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Volume 7 (1); March 25, 2017

Research Paper

Prevalence and Associated Risk Factors of Bovine Schistosomiasis in Northwestern Ethiopia.

Kassahun Gebremeskel A, Tayelgn Simeneh S and Addisu Mekuria Sh.

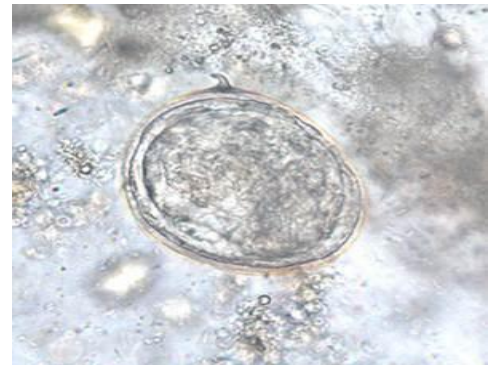
World Vet. J. 7(1): 01-04, 2017; pii:S232245681700001-7

ABSTRACT

Schistosomiasis is a parasitic disease caused by microorganisms from the genus *Schistosoma*. It has a huge negative impact on both economy and health worldwide. In this paper a cross-sectional study was conducted to evaluate the prevalence and associated risk factors of bovine schistosomiasis in north western Ethiopia with the objective of providing detailed information on bovine schistosomiasis prevalence in relation to animal and ecological related risk factors. The sampled animals were categorized under four associated risk factors which include: origin, sex, body condition score and age. Fecal samples were randomly collected from a total of 289 animals and *Schistosoma*'s eggs were identified by sedimentation technique. 69 (23.9%) fecal samples were revealed positive for *Schistosoma*. The highest (29.8%) prevalence rate was recorded at Keltafa district followed by Lalibella (25.9 %), Korench (19.1%) and kurbiha (14.0%). Animals categorized under medium body condition score has a relative high prevalence (25.7%) followed by poor (24.3%) and good body condition (21.7%) animals. In conclusion, the prevalence recorded among different selected study districts, sex, body condition score and age groups shows some degree of variability and insignificant ($p>0.05$), which resulted from the difference in abundance of marshy areas and rivers, animal's immunity and types of management system. Despite these variability factors, the disease has a great socio-economic impact that needs intervention.

Key words: Bovine, Prevalence, Schistosomiasis, Ethiopia

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Research Paper

Resistant Gene of Pseudomonas Aeruginosa in Mastitic Cattle; Biochemical and Immunological Parameters.

Awad Ibrahim N, El-Metwally Farag VM, Abd-El-Moaty AM and Magdy Atwa S.

World Vet. J. 7(1): 05-13, 2017; pii:S232245681700002-7

ABSTRACT

Mastitis is an important infectious disease of cattle and *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria stand out amongst those fundamental causative agents. The present study was intended to assess *P. aeruginosa* activity isolated from mastitic cattle milk samples in a veterinary hospital. It also was assessed some free radicals and immunological parameters in the milk and serum samples. Control samples were taken from apparently healthy cows, negative California Mastitis test. The results cleared that positive *P. aeruginosa* isolates were 34 of 100 milk samples. In vitro antibiotic sensitivity test indicated that 79.4%, 70.5% and 58.82% of the isolates completely resisted cefotaxime, penicillin and amikacin respectively. Also, the resistance to meropenem was 11.76% and 8.8% for carbapenem resistant isolate which completely resisted other classes of β -lactams. While enrofloxacin and gentamicin sensitivity reached to 76.47% and 73.5% respectively. The technique of the P.C.R was done for detection of MexR gene (in isolates resisted to more than three antibiotics) and Vim gene (in carbapenem resistant isolates). The biochemical results investigated that the nitric oxide and Mmalondialdehyde antioxidant levels were increased significantly while the cholesterol was decreased significantly in both serum and milk samples. Meanwhile, catalase and lysozyme were changed between groups and total protein and globulin had increased significantly in milk samples only. In conclusion, *P. aeruginosa* isolates including MexR and blaVim genes showed considerable percent of resistance to carbapenem group and other classes of β -lactam. In addition, the estimated biochemical and immunological parameters were affected in that case of mastitis in cattle. The results may encourage studies which are concerned with antioxidants treatment for mastitis in cattle. It may be a key for decreasing body resistance to antibiotics.

Key words: Mastitis, Cattle, *Pseudomonas aeruginosa*, PCR, Lysozyme

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Antimicrobial Resistant of *Pseudomonas aeruginosa* and biochemical study

Research Paper

Isolation and Characterization of Bacterial Species from Respiratory Tracts of Cattle Slaughtered in Addis Ababa City, Central Ethiopia.

Kassahun Gebremeskel A, Sisay Tesema T, Awukew Yegoraw A, Tesfaye Birhanu B and Addisu Mekuria Sh.

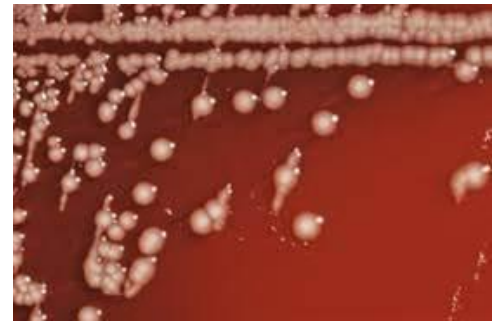
World Vet. J. 7(1): 14-20, 2017; pii:S232245681700003-7

ABSTRACT

The present study was an attempt to isolate and identify the diverse bacteria localizing pneumonic lungs and the associated tracheas of 50 slaughtered cattle at Addis Ababa Abattoirs enterprise, central Ethiopia, in both aerobic and anaerobic environments. 158 and 135 bacterial isolate was found in aerobic and anaerobic state, respectively using primary and secondary microbiological testes. Gram positive bacteria were the dominant bacteria in both conditions. The frequency of isolation increased from trachea down to the lung in both state indicating the bacterial role in the progress of bovine pneumonia. Most prevalently isolated bacteria from both aerobics and anaerobics conditions were *Staphylococcus* species, *Bacillus* species, *Mannheimia haemolytica* and *Pasteurella multocida*. Whereas the *Streptococcus* species, *E.coli*, *Klebsiella pneumoniae*, *Actinobacillus* species, *Micrococcus* species, *Arcanobacterium*, species *Neisseriaspecies*, *Acinetobacter* species, *Corynebacterium* species, *Bordetella* species, *Pseudomonas* species, and *Rhodococcus equi* were among the bacteria isolated.

Key words: Aerobic, Anaerobic, Bacteria, Bovine, Pneumonia

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Research Paper

Bovine Tuberculosis Prevalence, Potential Risk Factors and Its Public Health Implication in Selected State Dairy Farms, Central Ethiopia.

Ambaw Endalew M, Deresa B and Ameni Chimdi G.

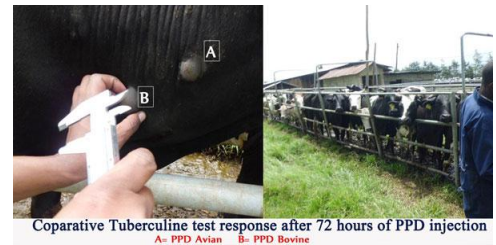
World Vet. J. 7(1): 21-29, 2017; pii:S232245681700004-7

ABSTRACT

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis* (*M.bovis*), is endemic in Ethiopia. However, its magnitude in cattle and human population are not well documented. A cross-sectional study was conducted on 720 apparently healthy dairy cattle kept in three different state owned farms in central Ethiopia to quantify the risk factors and determine the prevalence of BTB using (CIDT) Comparative Intra-Dermal Tuberculin Test from December 2013 to November 2014. Questionnaire survey was used to assess the risk factors and zoonotic implication of BTB. The prevalence of BTB was at 16.53% (95% CI 14.2-18.9) and It was significantly higher in crossbreed ($X^2= 54.76$; $P < 0.001$; OR=16.1; 95% CI=6.2-41.1) and animals older than 4 years ($X^2=34.51$; $P < 0.001$, OR =6.22; 95% CI=3.5-11.12). Moreover, the prevalence was also significantly higher in good body conditioned dairy cattle compared to poor body conditioned dairy cattle ($X^2=29.69$; $P < 0.001$; OR=2.45; 95% CI=1.1-5.7). The prevalence of BTB was also significantly varied among the reproductive status of the dairy cattle ($X^2 =18.10$; $P < 0.001$).The majority of the respondents consume raw milk (66.1% and raw meat (74.20%) respectively. There was statistically significant variation ($X^2 =12.51$; $P < 0.03$) in consumption habit between educated and non-educated dairy farm workers. The major risk factors for bovine tuberculosis in this study were breed and age of the dairy cattle. Consumption of raw milk and meat is still a common practice in the study farms. Culling of aged dairy cattle and continuous test and slaughter of infected cattle should be practiced at least in state owned dairy farms to decrease the risk of transmission. In addition to awareness creation of the public particularly the dairy farm workers on the zoonotic nature of tuberculosis is of utmost importance to control bovine tuberculosis.

Key words: Bovine tuberculosis prevalence, Dairy cattle, Farm worker, Risk factor

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Comparative Tuberculine test response after 72 hours of PPD injection
A- PPD Avian B- PPD Bovine

Research Paper

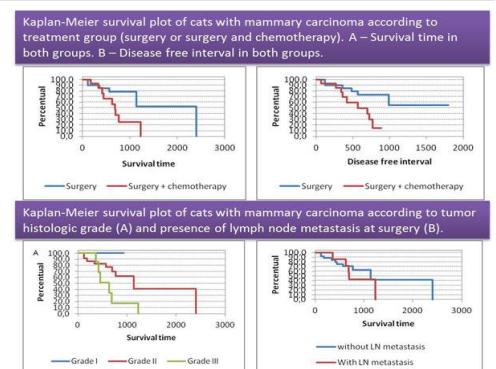
Retrospective Study on Survival Time of Cats with Mammary Carcinomas Undergoing Surgery Alone or with Adjuvant Chemotherapy.

Cunha SCS, Corgozinho KB, Souza HJM, Silva K, Leite J, Mello M and Ferreira AMR.

World Vet. J. 7(1): 30-35, 2017; pii:S232245681700005-7

ABSTRACT

This retrospective study was carried out to evaluate disease free interval (DFI) and survival time of cats with mammary carcinomas that underwent mastectomy (RM) and adjuvant chemotherapy (RMAC) in 35 cats to remove the neoplastic mammary chain and regional inguinal lymphadenectomy. According to performed treatment, the cats were divided into two groups. The RM group (21 cats) received no adjuvant therapy, and the RMAC group (14 cats) received chemotherapy with mitoxantrone or doxorubicin. Histopathological margins were considered complete in all cases. Eight cats had histologically confirmed lymph node involvement at the time of surgery. Three cases were classified as stage I, 21 cases as II and eight cases as III. Nine cats had tumor recurrence (four cats of RM group and five cats of RMAC group)



and 12 cats had distant metastasis to the lungs (six cats of each group). Mean and median survival times were 1625 and 2404 days in the RM group, while mean DFI was 815 days. In RMAC group, mean and median survival times were 719 and 690 days, while mean DFI was 549 days. Surgery remains the main treatment and more studies are necessary to evaluate the benefit of adjuvant chemotherapy.

Key words: Feline, Mammary carcinoma, Oncology, Surgery, Chemotherapy
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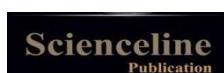
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Prevalence and Associated Risk Factors of Bovine Schistosomiasis in Northwestern Ethiopia

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ABSTRACT

Schistosomiasis is a parasitic disease caused by microorganisms from the genus *Schistosoma*. It has a huge negative impact on both economy and health worldwide. In this paper a cross-sectional study was conducted to evaluate the prevalence and associated risk factors of bovine schistosomiasis in north western Ethiopia with the objective of providing detailed information on bovine schistosomiasis prevalence in relation to animal and ecological related risk factors. The sampled animals were categorized under four associated risk factors which include: origin, sex, body condition score and age. Fecal samples were randomly collected from a total of 289 animals and *Schistosoma*'s eggs were identified by sedimentation technique. 69 (23.9%) fecal samples were revealed positive for *Schistosoma*. The highest (29.8%) prevalence rate was recorded at Keltafa district followed by Lalibella (25.9%), Korench (19.1%) and kurbiha (14.0%). Animals categorized under medium body condition score has a relative high prevalence (25.7%) followed by poor (24.3%) and good body condition (21.7%) animals. In conclusion, the prevalence recorded among different selected study districts, sex, body condition score and age groups shows some degree of variability and insignificant ($p>0.05$), which resulted from the difference in abundance of marshy areas and rivers, animal's immunity and types of management system. Despite these variability factors, the disease has a great socio-economic impact that needs intervention.

Key words: Bovine, Prevalence, Schistosomiasis, Ethiopia

INTRODUCTION

Parasitism represents a major challenge to development. Trematode parasitism is one of the major problems constraining of both animal and human productivity around the world (Jejaw et al., 2015). These parasites are found in vast water lodged and marshy grazing field, a condition anticipated for being ideal for the propagation and maintenance of the intermediate host (snails) and hence high prevalence of trematode infection occurred (Fromsa et al., 2011). Schistosomiasis (also called Bilharzias after the German tropical disease specialist (Lo and Lemma, 1973) is a parasitic disease caused by microorganism from the genus *Schistosoma*. The major species that cause animal schistosomiasis include: *Schistosomabovis*, *S. indicum*, *S. japonicum*, *S. matthei*, *S. intercalatum*, *S. nasale* and *S. rodhoni* (Jejaw et al., 2015). *S. bovis*, *S. matthei* and *S. intercalatum* are the most important species that can cause schistosomiasis in ruminants. The adult worms inhabit the mesenteric vessels of the definitive host and the intermediate forms develop into snails from the genus *biomphalaria*, *bulinus* and *monocephala* (Kassai, 1999).

Schistosomiasis is one of the 15 neglected diseases in tropics and it is the second parasitic disease next to malaria. It is endemic not only in sub-Saharan Africa, but also in the Middle East, Far East, South, Central America and the Caribbean (Taylor et al., 2007; Georg et al., 2003).

S. bovis has a localized distribution, which is commonly found in northern, eastern, south eastern and central part of Ethiopia. There are some reports which indicate the prevalence of schistosomiasis in different area of Ethiopia (Belayneh and Tadesse, 2014). However there are few documentation examples in north western Ethiopia. Therefore this study was conducted to fill this information gap with the objectives of estimating the prevalence and assessing the impact of host and ecological related risk factors for the occurrence of bovine schistosomiasis in north western part of Ethiopia.

MATERIAL AND METHODS

Study area

The study was conducted in four districts such as Ahuri-keltafa, Korench, Kurbiha and Lalibella which are 60km

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away from the city of Bahir Dar from November 2015 to April 2016. The areas cover a total of 217995 hectares of land. Geographically located at 11°37'N latitude and 37°25'E longitude with an altitude of 1500-2300 meter above sea level (m.a.s.), 1200-1600 mm rain fall and average temperature of 29.5°C recorded annually. Animal population in the areas include 204747 cattle, 763000 sheep, 35970 goats, 9980 horses, 5218 mules and 16570 donkey. About 70% of the land is featured by plain plateaus and covered by various bush formation low woods and some semi-humid and humid highland vegetation. The landscape is marked by the presence of Nile River, has a poor drainage system and annual over flooding during the rainy seasons which leave pockets of water bodies for long period of time during the dry season (CSA, 2008).

Study animals

The sampling units of the study were cattle of different body condition score, age, sex and origin that were found in the four districts. The age of each animal was estimated using the owner's response and dentition pattern as young or adult. The cattle had different body condition scores like, good, medium and poor depends on visualization and both sexes were involved in the study.

Study design, sampling and data collection

A cross-sectional study was conducted to estimate the prevalence of bovine schistosomiasis and its associated risk factors. This study was performed by coprological examination of samples, which were collected from systematically random selected animals. Samples of fresh faeces were collected directly from the rectum of the cattle. The collected samples were preserved by 10% formalin in a universal bottle with proper labeling of necessary information and then transported to the laboratory for further examination and analysis of *Schistosoma*'s eggs using sedimentation technique.

Data management and analysis

Data was stored in a Microsoft Excel spreadsheet and analyzed using SPSS version 16.0 statistical software. While descriptive statistics such as percentages were used to calculate prevalence rate, Chi-square test was used to evaluate the association between different parameters and P-value less than 0.05 considered as significant.

Ethical approval

This study is conducted according to the research ethics approved by the University of Gondar, Ethiopia and no animal was subjected to suffer.

RESULTS

From the total of 289 bovine species examined, 69 (23.9%) were infected by *Schistosoma*. The highest prevalence (29.8%) was observed in animals originated from Ahuri-keltafa district representing mid highland. As indicated by table 1 the prevalence of schistosomiasis at Ahuri-keltafa (29.8%) district was followed by lalibella (25.9%) and korench (19.1%) districts. As denoted by table 1 the prevalence based on sex was 24.3% in female whereas 23.5% was recorded in male bovines. Based on age as risk factor, the prevalence in adults were relatively higher (25.6%) than that of young bovines (21.6%). Moreover, highest prevalence was (25.7%) recorded in medium body condition score followed by poor (24.3%) and good (21.7%) body condition score animals. Despite all risk factors have some degree of visible effect on the prevalence of schistosomiasis, the difference between all risk factors was not statistically significant ($p > 0.05$).

Table 1. Prevalence and associated risk factors of bovine schistosomiasis around in north western Ethiopia from November 2015 to April 2016

| Risk factors | Category | No. examined | Positive | Prevalence (%) | P- value |
|----------------|-----------|--------------|----------|----------------|----------|
| Sex | Male | 153 | 36 | 23.5 | 0.88 |
| | Female | 136 | 33 | 24.3 | |
| Age | Adult | 164 | 42 | 25.6 | 0.43 |
| | Young | 125 | 27 | 21.6 | |
| Body Condition | Good | 106 | 23 | 21.7 | 0.79 |
| | Medium | 113 | 29 | 25.7 | |
| | Poor | 70 | 17 | 24.3 | |
| Origin | Keltafa | 84 | 25 | 29.8 | 0.16 |
| | Korench | 47 | 9 | 19.1 | |
| | Kurbiha | 50 | 7 | 14.0 | |
| | Lalibella | 108 | 28 | 25.9 | |

DISCUSSION

The overall prevalence of *Schistosoma bovis* found in this study area was at 23.9%. This prevalence rate is relatively lower as compared to previous studies, including 29% by Hailu (1999) in Bahir Dar area, north and central Ethiopia, 37.3% by Almaz and Solomon (2011) in Bahir Dar area, 27.13% and by Alemseged (2010) in Dembia district. But relatively higher than 17.4% by Yalelet (2004) in and around Bahir Dar, North western Ethiopia, 10.17% by Mersha et al. (2012), at South Gondar zone, Amhara national regional state, Ethiopia, Gewanie (1.5%) and Hawassa (5.5%) by Lo and Lemma (1973). Furthermore, as Abebe et al. (2011) reported the prevalence of schistosomiasis was 4.59% in Agaro and 10.11% Jimma area. The difference might be due to reasons such as irrigation practice, agro-ecology, animal management type, climatic variation and other geographical factors.

The present finding also disclosed higher prevalence (29.8%) in Keltafa district as compared to the other districts. The variation in the prevalence of the disease might be the presence of Nile, Kiltiand Biranti rivers which are the major water source to animals originated from Keltafa area and the presence of Gedelit, Gumara, Walkaye, Wulsi and Ahuri marshy range land from which animals graze in common. In support of this theory, Urquhart et al. (1996) has explained the importance of water bodies and marshy areas for the occurrence of schistosomiasis. Prevalence in Lalibella district was the second highly prevalent (25.9%). This result might be due to the presence of highly swampy communal range land called Flantimesk, Adissambamesk, Azenamesk and the presence of Zabziriver which passes across the Flantimesk communal range land. The remaining two districts such as Korench (19.1%) and Kurbiha (14.0%) have seasonal marshy areas and small rivers which would serve as the reservoir of the intermediate hosts (snail) and important for endemic nature of schistosomiasis.

In the present study the prevalence variation of schistosomiasis between the two sexes were at 23.5% in female and 24.3% in male. This data is in line with Tadesse et al. (2009) who reported the prevalence of bovine schistosomiasis 22.4% in male and 25.9% in female around Bahirdar city. This might be due to the fact that absence of difference in grazing behavior, animal management and grazing land for the two sexes.

Statistical analysis of this study indicated that body condition score had no influence on the prevalence of bovine schistosomiasis. The prevalence was relatively highest in medium body condition score animals (25.7%), comparing to poor (24.3%) and good (21.7%) condition score bovines. This result contradicted the result of Hailu (1999) and Belayneh and Tadesse (2014) who reported the prevalence of bovine schistosomiasis 22.4% in male and 25.9% in female at Bahirdar city. This Result contradict again with Abebe et al. (2011) who reports as the prevalence of schistosomiasis was 23.81% in poor body condition score animals, 3.92% in medium body condition score animals and 0.00% in good body condition score animals at Jimma and Agaro areas. The deviation might be due to variation in the accessibility of animals to the marshy area, animal management system and difference in the study districts.

The age of the study animals was classified as young (≤ 3 years) and adult (> 3 years) by dentition. The prevalence of schistosomiasis according to age as a risk factor were 21.6% and 25.6% in young and adult animals, respectively. This result agreed with the result of Belayneh and Tadesse (2014) and Abebe et al. (2011) who obtained the prevalence was 0.00% in young and 8.28% in adult animals at Jimma town municipal abattoir. However, this result disagrees with Taylor (2007) who reported that the prevalence of the disease is dependent on age.

CONCLUSION

The present study shows that different risk factors affect the prevalence of schistosomiasis. Although the different variability resulted from degree of a bundancy in marshy areas and rivers which act as reservoir for snail intermediate hosts, animal's immunity status, animal management system and other geographical and environmental factors, the disease has great socio-economic impact that needs an intervention.

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Competing interest

The authors have not declared any conflict of interest

Author's contributions

Addis Kassahun Gebremeskel designed and conducted analysis; Solomon Tayelgn Simeneh conducted the experiments, Shewangzaw Aaddisu Mekuria conducted edition.

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Resistant Gene of *Pseudomonas Aeruginosa* in Mastitic Cattle with Reference to some Biochemical and Immunological Parameters

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ABSTRACT

Mastitis is an important infectious disease of cattle and *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria stand out amongst those fundamental causative agents. The present study was intended to assess *P. aeruginosa* activity isolated from mastitic cattle milk samples in a veterinary hospital. It also was assessed some free radicals and immunological parameters in the milk and serum samples. Control samples were taken from apparently healthy cows, negative California Mastitis test. The results cleared that positive *P. aeruginosa* isolates were 34 of 100 milk samples. In vitro antibiotic sensitivity test indicated that 79.4%, 70.5% and 58.82% of the isolates completely resisted cefotaxime, penicillin and amikacin respectively. Also, the resistance to meropenem was 11.76% and 8.8% for carbapenem resistant isolate which completely resisted other classes of β -lactams. While enrofloxacin and gentamicin sensitivity reached to 76.47% and 73.5% respectively. The technique of the P.C.R was done for detection of MexR gene (in isolates resisted to more than three antibiotics) and Vim gene (in carbapenem resistant isolates). The biochemical results investigated that the nitric oxide and Mmalondialdehyde antioxidant levels were increased significantly while the cholesterol was decreased significantly in both serum and milk samples. Meanwhile, catalase and lysozyme were changed between groups and total protein and globulin had increased significantly in milk samples only. In conclusion, *P. aeruginosa* isolates including MexR and blaVim genes showed considerable percent of resistance to carbapenem group and other classes of β -lactam. In addition, the estimated biochemical and immunological parameters were affected in that case of mastitis in cattle. The results may encourage studies which are concerned with antioxidants treatment for mastitis in cattle. It may be a key for decreasing body resistance to antibiotics.

Key words: Mastitis, Cattle, *Pseudomonas aeruginosa*, PCR, Lysozyme

INTRODUCTION

Mastitis is one of the most predominant and expensive diseases in productive dairy cows. Almost half of all clinical cases are caused by Gram-negative bacteria, among these bacteria *P. aeruginosa* (Radostits et al., 2006; Pragasam et al., 2016). It was widely distributed in nature with an extraordinary physiological and metabolic adaptability. *Pseudomonas aeruginosa* was able to persist in both community and hospital settings for long time (Florentin et al., 2016). Moreover, its resistance for many antibiotics of clinical use, such as aminoglycosides, β -lactams and fluoroquinolones was a real problem. This reflected the great hazard to general health of public (Lister et al., 2009; Toval et al., 2015). Carbapenem was one of β -lactam class; the last therapeutic resource for control of bacterial infections and its use had been associated with emergence of carbapenem resistant bacteria (Pollini et al., 2013; Uruvoma, 2015).

The mexR gene was an important regulatory gene for multidrug resistant specially the β lactam group and was aimed to identify the carbapenem resistance by detecting β -lactams VIM gene (Suman et al., 2006). First class B of metallo- β -lactamases (MBLs) enzymes (produced by *P. aeruginosa*) were recorded in 1991 in Japan (Watanabe et al., 1991). There were main six families. However, the most frequent MBLs were Plasmid-mediated IMP-type carbapenemases (IMP) and Verona integron-encoded metallo- β -lactamase (VIM) (Toval et al., 2015; Zhao and Hu, 2015).

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The normal aerobic metabolism byproducts were involved mainly with the Reactive Oxygen Species (ROS) which occurred mainly in the mitochondria. However, under stress, they increased much more body's defenses ability and oxidative stresses take place leading to cell injury (Navasa et al., 2014). In a like manner was the reactive nitrogen species (derived from nitric oxide). In sum, they impaired some biological molecules, gave rise to several diseases and changed the milk composition (Markiewicz-Keszycka et al., 2013). Then, the health of highly producing dairy cow would worsened (Sordillo and Aitken, 2009). As well as, the malondialdehyde (MDA) was the main lipid-peroxidation stress marker. It supported the ROS production subsequently damaging the mammary tissues and reducing the immune state (Rahal et al., 2014). Antioxidants are substances that successfully trapping the oxidative intermediates preventing biomolecules oxidation or reducing the oxidized ones. It well stand against the oxidative damage (Sordillo et al., 2009). In bovine milk, catalase is the index of mastitis and the lysozyme is a bactericidal agent (Fox et al., 2015).

Studying bacterium, immunity and antibiotic resistance were very important for treatment and eradication of mastitis due to *P. aeruginosa* microorganism. The disease puts a great financial stress on the dairy industry (Ventola, 2015). Hence, many researchers focused on proteins biomarkers in the biological fluids as economic tests help to diagnose and treat livestock diseases (Oskoueian et al., 2016). This study was directed to record the percent of *P. aeruginosa* including resistant gene in mastitic milk of the studied area and determined some biochemical and immunological parameters in serum and milk to evaluate the cellular damage.

MATERIAL AND METHODS

Ethical approval

Animal handling was followed by animal ethics committee guidelines, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Animals and sampling

One hundred Holstein cows showing clinical signs of mastitis in different seasons at the educational veterinary hospital, Mansoura university, Dakahlia Governorate, Egypt were subjected to blood and milk sampling (in 2014 and 2015). Ten apparently healthy cattle, with negative California Mastitis test served as control. The age of the cattle ranged from 3 to 6 years. The samples were collected with hygienic precautions and were directly transported to the laboratories. The collected blood samples (in plain centrifuge tube) were placed in a slanted position (20 min at room temperature) for clotting. Milk and blood samples were centrifuged at 5000 revolutions per min (rpm) for 30 min and at 3000 rpm for ten min respectively. Serum and aqueous supernatant of milk were preserved at - 20 °C till used. The milk precipitate was used for bacteriological examination. Per the results of isolation and identification, ten milk and corresponding serum samples were randomly selected from *P. aeruginosa* mastitic samples (*P. aeruginosa* group) and were used for estimation of different biochemical and immunological parameters.

Bacteriological examination and identification of the isolates

An inoculum from each milk sample was inoculated into broth before plating on nutrient and MacConkey agar, then Pseudomonas agar base with cetrinide_nalidixic acid (C_N) supplement and blood agar. Then aerobically incubated (at 37 °C) the plates and examined after 24-48 h. Suspected colonies were described for their characteristic green pigmented colonies on nutrient and pseudomonas agar base with C_N supplement and for hemolytic activity on blood agar (Konemann et al., 1992). Gram stain and the test of oxidase were done (Macfaddin, 2000). Finally the suspected isolates were sent to the animal health research institute (Dokki, Cairo, Egypt) for further identification by VITEK2 compact system (based on biochemical identification via colorimetric technology), card 2GN (BioMérieux-France).

In vitro antimicrobial assay

Antimicrobial sensitivity test was assessed via disc diffusion method by means of Mueller-Hinton agar (Finegold and Martin, 1982). Antimicrobial discs (Oxoid) were penicillin, cefoperazone, cefotaxime, gentamicin, amikacin, enrofloxacin, meropenem and imipenem. After incubation (18-24 h), the visible clear zone of inhibition was determined (mm) following the interpretation chart guidelines (CLSI, 2014).

Extraction of the DNA

The DNA extraction has done utilizing Mini kit QIAamp DNA (Qiagen, Germany, GmbH) for manufacturer's proposals. In details, proteinase K (10 µL) and lysis buffer (200 µL) was added to the sample suspension (200 µL).

Then, it was incubated at 56 °C (10 min) at that time added the ethanol 100% (200 µL). The sample has been washed and centrifuged. Then, the elution buffer (100 µL) was eluted with the nucleic acid.

Amplification of the PCR

The used oligonucleotide primers (Metabion, Germany) were utilized in a reaction (25 µL) contained 12.5 µL of the EmeraldAmp Max PCR Master Mix (Takara, Japan), water (4.5 µL), DNA template (6 µL) and 1 µL of each primer (20 pmol concentration). This was done in an applied biosystem 2720 thermal cycler (Table 1).

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions in *P. aeruginosa* isolated from milk of Holstein mastitic cattle (3–6 years of age) in 2014-2015

| Target gene | Primers sequences | Amplified segment (bp) | Prim. denat. | Amplification (35 cycles) | | | Final exten. | Reference |
|--------------------------------|--------------------------|------------------------|--------------|---------------------------|-----------|---------|--------------|------------------------|
| | | | | Sec. denat. | Annealing | Exten. | | |
| <i>P. aeruginosa</i> blaVIM | TTTGGTCGCA TATCGCAACG | 500 | 94°C | 94°C | 50°C | 72°C | 72°C | (Amudhan et al., 2012) |
| | CCATTCAGCC AGATCGGCAT | | 10 min | 45 sec. | 45 sec. | 45 sec. | 10 min. | |
| <i>P. aeruginosa</i> mexR | GCGCCATGGC CCATATTCAG | 637 | 94°C | 94°C | 57°C | 72°C | 72°C | (Sanchez et al., 2002) |
| | GGCATTTCGCC AGTAAGCGG | | 5 min. | 45 sec. | 45 sec. | 45 sec. | 10 min. | |

Prim: primary, sec: secondary, denat: denaturation, exten: extension.

The PCR Products analysis

Electrophoresis was done for the PCR products separation using 1x TBE buffer had 1.5% of agarose gel (Applichem, Germany, GmbH) at room temperature by means of 5V/cm gradients. Then, analysis was done by loading the product (20 µL) into each gel slot. A gel pilot 100 bp and 100 bp, in addition to Ladder of the DNA (Qiagen, Germany, GmbH) were used for determination of the sizes of the fragment. Gel documentation system (Alpha Innotech, Biometra) was encountered for photography. The data were analyzed via computer program software.

Immunological and biochemical analysis

Serum Total Protein (TP) and serum albumin were estimated by means of readymade kits (Stanbio laboratory, USA). Globulin (TP minus albumin) and albumin globulin ratio (A/G) were calculated according to Kaneko et al., (1997). Cholesterol (Allain et al., 1974), triglycerides, (Fossati and Prencipe, 1982), catalase, Nitric Oxide (NO) and MDA were estimated using readymade diagnostic kits (Bio-diagnostic, Egypt). All parameters were calorimetrically estimated by means of semiautomatic spectrophotometer (BM-Germany 5010) according to the suppliers' kits protocols.

Catalase determination was based on the reaction of the catalase with specific quantity of H₂O₂. Stopping the reaction was done after one min by means of the catalase inhibitor (Aebi, 1984). The MDA determination based on malondialdehyde (one molecule) reaction with thiobarbituric acid (two molecules) in acidic environment (Satoh, 1978), while NO determination was done according to nitrite method. In presence of nitrite, subsequent substance coupled with N-(1-naphthyl) ethylenediamine in acidic media and the azo dye could be measured (Ignarro et al., 1987).

The turbidometric assay was done for determination of the lysozyme activity (Parry et al., 1965). In details, the lysozyme substrate was prepared using 0.75 mg/mL of micrococcus lysodeikticus (gram positive bacteria) lyophilized cells (Sigma, St. Louis, MO). Then the preparation was suspended in 0.1 M of the buffer of sodium phosphate/citric acid (pH 5.8). The substrate solution (175 mL) was placed into each microtiter plate well at 25 °C. The serum or supernatant of milk sample (25 µL) was added in duplicate. After 0 and 20 min, absorbance (at 450 nm wave length) was recorded via ELISA reader microplate (Bio TEC, ELX800G, USA). The lysozyme concentrations were recorded (µg/ml) corresponding to the standard curve obtained with lyophilized hen-egg-white-lysozyme (Sigma).

Statistical analysis

Serum and milk immunological and biochemical parameters were analyzed statistically using program, SPSS 17.0 for windows, Student's t test was used for determined the statistical significance. The difference between groups was considered significant at P < 0.05. The data were expressed as mean ± SE.

RESULTS

Prevalence and antibiotic sensitivity test of *P. aeruginosa* organism in milk samples

Out of all of the examined samples 34 (34%) were positive for *P. aeruginosa*. Isolates showed multidrug resistant to penicillin, cefotaxime and cefoperazone were 12 (35.29%). Further, the resistance for carbapenem group (meropenem and imipenem) was four (11.76%) isolates.

Antibiotic sensitivity test results were shown in table 2. There were 58.82% and 11.76% *P. aeruginosa* of the isolates were completely resist to amikacin and gentamycin respectively. Moreover 70.59%, 79.41% and 35.29% were completely resist to penicillin, cefotaxime and cefoperazone respectively. Otherwise, although about 11.76% were resistant to meropenem and 2.94% for imipenem.

Detection of mexR gene and MBL (blaVIM) gene

The results revealed positive amplification of the 637 bp of mexR from the extracted DNA of four (33.33%) isolates from 12 *P. aeruginosa* isolates which showing multidrug resistance. While three (75%) from four *p. aeruginosa* isolates (showed resistance to carbapenem group) were positive for blaVIM gene (Figure 1).

Immunological and biochemical analysis

Table 3 demonstrated the determined immunological and biochemical parameters results. Catalase, lysozyme, cholesterol, and triglycerides levels significantly (p value; 0.000, 0.001, 0.009 and 0.014 respectively) decreased in the serum of *P. Aeruginosa* group comparing with control group. Contrast to milk levels of catalase, lysozyme, TP and globulin increased significantly (p value; 0.000, 0.001, 0.197 and 0.237 respectively) however decreasing of milk A/G ratio. The oxidative stress (NO and MDA) activity increased significantly (p value; 0.006 for serum NO and 0.000 for others) in each serum and milk groups than their control ones.

Table 2. Results of antibiotic sensitivity test of milk samples (N = 34) of Holstein mastitic cattle (3–6 years of age) infected with *P. aeruginosa* in 2014-2015

| Antibiotic | Sensitive | | Intermediate | | Resistant | |
|--------------|-----------|------|--------------|-------|-----------|-------|
| | No | % | No | % | No | % |
| Meropenem | 24 | 70.6 | 6 | 17.65 | 4 | 11.76 |
| Imipenem | 31 | 91.2 | 2 | 5.88 | 1 | 2.94 |
| Penicillin | 2 | 5.88 | 8 | 23.53 | 24 | 70.59 |
| Cefoperazone | 3 | 8.82 | 19 | 55.88 | 12 | 35.29 |
| Cefotaxime | 0 | 0 | 7 | 20.59 | 27 | 79.41 |
| Amikacin | 6 | 17.7 | 8 | 23.53 | 20 | 58.82 |
| Gentamycin | 25 | 73.5 | 5 | 14.71 | 4 | 11.76 |
| Enrofloxacin | 26 | 76.5 | 8 | 23.51 | 0 | 0 |

Figure1. Detection of mexR gene and MBL (blaVIM) gene in *P. aeruginosa* isolated from milk of Holstein mastitic cattle (3-6 years of age) in 2014-2015.

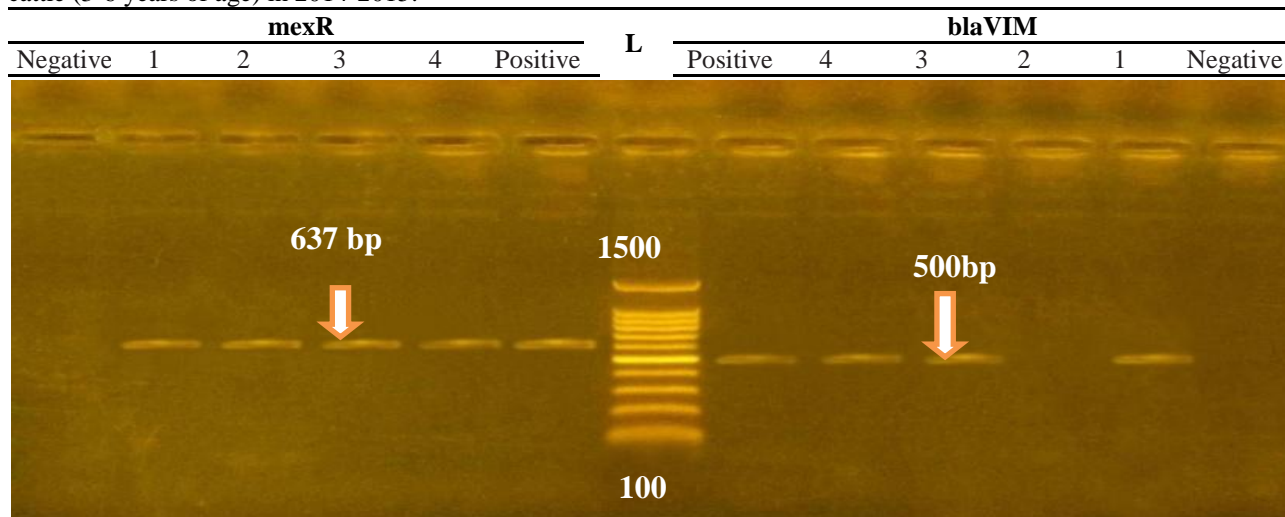


Table 3. Serum and milk biochemical and immunological parameters (Mean \pm S.E) of Holstein mastitic cattle (3-6 years of age) infected with *P. Aeruginosa* in 2014-2015

| Groups (N = 10) | NO μ mol/L | Ctalase nmol/ml | MDA nmol/ml | Lyso. μ g/ml | Chol. mg/dl | Trigly. g/dl | T.P. g/dl | Alb. g/dl | Glob. g/dl | A/G ratio |
|----------------------|------------------|-------------------|-------------------|---------------------|--------------------|-------------------|------------------|-----------------|------------------|------------------|
| Serum | | | | | | | | | | |
| Control | 1.21 \pm 0.23* | 11.75 \pm 0.28* | 4.41 \pm 0.73* | 37.69 \pm 2.00* | 117.2 \pm 17.75* | 43.00 \pm 4.16* | 5.88 \pm 0.21 | 1.53 \pm 0.10 | 4.35 \pm 0.24 | 0.37 \pm 0.04 |
| <i>P. Aeruginosa</i> | 6.65 \pm 1.71* | 7.64 \pm 0.65* | 10.32 \pm 1.33* | 15.65 \pm 4.84* | 54.83 \pm 12.06* | 27.96 \pm 3.63* | 6.55 \pm 0.45 | 1.59 \pm 0.09 | 4.97 \pm 0.44 | 0.34 \pm 0.03 |
| P Value | 0.006 | 0 | 0.001 | 0.001 | 0.009 | 0.014 | 0.197 | 0.685 | 0.237 | 0.569 |
| Milk | | | | | | | | | | |
| Control | 1.26 \pm 0.08* | 8.76 \pm 0.45* | 1.90 \pm 0.29* | 22.91 \pm 1.84* | 173.0 \pm 5.66* | 28.02 \pm 2.55 | 4.15 \pm 0.40* | 1.28 \pm 0.08 | 2.87 \pm 0.46* | 0.56 \pm 0.09* |
| <i>P. Aeruginosa</i> | 7.74 \pm 1.06* | 13.23 \pm 0.58* | 23.97 \pm 9.37* | 128.87 \pm 26.86* | 71.8 \pm 3.01* | 32.02 \pm 4.38 | 8.49 \pm 0.35* | 1.24 \pm 0.09 | 7.25 \pm 0.40* | 0.18 \pm 0.02* |
| P Value | 0 | 0 | 0.03 | 0.001 | 0 | 0.441 | 0 | 0.754 | 0 | 0.001 |

NO, nitric oxide; MDA, malondialdehyde; Lyso, lysozyme; chol, cholesterol; trigly, triglycerides; T.P, total protein; Alb, albumin; Glob, globulin; A/G ratio, albumin globulin ratio. Values with asterisk (*) represent significantly difference versus control in the same column at $p < 0.05$. Values are presented as mean \pm SE.

DISCUSSION

The *P. aeruginosa* is an environmentally abundant bacterium that causes severe disease among immune compromised hosts. It is one of the common cause of mastitis (Mcvey et al., 2013). The present resistance to penicillin tend to agree with Ranjan et al. (2010). Also, the high resistance of *P. aeruginosa* to cefoperazone and cefotaxime agreed with others (Akhoon et al., 2012 and Kotwal et al., 2016).

The resistant isolates against to carbapenem group were of a great concern due to it was the last therapeutic resource for controlling infections. It may attributed to carbapenemase produced by bacteria which involved in food producing animals and their surroundings (EFSA, 2013). The resistance to imipenem was agreed with Ohnishi et al., (2011) and (Kotwal et al., 2016). Contraries, *P. aeruginosa* that isolated from human, chickens, milk and calves lung were 100% sensitive to the same drug (Osman et al., 2012). Our result of multidrug resistance isolates included mexR gene may be due to mutation. That gene mutation increased the resistance to many antibiotics in samples of corneal keratitis scraping with *P.aeruginosa* in human (Suman et al., 2006). However, *mexAB* pump was not the cause of meropenem resistance (Pragasam et al., 2016).

Nitric oxide is one of the important biomarker for immunomodulation. In the same time, it has a main role in the cellular damage by means of ROS and proteolytic enzymes. This bad action was cleared in many diseases as mastitis (Lacasse et al., 2008 and Jeon et al., 2014). Present NO results were in harmony with Bastan et al. (2013) results who reported that NO concentration mainly depend on quarters infectious status by among bacterial species.

Catalase was one of the primary antioxidant enzymes that convert ROS to more stable molecules (Suntres, 2011). Lowering catalase activity in the serum may related to proliferation of consumed ROS, outcome of the toxins. Meanwhile its elevation in milk with lysozyme may attribute to the raised Somatic Cell Count (SCC). It was confirmed by increasing TP and globulins level in addition lowering of the A/G ratio. Catalase and MDA results were a reason for the oxidative damage in mastitis of dairy cows (Jhambh et al., 2013).

Lysozymes are vital constituents in the animals' innate immune system as they hydrolyzed major polymer of the bacterial cell wall. Gram negative bacteria had lysozyme inhibitors represented as virulence elements when interacting with the animal cell G (Callewaert et al., 2008). The current lysozyme results were different between serum and milk groups. The results were in concord with a case of *Staph aureus* mastitis in cow milk (Osman et al., 2010). Meanwhile serum lysozyme concentration increased in case of tuberculosis and *Staph aureus* mastitis in cow, in contrary with the level in milk whey (Ramadan et al., 2009). Though the impact of lysozyme genotypes for predicting mastitis was moderately low (Zaborski et al., 2016).

Regarding to the results of serum cholesterol and triglycerides levels may attribute to the suppressed appetite due to mastitis and the cow consuming insufficient nutrients. Triglyceride or cholesterol is dietary lipids or endogenous lipids which are produced by hepatocytes (Meyer et al., 1992). However, serum cholesterol level was decreased in two mastitic herds even with changing the feed program (Ohtsuka et al., 2006). Definite bacterial toxins increased the production of ROS, proinflammatory cytokines and biologically active lipids (Al Batran et al., 2013 and Rahal et al., 2014). Consequently, elevation of lipids, NO and MDA as well as reduction of catalase and lysozymes levels may be due to bacterial toxins.

As the resistance of *P. aeruginosa* to antibiotics increased dramatically, the maintenance of normal average of determined parameters (NO and MDA, catalase and lysozymes) could improve the immunity then the action of antibiotics will be maximized. Further studies were required for predicting the microbial mastitis from evaluating the immunological parameters.

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Competing interests

The authors have declared that no competing interest exists.

Author's contributions

All of authors participated in the idea, planning of the research, samples processing, drafting the article and approved final revision. Amany Mohamed Abd-El-Moaty and Verginia Mohamed El-Metwally Farag participated in bacteriological examination, identification and antimicrobial sensitivity test. Nermin Awad Ibrahim participated in DNA extraction, PCR analysis. Verginia Mohamed El-Metwally Farag performed immunological, biochemical, statistical analysis. Samar Magdy Atwa participated in diagnosis of the disease, samples (milk and blood) collection, collecting data and antimicrobial sensitivity test. Nermin Awad Ibrahim, Verginia Mohamed El-Metwally Farag and Amany Mohamed Abd-El-Moaty participated in writing the paper.

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Isolation and Characterization of Bacterial Species from Respiratory Tracts of Cattle Slaughtered in Addis Ababa City, Central Ethiopia

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ABSTRACT

The present study was an attempt to isolate and identify the diverse bacteria localizing pneumonic lungs and the associated tracheas of 50 slaughtered cattle at Addis Ababa Abattoirs enterprise, central Ethiopia, in both aerobic and anaerobic environments. 158 and 135 bacterial isolate was found in aerobic and anaerobic state, respectively using primary and secondary microbiological testes. Gram positive bacteria were the dominant bacteria in both conditions. The frequency of isolation increased from trachea down to the lung in both state indicating the bacterial role in the progress of bovine pneumonia. Most prevalently isolated bacteria from both aerobics and anaerobics conditions were *Staphylococcus* species, *Bacillus* species, *Mannheimia haemolytica* and *Pasteurella multocida*. Whereas the *Streptococcus* species, *E.coli*, *Klebsiella pneumoniae*, *Actinobacillus* species, *Micrococcus* species, *Arcanobacterium*, species *Neisseriaspecies*, *Acinetobacter* species, *Corynebacterium* species, *Bordetella* species, *Pseudomonas* species, and *Rhodococcus equi* were among the bacteria isolated.

Key words: Aerobic, Anaerobic, Bacteria, Bovine, Pneumonia

INTRODUCTION

The majority of the third world countries are located in the tropics and livestock production is very important to their economy (Thornton, 2010). Cattle population has been found to be the main components of livestock production in most farming systems in Sub-Saharan Africa (McDermott and Arimi, 2002). Ethiopia is one of the Sub-Saharan Africa countries that has the largest livestock population in Africa, with an estimate population of 54 million cattle, 25.5 million sheep and 24.06 million goat (Leta and Mesele, 2014).

The livestock productivity, despite its huge population size, remains marginal. This could be due to a number of constraints including poor genetic potential, high disease incidence, malnutrition, thermal stress and poor management conditions (Radostits et al., 2000). Among all diseases, affecting the respiratory system are generally the most important in every species of domestic animals (Thomson, 1998). The lung is continuously exposed to air that contain dust, bacteria, fungi, viruses and various noxious agents and defense against these potentially harmful materials is controlled by a complex of protective mechanisms. Stress factors such as change in weather condition, transportation, poorly ventilated housing and nutrition deficiencies have a predisposing role (Jerome, 1993).

Respiratory tract infection is one of the major causes of losses of productivity in beef as well as dairy production. Cattle are more prone to respiratory tract infection than other animals due to marked physiological problems and anatomical structures that predispose the respiratory tract to infection (Radostits et al., 2000). Cattle are susceptible to retrograde drainage from pharynx as a result to pulmonary hypertension, then their lung is well lobated and well lobulated that does not permit free air ventilation into adjacent structure (Taylor et al., 2010; Thomson, 1998).

Infectious pneumopathies are commonly attributed to *Mannheimia (Pasteurella) haemolytica*, which causes the most severe damage to the lung. In addition, bacterial agents such as *Mycobacterium*, *Mycoplasma*, *Haemophilus*, *Fusobacterium* and *Actinomyces* inflict damage on the pulmonary tissues of most domestic animals (Radostitis et al., 1994). Several infectious agents are commonly isolated from the respiratory tracts of clinically sick and healthy animals (Radostits et al., 2000). The few studies conducted in Ethiopia mainly focused on the bacterial flora of pneumonic lungs

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of sheep, cattle and camel (Mamo et al., 2011; Marru et al., 2013; Mekibeb et al., 2013; Shiferaw et al., 2006; Sisay and Zerihun, 2003). However, there are no documentations available that give brief information on aerobic and anaerobic bacterial agents that are responsible for cattle pneumonia in Ethiopia.

Therefore, the present study was designed to isolate and characterize aerobically and anaerobically growing bacteria from the respiratory tract of pneumonic cattle and to compare and contrast those bacteria colonizing the two anatomical sites (trachea and lung).

MATERIALS AND METHODS

Study area

The study was conducted at Addis Ababa abattoirs enterprise, central Ethiopia, from October 2010 to June 2011. Geographically Addis Ababa is located 9^o2' N and 38^o 42'E having an elevation of 2400M asl and mean annual rainfall of 1800mm which is in bimodal pattern. The long rainy season lays from June to September (kiremt) followed by a dry season from October to February (Bega) and a short rainy season from March to May (Belg). The city has an average minimum and maximum temperature of 10.7 and 23.6^oc respectively (NMSA, 2005).

Study animals

The study was conducted on 50 cattle at Addis Ababa abattoirs enterprise without referencing of age, sex of the animals at which ante mortem examination was conducted and passed for slaughter.

Study design and sampling

Fifty pneumonic lungs were selected randomly with their corresponding tracheas and sampled aseptically for microbiological examination. Samples were then kept separately provided with identification mark and transported to Addis Ababa University (AAU), School of Veterinary Medicine (SVM) microbiology laboratory.

Sample collection

Lung sample collection: Before lung and its corresponding tracheal sterile swab samples were collected, gross examination of pneumonic lung was undertaken. Lungs that were congested were assumed to be pneumonic without involving parasitic cause of pneumonia. From each pneumonic lung, lung tissue was aseptically collected. Sampling was undertaken according to the procedure recommended by Quinn et al. (1999). Lung tissues were removed and were put in a sterile screw capped universal bottle and those samples were separated, identified, and transported on ice box to AAU, SVM microbiology laboratory.

Tracheal swab samples collection: After slaughtering and separating of the offal from the carcass, the trachea of each pneumonic lung of cattle was grasped with tissue forceps and opened with sterile scalpel blade. Then, sterile cotton tipped swabs soaked with Tryptose Soya Broth (TSB) (OXOID, Hampshire, England) transport medium were inserted into the tracheal tube, and the mucus membrane was rubbed. From a trachea two swab samples were taken for aerobic and anaerobic culture. The collected tracheal swabs were putt inside test tubes containing TSB, and which were separated, identified, and transported to the microbiology laboratory. One group of tracheal swab samples were placed in a sterile anaerobic jar together with a flaming candle and the jar was closed and incubated at 37^oc for 24 hours. The other group of tracheal swab samples were placed in a rack and incubated at 37^ocfor 24 hours in an aerophilic incubator.

Bacterial isolation and characterization

Bacteriological Culturing

Lung: The lung parenchymal tissue was cut and cultured on blood agar (Oxoid, Hamshire, UK) which contain 7% sheep blood using sterile forceps. Each lung sample was inoculated into two plate containing blood agar medium, for aerobic and anaerobic culture. The anaerobic group was kept inside a sterile anaerobic jar containing flaming candle and incubated at 37^oc for 24 hours in Co₂ incubator while the aerobic group was incubated at 37^oc for 24 hours in an aerophilic incubator.

Tracheal swabs: After 24 hours of incubation in the TSB medium, both aerobically and anaerobically cultured tracheal swab samples were thoroughly agitated to aid mixing. A loop full of broth culture was taken and streaked over identified Petri-plates containing blood agar medium supplemented with 7% sheep blood (Quinn et al., 1994).

Morphological and cellular characterization: Pure culture colonies were subjected to Gram staining to study staining reaction and cellular morphology under light microscope, at 100×magnification and potassium hydroxide (KOH) test were done to avoid confusion in Gram stain reaction. Mixed colonies and Gram-negative bacteria were sub-cultured on both blood and MacConkey (Oxoid, Hamshire, UK) agar plates for further analysis. Pure cultures of single colony type, from both blood and MacConkey agar, were transferred onto nutrient agar-medium for a series of primary

tests such as Catalase , Oxidase, Motility, and Fermentative-Oxidative and secondary tests including triple sugar iron agar, citrate utilization test, methyl red test and indole test, following standard procedures described in Quinn et al. (1999).

Data analysis

Descriptive statistics were used to summarize the data generated from the study. The relative abundance of each species/genera was expressed as a percentage in comparison to the total number of isolates.

Ethical Approval

The study considered direct observation of slaughter animals in the abattoir and took appropriate samples for further microbiological experiments and no animal was subjected to suffering as a result of this study. Nevertheless, ethical approval was conducted by research ethical approval committee of Addis Ababa University, school of veterinary medicine, Ethiopia.

RESULTS

Aerobically isolated bacteria

Out of 100 specimens collected from trachea and the corresponding pneumonic lung (50 from each site), 100 (100%) harbored bacteria. In general, 158 different bacteria species were isolated from 100 infected specimens in aerobic condition. The proportion of Gram positive bacteria were dominated over Gram negative bacteria accounting about 58.8% while Gram negative were 37.97%.

The predominant species among the aerobic isolates were the *Staphylococcus* species with proportion of 27.85% frequently isolated from the lungs (58%) and the trachea (30%) followed by *Bacillus* species (17.18%), *Pasteurella multocida* (13.29%) and *Mannheimia haemolytica* which accounted for 10.13%. On the other hand, *Arcanobacterium* and *Acinetobacter* species (0.63% each) were among the least encountered bacterial genera in aerobic state.

As shown in table 1, the majority of the isolates colonized both trachea and descended to the lung with the exception of *Rhodococcus equi*, *Pseudomonas*, *Micrococcus*, *Bordetella*, *Acinetobacter* and *Arcanobacterium* species which were absent from tracheas but were isolated from the corresponding lungs, whereas *Klebsiella pneumonia* absent from lung but isolated from trachea. Among Gram negative bacteria the most frequently isolated and the least encountered bacteria were the *P. multocida* and *Acinetobacter* species respectively.

Table 1. Aerobically isolated bacteria from trachea and pneumonic lung of cattle slaughtered in Addis Ababa abattoirs enterprises, central Ethiopia from October 2010 to June 2011

| Bacterial isolates | Trachea | Lung | Frequency | Proportion |
|--------------------------------|---------------|---------------|------------|------------|
| Gram positive bacteria | No (%) | No (%) | | |
| <i>Streptococcus</i> species | 3(6) | 5(10) | 8 | 5.06 |
| <i>Bacillus</i> species | 8(16) | 19(38) | 27 | 17.18 |
| <i>Staphylococcus</i> species | 15(30) | 29(58) | 44 | 27.85 |
| <i>Micrococcus</i> | 0(0) | 7(14) | 7 | 4.43 |
| <i>Rhodococcus equi</i> | 0(0) | 6(12) | 6 | 3.88 |
| <i>Arcanobacterium</i> species | 0(0) | 1(2) | 1 | 0.63 |
| Gram negative bacteria | | | | |
| <i>Actinobacillus</i> species | 2 (4) | 3(6) | 5 | 3.25 |
| <i>Escherichia coli</i> | 4(8) | 4(8) | 8 | 5.06 |
| <i>Klebsiella pneumonia</i> | 2(4) | 0(0) | 2 | 1.27 |
| <i>Mannheimia haemolytica</i> | 8(16) | 8(16) | 16 | 10.13 |
| <i>Pasteurella multocida</i> | 14(28) | 7(14) | 21 | 13.29 |
| <i>Bordetella</i> species | 0(0) | 4(8) | 4 | 2.53 |
| <i>Pseudomonas aeruginosa</i> | 0(0) | 3(6) | 3 | 1.98 |
| <i>Acinetobacter</i> species | 0(0) | 1(2) | 1 | 0.63 |
| Unidentified bacteria | 1(2) | 4(8) | 5 | 3.16 |
| Total | 57 | 101 | 158 | 100 |

Anaerobically isolated bacteria

From the anaerobically cultured samples, strictly anaerobic bacteria were not isolated. Most of the bacteria were similar with aerobically isolated bacteria indicating that the bacteria were facultative anaerobes. Most frequently anaerobically isolated bacterial species were *Staphylococcus species* (35.56%), *Bacillus species* (21.48%) *Mannheimia haemolytica* (11.8%) and *Pasteurella multocida* (10.37%), these results are similar with that of the aerobically isolated bacteria except the percentage and frequency. *Arcanobacterium species*, *Neisseria species* and *Klebsiella pneumoniae* (0.74% each) were the least bacteria isolated from the anaerobic condition.

Similar with the aerobic isolates, as shown in table 2 most of the anaerobically isolated bacteria colonized the lung and bacteria that did not exist in the trachea were isolated from the lung including *Micrococcus species*, *Klebsiella Pneumoniae*, *Arcanobacterium*, *Neisseria* and *Corynebacterium species*. Like that of the aerobically isolated bacteria Gram positive bacteria dominated (65.2%) while Gram negative bacteria accounted for 33.33% in anaerobic situation.

In general, there was a general increment in the isolation rate as one goes down from the trachea toward the lung with the highest and lowest infection rate being the lung and trachea, respectively in both aerobic and anaerobic conditions. Most of the bacteria that were isolated aerobically have a larger frequency of isolation in anaerobic condition.

Table 2. Anaerobically isolated bacteria from trachea and pneumonic lung of cattle slaughtered in Addis Ababa abattoirs enterprises, central Ethiopia from October 2010 to June 2011

| Bacterial isolates | Trachea | Lung | Frequency | Proportion |
|--------------------------------|---------------|---------------|------------|------------|
| Gram positive bacteria | No (%) | No (%) | | |
| <i>Streptococcus species</i> | 1(2) | 3(6) | 4 | 2.96 |
| <i>Bacillus species</i> | 12(24) | 17(34) | 29 | 21.48 |
| <i>Staphylococcus species</i> | 15(30) | 33(66) | 48 | 35.56 |
| <i>Micrococcus species</i> | 0(0) | 3(6) | 3 | 2.22 |
| <i>Arcanobacterium species</i> | 0(0) | 1(2) | 1 | 0.74 |
| <i>Corynebacterium species</i> | 0(0) | 3(6) | 3 | 2.22 |
| Gram negative bacteria | | | | |
| <i>Escherichia coli</i> | 11(22) | 2(4) | 13 | 9.63 |
| <i>Klebsiella pneumoniae</i> | 0(0) | 1(2) | 1 | 0.74 |
| <i>Mannheimia haemolytica</i> | 15(30) | 1(2) | 16 | 11.85 |
| <i>Pasteurella multocida</i> | 8(16) | 6(12) | 14 | 10.37 |
| <i>Neisseria species</i> | 0(0) | 1(2) | 1 | 0.74 |
| Unidentified bacteria | 2(4) | 0(0) | 2 | 1.48 |
| Total | 64 | 71 | 135 | 100 |

DISCUSSION

The present study recognized both aerobically and anaerobically growing bacteria from the respiratory tract of 50 cattle slaughtered at Addis Ababa abattoirs enterprises. A total of 158 bacteria were isolated from both tracheas and lungs aerobically. The isolation of Gram positive bacteria in higher proportion than the corresponding Gram negative bacteria in this study agrees with studies of few workers such as Esra et al. (2009) who isolated from apparently healthy and unhealthy nasal cavities of Holstein cattle (65.68%).

Esra and Hakan (2010) isolated these aerobic bacterial floras from the nasal cavity in healthy Anatolian water buffalo calves in Turkey, Yimer and Asseged (2007) identified aerobic bacteria from pneumonic lung from sheep. GutDelete et al. (2009) reported aerobically similar bacteria from the upper respiratory tract of diseased donkey. Jenberie et al. (2012) isolated and identified similar bacteria from pneumonic lung of camels. The invariable isolation of those organisms from pneumonic lungs of various animal species may indicate their significance in different respiratory system problems in different animals.

In parallel with the aerobic isolates, all the bacteria recovered from the anaerobic culture were facultative anaerobes and were similar to those bacteria isolated from aerobic condition. A total of 135 facultative bacteria were isolated and in contrast with the aerobically isolated, the frequency of isolation of some of these bacteria increased comparing to aerobically isolated bacteria. In line with this study Chirino-Trejo et al. (1983) reported similar facultative bacteria from pneumonic lung of cattle in Canada. In the present study unlike to Chirino-Trejo et al. (1983), strictly anaerobic bacteria were not isolated.

The higher isolation rate in this study is probably associated with the anatomy and physiology of bovine lung. Bovine lung is highly lobated and lobulated whereas small ruminants' lung is highly lobated and poorly lobulated. This structure of bovine lung doesn't allow collateral ventilation (Thomson, 1998). Moreover, bovine lung is prone to respiratory hypertension due to retrograde drainage from the pharynx and it has a low tidal volume and relatively small size that predispose it to respiratory infection (Taylor et al., 2010).

Among the total isolates, the *Staphylococcus* species were the predominant bacteria in both conditions and have high proportions in the lung and trachea. In agreement with this study *Staphylococcus* species were isolated and reported in higher proportion in the previous few studies aerobically. Esra et al. (2009) isolated these bacteria from unhealthy Holstein cattle nasal cavities with high frequency and percentage (33.3%). The dominance of these bacteria is also in line with the study conducted by Megra et al. (2006) who isolated aerobic bacteria flora of the respiratory passage of healthy goats. These indicate there is a likelihood of association between these bacteria and pneumonic syndrome of lung. The bacteria are commensally living in the mucous membrane of the upper respiratory tract of animals and are opportunistic pathogens (Quinn et al., 1994).

Bacillus species were the second abundant bacteria harvested with higher frequency in both situations. Different workers at different times isolated these species of bacteria with variable rates of isolation aerobically. It was isolated by Esra et al. (2009) (16.70%) from unhealthy Holstein cattle, Chirino and Prescott (1982) were unable to isolate these bacteria anaerobically. The high localization of these bacteria in the lung may play an important role in the progress of bovine pneumonia.

In the present study, *M. haemolytica* and *P. multocida* isolated with a relatively higher proportion and the result is similar to the report obtained from Garedew et al. (2010) who isolated these bacteria from Maedi-Visna affected sheep in Ethiopia with the rate of 7.89% and 7.02% of *M. haemolytica* and *P. multocida*, respectively. While anaerobically, *M. haemolytica* and *P. multocida* were reported by Chirino and Prescott (1982) at the rate of 13.68% and 18.94%, respectively. These bacteria are commensally living in the upper respiratory tract and all *Pasteurella* species are probably extracellular parasites with various stresses, including concurrent viral infections predisposing to infections as in shipping fever. They play a part in shipping fever and cause pneumonia as a primary or a secondary etiology. *M. haemolytica* produces a soluble cytotoxin (leukotoxin) that has a role in breaching the lung's primary defense mechanism by its action on the alveolar macrophage and other leukocytes of ruminants (Quinn et al., 1999).

Despite their few proportion, *Streptococcal* species were isolated from the lungs and tracheas of pneumonic cattle and the result observed in the present study is in agreement with Obasiet al. (2010) who reported an isolation rate of 8.2% from caprine pneumonic lungs and Chirino and Prescott (1982) obtained related results of 6.31% from each aerobic and anaerobic culture of pneumonic cattle. These bacteria are resident flora of the upper respiratory tract mucous membrane and are commonly associated with suppuration and abscess formation (Quinn et al., 1994).

The isolation of *E. coli* in this investigation is in agreement with Esra et al. (2009) who isolated the bacteria from unhealthy Holstein nasal cavities at the rate of 10%. Unlike to this study the isolation of Chirino and Prescott (1982) has a relatively higher isolation rate (11.5% and 20%) of aerobic and anaerobic isolate, respectively from pneumonic cattle in Ontario, Canada. The isolation of this bacterium in this study correlates with the natural habitants of *E. coli* which can survive in faecal particles, dust and water for weeks and month (Quinn et al., 1999). The finding of *E. coli* from pneumonic lungs suggests that *E. coli* is considered as a secondary invader in the bovine pneumonia and contribute to the pneumonic progress.

Micrococcus species, which are the normal flora of respiratory tract, were isolated and the obtained result coincided with that of Esra et al. (2009) who isolated *Micrococcus* species with rate of 3.33% from unhealthy Holstein cattle nasal cavities. *Micrococcus* spp. are assumed to be non-pathogenic (Carter, 1984). However, in most of the infections along with other pathogens, it may flare up and act as a secondary invader.

Corynebacterium species were isolated anaerobically from this study. In the previous time these bacteria were isolated by Chirino and Prescott (1982) in similar condition but with relatively high isolation rate (26.9%). According to Quinn et al. (1994) these are pyogenic bacteria be able to cause a variety of suppurative condition.

Among the total aerobically isolated bacteria, *Rhodococcus equi* was also reported. This is supported by a report by Nesibu et al. (2010), who isolated this bacterial spp. with a rate of 5.3%. Since *R. equi* is an inhabitant of soil and intestinal tracts of animals, it can replicate at warm temperatures in soil enriched with faeces of herbivores (Quinn et al., 2002) and likely to find this bacteria in the respiratory tract of animals. The bacteria are known to cause suppurative foal pneumonia and suppurative disease formation in domestic animals (Thomson, 1998).

Among the least aerobically isolated bacteria in this study were *Acinetobacter* and *Arcanobacterium* species having an equal isolation rate. Whereas *Klebsiellapneumoniae*, *Arcanobacterium* and *Neisseria* species are the least bacterial isolate anaerobically. *Arcanobacterium* species are present on the mucous membrane of the host animals, often in the oral cavity and nasopharynx. The infection of these bacteria is endogenous and most of the species cause pyogranulomatous reaction in animal tissues and the isolation of this bacterium from the lower and upper respiratory

tract indicates its role as a secondary invader in low respiratory tract infection (Quinn et al., 1999). Whereas, *Acinetobacter* species are commonly found in soil, sewage, water, food and milk and are part of the normal flora of human and animal and can cause nosocomial infections especially in immunocompromised patients (Quinn et al., 1994). On the other hand, *Klebsiella pneumoniae* is an inhabitant of the intestinal tract of animals as well as oil and saw dust, faecal contamination of the environment accounts wide distribution of the organism and contribute to the occurrence of opportunistic infection (Quinn et al., 1999).

CONCLUSION

Although they are normal flora in the upper respiratory tract, they may descend and involve as a primary and a secondary etiological agent in the progress of bovine pneumonia and bovine respiratory disease complex. Risk factors like suffocation, shipping, immunity compromise, malnutrition, other respiratory diseases complication and inappropriate management of animals provide ideal opportunities for these bacteria to cause respiratory diseases and aggravation. Therefore, appropriate prevention methods should be established and identifying the most pathogenic species guarantee future studies.

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Competing interest

The authors have not declared any conflict of interest

Author's contributions

Addis Kassahun Gebremeskel carried out the sample collection, experiments, analysis and writing; Addisu Awukew Yegoraw and Biruk Tesfaye Birhanu collect samples; Tesfaye Sisay Tesema designed the experiments; Shewangzaw Addisu Mekuria conducted edition.

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Bovine Tuberculosis Prevalence, Potential Risk Factors and Its Public Health Implication in Selected State Dairy Farms, Central Ethiopia

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ABSTRACT

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis* (*M.bovis*), is endemic in Ethiopia. However, its magnitude in cattle and human population are not well documented. A cross-sectional study was conducted on 720 apparently healthy dairy cattle kept in three different state owned farms in central Ethiopia to quantify the risk factors and determine the prevalence of BTB using (CIDT) Comparative Intra-Dermal Tuberculin Test from December 2013 to November 2014. Questionnaire survey was used to assess the risk factors and zoonotic implication of BTB. The prevalence of BTB was at 16.53% (95% CI 14.2-18.9) and It was significantly higher in crossbreed ($\chi^2= 54.76$; $P<0.001$; OR=16.1; 95% CI=6.2-41.1) and animals older than 4 years ($\chi^2=34.51$; $P<0.001$, OR =6.22; 95% CI=3.5-11.12). Moreover, the prevalence was also significantly higher in good body conditioned dairy cattle compared to poor body conditioned dairy cattle ($\chi^2=29.69$; $P<0.001$; OR=2.45; 95% CI=1.1-5.7). The prevalence of BTB was also significantly varied among the reproductive status of the dairy cattle ($\chi^2=18.10$; $P<0.001$). The majority of the respondents consume raw milk (66.1% and raw meat (74.20%) respectively. There was statistically significant variation ($\chi^2=12.51$; $P<0.03$) in consumption habit between educated and non-educated dairy farm workers. The major risk factors for bovine tuberculosis in this study were breed and age of the dairy cattle. Consumption of raw milk and meat is still a common practice in the study farms. Culling of aged dairy cattle and continuous test and slaughter of infected cattle should be practiced at least in state owned dairy farms to decrease the risk of transmission. In addition to awareness creation of the public particularly the dairy farm workers on the zoonotic nature of tuberculosis is of utmost importance to control bovine tuberculosis.

Key words: Bovine tuberculosis prevalence, Dairy cattle, Farm worker, Risk factor

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic infectious disease of animals characterized by the formation of granulomatous lesions in tissues and organs, more significantly in the lungs, lymph nodes, intestine and kidney (Domingo et al., 2014). It is caused by slowly growing non-photochromogenic bacilli, *Mycobacterium bovis* members of the *M. tuberculosis* complex. *Mycobacterium bovis* is the most universal pathogen among mycobacteria and affects many vertebrate animals of all age groups including humans. Cattle, goats and pigs are found to be most susceptible, while sheep and horses are showing a high degree of natural resistance (Radostits et al., 2000; Thoen et al., 2006). Bovine tuberculosis causes tremendous loss to the dairy industry. Studies are showing that BTB causes 30-50 %, 5-25% and 6-12 % loss in dairy and beef production. The loss attributed to BTB due to culling was 30-59 % (Radostits et al., 2000).

Tuberculosis (TB) is among the top public health threats globally, particularly in developing countries (WHO, 2007). Human TB is still a major cause of death worldwide in general and in the high TB-burden regions in particular (WHO, 2007). Even though *Mycobacterium tuberculosis* is the most common cause of human TB, an unknown proportion of cases are due to *M. bovis*. Bovine Tuberculosis (BTB) is a principal zoonotic problem transmitted to humans primarily through consumption of infective milk and other animal products obtained from infected cattle and/or

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occasionally by respiratory route (Ashford et al., 2001). The main risk factors for *M. bovis* infections in humans are poverty, malnutrition, HIV infection, consumption of raw milk and close contact with livestock (Ayele et al., 2004; Cosivi et al., 1998). All people are susceptible to BTB; however, dairy farm workers, veterinarians, farmers, abattoir workers and people who consume contaminated raw milk and meat, and those who are in close contact with infected animals and animal products are at higher risk for zoonotic tuberculosis. A study in central Ethiopia indicated that cattle owners who consumed raw milk were at greater risk of having active tuberculosis than those who consumed boiled milk (Ameni et al., 2007; Ayele et al., 2004).

The endemic nature of bovine tuberculosis in Ethiopia was reported since 1967 by Food and Agricultural Organization (FAO, 1967). The prevalence of BTB in the country especially central Ethiopia ranged from 3.4% in smallholder production systems to 50% in intensive dairy production system (Ameni et al., 2007; Ameni et al., 2001; Asseged et al., 2000). Research conducted in Addis Ababa on cross breed cattle showed that the prevalence of BTB was 34.1% (Wondewosen et al., 2010); however, still there is lack of knowledge about the actual prevalence and distribution of the disease at a national level. In addition, large size dairy farms are kept by Ethiopian Institute of Agricultural Research (EIAR) with significant contribution to the local milk supply chain and heifer distribution to small holder milk producers. However, the status of these dairy farms with regard to BTB was not known. Thus, this study was initiated to investigate the status of bovine tuberculosis and its risk factors in those state owned dairy farms.

MATERIALS AND METHODS

Ethical approval

The research work was conducted according to Organization International Epizootics (OIE, 2010) principles of the use of animals in research and education. As much as possible we prevent, alleviate and minimize pain, suffering, and distress and enhance welfare for the animals used for research during tuberculin test. Regarding questionnaire survey consent form were prepared and asked the respondents either they are voluntary to participate for this study. Only the voluntary one were selected and signed on the consent form before interview were conducted.

Study farms

The study was conducted at three dairy farms managed under (EIAR), at Holeta and Bishoftu agricultural research centers. Holeta agricultural research Center has two dairy farms found in Holota and Ada Berga. Holeta is located at 34 km West of Addis Ababa, at 09°02'N latitude and 38°34' E longitudinal and altitude ranges 2060 to 3380 meter above sea level with an average temperature of 21°C and 900 to 1100 mm annual rainfall with bimodal pattern. Ada Berga is also located at 64 km West of Addis Ababa at altitude of 2435 meter above sea level, with average annual temperature of 17.5°C and rainfall 1143 mm. Bishoftu, former Debre Zeit, is located at 45km South East of Addis Ababa and a latitude of 8°44' N and 39°02' E longitude with a latitude of 1900 meter above sea level(m.a.s.l). The annual rainfall of Bishoftu is 800 mm with mean minimum and maximum temperatures of 8.9 and 28.3°C, respectively (EIAR, 2013).

Study animals

A total of 720 dairy cattle: 363, 243 and 114 were sampled from Holeta, Adaberga and Bishoftu dairy farms respectively. The sampled dairy cattle consisted of Jersi, Boran, and Holstein crosse (HolsteinX Boran) breeds. Forty eight percent of the tested dairy animals were cross breeds and the remaining 30% and 22% were Boran and Jersey breeds respectively. Young animals, less than six months and cows one month before and one month after parturition were not included in this study.

Study design and sampling methods

The study design was cross-sectional. The three dairy farms were purposively sampled for screening. All eligible dairy cattle in the farms were included. During the screening test, the breeds, age, sex, reproductive status and the Body Condition Score (BCS) of the dairy cattle were recorded. BSC was classified into poor, medium and good after physical observation of vertebral column, spines, tuber coxae and ribs of the animal (Ameni et al., 2007; Ameni et al., 2001; Nicholson and Butterworth, 1986).

Comparative Intra-Dermal Tuberculin Test

Comparative Intra-Dermal Test (CIDT) was conducted on 720 dairy cattle. The skin of the middle of the neck of each dairy cattle on the right side was shaved at two sites 12 cm apart, cleaned and checked for any injury. The skin thickness was measured with a caliper before the tuberculin was injected. Aliquots of 0.1 ml of 3000 International Units (IU) per Milliliter (MI) of bovine purified Protein derivative (PPD) (Prionics Lelystad B.V. Platinastraat 338211 A R Lelystad, Netherlands), and 0.1 ml of 2,500IU/ml of avian PPD (Prionics Lelystad B.V. Platinastraat 338211 A R

Lelystad, Netherlands) was injected into the dermis at these sites by using insulin syringe and needle. After 72 hrs, the thicknesses of the skin at the injection sites were measured again, using caliper (OIE, 2009). The results were interpreted in accordance with the standards and recommendations of OIE (2009) cut off value. The reaction was interpreted as positive, inconclusive and negative. When the differential increase in skin thickness at the bovine PPD injection site was greater than that of the avian PPD injection site by 4 mm, the cattle was considered positive for mycobacterial species infection other than avian type. It was considered inconclusive when the increase in skin thickness at the bovine PPD injection was greater than that of avian PPD injection site by values between 1 and 4mm. The result was considered negative when skin thickness at the bovine PPD injection site was less than or equal to the increase in the skin reaction at the avian PPD injection site which was less than one.

Questionnaire survey

Face to face interview of dairy farm workers having different responsibilities in the farm were conducted. A total of 62 voluntary respondents from the three dairy farms were interviewed. Fifty six of them were Dairy Farm Workers (DFW) and six were assistant veterinarians (animal health assistants). In addition, one farm manager was also interviewed. The questionnaire was pre-tested and administered to the respondents after having informed verbal consent to assess knowledge and practices of the respondents on BTB and also assess the risk factors associated with the occurrence of the disease. The questions were specifically focused on assessing the respondent's awareness on BTB and its means of transmission from cattle to humans and vice versa. Recent history of TB cases in the family and the type of TB (if present) and history of persistent coughing for long time 2-3 weeks were assessed.

Data analysis

Data like skin thickness, ID, age, sex, breed, BCS, reproductive status of cattle, and farm location were collected. Data were entered into excel spread sheet 2007. Individual animal prevalence was determined as the number of reactors per 100 animals tested. The association of different risk factors with prevalence of BTB was analyzed using Pearson chi-square (χ^2) test. Multivariable logistic regression analysis was used to assess the strength of association between the outcome variable (prevalence) and the various explanatory variables (age, sex, and breed reproductive status and body condition scoring and farm location). Data were coded and analyzed using SPSS software packages 16. When assumptions of Chi-square failed Fisher's exact test was used. Odds ratio was calculated to assess strength of association of different risk factors to the occurrence of BTB in cattle. A confidence level of 95% and the level of significance at p-value <0.05 was used in all statistical analysis.

RESULTS

Prevalence of bovine tuberculosis

The overall prevalence of BTB in the three dairy farms was 16.5% (119/720) with 95% CI 14.2-18.9. The prevalence was highest in Bishoftu dairy farm and lowest in Adaberga dairy farm. Thus, there was statistically significant ($\chi^2= 56.85$; $P<0.001$) variation in the prevalence of BTB among the three farms (Table 1).

Potential risk factors for the prevalence of bovine tuberculosis

Association between different host related explanatory variables (age, sex, breed, reproductive status, body condition scoring) and prevalence of BTB was depicted in (Table 2). The prevalence of BTB was significantly ($\chi^2=54.76$; $P<0.001$) higher in cross breed cattle than in zebu cattle. A statistically significant variation ($\chi^2=34.51$; $P<0.001$) in the prevalence of BTB was observed between age groups. Moreover, CIDT positivity was statistically significant ($\chi^2 =4.22$; $P<0.05$) in cattle with good body condition than those with poor body condition. The prevalence of BTB was also affected ($\chi^2 =18.10$; $P<0.001$) by reproductive status of the dairy cattle. No significant variation ($\chi^2= 1.74$; $P>0.05$) was observed between male and female dairy cattle.

The strength of association of different risk factors to the prevalence of BTB using multivariable logistic regression analysis was described in (Table 3). Dairy cattle kept in Bishoftu and Holeta were more likely to be infected than in Adaberga (OR=24.2; 95% CI=8.8-66.7). The tuberculin skin test result of this study showed that as age of the cattle increases, they become more likely to be infected with BTB. Dairy cattle older than eight years were six times more likely to be infected with BTB than younger dairy cattle (OR=6.22; 95% CI=3.5-11.12). The odds of BTB prevalence were higher in cross breed than in Jersey cattle. Thus, cross breed cattle were 16 times more likely to be infected with *M. bovis* than Jersey (OR=16.1; 95% CI=6.23-41.13). Animals with good body condition were two times more likely to be tuberculin positive than those with poor body condition (OR=2.45; 95% CI=1.1-5.7).

Questionnaire survey

Participant demographics: According to the respondents' response, 71% of the respondents were urban while the rest 29% live in rural area. The majority (66%) of the respondents were male. Most of them (91.9%) are Orthodox Christian by religion. The greater proportion of the respondents has no formal education (33.87%). Ninety percent (90%) of the respondents were dairy farm workers with a minimum and maximum age of 21 and 70 years. The mean, minimum and maximum working experiences of the respondents in the farm were, 14.7, 1 and 36 years, respectively. Table 4 shows the demographic characteristics of the respondents who were interviewed as part of the study.

Knowledge and food of animal origin consumption habit of the respondents: The knowledge and perception of respondents in relation to BTB transmission were depicted in Table 5 below. Among 62 respondents, 38.7% of them knew that cattle can be infected with tuberculosis, from these, 25.8% mentioned that BTB is transmitted from animals to humans where as 1.6 % knew reverse zoonosis. Only 12.9% of the respondents mentioned the clinical sign of tuberculosis in humans and animals. About 24% of the respondents have known about the transmission of BTB from animals to humans via consumptions of raw animal product and by products.

We found that 66.1% of the respondents have the habit of raw milk consumption and only 33.9% of the respondents boil before consumption. All respondents consume soured milk products without heat treatment. Consumption habit of respondents is described under tables 6 and 7. Regarding meat consumption, 74.2% of the respondents consume raw meat. This questionnaire survey showed that habits of raw milk consumption was significantly influenced ($P < 0.05$) by occupation and education level of the respondents (Table 6). The habit of meat consumption seemed to be not affected by the level of education (Table 7).

Table 1. Association of bovine tuberculosis prevalence among the three dairy farms in central Ethiopia from December 2013 to November 2014

| Dairy farm location | No tested | No positive (%) | χ^2 | P-value |
|---------------------|-----------|-----------------|----------|---------|
| Bishoftu | 114 | 31(27.2) | 56.9 | 0.001 |
| Holeta | 363 | 83(22.9) | | |
| Adaberga | 243 | 5(2.1) | | |

Table 2. Association between different variables and result of comparative intra dermal tuberculin test in central Ethiopia from December 2013 to November 2014

| Variables | No of animals tested | No % positive | χ^2 | P-value |
|----------------------------|----------------------|---------------|----------|---------|
| Sex | | | 1.74 | 0.22 |
| Mala | 32 | 8(25) | | |
| Female | 688 | 111(16.13) | | |
| Breed | | | 54.76 | 0.001 |
| Cross holestin × boran | 346 | 93(26.90%) | | |
| Boran reed | 216 | 22(10.19) | | |
| Jersi | 156 | 5(3.14%) | | |
| Age | | | 34.51 | 0.001 |
| >8years | 90 | 32(35.56%) | | |
| 4-8years | 245 | 47(19.18%) | | |
| <4 years | 385 | 40(10.39%) | | |
| Body Condition | | | 29.69 | 0.001 |
| Good | 339 | 83(24.48%) | | |
| Medium | 322 | 29(9.01%) | | |
| Poor | 59 | 7(1.20%) | | |
| Reproductive status | | | 18.10 | 0.001 |
| Lactating | 241 | 37(15.35%) | | |
| Pregnant | 96 | 22(22.92%) | | |
| Dry | 78 | 23(29.49%) | | |
| Heifer | 260 | 29(11.33%) | | |
| Others | 45 | 8(17.78%) | | |

Calves older than 6 months and younger than one year

Table 3. Multivariable logistic regression analysis of tuberculin reactivity and risk factors among dairy cattle in the farms in central Ethiopia from December 2013 to November 2014

| Risk factor | No examined | No % positive | Odds ratio 95% CI | P-value |
|----------------------------|-------------|---------------|-------------------|---------|
| Dairy farm | | | | |
| Bishoftu | 114 | 31(27.19) | 24.20(8.80-66.70) | |
| Holota | 363 | 83(22.68) | 12.71(5.0-32.30) | |
| Adaberga | 243 | 5(2.1) | 1 | |
| Breed | | | | |
| Cross HXB | 346 | 93(26.90%) | 16.1(6.23-41.13) | 0.001 |
| Boran reed | 216 | 22(10.19) | 3.87(1.41-10.62) | |
| Jersi | 156 | 5(3.14%) | 1 | |
| Age | | | | |
| >8years | 90 | 32(35.56%) | 6.22(3.50-11.12) | 0.001 |
| 4-8years | 245 | 47(19.18%) | 2.2(1.36-3.54) | |
| <4 years | 385 | 40(10.39%) | 1 | |
| Body Condition | | | | |
| Good | 339 | 83(24.48%) | 2.45(1.04-5.74) | 0.001 |
| Medium | 322 | 29(9.01%) | 0.66(0.27-1.63) | |
| Poor | 59 | 7(1.20%) | 1 | 0.001 |
| Reproductive status | | | | |
| Lactating | 241 | 37(15.35%) | 0.57(0.19-1.67) | |
| Pregnant | 96 | 22(22.92%) | 3.64(1.16-11.45) | |
| Dry | 78 | 23(29.49%) | 3.85(1.16-12.73) | |
| Heifer | 260 | 29(11.33%) | 1.18(0.47-2.96) | |
| Others | 45 | 8(17.78%) | 1 | |

Others= Calves older than 6 months and younger than one year, H=Holstein Frisian B= boran breed

Table 4. Socio demographic characteristics of respondents in the three dairy farms, in central Ethiopia central Ethiopia from December 2013 to November 2014

| Characteristics | Total number interviewed (%) |
|------------------------|------------------------------|
| Farm | |
| Bishoftu | 14(22.6) |
| Holota | 34(54.8) |
| Adaberga | 14(22.6) |
| Residence | |
| Urban | 44(71) |
| Rural | 18(29) |
| Sex | |
| Male | 41(66.1) |
| Female | 21(33.90) |
| Marital status | |
| Married | 47(75.8) |
| Single | 10(16.1) |
| Divorced | 3(4.8) |
| Widowed | 2(3.2) |
| Religion | |
| Ortodox | 57(91.9) |
| Muslim | 2 (3.2) |
| Protestant | 3(4.8) |
| Education | |
| No formal education | 21(33.9) |
| Primary | 20(32.3) |
| Secondary high school | 12(19.4) |
| Tertiary | 9(14.5) |
| Occupation | |
| Dairy farm workers | 56(90.3) |
| Farm Manager | 1(1.6) |
| Assistant veterinarian | 5(8.1) |

Table 5. Knowledge and perception of respondents on zoonotic tuberculosis

| Knowledge | No respondents interviewed | No and (%) Respondents Knowledge |
|---|----------------------------|----------------------------------|
| Know MTB | 62 | 51(82.3) |
| Know BTB | 62 | 24(38.7) |
| Know BTB is zoonosis | 62 | 16(25.8) |
| Know raw milk is vehicle for <i>M. bovis</i> | 62 | 13 (21.0) |
| Know raw meat is vehicle for <i>M. bovis</i> | 62 | 14(22.6) |
| Know yogurt is as vehicle for <i>M. bovis</i> | 62 | 10(1.6) |
| Know clinical sign of tuberculosis in human | 62 | 8(12.9) |
| Know the transmission of BTB to human | 62 | 14(24%) |
| Know reverses zoonosis | 62 | 10(1.6) |

Table 6. Association of milk consumption habits of respondents among different risk factors in the study farms, central Ethiopia from December 2013 to November 2014

| Risk Factors Category | Milk consumption habit | | χ^2 | P-value |
|------------------------|------------------------|-------------|----------|---------|
| | Raw milk | Boiled milk | | |
| Residence | | | 1.3 | 0.38 |
| Urban | 31(50) | 13(21) | | |
| Rural | 10(16.13) | 8(12.9) | | |
| Education | | | 12.5 | 0.03 |
| no formal education | 15(24.19%) | 6(9.68) | | |
| Primary | 12(19.35%) | 8(12.90) | | |
| Secondary high school | 11(17.74%) | 1(1.61%) | | |
| Tertiary | 3(4.84%) | 6(9.6%) | | |
| Occupation | | | 9.6 | 0.048 |
| Dairy farm workers | 40(64.52%) | 16(25.81%) | | |
| Farm Manager | 1(1.61%) | 4(6.45%) | | |
| Assistant veterinarian | 0.0 | 1(1.61%) | | |
| Sex | | | 0.3 | 0.78 |
| Male | 28(45.16%) | 13(20.97%) | | |
| Female | 13(20.97%) | 8(12.90%) | | |

Table 7. Association of different factors to meat consumption habit of respondents in the study farms in central Ethiopia from December 2013 to November 2014

| Risk Factors Category | Milk consumption habit | | χ^2 | P-value |
|------------------------|------------------------|-------------|----------|---------|
| | Raw milk | Cooked milk | | |
| Residence | | | 0.8 | 0.5 |
| Urban | 34(54.84%) | 10(16.12%) | | |
| Rural | 12(19.35%) | 6(9.68%) | | |
| Education | | | 3.1 | 0.6 |
| no formal education | 17(27.42%) | 4(6.45%) | | |
| Primary | 13(20.98%) | 7(11.29%) | | |
| Secondary high school | 10(16.12%) | 2(3.23%) | | |
| Tertiary | 6(9.68%) | 3(4.8%) | | |
| Occupation | | | 0.9 | 0.4 |
| Dairy farm workers | 41(66.13%) | 15(24.19%) | | |
| Farm Manager | 4(6.45%) | 1(1.61%) | | |
| Assistant veterinarian | 1(1.61) | 0 | | |
| Sex | | | 5.1 | 0.4 |
| Male | 32 (51.61%) | 13(20.97%) | | |
| Female | 14 (22.58%) | 9(14.52%) | | |

Fisher's exact test= significance level $P < 0.05$

DISCUSSION

The overall animal level prevalence of BTB (16.5%) which is similar to the previous findings from central Ethiopia 17.5%,16.1 % 18.7%,13.5%,11.4% respectively (Alemu,1992; Regassa, 2005; Shitaye et al., 2007; Ameni et al., 2007; Rebuma et al.,2012; Biru et al., 2014). The prevalence of BTB at farm level was higher compared to overall prevalence, 27.2% at Bishoftu and 22.9% in Holeta dairy farms. This finding concurs with previous studies conducted in Zway, Holeta, Ambo and Bishoftu (Ameni et al., 2003b; Kiros, 1998). Lower prevalence of 2.14% was recorded in Jersey breeds at Adaberga Dairy Farm which was similar to previous study conducted in Assela, 3.5% (Redi, 2003). Generally, the prevalence report in this study was lower compared to the previous report (46.16%; n=860) (Ameni et al., 2003b) and 34.34% (n= 11320) (Wondewosen et al., 2010) from Addis Ababa dairy farms kept under intensive management system. The possible reason for the lower prevalence of BTB in this study could be the semi-intensive management system of the studied farms which might decrease the risk of transmission. Higher prevalence of BTB was reported so far by different authors in intensive production system than semi-intensive and extensive production system (Ameni et al., 2003b; Ameni et al., 2007; Cleaveland et al., 2007). Intensification and overcrowding of animals cause stresses and also make favorable condition for the transmissions of *M.bovis* between animals. Since major transmission route is respiratory and husbandry system of the animals could affect the tuberculin skin test prevalence (Ameni et al., 2007).

In this study, the prevalence of BTB was significantly higher in Holstein Boran cross breeds than local zebu cattle managed under similar husbandry system. Ameni et al. (2007) also reported a lower prevalence of BTB in local zebu than either exotic breeds or exotic crosses. Regarding body condition scoring, tuberculin reactivity was significantly higher in cattle in good physical condition than poor body conditioned animals which was 83(24.48%) ($\chi^2= 29.70$; $p=0.001$) which is similar to the previous study (Ameni et al., 2001, Ameni et al., 2005, Ameni et al., 2007; Kiros,1998 and Wondewosen et al., 2010). This could be because the tuberculin reaction is dependent on immune-competence, which in turn may be associated with the physical condition of the animals such that animals with better physical condition, in good level of nutrition in terms protein-energy and micronutrients, are immune-competent and thus give a better reaction to comparative tuberculin skin test (Ameni et al.,2007; Pollock and Neill, 2002). But animals with poor physical condition could be immune-compromised, and hence may not react to tuberculin although they might have been infected by mycobacteria.

The prevalence of BTB was significantly higher in adult and older cattle than the young animals. Similarly, previous studies in Ethiopia, Canada and Northern Ireland indicated increased incidences of bovine TB with increased age (Ameni et al., 2007; Rebuma et al., 2012). The reason could be, as suggested earlier by Mackay and Hein (1989), the possible influence of gamma delta T cells ($\gamma\delta$ T cells), which are predominantly found in the circulation of young calves shown to have an anti-mycobacterial role. It has been suggested that in older animals can be explained increased incidence of TB by a waning (decreasing) of protective capability in aging animals as experimentally confirmed in the murine system (O'Reilly and Daborn, 1995). Furthermore, it could be also be due to the increase in the probability to encounter *M.bovis* with a longer period of life (Barwinek and Taylor, 1996). The difference in results between ages of cattle could also be a result of the slow progression (latent infection) of disease to a detectable level. In contrast immature and very old animals rarely react to tuberculin inoculation regardless of the status of infection; the level of reaction is directly related to maturation and wasting of organs of immune system (Buddle et al., 2003)

Prevalence of bovine tuberculosis was significantly influenced by the reproductive stage of female animals. This could be because some of the reproductive stages (late pregnancy and early lactation) are stressful and thus reduce the response of the female animal to tuberculin skin test. This result is similar to the previous study (Ameni et al., 2005). Although the difference was not statistically significant, the prevalence of the disease was higher in males than female cattle in this study. It could be because of the relatively small number of male cattle included in this study. The result of questionnaire survey showed the habit of raw milk and meat consumption was 66.1% (41/62) and 74.20 % (21/62) respectively which is higher than the previous report (Ameni et al., 2003a). On the other hand, habit of consuming raw milk was lower when we compare with the other study report (Ameni et al., 2005). Consumption of raw milk was significantly higher ($\chi^2=12.51$; $p<0.05$) in respondents who has no formal education. The result of raw milk consumption was significantly higher in dairy farm workers ($\chi^2 = 9.61$; $p<0.05$) than assistant veterinarians and farm manager. Most of the respondents in the study farms were none educated formally and were lucky to have become aware about bovine tuberculosis. On the other hand, meat consumption habit was not influenced by education level, occupation type and residence of the respondent which is different from previous study (Ameni et al., 2003a). Consumption of raw meat is a well come tradition in Ethiopia regardless of knowledge.

CONCLUSION

In conclusion bovine tuberculosis is distributed throughout the study farms. At least on dairy cattle were positive to bovine tuberculosis in all tested dairy farms. The prevalence of tuberculosis was influenced by breed, age, and body

condition, reproductive status and location of the dairy farm. Holstein cross breed, with old aged, good body conditioned dairy cattle were at higher risk to be reactor to tuberculin skin test. Most of the respondents consume raw animal products and by products regardless of residence, sex, occupation and education level. Raw milk consumption was influenced by level of education and occupation type. Dairy farm workers consume more raw milk when compared to other groups. Knowledge of the respondents on BTB transmission to humans was affected by education level and residence of the respondents. As level of education increases the awareness level of the respondents on zoonotic importance of BTB increases and respondents living in urban areas were more aware about the transmission of BTB to humans.

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Competing interests

The authors have declared that no competing interest exists in relation to this manuscript.

Author's contribution

Mulualem Ambaw Endalew designed the experiment, collected data and tuberculin testing, data management, entry, analysis and paper writing. Benti Deresa Gelalcha designed the experiment, manuscript write up, commenting and approval. Gobena Ameni chimdi wrote, commenting and approval of the paper.

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Retrospective Study on Survival Time of Cats with Mammary Carcinomas Undergoing Surgery Alone or with Adjuvant Chemotherapy

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ABSTRACT

This retrospective study was carried out to evaluate disease free interval (DFI) and survival time of cats with mammary carcinomas that underwent mastectomy (RM) and adjuvant chemotherapy (RMAC) in 35 cats to remove the neoplastic mammary chain and regional inguinal lymphadenectomy. According to performed treatment, the cats were divided into two groups. The RM group (21 cats) received no adjuvant therapy, and the RMAC group (14 cats) received chemotherapy with mitoxantrone or doxorubicin. Histopathological margins were considered complete in all cases. Eight cats had histologically confirmed lymph node involvement at the time of surgery. Three cases were classified as stage I, 21 cases as II and eight cases as III. Nine cats had tumor recurrence (four cats of RM group and five cats of RMAC group) and 12 cats had distant metastasis to the lungs (six cats of each group). Mean and median survival times were 1625 and 2404 days in the RM group, while mean DFI was 815 days. In RMAC group, mean and median survival times were 719 and 690 days, while mean DFI was 549 days. Surgery remains the main treatment and more studies are necessary to evaluate the benefit of adjuvant chemotherapy.

Key words: Feline, Mammary carcinoma, Oncology, Surgery, Chemotherapy

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INTRODUCTION

Mammary tumors are usually malignant in cats, with high metastatic rate. Distant metastasis can occur to the lungs, pleura, liver, diaphragm, adrenal gland, spleen, kidney, uterus and ovary (Macewen et al., 1984; Ito et al., 1996; Castagnaro et al., 1998; Viste et al., 2002; Borrego et al., 2009; Gimenez et al., 2010; Matos et al., 2012; Morris, 2013; Mills et al., 2015; Campos et al., 2016). Neutered animals are less likely to develop tumors than intact cats (Misdorp et al., 1992).

Surgery is the main treatment for mammary tumors in cats. Complete surgical intervention may be adequate for treatment of small tumors. However, for cats with larger tumors, postoperative survival is reported to be <1 year and many of these cats die from metastatic disease. Adjuvant chemotherapy postoperatively may increase survival time in these cases (Mcneill et al., 2009).

Tumor size, extent of surgery, histologic grade, lymph node involvement, lymphovascular invasion, tumor size and tumor grade have been described as prognostic factors. Other factors that influence disease-free interval and survival are clinical staging, histologic subtype, molecular subtyping, overexpression of Her2, mitotic index, development of metastatic disease and location of metastatic disease (Macewen et al., 1984; Ito et al., 1996; Castagnaro et al., 1998; Viste et al., 2002; Gimenez et al., 2010; Matos et al., 2012; Morris, 2013; Mills et al., 2015; Campos et al., 2016; Marques et al., 2016; Soares et al., 2016).

The objective of this study was to investigate disease free interval (DFI) and survival time (ST) of cats with mammary carcinomas that underwent radical unilateral mastectomy (RM) or radical unilateral mastectomy with adjuvant chemotherapy (RMAC).

MATERIALS AND METHODS

A retrospective study evaluated 35 cats diagnosed with mammary carcinoma from August 2013 to August 2016, regardless of breed, age or reproductive status, were studied. Cats with distant metastasis at diagnosis and / or unresectable tumors were excluded of the study.

All cats underwent tumor staging before surgery including complete history, physical examination, measurement of tumors, Complete Blood Count (CBC), serum biochemistry profile, three view thoracic radiographs and abdominal ultrasound. The animal's reproductive status were classified as early spayed (ovariohysterectomy was performed less than 1 year old), late spayed (more than 1 year old) or intact. Oncologic examination included palpation of mammary nodules, gland localization, mass measurement and regional lymph node palpation.

An aggressive treatment (radical unilateral mastectomy or radical bilateral mastectomy in steps) was performed in all cases, and no aspiration or biopsies were performed prior to surgery. All cats underwent radical unilateral mastectomy. If tumors were present at both chains, staged bilateral mastectomy was performed (the other mammary chain was removed 4-8 weeks after the first surgery). A three-centimeter margin was obtained around tumors. For staging purposes, regional inguinal lymphadenectomy was performed in all cases. Axillary lymphadenectomy was performed if the lymph node was enlarged or visible during surgery. After histopathology, cats were classified according to the WHO TNM staging system (Owen, 1980).

The animals were divided in two groups, according to treatment. The RM group received no adjuvant therapy. The RMAC group received chemotherapy starting at the time of suture removal (15-30 days after surgery). The chemotherapy regimen consisted of four doses of doxorubicin (ADRIBLASTINA; Pfizer, Rio de Janeiro) administered at a dose of 20 mg/m², as a slow intravenous (IV) injection, once every 3 weeks. Mitoxantrone (EVOMIXAN; Evolabis, São Paulo) was administered at a dose of 6 mg/m², as a slow intravenous (IV) injection, once every 3 weeks in cats with evidence of renal disease. Ondansetron (VONAU; Biolab, Rio de Janeiro) was administered to all cats, at a dose of 0.5 mg/kg q 12 h, orally in the first seven days after chemotherapy, in order to prevent nausea.

Local recurrence was defined as the presence of a mass in the site of surgery (removed mammary chain) and new tumor was defined as presence of a mass in the other mammary chain, and these were determined by physical examination. Thoracic radiographs were performed every three months, or when there was clinical evidence of metastasis (dyspnea and/or cough). Cats that developed local recurrence or new tumors after treatment were offered surgery and those in which distant metastasis were found were advised for euthanasia when there was poor life quality.

All cases were included in the statistical analysis. The DFI was defined as the time from surgery until the development of local recurrence or metastatic disease. Survival was defined as the time from the original surgery until death from any cause. Median DFI and ST were determined by the use of the Kaplan–Meier product-limit method. Results shown are median number of days with 95% confidence intervals. The effect on survival and DFI of both groups, tumor size and regional metastasis were examined using Kaplan–Meier survival analysis with logrank and Wilcoxon tests.

Ethical Approval

This project was approved by CEUA (Comitê de Ética no Uso de Animais) of Fluminense Federal University, Brazil with the protocol n. 548.

RESULTS

Thirty-five cats with histologically confirmed mammary carcinoma were studied from August 2013 to August 2016. All cats were female and age ranged from 5 to 14 years old (mean 10 years). Twenty-five cats (71%) were mixed breed, six (17%) were Siamese, three (9%) were Persian and one (3%) was British shorthair. Mean weight was 4.32 kg. Nine cats (26%) were intact, four cats (11 %) were early spayed, 14 cats (40%) were late spayed and eight cats (23%) were spayed at unknown date.

Twenty-eight cats had a single tumor and seven cats had multiple tumors. Nodules were located in the caudal glands in 26 cases, in the cranial glands in eight cases and were multiple and poorly circumscribed in four cases. Eight cats (23%) had histologically confirmed lymph node involvement at the time of surgery. Three cases (9%) were histologically classified as stage I, 24 cases (69%) as stage II and eight cases (22%) as stage III.

None of the cats had prior surgeries for mammary tumors. Twenty-seven cats underwent unilateral mastectomy (77%) and eight cats underwent bilateral mastectomies performed in different surgeries (23%). Ovariohysterectomy was performed in all intact cats at the time of surgery prior to tumor removal. Histopathological margins were considered complete in all cases.

Ten cats had evidence of renal disease at the time of diagnosis based on sonographic abnormalities or evaluation of urinalysis, but only three cats had azotemia. These cats received subcutaneous fluid therapy (150 mL three times a week) during chemotherapy. Mitoxantrone was used instead of doxorubicin in these cats. Twenty-one cats were included in the RM group and 14 cats were included in the RMAC group. Two cats (14%) received doxorubicin chemotherapy and 12 cats (86%) received mitoxantrone chemotherapy. The most frequent adverse events of chemotherapy were azotemia (seven cases), leukopenia (four cases), anorexia (two cases) and vomiting (one case).

Nine cats (26%) had tumor recurrence (four cats of RM group and five cats of RMAC group) and 12 cats (24%) had distant metastasis to the lungs (six cats of each group). Seventeen cats of the study are dead, 13 because of disease progression or distant metastasis. The other four cats died due to unrelated causes (renal disease, hepatic lipidosis, soft tissue sarcoma and unknown cause). Eighteen (18/35) cats are still alive and being monitored, most of them free of disease. Mean and median survival times were 1625 and 2404 days in the RM group, while mean DFI was 815 days (Table 1). In the RMAC group, mean and median survival times were 719 and 690 days, while mean DFI was 549 days (Table 2 and Figure 1).

High grade tumors had significantly lower survival times and DFI. Grade III tumors had mean ST and DFI of 637 and 471 days, respectively, whereas grade II tumors 1405 and 756 days. Regional metastasis was also correlated to ST and DFI, as cats with metastasis to lymph node at the time of surgery had lower survival times, but there were no statistical significance (Figure 2). Tumor size was not correlated to prognosis.

Table 1. Age, breed, reproductive status, affected gland, histopathology, histological grade, regional metastasis, disease free interval, survival time and evolution of cats with mammary carcinoma treated with surgery alone (RM)

| Cat | Age | Breed | Reproductive status | Gland | Histopathology | Grade | LN | DFI | ST | Status |
|-----|-----|-------------------|---------------------|----------|-------------------|-------|----------------|------|------|----------------|
| 1 | - | Persian | Unknown spayed | A | Adenocarcinoma | I | N ₀ | 620 | 620 | Alive |
| 2 | - | Mixed | Early spayed | I | Adenocarcinoma | II | N ₀ | 568 | 568 | Alive |
| 3 | 9 | Mixed | Intact | I | Adenocarcinoma | II | N ₀ | 630 | 630 | Alive |
| 4 | 12 | Mixed | Early spayed | I | Adenocarcinoma | II | N ₀ | - | 1080 | Dead (Sarcoma) |
| 5 | 13 | Mixed | Unknown spayed | I | Adenocarcinoma | II | N ₀ | 960 | 960 | Alive |
| 6 | - | Mixed | Unknown spayed | I | Adenocarcinoma | II | N ₀ | 781 | 781 | Alive |
| 7 | 12 | Persian | Late spayed | I | Carcinoma in situ | I | N ₀ | 911 | 911 | Alive |
| 8 | - | Siamese | Unknown spayed | I | Adenocarcinoma | II | N ₀ | 633 | 633 | Alive |
| 9 | 10 | Mixed | Intact | A | Adenocarcinoma | II | N ₀ | 596 | 596 | Alive |
| 10 | 13 | Mixed | Intact | A | Adenocarcinoma | II | N ₀ | 473 | 473 | Alive |
| 11 | 11 | Mixed | Late spayed | I | Adenocarcinoma | III | N ₁ | 291 | 291 | Alive |
| 12 | - | Mixed | Unknown spayed | I | Adenocarcinoma | II | N ₀ | 120 | 120 | Dead (D) |
| 13 | 8 | Mixed | Intact | T, I | Adenocarcinoma | III | N ₁ | 360 | 360 | Dead (D) |
| 14 | 11 | Mixed | Late spayed | T | Adenocarcinoma | II | N ₀ | - | 7 | Dead (U) |
| 15 | 13 | British shorthair | Late spayed | T, A | Adenocarcinoma | II | N ₀ | 120 | 120 | Dead (D) |
| 16 | 14 | Siamese | Late spayed | A | Adenocarcinoma | II | N ₀ | 480 | 2404 | Dead (D) |
| 17 | 7 | Mixed | Late spayed | Multiple | Adenocarcinoma | II | N ₀ | 570 | 570 | Dead (D) |
| 18 | 5 | Persian | Early spayed | I | Adenocarcinoma | II | N ₀ | 1800 | 1800 | Alive |
| 19 | 8 | Mixed | Unknown spayed | I | Adenocarcinoma | I | N ₀ | 935 | 935 | Alive |
| 20 | 9 | Mixed | Intact | I | Adenocarcinoma | II | N ₀ | 990 | 1140 | Dead (D) |
| 21 | 12 | Mixed | Intact | Multiple | Adenocarcinoma | II | N ₀ | 999 | 999 | Alive |

*A: Abdominal gland; I: Inguinal gland; T: Thoracic gland; LN: Lymph node metastasis; D: Died from disease progression or metastasis; U: Died from unknown cause.

Table 2. Age, breed, reproductive status, affected gland, histopathology, histologic grade, regional metastasis, disease free interval, survival time and evolution of cats with mammary carcinoma treated with surgery and chemotherapy (Group RMAC).

| Cat | Age | Breed | Reproductive status | Gland | Histopathology | Grade | LN | Chemotherapy | DFI | ST | Status |
|-----|-----|---------|---------------------|----------|-------------------------|-------|----------------|--------------|-----|------|------------|
| 1 | 13 | Mixed | Early spayed | T | Adenocarcinoma | III | N ₀ | Mitoxantrone | 270 | 450 | Dead (D) |
| 2 | 11 | Siamese | Intact | A | Adenocarcinoma | II | N ₁ | Mitoxantrone | 700 | 700 | Dead (D) |
| 3 | 10 | Mixed | Late spayed | T, A | Adenocarcinoma | II | N ₀ | Doxorubicin | 70 | 180 | Dead (D) |
| 4 | 14 | Mixed | Intact | I | Adenosquamous carcinoma | III | N ₁ | Mitoxantrone | 570 | 630 | Dead (D) |
| 5 | 11 | Siamese | Late spayed | T | Adenocarcinoma | III | N ₀ | Mitoxantrone | 420 | 420 | Dead (CRF) |
| 6 | 12 | Mixed | Late spayed | T | Adenocarcinoma | II | N ₀ | Mitoxantrone | 379 | 379 | Alive |
| 7 | 10 | Mixed | Late spayed | I | Adenocarcinoma | III | N ₀ | Mitoxantrone | 398 | 398 | Alive |
| 8 | 10 | Siamese | Unknown spayed | I | Adenocarcinoma | II | N ₁ | Mitoxantrone | 743 | 743 | Alive |
| 9 | 13 | Mixed | Late spayed | T | Adenocarcinoma | III | N ₁ | Mitoxantrone | 360 | 690 | Dead (D) |
| 10 | 13 | Siamese | Late spayed | A | Adenocarcinoma | II | N ₀ | Mitoxantrone | 340 | 340 | Dead (D) |
| 11 | 10 | Mixed | Late spayed | I | Adenocarcinoma | III | N ₁ | Mitoxantrone | 720 | 1230 | Dead (D) |
| 12 | 7 | Mixed | Unknown spayed | Multiple | Adenocarcinoma | II | N ₀ | Mitoxantrone | 770 | 770 | Dead (HL) |
| 13 | 13 | Mixed | Late spayed | A | Adenocarcinoma | II | N ₁ | Doxorubicin | 886 | 886 | Alive |
| 14 | 10 | Mixed | Intact | Multiple | Adenocarcinoma | II | N ₀ | Mitoxantrone | 226 | 226 | Alive |

*A: Abdominal gland; I: Inguinal gland; T: Thoracic gland; LN: Lymph node metastasis; D: Died from disease progression or metastasis; CRF: Died from chronic renal failure; HL: Died from hepatic lipidosis

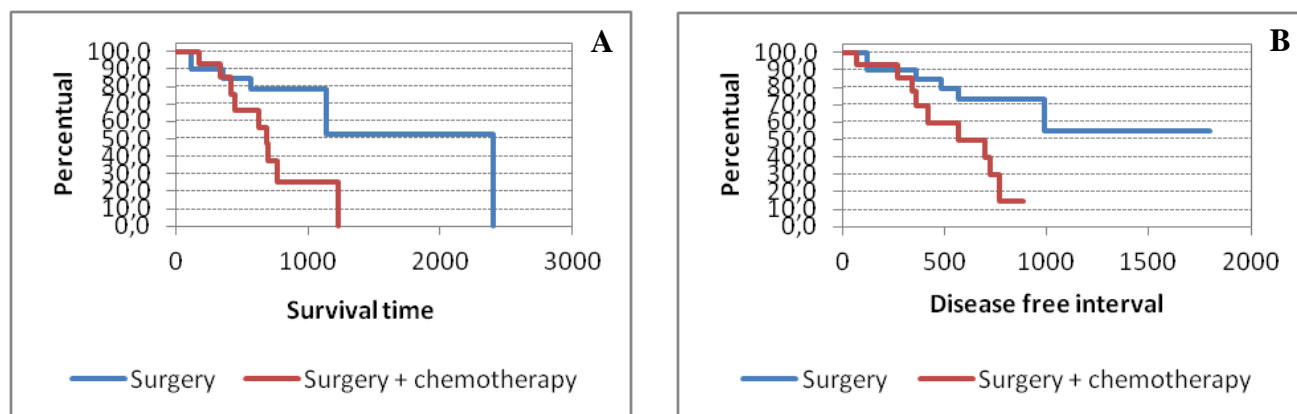


Figure 1. Kaplan-Meier survival plot of cats with mammary carcinoma according to treatment group (surgery or surgery and chemotherapy). A: Survival time in both groups. B: Disease free interval in both groups

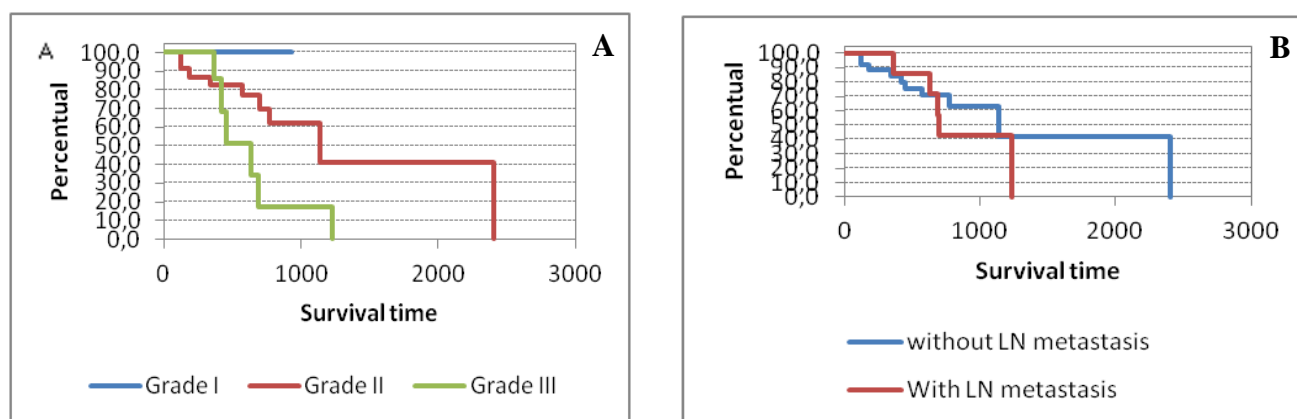


Figure 2. Kaplan-Meier survival plot of cats with mammary carcinoma according to tumor histologic grade (A) and presence of lymph node metastasis at surgery (B)

DISCUSSION

The mean age of cats in this study is similar to that reported in other studies (Borrego et al., 2009; Cunha et al., 2015). Intact or late spayed cats were the majority of cases in this study, suggesting that early ovariohysterectomy reduces the risk of developing a mammary tumor in cats (Misdorp et al., 1992; Overley et al., 2003).

Adjuvant chemotherapy is commonly recommended for the treatment of feline mammary tumors. However, few studies have evaluated the benefit of adjuvant chemotherapy (Jeglum et al., 1985; Novosad et al., 2006; Borrego et al., 2009; Mcneill et al., 2009; Campos et al., 2014; Cunha et al., 2015). The mean survival time of cats with mammary tumors undergoing surgery alone is only 10–14 months, with the vast majority of cats dying of metastatic disease (Castagnaro et al., 1998). Cats subjected to radical surgery plus doxorubicin had median survival times and median disease free interval of 448 and 255 days respectively in one study (Novosad et al., 2006) and disease free interval and survival time were 269 and 460 respectively when a cox-2 inhibitor was added (Borrego et al., 2009). In the present study, mean and median survival times were 1625 and 2404 days in RM group, with mean DFI of 815 days. In RMAC group, mean and median survival times were 719 and 690 days, with mean DFI of 549 days. There was no benefit in ST and DFI of cats that received adjuvant chemotherapy. A possible hypothesis is that the RMAC group had 42,8% mammary carcinomas staged as grade III, while the RM group had only 9,5% grade III tumors. High grade tumors would have a poorer prognosis despite adjuvant treatment (Seixas et al., 2011).

High grade tumors had significantly lower survival times and DFI, as previously reported (Preziosi et al., 2002; Seixas et al., 2011; Mills et al., 2015). Grade III tumors had mean ST and DFI of 637 and 471 days, respectively, whereas grade II tumors 1405 and 756 days. Most of cats with grade III tumors also had lymph node metastasis and were therefore submitted to adjuvant chemotherapy. Chemotherapy might have improved ST in these cases (Campos et al., 2014), but this data can't be analyzed in this study as only two cats with grade III tumors did not receive chemotherapy and ST couldn't be compared.

Eight cats (23%) had histologically confirmed lymph node involvement at the time of surgery. Three cases (9%) were histologically classified as stage I, 24 cases (69%) as stage II and eight cases (22%) as stage III. Cats with metastasis to lymph node at the time of surgery had lower survival times, but there was no statistical significance. The presence of lymph node metastases also showed statistically significant negative correlation with survival in previous studies (Mills et al., 2015). Tumor size was not correlated to prognosis in this study, which differs from Viste et al., 2002.

Cats with evidence of renal disease are not good candidates for doxorubicin chemotherapy because of its nephrotoxicity properties (O'Keefe et al., 1993). An alternative option for these cases is mitoxantrone (Cunha et al., 2015). Nevertheless, some cats (six cats) developed azotemia. Mitoxantrone should be administered with caution, especially in cats with renal disease. In conclusion, surgery remains the main treatment and adjuvant chemotherapy has not been proven to be of benefit. It may have a role in grade III tumors and/or cats with lymph node metastasis, yet to be defined.

Competing Interests

The authors have no competing interests to declare.

Author's contribution

The authors Simone Cunha, Katia Corgozinho and Heloisa Souza were responsible for the clinical, oncological, surgical and chemotherapeutic treatment of the cats, as well as the article writing. The authors Kassia Silva, Juliana Leite, Marcela Mello and Ana Ferreira performed the histopathology, and review of the manuscript.

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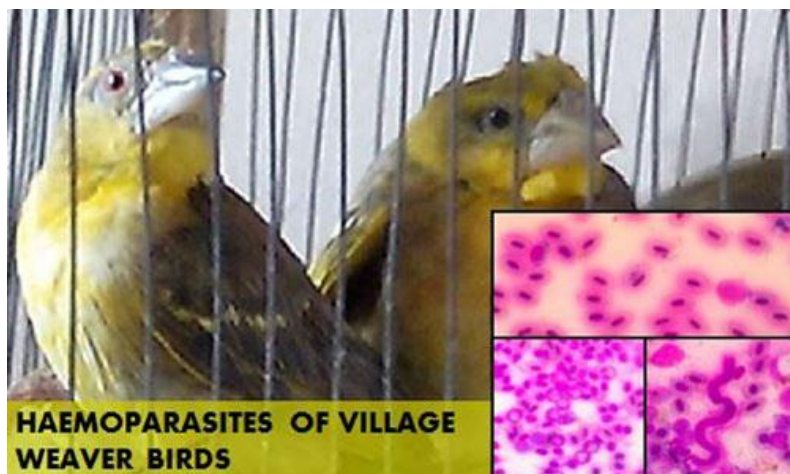
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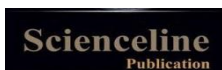
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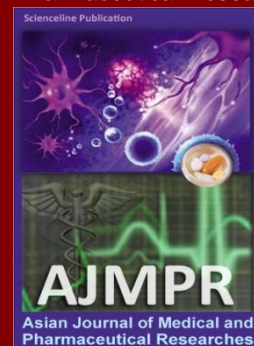
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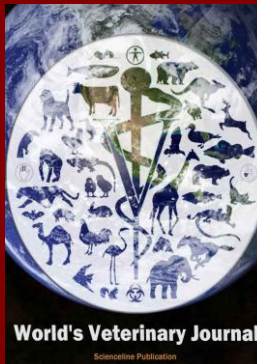
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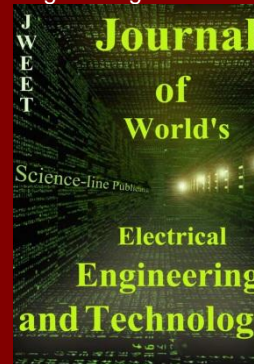
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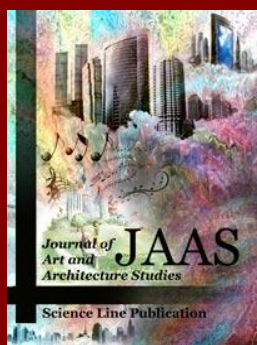
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