		
Human disease transmit to animals	Yes	1	2	0	4	19.472	0.000
	No	34	29	24	6		

respectively. All respondents clean milk handling containers before and after use. In the present study, 95% the farmers did not practice milk cooling after milking, because of lack of facilities for cooling milk, which is a serious problem to hygienic milk production. Contrary to the present findings, Benta and Abtamu (2011) reported that 50% of the farmers in Wolayta Sodo cooled milk immediately after milking. This is might be because, farmers in the study area lack facility for cooling and storing of milk. Quinn et al. (2002) reported that cooling milk after milking reduces risk of the growth of both pathogenic and spoilage bacteria.

In this study, most of the respondents (57.5%) consume milk after boiling it, 23.4% consume raw milk as milked and 19.1% consume milk after processing (yogurt). Contrary to the results of the present study, Duguma and Geert (2015) reported that most (92.6%) of the farmers in Jimma boil milk before consumption, 3.7% also indicated that they consume raw milk, Zelalem and Faye (2006) reported that 45% of the respondents did not boil milk before consumption. This might be due to habitual practice of famers that they prefer taste of milk boiled.

With regards to farmers' knowledge about milk-borne zoonoses, they were aware of tuberculosis (38.89%), mastitis (33.33%), anthrax (19.84%), brucellosis (6.35%), and salmonellosis (1.6%). The results of the current study revealed that majority (38.89%) of the farmers were more aware of bovine tuberculosis than other milk-born zoonoses due to its frequent occurrence in the study area.

In agreement with this study, the findings by Stanly (2012) showed that farmers were more knowledgeable about tuberculosis as compared to brucellosis (74.3 vs. 2.9%) in north Malawi. Girma et al. (2012) reported that in Addis Ababa, 88.54 and 49.48% of the respondents were aware of bovine tuberculosis and brucellosis, respectively. In the present study, farmers lacked awareness on anthrax (80.16%), brucellosis (93.65%), mastitis (66.67%), tuberculosis (61.11%) and salmonellosis (98.4%) as milk-borne zoonoses. Similar observations were made by Ekuttan (2005) who showed in Kenya that dairy farmers lacked knowledge on specific milk-borne zoonoses.

The results of the present study revealed that respondents had low level of awareness on milk-borne zoonoses, except mastitis and tuberculosis, which are commonly available in the study area. This is in agreement with the findings of Belay et al. (2012) and Jergefa et al. (2009) in Ethiopia, and Munyeme et al. (2010) in Zimbabwe.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS: CSA, Central Statistics Authority; **CAC,** Codex Alimentarius Commission; **FSA,** Food Standards Agency; **NMSA,** National Meteorological Services Agency; **IMPS,** improving productivity and market success; **ILCA,** International Livestock Centre for Africa; **SPSS,** Statistical Package for Social Sciences.

REFERENCES

Ashenafi M, Beyene F (1994). Microbial load, microflora, and keeping

quality of raw and pasteurized milk from a dairy Farm. Bull. Anim. Health Prod. Afr. 42:55-59.
Azage T (2004). Urban livestock production and gender in Addis Ababa, urban agriculture Magazine number 12. Proc. 14th Annual Conference of the Ethiopian Society of Animal production (ESAP). September 5-7, 2006. Addis Ababa, Ethiopia.
Barros LSS, Sóglia SLO, Ferreira MJ, Rodrigues MJ, Branco MPC (2011). Aerobic and anaerobic bacteria and Candida species in crude milk. J. Microbiol. Antimicrob. **3**:206-212.
Bartlett JE, Kotrlik JW, Higgins CC (2001). Organizational Research: Determining Appropriate Sample Size in Survey Research. Inform. Technol. Learn. Perform. J. 19(1):43.
Belay D, Yisehak K, Geert PJ, Janssens C (2012). Survey of major diseases affecting dairy cattle in Jimma town, Oromia, Ethiopia. Glob. Vet. **8**:62-66.
Benta D, Abtamu T (2011). Study on Prevalence of Mastitis and its Associated Risk Factors in Lactating Dairy Cows in Batu and its Environs, Ethiopia. Glob. Vet. 7(6): 632-637.
Bereda A, Yilma Z, Nurfeta A (2012). Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, Southern Ethiopia. Wudpecker J. Agri. Res. 1(11):459-465.
Bereda A, Yilma Z, Nurfeta A (2014). Dairy Production System and Constraints in Ezha Districts of the Gurage Zone, Southern Ethiopia. Glob. Vet. 12(2):181-186.
Bertu WJ, Depar M, Gusi AM, Ngulukun SS, Leo S, Jwander LD (2010). Prevalence of brucella antibodies in marketed milk in Jos and environs. Afr. J. Food Sci. 4(2): 062-064.
Bruktawit S (2016). Physicochemical properties and microbial quality of cow milk collected from selected subcity of Addis Ababa, Ethiopia. pp.65-78.
Central Statistics Authority (CSA) (2014). Federal Democratic Republic of Ethiopia Central Statistics Authority, Agricultural sample survey.Vol. II. Report on livestock and livestock characteristics. Statistical Bulletin. Addis Ababa, Ethiopia.
De Buyser ML, Dufour B, Maire M, Lafarge V(2001). Implication of milk and milk products in food-borne diseases in France and in different industrialized countries. Int. J. Food Microbiol. 67(1):1-17.
DeGraaf T, Romero Zuniga, Cabalellero M, Dwinger RH (1997). Microbiological quality aspects of cow's milk at a smallholder cooperative in Turrialba, Costa Rica. Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux 50(1):57-64.
Duguma B, Geert PJ (2015). Assessment of Dairy Farmers' Hygienic Milking Practices and Awareness of Cattle and Milk-Borne Zoonoses in Jimma, Ethiopia. Food Sci. Quality Manag. 45.
Ekuttan CE (2005). Biological and chemical health risks associated with smallholder dairy production in Dagoretti Division. Nairobi, Kenya (Unpublished MSc Thesis, Department of Community Health, University of Nairobi, Kenya).
Getachew F (2003). A review of small scale dairy sector in Ethiopia. FAO prevention of food losses programme. Milk and milk products. Post-harvest losses and food safety in Sub-Saharan Africa and Near East.
Girma S, Zewde G, Tafess K, Jibat T (2012). Assessment of awareness on food borne zoonoses and its relation with veterinary public health
services in and around Addis Ababa, Ethiopia. J. Public Health Epidemiol. 4(2):48-51.
Godefay B, Molla B (2000). Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa. Berliner und Munchener Tierarztliche Wochenschrift, 113(7-8):276-278.
International Livestock Centre for Africa (ILCA) (1990). Annual Report and Programme Highlights. ILCA, Addis Ababa, Ethiopia. ISSN 1014-9015.
Improving Productivity and Market Success (IPMS) (2005). Ethiopian Farmers Project Working Paper 9, ILRI (International Livestock Research Institute), Nairobi, Kenya. P 62.
Jergefa T, Kelay B, Bekana M, Teshale S, Gustafson H, Kindahl H (2009). Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. Revue scientifique et technique (International Office of Epizootics) 28(3):933-943.
Kedija H, Azage T, Mohammed YK, Berhanu G (2008). Traditional cow and camel milk production and marketing in agropastoral and mixed crop–livestock systems: The case of Mieso District, Oromia Regional State, Ethiopia. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 13. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 56:1-3.
Milligo V, Ouedraogo GA, Agenas S, Svennersten-Sijaunja K (2008). Survey on dairy cattle milk production and milk quality problems in peri-urban areas in Burkina Faso. Afr. J. Agric. Res. **3**:215-224.
Munyeme M, Muma JB, Munang'andu HM, Kankya C, Skjerve E, Tryland M (2010). Cattle owners' awareness of bovine tuberculosis in high and low prevalence settings of the wildlife-livestock interface areas of Zambia. BMC Vet. Res. **6**:21.
Quinn PJ, Carter ME, Markey B, Carter GR (2002). Actinobacillus species. In Veterinary Microbiology and Microbial Disease.
Sintayehu Y, Beyene F, Tegegne A, Gebremedhin B (2008). Dairy production, processing and marketing systems of Shashemene – Dilla area, South Ethiopia.
Stanly FT (2012). Smallholder dairy farming in northern Malawi: husbandry practices, constraints and prevalence of major production and zoonotic diseases. (Unpublished PhD Thesis). Institute of Animal Breeding and Husbandry, ChristianAlberchtes-Universtaete zu Kiel, Germany. P 89.
Taylor LH, Ltham SM, Wopolhouse ME (2000). Risk factors for human disease emergence. Transactions of Royal London Society of Biological Sciences 356:983-989.
Teshager A, Duguma B, Tolemariam T (2013). Socioeconomic and Farm Characteristics of Smallholder Cattle Producers in Ilu Aba Bora Zone of Oromia Regional State, South Western Ethiopia. Glob. Vet. 10(5):607-613.
World Health Organization (WHO) (2009). Annual report. Zoonoses and veterinary public health. Brucellosis (WHO Document Production Services, Geneva, Switzerland).
Yitaye A, Wurziger M, Azage T, Zollitsch W (2007). Urban and peri-urban farming system and utilization of the natural resources in the north Ethiopian highlands: In proceedings of Conference on International Agricultural Research for Development, 9-11 October 2007, University of Gottingen, Germany.
Yitaye A, Zollitsch W, Wurzinger M, Azage T (2008). Characterization and analysis of the urban and peri-urban dairy production systems in the North western Ethiopian highlands. A thesis submitted to BOKU – University of Natural Resources and Applied Life Sciences, Vienna, Austria for the award of Doctor *Rerum anturalium* technicarum (Doctor of Natural and Technical Sciences), Vienna, October 2008.
Zelalem Y (2003): Sanitary conditions and microbial qualities of dairy products in urban and peri-urban dairy shed of the central Ethiopia. DEA. Lyon, France.
Zelalem Y (2009). Microbial Properties of Ethiopian Marketed Milk and Milk Products and Associated Critical Points of Contamination: An Epidemiological Perspective, Addis Ababa, Ethiopia. pp. 298-322.
Zelalem Y, Faye B (2006). Handling and microbial load of cow's milk and irgo-fermented milk collected from different shops and producers in central highlands of Ethiopia. Ethiop. J. Anim. Prod. **6**(2):67-82.
Zinsstag J, Schelling E, Roth F, Bonfoh B, de Savigny D, Tanner M (2007). Human benefits of animal interventions for zoonosis control. Emerging Infect. Dis. 13(4):527.

academicJournals

Vol. 10(2), pp. 55-59, February 2018
DOI: 10.5897/JVMAH2017.0603
Article Number: 74D55C855561
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Study on veterinary antibiotic drugs handling and utilization in and around Holeta

Seblewongel Ayichew Megerrsa and Taddesa Bezu Ashine*

School of Veterinary Medicine, Wolaita Sodo University, Ethiopia.

Received 8 June, 2017: Accepted 13 July, 2017

A cross-sectional study was conducted to assess the antibiotics drug handling and utilization practices of the animal owners from November 2016 to April 2017. A total of 384 randomly selected animal owners in and around Holeta were interviewed using semi-structured questionnaires. Most of the respondents (animal owners) were educated (59.4%), whereas less than half of them (40.6%) were uneducated. The prevalence on the challenges faced by their animals was 25, 42.2, and 32.8% due to disease, feed shortage and both disease and feed shortage, respectively. A total of 6.5% of the respondents purchased antibiotics from private pharmacy and brought to their house by carrying them in pockets, while 3.9 and 2.9% of the respondents store drugs on the shelf and floor up to three months, respectively. Drug administration activity was performed by non-professional personnel simply by guessing. 66.1% of the respondents knew about withdrawal period of antibiotics whereas 33.9% of them did not know about withdrawal period of antibiotic drugs. At the time of drug administration, 12.8% sold the milk, 86.7% gave the milk for calf and 0.5% of them used the milk for home consumption without maintaining its withdrawal period. A few number of animal owners (2.6%) injected their animals below normal dosage resulting to resistance of the disease to the antibiotics. Majority of the animal owners (93.5%) used veterinary professional service to their diseased animals for treatment whereas a few number of the respondents (6.5%) purchased antibiotics from private pharmacy without prescription of the veterinarian for self-treatment of their animals. In conclusion, this study result revealed that there is improper handling and utilization practice of veterinary antibiotic drugs in the study area. It is recommended that training should be given for the animal owners on the appropriate handling and utilization practice of veterinary drugs and continuous follow up by the stake holders should be undertaken.

Key words: Antibiotics drug, animal owners, Holeta, veterinary.

INTRODUCTION

Ethiopia is a leading country in the number of animal population in the African continent. However, the output in terms of contributions to the improvement of the livelihood of animal owners and for the growth of the national economy is at a lower stage compared to the vast resource on hand. Poor Animal health service and

E-mail: taddesagebisa@gmail.com. Tell: 0909477516.

lack of properly utilizing veterinary antibiotics are among the main contributing factors for the poor utilization of the resources (Flynn, 2012).

A drug is any substance that when inhaled, injected, smoked, consumed, absorbed via a patch on the skin, or dissolved under the tongue causes a physiological change in the body. In pharmacology, a pharmaceutical drug is a chemical substance used to treat, cure, prevent, or diagnose a disease or to promote well-being (Flynn, 2012). Veterinary drugs are such substances as are made to treat, prevent or diagnosis diseases in animals that belong to different chemical classes and therapeutic areas, for example antibiotics, anti helminthics, non-steroidal anti-inflammatory drugs (NSAIDs), etc (Hirsh and Zee, 1999). An antibiotic class can be defined as a group of agents with a similar mechanism of action, regardless of chemical structure (Addah et al. 2009). Antibiotic drugs have been widely used globally in animals for more than 50 years, with tremendous benefits in animal production and economic development (Flynn, 2012).

There are many risks derived from irrational use of antibiotics resulting in environmental contamination with original substances or derivatives, indirect impact on health via resistant micro-organisms, direct organic damage and the influences on the biotic environment are a matter of concern (Thawani, 2010).

Major economic loses and animal welfare problems could arise in veterinary medicine, because antimicrobial resistance has been found to cause therapy failure and higher mortality and morbidity rate (Acar, 1997). The provision of quality animal health-care necessitates the availability of safe, effective and affordable antibiotics of the required quality, in adequate quantity at all times, and presented, dispensed and used rationally (Acar, 1997).

In and around Holeta, West Shoa Zone of Oromia Regional State, there is little information regarding, veterinary antibiotic drugs handling and utilization practice and there is no published material concerning this title.

Therefore, the objective of the study was to assess the antibiotic handling and utilization practices of the farmers and to assess the challenges that enforced the animal owners to buy antibiotics.

MATERIALS AND METHODS

Study area

The study was conducted from November 2016 to April 2017 in and around Holeta. The site is located at 9º 3' N latitude and 38º 30' E longitudes, about 33 km West of Addis Ababa along the main road to Ambo in Oromia Regional State. The study area has an altitude of 2400 m above sea level and receives an average annual rainfall of about 1000 mm.

The mean minimum and maximum temperatures are 6 and 22ºC respectively. Regarding season, there are three seasons: short rainy season (March to May), long rainy season (June to September) and dry season (October to February).

Study design

Cross-sectional questionnaire - based study was carried out from November 2016 to April 2017 in and around Holeta. Data collection questionnaire format was developed and animal owners were interviewed to assess handling and utilization of veterinary antibiotic drugs used by them for treatment of animal diseases using a simple random sampling method.

Sample size determination

The sample size for collecting the questionnaire data was determined by using formula as indicated on (Thrusfield, 2005):

$$n = \frac{z^2\, p\,(1-p)}{w^2}$$

Where, Z is the confidence level, P is the estimate of the population, and W is the margin of error. (When: Z = 1.96, P = 0.5, and W = 0.05).

$$N = \frac{(1.96)^2\,(0.5)(1-0.5)}{(0.05)^2}$$

Based on the above formula the total numbers of animal owners selected for questionnaire survey was 384.

Study population

Animal owners (384) from randomly selected kebeles of in and around Holeta, were considered for this study.

Data collection and analysis

Questionnaire administration was done by local language to gather Information through semi-structured questionnaire (annex) from animal owners and the collected data from questionnaires were entered into Microsoft Excel spread sheet version 2010 and analyzed using SPSS Version 20 for descriptive statistics in percentage.

RESULTS

The present study revealed that greater than half of the respondents (59.4 %) in and around Holeta were educated whereas less than half of them (40.6%) were uneducated. The prevalence on the challenges faced by their animals was 25, 42.2, and 32.8% due to disease, feed shortage and both disease and feed shortage, respectively. A small number of farmers (6.5 %) in the study area purchased antibiotics from private pharmacy to treat their animals. 6.5% of the respondents purchased antibiotics from private pharmacy and brought to their house by carrying them in the pocket. 3.9 and 2.9% of the respondents store drugs on the shelf and floor up to three months, respectively. Drugs administration activity was performed by non-professional person simply by

Table 1. Antibiotics handling practice of animal owners.

Parameter	Category	Number	Percentage
Education level	Illiterate	156	40.6
	Below grade 8	174	45.3
	Above grade 8	54	14.1
Challenges on animal health	Disease	96	25.0
	Feed shortage	162	42.2
	Disease and feed shortage	126	32.8
Purchase of drugs	No	359	93.5
	Yes	25	6.5
Drug administered by	Not applicable	359	93.5
	Non professional	25	6.5
problem during drug administration	Not applicable	374	97.4
	Swelling of injected site	10	2.6
means of drug transportation	Not applicable	359	93.5
	Carrying in pocket	25	6.5
Store before administration	Not applicable	359	93.5
	Yes	25	6.5
Place of store	Not applicable	359	93.2
	On shelf	15	3.9
	On floor	10	2.9
Duration of drug store	Not applicable	359	93.2
	One month	10	2.6
	Two month	11	2.9
	Three month	4	1.3
Proper dose administration	Not applicable	359	93.2
	From leaflet	3	0.8
	Advice from professional	20	5.2
	By guess	2	0.5

guessing and information obtained from other professional (Table 1).

Most of the respondents (66.1%) have awareness about the withdrawal period of antibiotics whereas 33.9% of the respondents did not know about withdrawal period of drugs. Out of 384 respondents, 12.8% sold the milk, 86.7% gave milk for calf and 0.5% of them used milk for home consumption at the time of drugs administration. A few numbers of respondents (2.6%) injects their animals below the normal dosage resulting drug resistant disease to their animals (Table 2).

DISCUSSION

The handling and utilizing practice of antibiotics used in animal owners have been implicated in the development and spread of resistant bacteria phenotypes that is affecting the therapeutic efficacy of current antibiotics (Silbergeld et al., 2008; Sanford et al., 2009). This study aimed at assessing the practices of handling and utilization of veterinary antibiotics as a case study.

This study revealed that animal owners are faced with many challenges regarding their animals including feed shortage and disease. These challenges were enumerated from many sources such as lack of sufficient and standard nutrition, poor husbandry practices, inadequate animal health services such as treatment practices and disease control activity. Many of the animal owners in the study area were advised and supported by the professionally skilled person to treat their diseased animals, while some others bought antibiotics from private pharmacy and treated their animals by themselves.

Table 2. Antibiotics utilization practice of farmers.

Parameter	Category	Number	Percentage
Awareness of withdrawal period	No	130	33.9
	Yes	254	66.1
Information of withdrawal period	Not applicable	130	33.9
	From professionals	254	66.1
Use of milk after drug administration	For sell	49	12.8
	For calf	333	86.7
	For home consumption	2	0.5
Dosage below the normal	Not applicable	358	93.2
	No	22	5.7
	Yes	4	1.0
Response of below dosage	Not applicable	380	99.0
	Not cured	4	1.0
	Not available	25	6.5

The primary purpose of veterinary antibiotic drugs is to safeguard the health and welfare of animals with well diagnosed disease and well prescribed drugs under the supervision of veterinarian and professional person. Lack of practice to go to veterinary professionals, when their animals were sick and ineffective selection of veterinary drugs was the major problem of veterinary antibiotics handling and utilization. As a result, antibiotics were tried without proper diagnosis (Laxminarayan et al., 2013).

The trend of antibiotic administration by the respondents (6.5%) indicates improper practice of antibiotic utilization. The underperformance of some of the antibiotics as reported by the animal owners could be due to lack of awareness about the handling and utilization of antibiotics. The storage conditions of antibiotics in the animal owners were suboptimal because the storage environments of the antibiotics were prone to temperature fluctuations which hastens antibiotic decomposition, reducing its concentrations and efficacy (keke et al., 1999) thus promoting resistance in exposed bacteria (Laxminarayan et al., 2013; Teuber, 2001).

The major considerations for proper usage of antibiotics drugs, which are a main concern of modern medicine, are to select the optimal drugs at the proper dosage and duration, to minimize the emergence of resistance and to provide health services at a reasonable cost (Pang et al., 1994) whereas the end users of antibiotics were unable to select proper dosage and duration of treatment in the study area indicating improper utilization of antibiotic drugs. The knowledge of many animal owners concerning antibiotics withdrawal periods and dosages was found to be low. Moreover, the animal owners depended more on fellow animal owners than veterinarians for antibiotic knowledge, which resulted in the use of the same antibiotics and similar handling practices among animals in close proximity or within the same district. Poor dosing practices, for example, were common when an antibiotic failed to treat an infection. The animal owners lacked adequate measuring instruments to mete out correct dosages. In this study area there were lack of knowledge and practice concerning antibiotics dosing and withdrawal period as the same as reported earlier (Addah et al., 2009).

Ideally, good antibiotic prescribing practice should reflect the use of the most effective, least toxic, and least costly antibiotic for the precise duration of time needed to cure the infection (Gyssens, 2001) whereas the animal owners bought antibiotics without prescription in the study area that. Generally, majority of the animal owners in and around Holeta were provided veterinary professional service on animal disease treatment whereas a few number of the farmers purchased antibiotics from private pharmacy without prescription for self-treatment of their animals indicating improper handling and utilization practice of antibiotic drugs.

Abbreviations: NSAID, Non-steroidal anti-inflammatory drugs; **MICs,** minimum inhibitory concentrations; **MBCs,** minimum bactericidal concentrations; **CAFOs,** concentrated animal food operations; **HARC,** Holeta Agriculture Research Center.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Acar JF (1997). Consequence of bacterial resistance to antibiotics in medical practice. Clin. Infect. Dis. 24(Supplement_1):S17-S18.

Addah W, Baah J, Tia S, Okine E (2009). Knowledge and practices of smallholder farmers and herdsmen in the use of acaricides and gastrointestinal anthelminthes in Ghana. Livest. Res. Rural Dev. 21:11.

Flynn WT (2012). The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing animal's center for Veterinary Medicine (HFV-1). Food and Drug Administration. Department Of Health and Human Services. http://www.fda.gov/downloads/animal veterinary /guidancecomplianceenforcement/.guidance for industry/ucm.21:6-9.

Gyssens IC (2009). Quality measures of antimicrobial drug use. Int. J. Antimicrobial Agents 17(1):9-19.

Hirsh DC, Zee YC (1999). Veterinary microbiology.1st ed. Blackwell Publishing Company. California. pp. 48-57.

Laxminarayan R, Duse A, Wattal C (2013). Antibiotic resistance-the need for global solutions. Lancet Infect. Dis. 13(12):1057-1098.

Okeke N, Lamikanra A, Edelman R (1999). Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerging Infect. Dis. 5(1):18-27.

Pang Y, Brown BA, Steingrube VA, Wallacer RJ, Roberts MC (1994). Tetracycline resistance determinants in Mycobacterium and Streptomyces species. Antimicrobial Agents Chemother. 38(6):1408-1412.

Sanford JC, Mackie RI, Koikeetal S (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38(3):1086-1108.

Silbergeld EK, Graham J, Price BL (2008). Industrial food animal production. antimicrobial resistance and human health. Ann. Rev. Public Health. 29:151-69.

Teuber M (2001). Veterinary use and antibiotic resistance. Curr. Opinion Microbiol. 4(5):493-499.

Thawani V (2010). Rational use of medicine achievements and challenges. Indian J. Pharmacol. 42:63-64.

Thrusfield M (2005). Veterinary Epidemiology, 3rd ed. Blackwell Science Ltd. Edinburgh, UK. pp. 228-247.

academicJournals

Vol. 10(2), pp. 60-66, February 2018
DOI: 10.5897/JVMAH2017.0637
Article Number: 5713B2D55567
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Evaluation of the safety and efficacy of combined Newcastle disease, fowl pox and fowl typhoid vaccine under laboratory condition

Hana Tadesse[1*], Senait Belete[2] and Benti Deressa[3]

[1]School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia.
[2]National Veterinary Institute (NVI), Debre Zeit, Ethiopia.

Received 15 August, 2017: Accepted 18 December, 2017

An experimental study was conducted on day-old chicks to evaluate the safety and efficacy of combined Newcastle diseases (ND), fowl pox (FP) and fowl typhoid (FT) vaccine. The vaccine was prepared using the Lasota strain of Newcastle disease virus (NDV), the FP strain of fowl pox virus (FPV) and the 9R strain of FT. The vaccine was found safe, as no clinical signs or mortalities were observed. Post vaccination Haemaglutination Inhibition (HI) titre for ND was above the required protection level (≥1:16) and its geometric means (GM) were 0.0098, 0.0063 and 0.0059 for group one, two and three respectively, who received conventional and combined vaccine. The difference in GM between the three vaccinated groups were not significant (*p*=0.544). The trivalent combined vaccine did not show significant difference in the HI titre result among the groups that were given conventional vaccines and the other two experimental groups which received trivalent vaccine (*p*=0.257). From 75 samples, 73 (97.3%) were positive for FT through rapid slide agglutination test (RSAT). The chicks were challenged separately for the three diseases using the specific pathogens. Both combined and conventional vaccine conferred protection upon challenge. For ND challenge, 93.3% (*n*=14/15) of the control groups died. From FT and FP control groups 86.6% (*n*=13/15) and 20% (*n*=3/15) respectively died up on challenge. Both combined and conventional vaccine type conferred a similar and good level of protection. However, the use of combined vaccine has considerable advantage particularly in terms of convenience and cost effectiveness to control multiple diseases through simple immunization schedule. Further studies were recommended on the development of combined avian vaccines in Ethiopia.

Key words: Newcastle disease, fowl pox, fowl typhoid, experimental study, combined vaccine.

INTRODUCTION

Infectious and non-infectious diseases are major threat to human and animal life throughout the globe. The number of people and animals dying due to infections are far greater than any other reason in the world every year. With

*Corresponding author. E-mail: tadessehanna@ymail.com.

increasing industrialization and intensification of rearing systems, the disease pattern in domestic fowl is changing. There are increasing problems and thus increased risk of disease entry to local chicken by movement of infected birds or contaminated products or materials from other places (Desalew, 2012).

The major endemic diseases, which constitute major constraints to poultry production in Ethiopia, include Newcastle disease caused by virulent strains of avian Paramyxovirus type 1 (APMV-1) serotype of the genus Avulavirus belonging to the subfamily *Paramyxovirinae*, family *Paramyxoviridae*, Fowl pox caused by Avian poxvirus, and Fowl typhoid caused by the highly pathogenic chicken-adapted *S. enterica* biotype Gallinarum. These diseases are prevalent in different parts of Ethiopia and pose significant economic problems to poultry production (Hailu, 2012).

In all countries where those diseases occur, vaccination is accepted as the method of control for the prevention of the diseases expansion (Petra and Karen, 2012). In Ethiopia, individual or separate vaccination program of different livestock diseases has been practiced for years (Gelagay et al., 2012). The individual vaccination strategies has several constraints like stress during individual handling, vaccination cost, number of inoculations, compliance to the vaccine schedule and logistic costs.

In order to increase the probability of early control and/or eradication of the most important diseases of poultry at national level and reach the target vaccination coverage, the production of combined vaccine is very advantageous both from the technical and economic point of view. Therefore, the objectives of the present study was to compare antibody production level of national veterinary institute (NVI) produced Newcastle disease, fowl pox and fowl typhoid conventional vaccines with the experimental combined vaccine and to evaluate the safety and efficacy of newly produced combined vaccine under laboratory condition.

MATERIALS AND METHODS

Experimental animals

A total of SPF 135 white leghorn day-old chicks were raised under intensive management system and used in all the vaccination experiments. The chicks' house was fumigated with formalin before the introduction of chicks and bedded with disinfected wood shavings.

Experimental design

Randomized controlled design was used. Experimental chicks were randomly placed in four vaccine groups (*n*= 30 chicks /group) (Table 1), identified by leg band. Forty-five chicks were used as a control for the three treatment groups (*n*= 15 control chicks /group).

Master seed management

All vaccine seed strains were supplied by African Union Pan African Veterinary Vaccine Center (AU-PANVAC). All the vaccine seed strains were live-attenuated (Lasota, FPV and *Salmonella Gallinarum* 9R).

Production of experimental combined vaccine

ND (10^7 EID_{50}/ml) and FP (10^3 $TCID_{50}$/ml) vaccines were prepared using specific pathogen free eggs (SPF) while FT vaccine was produced by using *Staphylococcus gallinarum* 9R strain on *S. Gallinarum* broth (5×10^7 CFU) medium (World Organization for Animal Health (OIE), 2015). The vaccines were produced by mixing one part of fowl pox virus (FPV) (500 ml), and two parts of each Newcastle disease virus (NDV), (1000 ml) and FT (1000 ml). Likewise, 2500 ml of freeze-drying media (Lactalbumin Hydrolysate 5% and sucrose 10%) were prepared before mixing. Totally, 5000 ml solution was mixed and dispensed into sterile glass vials with 2.5 ml quantities per ampoule by using a sterile calibrated automatic syringe. The titer was expressed on the bases of embryo mortality for ND, cytopathic effect for FP and culture turbidity for FT; and calculated by spearman-Karber method (Kiril et al., 2017). The safety, titration and the sterility of each vaccine was checked separately before combining according to World Organization for Animal Health (OIE) (2015).

Validation of the vaccine

After lyophilization, each vial was checked for vacuum with vacuum tester. In addition, freeze-dried vaccine was checked for sterility and titration according to World Organization for Animal Health (OIE) (2015)

Safety test

The safety test was carried out by using 10 seven day-old chicks, each of them was inoculated through eye-drop, wing web, and subcutaneous routes of inoculation with single dose of the combined vaccine. After inoculation, they were clinically checked for 3 weeks to determine the presence of local and/or systemic adverse reactions, which may develop after vaccination. Moreover, 5 six week-old chicks received 10 doses of the combined vaccines by the same route and observed for 3 weeks (World Organization for Animal Health (OIE), 2015).

Serum collection

The blood samples were collected randomly from experimental groups of chicks to assess the immunity level prior to vaccination and after vaccination at day 21.The blood samples were collected from different groups of chicks from their wing vein by using 3ml sterile syringe. After collection, the syringe was kept in slanting position over-night in order to collect the serum samples, the sera were collected and inactivated in water bath at 56°C for 30 min. After inactivation, the sera samples were tested, to determine the antibody level by using hemagglutination inhibition test for Newcastle disease and rapid slide agglutination test for fowl typhoid (World Organization for Animal Health (OIE), 2015)

Experimental grouping

The chicks in group one were vaccinated with HB1and Lasota conventional vaccines at 7 days and 5 weeks of age for Newcastle diseases (ND) through eye drop; at 9 weeks of age FP vaccine through wing web and, at 10 weeks of age with FT vaccine through subcutaneous route (World Organization for Animal Health (OIE), 2015)Group two and three were vaccinated two times with combined

Table 1. Vaccine type, age, dose and number of chicks within a group.

Group	Vaccination age	Type of vaccine	Dose	Booster	No. of chicks
1	7 days and 5 weeks booster	HBI	10^7	1× HBI	30
		Lasota	10^7	1×Lasota	
	9 weeks	FP	10^3	1x FP	
	10 weeks	9R (FT)	10^7	1x FT	
2	7 days and 8 weeks booster	Combined	-	2×combined	30
		Combined	-		
3	14 days, 9 weeks booster	Combined	-	2×combined	30
		Combined	-		45

vaccine at 1st and 2nd vaccination, the chicks of the group two received the vaccine at 7 days of age and boosted at 8 weeks of age; group three received the vaccine at 14 days of age and boosted at 9 weeks of age (Table 1).

Challenge pathogens and experiment

Virulent strains of velogenic NDV, FPV and FT were obtained from the NVI Research and Diagnostic laboratory, which is previously collected and confirmed positive for the three diseases, from different regions of Ethiopia during different outbreaks and given to chickens at a titre of 10^6 EID_{50}/bird, 10^5TCID_{50}/bird and 10^7CFU/bird, respectively. The titres of the challenge viruses and bacteria were checked before challenge according to the NVI's standard operating procedure (SOP). The challenge test was conducted separately for ND, FP and FT. Ten chicks (n=10) from each treatment group for each pathogens and five (n=5) from control groups for each pathogens were challenged 5 weeks after the last vaccination and they were kept under strict quarantine. They were observed for disease symptoms and gross pathological lesions for 14 days post-challenge. Detail clinical and post-mortem examination were conducted especially on birds showing clinical signs of those diseases.

Statistics

Data collected were entered into micro soft (MS) excel spread sheets and statistical package for social science (SPSS) version 20 was used to analyze the data. Descriptive statistics were used to analyze the variation in mean antibody titer and occurrence of disease following challenge trial among treatment groups. A 5 % absolute precision and 95% confidence interval was used and level of significance was set at p =0.05.

RESULTS

Safety

In safety trial, birds were euthanized at six weeks post-vaccination; necropsies were performed and samples were taken and tested. No abnormal clinical signs or mortalities were observed either in the group of seven-day old chicks of the safety group, which received one dose of vaccine or in the six week-old birds receiving ten doses at different routes of inoculation of the vaccine.

Serological response to the vaccine

For efficacy of the trivalent vaccine, a total of 75 sera samples were randomly collected from vaccinated birds (group one, group two and group three) and the geometric mean HI titre and rapid slide agglutination test (RSAT) were observed for ND and FT, respectively. Fowlpox is confirmed through challenge. In all vaccinated groups of chicks, the HI antibody levels were above the required protection level which is 1:16 for NDV (World Organization for Animal Health (OIE), 2015). The analysis of the data showed that the trivalent vaccine did not show significant difference (p=0.544) in the GM HI titre between groups (Table 2). The lowest HI titre was 1:16 (group one and two) and the highest HI titer was 1:2048 (group two and three). The trivalent combined vaccine did not show significant difference (p=0.257) in the HI titre result among the groups that were given conventional NVI produced vaccines and the other two experimental groups which received trivalent vaccine. From 75 samples, 73 (97.3%) were positive for FT through rapid slide agglutination test (RSAT) and test results observed among the groups (one, two and three) that were given one group with conventional NVI produced vaccines and two groups experimental trivalent vaccine were not significantly different (p=0.618) (Table 3).

Response to challenge

The challenge protection produced by vaccinated groups in comparison to the unvaccinated control groups showed that, from 90 birds challenged only 3 birds (3.3%) died and 87 birds (96.7%) survived from vaccinated groups of chicks after challenge with virulent local strains of respective virus and bacteria species (Table 4). From 45 unvaccinated controls challenged, 32 birds died (71.1 %) only 13 birds survived (28.9%); among those survivors 12 of them were FP survivors and showed typical clinical sign of FP (Figure 3). In terms of challenge protection, significant difference (P=0.001) was observed among vaccinated groups in comparison to that of unvaccinated control groups. However, there was no significant difference in terms of

Table 2. Hemagglutination inhibition titre of NDV.

Variable	GMT	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
Group 1									
No.	0.0098	1	3	6	9	5	1	-	-
Percentage		4	12	24	36	20	4	-	-
Group 2									
No.	0.0063	1	3	5	4	5	5	1	1
Percentage		4	12	20	16	20	20	4	4
Group 3									
No.	0.0059	-	3	5	5	6	4	1	1
Percentage		-	12	20	20	24	16	4	4

Table 3. Rapid slide agglutination test for fowl typhoid (RSAT).

Group	RSAT	
	Negative (%)	Positive
Group 1	1 (4.0)	24 (96.0)
Group 2	1 (4.0)	24 (96.0)
Group 3	0 (0)	25 (100.0)
Total	2 (2.7)	73 (97.3)

Table 4. ND, FP and FT, vaccinated and control groups challenge survived and died.

Pathogen	Group 1		Group 2		Group 3	
	Conventional {No. (%))	Control {No. (%))	Combined {No. (%))	Control {No. (%))	Combined {No. (%))	Control {No. (%))
Newcastle disease						
Survived	9(90)	0(0)	9(90)	1(20)	10(100)	0(0)
Died	1(10)	5(100)	1(10)	4(80)	0(0)	5(100)
Fowl Typhoid						
Survived	9(90)	2(40)	10(100)	0(0)	10(100)	0(0)
Died	1(10)	3(60)	0(0)	5(100)	0(0)	5(100)
Fowl Pox						
Survived	10(100)	4(80)	10(100)	5(100)	10(100)	3(60)
Died	0(0)	1(20)	0(0)	0(0)	0(0)	2(40)

post-challenge protection (p >0.05) between NVI produced conventional and trivalent combined vaccine.

DISCUSSION

The three diseases (ND, FP and FT) are a major problem of poultry industry in Ethiopia (Hailu, 2012). In the present study, the production of improved vaccine was achieved successfully. The experiment showed treatment has no pathogenicity effect on vaccinated chicks. Unvaccinated control group of chicks have shown clinical sign of the disease during challenge experiment (Figures 1, 2, 3). The outcome of infection and interaction between the two viral and one bacterial strain indicated that there is no potential interference with the replication of the pathogenic strains. Mayahi et al. (2013) showed that the use of polyvalent vaccines combined in the manufacturing laboratory can attenuate the interference between these viruses when compared to vaccines associated just before vaccination.

The trial compared that chicks received the conventional vaccines (group one) and combined vaccine (group two and three); group three provided best antibody response. From the results of the challenge tests, the vaccine was effective without any evidence of interference. The vaccine was found to be safe for seven day-old and 6 week-old

Figure 1. Prostration of head and neck of a bird that died from NDV infection.

Figure 2. Diarrhoea of a control bird that died from FT infection.

chicks. Zou et al. (2013) reported similar arguments; in their findings the results of the challenge tests and performances of birds showed that all the three vaccine was found to be safe even for one-day old chicks in the first week of vaccination.

Ayala et al. (2016) observed that Lasota is much more immunogenic than the Hitchner B1 and strain V4. A number of researchers have reported that live ND vaccines give better protection and health status than killed vaccines. The use of live vaccines is preferred for priming the birds as it produces local immunity in the mucosal membrane of the conjunctiva, thus providing immediate protection on subsequent exposure with field virus challenge (Patti et al., 2013; Taebipour et al., 2017). In the present experiment, in all vaccinated groups of chicks, the hemagglutination inhibition antibody levels were above the required protection level which is 1:16; it assure the criterion set by World Organization for Animal Health

Figure 3. A lesion on the comb and wattle of a control bird that died from FP virus.

(OIE) (2015).

Furthermore, experience in Ethiopia had shown that Lasota vaccine confers immunity for 6 months when administered at 5 weeks of age. The results of vaccine trials in Ethiopia showed that conventional (HitchnerB1 and Lasota) and the thermo stable ND-I2 vaccines give similar antibody response and protection against challenge when given via the ocular and the drinking water route (Mayers et al., 2017). In this study, the HI titre 1:16 was considered protective and it was comparable to the results of previous findings (Ayala et al., 2016; Majid, 2014) that reported birds with HI titers 1:16 were protected against challenge with a virulent strain of NDV.

In the present study, the FP strain of FPV was used. The production of good immunity levels by administration of FP vaccine via wing web was confirmed by means of challenge experiment. From 45 birds challenged, none of 30 birds from the vaccinated group were died but only 3 (20%) birds from the control groups were died and 12 (80%) survived with typical clinical sign of the disease (Figure 3), this result is similar to the findings of Meseko et al. (2012). There is no obvious clinical signs were detected among all vaccinated birds twenty-one days post challenge. As for the control group, typical clinical signs of fowl pox (scabs on comb, wattles and legs) were observed seven days post challenge.

Mortality in highly susceptible chicks exposed to virulent strains of *S. Gallinarum was* limited by SG9R vaccine (Wigley, 2017). Chetan et al. (2014)and Łaniewski et al (2014) showed that a 9R vaccine provided excellent protection and is safe for vaccination of 4 week-old chicks. Indeed, in the present study, birds that received the SG9R strain by subcutaneous route showed no evidence of disease.

An ideal vaccine should promote protection of birds against mucosal and systemic infection by effectively stimulating both immune responses (Revolledo and Ferreira, 2012). In the present study, the strain used for *S. Gallinarum* was 9R. 9R is the rough strain that originated from the smooth strain 9S (Paweł et al., 2014). The 9R strain does not contain the somatic antigen characteristics as the smooth forms *of S. Gallinarum* due to the loss of some lipopolysaccharide. The change in lipopolysaccharide reduced the virulence of the strain (Bérto et al., 2015; Immerseel et al., 2013).

In the study findings, out of 75 samples, 73 (97.3%) were positive for FT through RSAT. The antibody response of birds vaccinated with conventional and combined vaccines was very effective. In addition, there were no statistically significant differences in the protection efficacy or immune responses between group one, two and three. In this study, the best protection was observed in group three. The results indicate that the application of combined experimental vaccine is safe when vaccinated at 10 weeks of age. There were no detected clinical signs of disease or mortality due to the vaccine strain during the monitoring period of the safety trial. This result is similar to the findings of Atul et al. (2012) who tested the safety of the SG9R vaccine when administered via injection.

Vaccination of group one with conventional vaccine; group two and three with combined vaccine showed protection rate against challenge with the wild-type SG observed 14 days after one-dose vaccination. Twenty-nine (96.6%) birds survived among vaccinated groups, while from the control non-vaccinated group, 13(86.6%) birds died and this was the same as the findings of Chetan et al. (2014).

In conclusion the combined trivalent vaccine used in this

study was found to be safe as no abnormal signs or mortality was observed during safety test throughout the monitoring period. There was no statistically significant variation in the efficacy of the vaccine between the three experimental groups which received conventional NVI produced vaccines and the experimental combined vaccine.

The combined vaccine gave a similar level of protection to the conventional one based on HI, RSAT and challenge protection test for FP. Immunized animals remained apparently healthy without any signs of illness after experimental challenge with each of three pathogens. However, the use of combined vaccine can facilitate greater convenience, bring down the cost of vaccination significantly, reduce stress to the animal, and reduce vaccination time and logistic costs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to The National Veterinary Institute (NVI) of Ethiopia and Jimma University, College of Agriculture and veterinary Medicine for mobilizing and organizing the necessary facilities that enabled us complete this work successfully.

REFERENCES

Atul AC, Chetan VJ, Sam Woong K, John HL (2012). Construction of a Salmonella Gallinarum ghost as a novel inactivated vaccine candidate and its protective efficacy against fowl typhoid in chickens. Vet. Res. 43(1):44.

Ayala AJ, Dimitrov KM, Becker CR, Goraichuk IV, Arns CW, Bolotin VI, Ferreira HL, Gerilovych AP, Goujgoulova GV, Martini MC, Muzyka DV (2016). Presence of Vaccine-Derived Newcastle Disease Viruses in Wild Birds. Presence of vaccine-derived Newcastle disease viruses in wild birds. PloS one 11(9):e0162484.

Bérto LD, Beirão BCB, Filho TF, Ingberman M, Fávaro Jr C, Tavella R, de Mesquita Silva RB, Caron LF (2015). Live and Inactivated Salmonella Enteritidis Vaccines: Immune Mechanisms in Broiler Breeders. World J. Vaccines 5(04):155.

Chetan VJ, Atul AC, John HL (2014). Generation of a safety enhanced Salmonella Gallinarum ghost using antibiotic resistance free plasmid and its potential as an effective inactivated vaccine candidate against fowl typhoid. Vaccine 32(9):1093-1099.

Desalew TT (2012). Management practices, productive performances and egg quality traits of exotic chickens under village production system in East Shewa, Ethiopia: College of veterinary medicine and agriculture, Addis Ababa University.

Gelagay A, Nigussu F, Shiferaw J, Gedlu M, Teshale S, Haileleul N (2012). Study on immunogenicity of combined sheep and goat pox and peste des petitis ruminant's vaccines in small ruminants in Ethiopia. Afr. J. Microbiol. Res. 6(44): 7212-7217.

Hailu M (2012). Review on major viral diseases of chickens reported in Ethiopia. J. Infect. Dis. Immun. 4(1):1-9.

Immerseel FV, Studholme DJ, Eeckhaut V, Heyndrickx M, Dewulf J, Dewaele I, Hoorebeke SV, Haesebrouck F, Meirhaeghe HV, Ducatelle R, Paszkiewicz K, Titball RW (2013). Salmonella Gallinarum field isolates from laying hens are related to the vaccine strain SG9R. Vaccine 31(43):4940-4945.

Kiril MD, Claudio LA, Qingzhong Yu, Patti JM (2017). Newcastle disease vaccines-A solved problem or a continuous challenge? Vet. Microbiol. 206:126-136.

Łaniewski P, Mitra A, Karaca K, Khan A, Prasad R, Curtiss R, Roland KL (2014). Evaluation of Protective Efficacy of Live Attenuated Salmonella enteric Serovar Gallinarum Vaccine Strains against Fowl Typhoid in Chickens. Clin. Vaccine Immunol. 21(19):1267-1276.

Majid B (2014). The response of ducks to V4 Newcastle disease virus and its transmission to contact ducks and domestic chickens. In Veterinary research forum: An international quarterly journal faculty of veterinary medicine, Urmia University, Urmia, Iran. 5(2):145.

Mayahi M, Talazadeh F, Aslahi H (2013). Effect of the commercial mixed live Newcastle disease and infectious bronchitis vaccines and the use of two separate vaccines given simultaneously on systemic antibody responses in chickens. Iran. J. Virol. 7(3):17-21.

Mayers J, Mansfield KL, Brown IH (2017). The role of vaccination in risk mitigation and control of Newcastle disease in poultry. Vaccine. 35(44):5974-5980.

Meseko CA, Shittu IA, Akinyede O (2012). Seroprevalence of fowl pox antibody in indigenous chickens in Jos North and South council areas of Plateau State, Nigeria: Implication for vector vaccine. ISRN Vet. Sci. 2012:154971.

Patti JM, Claudio LA, John El A, Kristi MD, Sean CC, Zijing G, Darrell RK (2013). Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. Dev. Comp. Immunol. 41(4):505-513.

Petra O, Karen R (2012). The current challenges for vaccine development. J. Med. Microbiol. 61:889-894.

Revolledo L, Ferreira AJP (2012). Current perspectives in avian salmonellosis: Vaccines and immune mechanisms of protection. J. Appl. Poult. Res. 21:418-431.

Taebipour MJ, Dadras H, Nazifi S, Afsar M, Ansari-Lari M (2017). Evaluation of blood monocyte and lymphocyte population in broiler chicken after vaccination and experimental challenge with Newcastle disease virus. Vet. Immunol. Immunopathol. 190:31-38.

Wigley P (2017). Salmonella enterica serovar Gallinarum: addressing fundamental questions in bacteriology sixty years on from the 9R vaccine. Avian Pathol. 46(2):119-124.

World Organization for Animal Health (OIE) (2015). Manual of diagnostic tests and vaccines for terrestrial animals. Part. 2: 1-17.

Zou YJ, Liu Y, Wang YQ, Wu J, Gong FL, Ma GH (2016). Immune Effect of HTCC Hydrogel Microspheres and the Blending Emulsion of Oil and Microspheres as New Adjuvants of Combined Vaccine against Newcastle Disease and Avian Influenza. Available at: http://www.jproeng.com/EN/abstract/abstract2719.shtm

academicJournals

Vol. 10(2), pp. 67-71, February 2018
DOI: 10.5897/JVMAH2017.0658
Article Number: AEDB3E655568
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Telediagnosis: Parasitological experiences in wild ruminants of South African preserves

Gianluca Pio Zaffarano[1], Benedetto Morandi[1*], Alessia Menegotto[2], Fabio Ostanello[1] and Giovanni Poglayen[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (BO), Italy.
[2]Conservation Global Agency for Environmental Gain npc, Company # 2010/018132/08, P.O. Box 2791, Knysna 6570, Garden Route, South Africa.

Received 20 October, 2017: Accepted 30 November, 2017

A survey on wild ruminants' health status of any South African preserves was attempted, assessing body condition score (BCS) through tele-diagnosis. The wildlife BCS was linked to the presence of gastrointestinal parasites that should be recognized, counted and statistically evaluated. For this purpose, we examined 103 faecal samples of wild ruminants from 6 South African preserves. For practical reasons, the animals were divided into two macro-categories: small and large ruminants. The results obtained showed a prevalence of 78.1 and 15.6% in large ruminants for gastrointestinal strongyles (GIS) and coccidian, respectively, while small ruminants showed 92.3% due to GIS and 30.8% for coccidia. No statistically significant difference in the prevalence among the preserves was detected; on the other hand, a low value of BCS corresponds to a greater presence of parasites with statistics difference in the macro-categories (small ruminant x^2=5.238; P=0.020; large ruminant x^2= 15.215; P<0.001) and sex classes (male x^2=5.409; P=0.020; female x^2 =17.350; P<0.001). For these reasons, our results provide a practical feedback for the management preserves. The present paper is fully part of the limited experiences of telediagnosis in a conservation perspective. Based on the results obtained, we decided to organize a project that could limit and assess the risk factors in the management of these activities in the South African context.

Key words: Wild ruminants, telediagnosis, parasites, body condition scores, South African preserves.

INTRODUCTION

In recent past, Veterinary Medicine has focused its interest on involving wild animals not only as single head fenced in captivity and therefore clinically similar to domestic one, but also as free-living populations. All these are meant to protect biodiversity and curtail the possible spread of pathogens, and zoonotic diseases. These preliminary considerations suggest transferring the clinical approach proposed by Bologna Academy (Messieri and Moretti, 1982) and more recently by Cambridge Academy (Jackson and Cockcroft, 2002),

*Corresponding author. E-mail: benedetto.morandi2@unibo.it. Tel: +39 0512097058. Fax: +39 0512097039.

Figure 1. Images of animals and environments in the South African preserves investigated.

simplifying and adapting them to wild ruminants in game preserves of South Africa. These are wild farms suitable for the conservation, including breeding of species of local wildlife particularly valuable, from economic, touristic or endangered point of view. Their management is quite particular: wild ruminants are fenced on many hectares of land and continuously exchanged with other preserves. Considering that from this wild farm parasitological information are lacking and also domestic ruminants are raised close to wild ones, we suggested transferring the clinical approach cited adapting them to wild ruminants by a visual system for scoring body condition (telediagnosis). In the international literature, we have found four specific papers of this non-invasive method to define health status: two in Asian Elephants (*Elephas maximus* L., 1758) (Ramesh et al., 2011; Wijeyamohan et al., 2015) and two on wild ruminants, in particular Bassano et al. (2003) on *Ovis canadensis* (Shaw, 1804) and *Capra ibex* (L., 1758) and Pfeifer (2015) *Cervus elaphus* (L., 1758).

The aim of this study was to survey the health status of wild ruminants by telediagnosis. This was evaluated by scoring body condition. Body condition score (BCS) is a subjective tool to assess the amount of metabolizable energy stored in body fat (primarily subcutaneous) and muscle tissues of a live animal (Edmonson et al., 1989; Burkholder, 2000; Alapati et al., 2010). Body condition is an index of an animal's health (Terranova and Coffman, 1997). An increase or decrease in body condition could mean a change in quality of management or environment in which an animal lives (Figure 1).

The wildlife BCS should be linked to the presence of gastrointestinal parasites that should may be recognized, counted and statistically evaluated.

These described assumptions have had to adapt to the preserves logistical and laboratory requirements provided. Another purpose to study the parasitism of wild ruminants should be to help their management by rangers.

MATERIALS AND METHODS

Study area

Our survey was done in 6 preserves in the Eastern region of Garden Route, Republic of Sud Africa (Figure 2) during February 2016. The area has soil and weather characteristics that allow arid lands mixed with wetlands, characterized by particular kind of bush (named fynbos), especially suitable for game preserve activity aimed to the conservation of autochthonous flora and fauna.

Animals

Overall, we have had the opportunity to work with 103 animals belonging to 15 different ruminant species (Table 1). The adjustment of the clinical procedures applied to domestic animals provides general appearance and physical examination, excluding the medical history, since in wildlife it is impossible to know the history of individuals. The animals were identified through an optical instrument (field glass Olympus 10X50) at dropping time, later they were photographed and then classified according to sex (male, female) and category (small or large ruminants). The sex was determined in 102 animals, 34 males and 68 females, in one instance it was not possible because it was a very young individual and hidden from the herd. BCS was evaluated analysing the ribs, spine, hip bone/rump, tail head and belly, according to the method described by Pfeifer (2015). Randomly, the classification was simplified by grouping the animals into two main categories: emaciated/medium and good/excellent. Faecal samples were collected off the ground, marked with a serial number, scientific and common names of the species. Collected samples were stored in a

Figure 2. Study area with the six investigated preserves: red star (Garden Route, 34°12'31''S; 21°38'00''E), white (Wolwedans, 34°01'48''S; 21°59'40''E), yellow (Gondwana, 34°04'51''S; 21°54'40''E), orange (Hartenbos, 34°02'41''S; 21°59'41''E), light blue (Bergsig, 34°05'32''S; 22°02'06''E) and green (Plettenberg, 33°56'43''S; 23°21'00''E).

Table 1. Animal species and categories considered.

Category	Species	Number
Large ruminant	Giraffe (*Giraffa camelopardalis* L., 1758)	9
	Blu Wildebeest (*Connochaetes taurinus* Lichtenstein, 1812)	10
	Waterbuck (*Kobus ellipsiprymnus* Ogilby, 1833)	3
	Orix (*Oryx gazzella* L., 1758)	3
	Eland (*Taurotragus oryx* Pallas, 1766)	20
	Buffalo (*Syncerus caffer* Sparrman, 1779)	7
	Kudu (*Tragelaphus strepsiceros* Pallas, 1766)	2
	Sable Antelope (*Hippotragus niger* Harris, 1838)	7
	Black Wildebeest (*Cannochaetes gnou* Zimmermann, 1780)	3
	Total large ruminant	64
Small ruminant	Bontebok (*Damaliscus pygargus* Pallas, 1767)	11
	Gray rhebok (*Pelea capreolus* Forster, 1790)	1
	Red Hartebeest (*Alcelaphus buselaphus* Pallas, 1766)	4
	Impala (*Aepyceros melampus* Lichtenstein, 1812)	16
	Springbok (*Antidorcas marsupialis* Zimmermann, 1780)	6
	Blesbuck (*Damaliscus pygargus phillips* Harper, 1939)	1
	Total small ruminant	39
	Total	103

cooler, transported in a few hours in a refrigerator (+ 4°C), and then in the laboratory examined.

Examined samples

Stool samples were referred for qualitative and quantitative coprological evaluation. It was realized with an alternative tool that stocks parasitic forms without centrifugal step (Mini- FLOTAC, Silva et al., 2013; Godber et al., 2015), using a floatation solution (specific gravity 1.3).

Statistical analysis

The study of prevalence for coccidia and gastrointestinal strongyles (GIS) was evaluated by comparing the sampling area, sex, and category (small or large ruminants) using chi-square test (χ^2). All statistical analyses were performed using the software SPSS 23.0 (IBM SPSS Statistics, New York, United States).

RESULTS AND DISCUSSION

Qualitative results

Overall, 86 of 103 (83.5%) analysed faecal samples were positive for parasites. Specifically, 86 samples were positive for gastrointestinal strongyles (GIS); and 22

Table 2. Relationship between the four macro-categories considered.

Ruminant	GIS (Prevalence%)	Coccidia (Prevalence%)
Large ruminant	50/64 (78.1%)	10/64 (15.6%)
Small ruminant	36/39 (92.3%)	12/39 (30.8%)

Table 3. Prevalence of the two different parasites categories in the investigated preserves.

Game preserve visited (animals sampled)	GIS (Prevalence %)	Coccidia (Prevalence %)
Bergsig (14)	12 (85.7%)	3 (21.4%)
Garden Route (29)	26 (89.7%)	8 (27.6%)
Gondwana (35)	29 (82.9%)	6 (17.1%)
Hartenbos (9)	7 (77.8%)	Not found
Plettenberg (8)	7 (87.5%)	3 (37.5%)
Wolwedans (8)	5 (62.5%)	2 (25.0%)

Table 4. Statistically significant differences between animal categories related to BCS.

Animal categories	Emaciated/Medium (%)	Good/ Excellent (%)	
Small Ruminant	31 (96.9%)	5 (71.4%)	X^2=5.238; P=0.020
Large Ruminant	33 (97.1%)	17 (56.7%)	X^2= 15.215; P<0.001
Total	64 (97%)	22 (59.5%)	X^2=24.207; P<0.001

Table 5. Statistically significant differences between sex related to BCS.

Sex	Emaciated/Medium (%)	Good/Excellent (%)	
Male	14 (93.3%)	11 (57.9%)	X^2=5.409; P=0.020
Female	49 (98.0%)	11 (61.1%)	X^2=17.350; P<0.001
Total	63 (96.9%)	22 (59.5%)	X^2=23.827; P<0.001

(21.85%) of these were also positive for oocysts of coccidia. Two samples tested positive for whipworm and tapeworm eggs respectively (0.97%). Parasites prevalence was not statistically different (P>0.05) between small ruminants and large ruminants (Table 2).

Statistically significant difference in the prevalence among the preserves was not detected (Table 3). However, there was a lower prevalence, albeit without statistical significance, of GIS in Wolwedans and lack of coccidia in Hartenbos. Even between sexes the parasitism seems to be equal.

Quantitative results

If we take into account the quantitative results, positivity at least one parasite (egg/oocyst), a statistically significant difference emerges for BCS levels and sex (Tables 4 and 5). In one head only positive for GIS we observed diarrhoea.

The lack of previous surveys, the preserves management characteristics and the logistic difficulties led as to modify our initial project. This resulted during data elaboration to consider only the macro categories of ruminants (large and small) and other parasites (GIS and coccidia). For this purpose, it was particularly useful having available a diagnostic tool that allowed a field activity. Both macro categories created reflect the reality of the hosts/parasite/environment situation in the surveyed areas. The absence of the lower category of BCS supports the hypothesis of a natural predation by carnivore. Despite this simplification, our experience allows validating some results by the statistic help, which excludes the results randomness.

Also without the statistic help, the two parasites categories' prevalence in large and small ruminants was higher anyway. This outcome should be justified in that large African ruminants like diet of trees and bushes that do not favor oro-faecal transmission cycle, characteristic of gastrointestinal parasites. According to the preserves' situation, the different parasites' prevalence could depend on Wolwedans in that it is organized like a true breeding unit (few hectares and small yards) with all characteristics management procedures, while the

particularly dried environment of Hartenbos could limit the coccidian transmission that needs humidity to reach the infectivity stages. We did not find prevalence differences between sexes, but this was evident in both categories when related to BCS linked parasite prevalence both for GIS and coccidia. The presence of these parasites is significantly associated in both sexes. This data appear particular interesting for the characteristics of the preserves studied; one could benefit from the information relative to the crucial influence of parasites and BCS being able to hypothesize specific control activities.

Conclusion

For this reason, our results although limited in numbers and of simplified approach could have a practical feedback for the preserves management. In fact, if a bad BCS is related to the higher parasites presence same animals should be treated avoiding its loss and at the same time not interfere with the natural distribution of the parasites (Wilson et al., 2002). For a practical purpose, the animals that could benefit from treatment could be those fenced in small pens or captured for transport.

Future updating should reduce the two macro categories correctly recognising the host species and identify parasites found in dead animals. In this regard, it is extremely interesting the experience carried out in the Limpopo National Park (South Africa) by Van Wyk and Boomker, (2011) where it was possible to isolate and identify the parasites species and the conclusions refer to the importance of parasites in the transfer animal, well known at our latitudes (Lanfranchi et al., 2003).

The present paper is full part of the limited experiences of telediagnosis in a conservation perspective. Based on the results obtained, we decided to organize a project that could limit and assess the risk factors in the management of these activities in the South African context.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors would like to thank Natalie Bellet for English language revision.

REFERENCES

Alapati A, Kapa SR, Jeepalyam S, Rangappa SM, Yemireddy KR (2010). Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. J. Vet. Sci. 11:1-8.

Bassano B, Von Hardenberg A, Pelletier F, Gobbi G (2003). A method to weigh free-ranging ungulates without handling. Wildl. Soc. Bull. 31:1205-1209.

Burkholder WJ (2000). Use of body condition scores in clinical assessment of the provision of optimal nutrition. J. Am. Vet. Med. Assoc. 217:650-654.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G (1989). A body condition scoring chart for holstein dairy cows. J. Dairy Sci. 72:68-78.

Godber OF, Phythian CJ, Bosco A, Ianniello D, Coles G, Rinaldi L, Cringoli G (2015). A comparison of the FECPAK and Mini-FLOTAC faecal egg counting techniques. Vet. Parasitol. 207:342-345.

Jackson PGG, Cockcroft PD (2002). Clinical examination of farm animals Blackwell Science Ltd, Oxford.

Lanfranchi P, Ferroglio E, Poglayen G, Guberti V (2003). Wildlife Veterinarian, Conservation and Public Health. Vet. Res. Comm. 27:567-574.

Messieri A, Moretti B (1982). Semiologia e Diagnostica medica Veterinaria Libreria Universitaria L. Tinarelli Bologna. (In Italian).

Pfeifer A (2015). Differences in Body Condition of Elk, *Cervus elaphus*, by Location in Yellowstone's Northern Range' Poster produced by University of Washington School of Environmental & Forest Sciences.

Ramesh T, Sankar K, Quereshi Q, Kalle R (2011). Assessment of Wild Asiatic Elephant (*Elephas maximus indicus*) Body Condition by Simple Scoring Method in a Tropical Deciduous Forest of Western Ghats, Southern India. Wildlife Biol. Pract. 7:47-54.

Silva LMR, Vila-Vicosa MJM, Maurelli MP, Morgoglione ME, Cortes HCE, Cringoli G, Rinaldi L (2013). Mini-FLOTAC for the diagnosis of *Eimeria* infection in goats: An alternative to McMaster. Small Rumin. Res. 114:280-283.

Terranova CJ, Coffma BS, (1997) Body weight of wild and captive lemurs. Zoo Biol.16:17-30.

Van Wyk IC, Boomker J (2011) Parasites of South African wildlife. XIX. The prevalence of helminths in some common antelopes, warthogs and a bushpig in the Limpopo province, South Africa. Onderstepoort J. Vet. Res. 78(1):1-11.

Wijeyamohan S, Treiber K, Schmitt D, Santiapillai C (2015) A Visual System for Scoring Body Condition of Asian Elephants (*Elephas maximus*). Zoo Biol. 34:53-59.

Wilson K, Bjornstad ON, Dobson AP, Merler S, Poglayen G, Randolph SE, Read AF, Skorping A (2002). Heterogeneities in macroparasite infectious: patterns and processes. in Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP (eds), "The Ecology of Wildlife Disease", Oxford University Press.

Journal of Veterinary Medicine and Animal Health

Related Journals Published by Academic Journals

- *Journal of Parasitology and Vector Biology*
- *Journal of Cell Biology and Genetics*
- *Journal of Infectious Diseases and Immunity*
- *Journal of Public Health and Epidemiology*
- *Medical Case Studies*
- *Journal of Medical Laboratory and Diagnosis*
- *Journal of Clinical Virology Research*

academicJournals