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Dedication

This first issue of the University of Bahri Journal of Veterinary Sciences is dedicated to the soul of Dr. Abdelwahab Elkitayabi who died in April 2021. The late Elkitayabi was a member of the Editorial Board. This work wouldn't be possible without his great contribution.

RESEARCH ARTICLE

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Internal and external biosecurity practices in poultry layer farms in Nyala, South Darfur State, Sudan

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KEYWORDS

Internal biosecurity
External biosecurity
Manure
Dead birds

ABSTRACT

The present study was conducted to evaluate quantitatively the internal and external biosecurity practices in layer farms in Nyala, South Darfur State, Sudan. Twenty-five commercial layer farms were investigated. Data were collected by means of a questionnaire. The respondents included farm owners, farm management and veterinarians. The overall biosecurity of the poultry farms was set up in two major categories, an internal and an external one. The two categories comprised a total of thirteen subcategories, each of which was given a score that ranged from 0.00 to 1.00. The overall score of the biosecurity in layer farms was 0.50. The score of the external biosecurity was 0.53 whereas that of the internal biosecurity was 0.64. The difference between the external and the internal biosecurity score was insignificant ($P>0.05$). No significant correlation ($P>0.05$) was observed between the scores of the external and the internal biosecurity. The highest score in the external biosecurity was observed in export of live animals, whereas the highest score in the internal biosecurity was found in disease management. Acceptable levels of biosecurity were found in measures related to the purchase of one day-old-chicks, as only 4% of the farms had poor practices. Strong positive correlation (0.48) was found between both removal of manure and dead animals, and infrastructure and biological vectors. The removal of manure and dead animals has also shown positive correlation (0.42) in relation to the entrance of visitors. In conclusion, the present study revealed poor biosecurity practices in layer farms in Nyala. More attention is recommended to raise the awareness of supervisors as well as farm owners on the importance of applying good farm biosecurity measures.

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INTRODUCTION

Poultry production has become one of the most popular and visible enterprises (Paul *et al.*, 2004). It is characterized by a huge diversity of production systems, with different scales of production, bird species, preventive measures, production inputs and outputs (Van Steenwinkel *et al.*, 2011).

In general, profitable poultry industry is always characterized by quick body gain and high egg production with less utilization of feed (Paul *et al.*, 2004). However, disease outbreaks will predominantly result in economic losses for individual farmers (Gelaude *et al.*, 2014). In order to tackle such issue, biosecurity has been considered as an essential component of modern flock health program.

Biosecurity is defined as a set of preventive measures designed to minimize the transmission of infectious diseases between and within farms (Dorea *et al.*, 2010).

In Sudan, few studies have carried out on biosecurity status in poultry farms (Mahmoud *et al.*, 2014; Tabidi *et al.*, 2014; Maisa, 2017). This is in addition to the fact that these studies were almost carried out in Khartoum State. Moreover, literature search has revealed no available data concerning biosecurity in poultry farms in Western Sudan, in particular in Nyala, the capital city of South Darfur State. Therefore, the present study was conducted to draw baseline information on the biosecurity status of poultry farms in the vicinity of Nyala city, South Darfur State, Sudan.

MATERIALS AND METHODS

Type, duration, and area of the study

The study was a cross sectional that was carried out during the period from August 2019 to December 2019 in Nyala city, the capital state of South Darfur.

Data were collected from 25 commercial layer farms which represented all the farms in the study area. Data on the internal and the external biosecurity measures were collected by means of a questionnaire that was designed according to the guidelines given by Gelaude*et al.* (2014). The questionnaire was conducted during the farm visits and each farm was visited every 3 days. The respondents were farm owners, farm managers and veterinarians.

The questionnaire included two main categories, an internal and an external biosecurity. Each category consisted of a set of subcategories. The external biosecurity consisted of 10 subcategories which were purchase of one day old chicks, source of feed, source of potable water, exports of live animals, feed supply, removal of manure and dead animals, entrance of visitors and personnel, supply of materials, infrastructure and biological vectors, location of farms. The internal biosecurity consisted of 3 subcategories which were disease management, cleaning and disinfection, materials and measures between compartments.

A score ranging from 0.0 to 1.0 was given to each subcategory. The scores were ranked as follows:<50% poor; 0.50 to0.70 good; 0.70 to 0.90 very good; 0.90 to 0.10excellent. The scores of the internal and the external as well as the overall biosecurity of each farm were calculated.

Data analysis

Data were analyzed by Statistical Packaging for the Social Sciences software program (SPSS, version 21 for Windows). Descriptive statistical analysis was applied on the collected data. The difference between the external and the internal biosecurity scores was applied using Student's T-test. The correlation between the external and the internal biosecurity scores was assessed by using Spearman's Rho Coefficient correlation test. The test was also conducted to examine the correlations between the subcategories of both biosecurity. Significant differences/correlations were reported when $p<0.05$.

RESULTS

All of the 25 farms examined in this study were open-floor system. The majority of the farms (96 %) had 1 to 5 houses; only one farm (4%) had more than 5 houses. The number of workers per farm was less than 5 in all farms.

The overall biosecurity score in layer farms in Nyala was 0.56. The score of external biosecurity was 0.53 whereas that of internal biosecurity was 0.64 (Table 1). The difference between the external and internal biosecurity scores was insignificant

($p>0.05$). In addition, no significant correlation ($p>0.05$) was observed between the scores of external and internal biosecurity.

Table 1: Ranking of the scores (Mean ±SD) of biosecurity in layer farms in Nyala, South Darfur (N= 25).

Item	Score	Rank
External biosecurity	0.53 ± 0.05	Good
Internal biosecurity	0.64 ±0.06	Good
Overall Biosecurity	0.56 ± 0.04	Good

SD: Standard deviation

The overall biosecurity was good in 88% of the farms and poor in 12% of them. Sixty eight percent of the farms has shown good external biosecurity practices whereas 72 % internal biosecurity has shown good practices (Fig. 1). Poor external biosecurity measures were observed in 32% of the farms. Most of the farms (88%) revealed acceptable score (good) for overall biosecurity but none of them displayed high level (excellent) of biosecurity (Figs. 1 and 2).

Table 2 displays the scores and ranks of the subcategories of the external and the internal biosecurity. Among the subcategories of external biosecurity, excellent practices (score 0.95) were recorded in export of live animals. Very good biosecurity measures were observed in location of the farm (score 0.71) and disease management (0.85) whereas good measures were seen in the subcategory purchase of one day old chicks (0.66), feed supply (0.59) and infrastructure and biological vectors (score 0.52). The remaining subcategories of external biosecurity had poor scores. The internal biosecurity demonstrated optimal score (0.85) but poor measures related to the remaining two subcategories were evident by low scores (0.45 and 0.30).

Table 3 shows the ranking of biosecurity subcategories in layer farms. The external biosecurity showed that all farms (100%) had poor biosecurity practices in relation to the source of potable water, and supply of materials subcategories. Nighty six percent revealed poor practices in terms of removal of manure and dead animals whereas 4% of the farms showed good practices. Poor practices related to entrance of visitors and personnel were also evident in 92 % of the farms whereas 8% of the farms showed good practices. About two third of the farms (64%) have shown poor measures of source of feed and 36% of them showed good measures. Different levels of biosecurity were seen in purchase of one day old chicks, in which only 4% of the farms had poor practices, 48% had good practices, 40% very good practices and the remaining 8% had excellent practices. High level of external biosecurity measures was only observed in the subcategory exports of live animals; 68% of the farms showed excellent practices, 24% showed very good practices but none of them exhibited poor practices. Regarding the internal biosecurity, all farms (100%) showed poor measures related to materials and measures between compartments. Poor cleaning and disinfection measures were also recorded in 92% of the farms. The implementation of disease management was excellent in 40% of the farms and very good in 56% of the farms and good in 4% of the farms.

Table 4 presents correlation between the scores of different subcategories of biosecurity. Positive correlation was observed between both purchase of one day old chicks and source of feed (0.41), and export of live animals (0.42) ($p<0.05$). In contrast, purchase of one day old chicks negatively correlated with materials and measures between compartments (- 0.40) ($p<0.05$). Export of live animals showed negative correlation between both feed supply (0.53) ($p<0.01$), along with materials and measures between compartments (-0.45) ($p<0.05$). Feed supply positively correlated with three subcategories, namely entrance of visitors and personnel (0.41), cleaning and disinfection (0.40), and materials and measures between compartments (0.43) ($p<0.05$). Removal of manure and dead animals demonstrated positive correlation with entrance of visitors and personnel (0.42), and infrastructure and biological vectors (0.48) ($p<0.05$). Cleaning and disinfection also showed positive correlation (0.41) with materials and measures between compartments ($p<0.05$).

Table 2: The overall ranking of the scores (Mean ±SD) of biosecurity subcategories in layer farms (N= 25) in Nyala, South Darfur.

Biosecurity	Subcategory	Score	Rank
External biosecurity	Purchase of one day old chicks	0.66±0.14	Good
	Source of feed	0.31±0.24	Poor
	Source of potable water	0.36±0.08	Poor
	Exports of live animals	0.95±0.16	Excellent
	Feed supply	0.59 ±0.12	Good
	Removal of manure and dead animals	0.31±0.15	Poor
	Entrance of visitors and personnel	0.27 ±0.10	Poor
	Supply of materials	0.22±0.25	Poor
	Infrastructure and biological vectors	0.52±0.13	Good
	Location of the farm	0.71±0.14	Very good
Internal biosecurity	Disease Management	0.85±0.10	Very good
	Cleaning and disinfection	0.45 ±0.08	Poor
	Materials and measures between compartments	0.30 ±0.25	Poor

SD: standard deviation; Poor: < 0.50 score; Good: score 0.50 to 0.70; Very good: score 0.70 to 0.90; Excellent: score 0.90 to 1.0

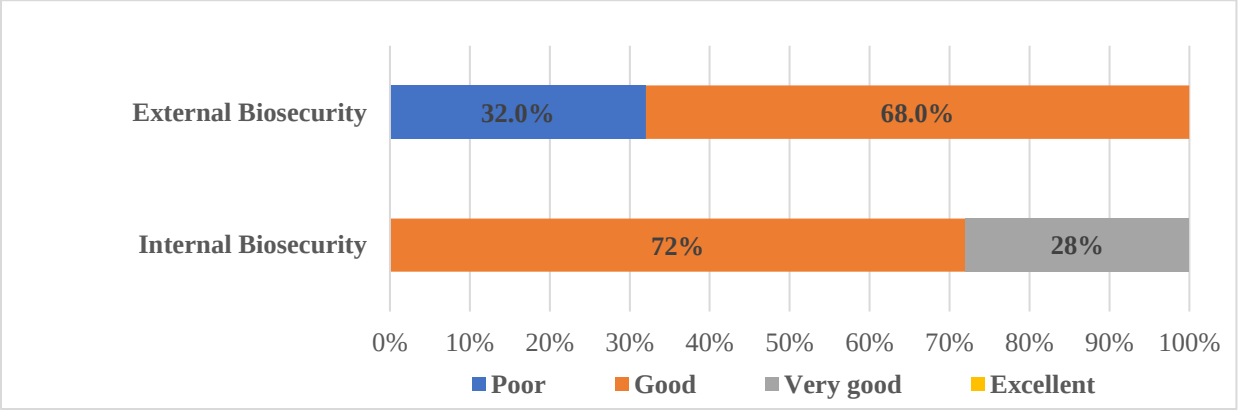

Figure 1: Ranks of the external and the internal biosecurity scores of layer farms in Nyala, South Darfur.

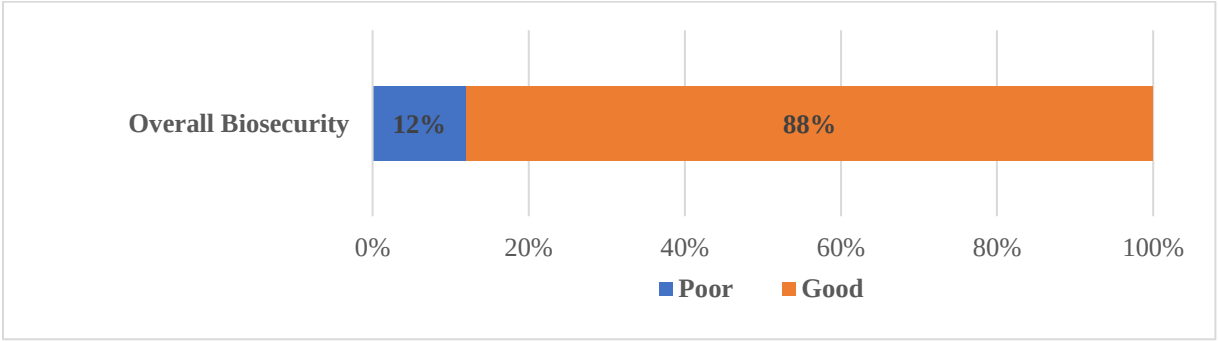

Figure 2: Ranks of overall biosecurity scores of layer farms in Nyala, South Darfur.

Table 3: Percentage of the ranks of biosecurity subcategories in layer farms (N= 25) in Nyala, South Darfur.

Biosecurity	**Subcategory**	**Rank**			
		P	**G**	**VG**	**E**
External biosecurity	Purchase of one day old chicks	4%	48%	40%	8%
	Source of feed	64%	36%	-	-
	Source of potable water	100%	-	-	-
	Exports of live animals	-	8%	24%	68%
	Feed supply	16%	60%	24%	-
	Removal of manure and dead animals	96%	4%	-	-
	Entrance of visitors and personnel	92%	8%	-	-
	Supply of materials	100%	-	-	-
	Infrastructure and biological vectors	40%	56%	4%	-
	Location of the farm	16%	40%	40%	4%
Internal biosecurity	Disease Management	-	4%	56%	40%
	Cleaning and disinfection	92%	8%	-	-
	Materials and measures between compartments	100%	-	-	-

P: poor (< 0.50); **G:** good (0.50-0.70); **VG:** very good (0.70-0.90); **E:** excellent (0.90-1.0).

Table 4: Correlation between biosecurity subcategories in layer farms (N= 25) in Nyala, South Darfur

Subcategories of biosecurity	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. Purchase of one day old chicks	-								
2. Source of feed	0.41*	-							
3. Exports of live animals	0.42*	- 0.02	-						
4. Feed supply	0.14	- 0.10	- 0.53**	-					
5. Removal of manure and dead animals	- 0.01	- 0.01	- 0.06	0.23	-				
6. Entrance of visitors and personnel	0.18	- 0.16	- 0.10	0.41*	0.42*	-			
7. Infrastructure and biological vectors	- 0.11	- 0.10	-0.05	- 0.27	0.48*	0.03	-		
8. Cleaning and disinfection	0.23	0.33	- 0.35	0.40*	0.06	0.35	- 0.04	-	
9. Materials and measures between compartments	- 0.40*	- 0.45*	- 0.42*	0.43*	0.10	0.26	0.03	0.41*	-

*p<0.05; ** p<0.01

DISCUSSION

The present study established for the first time the evaluation of the biosecurity in poultry farms in Darfur region, Sudan. The study utilized the scoring system published by Gelaude *et al.* (2014). However, it is not the first time to use such quantification system in Sudan, as it was previously used in the evaluation of biosecurity in layer farms in Khartoum State (Elhassan *et al.*, 2020). The unique feature of the scoring system is that not only it enables the quantification of biosecurity in poultry farms, but also takes the relative importance of the different biosecurity aspects into account (Gelaude *et al.*, 2014).The overall biosecurity in this study was good in 88% of the farms and only poor in 12% of them. Nevertheless, none of the investigated farms had excellent or even very good score.

Studies carried out on broiler farms in Europe by Van Limbergen *et al.* (2017) and Elhassan *et al.* (2020) in layer farms in Sudan, revealed that the score of external of biosecurity was remarkably lower than the score of internal biosecurity. It is plausible that broiler farmers obtained a clear benefit from improving internal biosecurity, such as implement a higher standard of hygiene in the broiler house, and consequently achieved high performance of animals (Postma *et al.*, 2016; Van Limbergen *et al.*, 2017). However, the present findings did not show significant differences between the levels of internal and external biosecurity. The buying of animals from different farms sources is considered as a greater risk of introduction of disease-causing agents. (Elhassan *et al.*, 2020). In the present study, acceptable different levels of biosecurity were seen in the purchase of one day old chicks. This may show the improved awareness among farm owners with biosecurity measures in terms of introducing new chicks.

The animal transport vehicles can also contribute to the disease-causing agents. (Hege *et al.*, 2002). In this study, the subcategory exports of live animals displayed the highest score. This indicates the high awareness among farm owners on biosecurity measures of cleaning and disinfection of the vehicles.

It is well known that the share of equipment between the stables or farms would certainly lead to greater risk of introduction of the disease-causing agents (Tabidi *et al.*, 2014; Lister *et al.*, 2008). In the present study, the subcategory supply of materials displayed the lowest biosecurity score as compared to other subcategories either in external or internal biosecurity. Similar findings have been reported by Elhassan *et al.* (2020) in layer farms.

Regarding the internal biosecurity in this study, disease management displayed the highest score as compared to other practices. This is in agreement with the findings given by Van Limbergen *et al.* (2017). The score of disease management might indicate the sufficient awareness of the adverse effects of poultry diseases amongst farm owners and supervisors. It is of great importance to apply biosecurity measures related to disease management such as isolation of infected birds, vaccination, and removal of dead birds (Gelaude *et al.*, 2014).

The present findings revealed not only a high biosecurity score of export of live animals, but also its strong negative correlation with the biosecurity score of feed supply. A possible explanation for the negative correlation might be due to the lack of applying simultaneous strict biosecurity measures on movements of both to and from the farm, indicating that farmers are applying strict measures on one direction only.

The present study showed poor biosecurity score in terms of removal of manure and dead animal as well as infrastructure and biological vectors. It is evident that dead birds and litter can be highly contaminated with pathogens (Lister *et al.*,2008). In addition, the aforementioned subcategories of external biosecurity in this study demonstrated strong positive correlation to each other. The positive correlate on could be attributed to that both subcategories are influenced by common factor, the lay out and construction of the farm. Further studies are needed to support this assumption.

Similarly, the present investigation showed that layer farms had acceptable level of cleaning and disinfection practices which positively correlated with the subcategory materials and measures between compartments. It seems that such

subcategories are correlated to each other because they may share the same hygiene measures.

CONCLUSIONS

The study showed low score of overall biosecurity practices applied to layer farms in Nyala City, South Darfur State, Sudan. The scores of external and internal biosecurity were more or less the same. More attention is recommended to raise the awareness of farm owners and supervisors on the optimum biosecurity measures and their impact on the overall flock health.

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RESEARCH ARTICLE

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Green method of synthesis silver nanoparticles as using fenugreek seeds extract (*Trigonella foenum-graecum*) and its application as antibacterial agent

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KEYWORDS

Biosynthesis
Fenugreek seed
Plant extract
Phagocytosis
Silver nanoparticles

ABSTRACT

In the present research, spherical silver nanoparticles (AgNPs) of 33-75 nm size have been synthesized using AgNO3 solution and aqueous extract of Fenugreek plant seeds as a reducing agent. The principle is based on the reduction of AgNO3 by the extract of fenugreek seeds. The nanoparticles were characterized and investigated by X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). The size and shape of the nanoparticles were found to be sensitive to the quantity of the extract. Silver nanoparticles are broad-spectrum antibacterial agents and the internalization of nanoparticles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor mediated endocytosis. This approach is not only of a green rapid synthesis kind and considered as a better alternative to chemical synthesis, but also found to be effective for large scale synthesis of silver nanoparticles.

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INTRODUCTION

In recent years, nanotechnology plays an important role in our day to day life as a result of not only engineer form and size of metal however the essential properties such as chemical, physical, mechanical, optical and particles change, and may additionally be modified (Alqudami and Annapoorni, 2007). Nanotechnology has succeeded in numerous fields like health care, food and feed, cosmetics, energy science, electronics, mechanics, space industries, environmental health, bioscience, chemical industries, drug and sequence delivery (Korbekandi and Iravani 2012). Nanotechnology has exuberantly been used for the treatments of cancer and other diseases (Brigger *et al.*, 2012). Recently, the green chemistry that aims to cut back or eliminate substances dangerous to human health and also the surroundings within the style, development and implementation of chemical processes and merchandise is changing into additional and additional vital (Poliakoff *et al.*, 2002). The use of nanoparticles is gaining importance in the present century, as they possess definite chemical, optical and mechanical properties. Metal nano- particles are of importance because of their potential applications in chemical change, photonics, biomedicine, antimicrobial activity and optics (Govindraju *et al.*, 2009).

The employment of phytochemicals within the synthesis of nanoparticles is a vital mutualism between nanotechnology and green chemistry (Huang *et al.*, 2007). To accommodate the twelve principles of green chemistry, several researches tried to avoid or reduce the uses of dangerous chemicals and solvents, like exploitation natural materials rather than ancient cyanogenic chemicals (Raveendran *et al.*, 2003). All components of the plant like leaf, stem, flower, seed and skin of the fruits were used earlier for the synthesis of silver nanoparticles (AgNPs). Plants are used for the synthesis of nanoparticles were coated by the plant extract that has medical edges and may be used as drug and cosmetic applications (Mallikarjunaa *et al.*, 2011).

Fenugreek was reported to possess gastro protecting result, antimicrobial activities, anticancer effect, employed in treatment of arthritis, reducing weight, increasing milk production and may regulate gland disease (Jasim, 2014). Fenugreek is a self-pollinating annual leguminous bean which belongs to Fabaceae family (Balch, 2003). Fenugreek seeds are the most vital and helpful part of fenugreek plant. The fenugreek, plant mainly shows the presence of saponin and alkaloids are anti-nutritional factors (Jani *et al.*, 2009). The rationale for choosing plant biosynthesis is to avoid problems of using dangerous substances and toxic reducing agents. To avoid the problem of agglomeration of Ag NPs in solution, it had been suspended in high salt concentration for clinical uses such as drug delivery.

The current investigation focuses on the aqueous seeds extract of Fenugreek used to synthesize AgNPs and evaluated for its antimicrobial activities by confirming the mechanism.

MATERIALS AND METHODS

Preparation of Dried Biomass

The seeds of Fenugreek local name (Helba) were collected from Omdurman market, Khartoum, Sudan. The seeds were thoroughly washed with deionized distilled water and crushed. The powder was further used for preparation of 10 g/L aqueous seeds extract. This extract was filtered with filter paper and stored at 4ºC until further use for present investigation.

Chemicals

Silver nitrate (AgNO3) was purchased from Lab Course Trading Enterprises, Khartoum Sudan and used without further purification. Deionized distilled water was used throughout the experiment. All other chemicals were of analytical grade.

Synthesis of nanoparticles

For biosynthesis of nanoparticles, 2.0 ml plant seeds extract were mixed with 25 ml of freshly prepared silver nitrate 10-3 M AgNO3 solution that was prepared in 250 mL of deionized water in a sterile conical flask and kept in dark place at room temperature. The reaction mixture was incubated for 30 min or until colour change to dark pink observed. The nanoparticles then synthesized by drying at 90oC.

Characterization of nanoparticles

The synthesized AgNPs were characterized by Scanning Electron Microscopy (SEM) and X- ray diffraction analysis

(XRD), by following the standard method (Nethradevi *et al*., 2012).

Test Organisms

The test organisms used for antimicrobial analysis of AgNPs extracts were four different species of bacteria. Pure isolates of these organisms were obtained from the Department of Microbiology, University of Bahri, Sudan. The bacteria include: *Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis* and *Escherichia coli.*

Antibacterial activity

The agar diffusion method was used for the antimicrobial activity study. Four types of agars were used in this experiment, sterile nutrient agar, Mac Conky, Eosin methylene blue (EMB) and mannitol salt agar. Six millimeters (mm) diameter wells were bored into the agar with sterile cork borer and filled with 0.4 ml. of various dilutions of the extracts: 40%, 60%, 80% and 100% in distilled water. The petri dishes were incubated at 37ºC for 24hrs. At the end of incubation, the zones of inhibition that developed were measured in (mm) with the help of a transparent ruler. Distilled water was used as negative control, and Ciprofloxacin was used as a positive one. Each sample repeated three times (A, B, and C), Diameter of zones inhibition ≥10 mm exhibited by plant extract was considered active (Alshafei *et al*., 2016).

Statistical analysis

Tests were performed in triplicate, and the results are expressed as means ± the standard errors of the means.

RESULTS

The current research work, biosynthesis of AgNPs was carried out using the aqueous extract of fenugreek seeds *(Trigonella foenum -graecum*) as reducing and capping agent. The principle is based on the reduction of $AgNO_3$ by the extract of fenugreek seeds.

Microscopic techniques

The scanning electron microscopy (SEM) and X- ray diffraction analysis (XRD) image of the nanoparticles presented the topography of the particle was showed in figure (1). SEM photograph of silver nanoparticles clearly indicates that synthesized silver nanoparticles have average size less than 100 nm, with different shape.

Table 1: Antimicrobial pattern of AgNPs on *Klebsiella pneumonia*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean ±SE
1	100%	36	17	18	16	17 ±0.6
2	80%	36	15	15	15	15 ± 0.0
3	60%	36	15	15	12	14 ± 1.7
4	40%	36	13	12	17	14 ± 1.7

Conc. = Concentration, (%) = Percentage, (mm) = millimeter zone of inhibition, (SE) = Standard Error

Table 2: Antimicrobial pattern of AgNPs on *Escherichia coli*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean ± SE
1	100%	25	17	16	15	16 ±0.6
2	80%	25	15	14	15	14.7 ±0.3
3	60%	25	16	17	15	16 ±0.6
4	40%	25	20	15	16	17 ±1.5

Conc. = Concentration, (%) = Percentage, (mm) = millimeter zone of inhibition, (SE) = Standard Error

Results of antimicrobial examination of the different concentration of the extract on the microbial isolates are shown in tables 1, 2 and 3 and figure 2. The antimicrobial activity of the AgNPs were evaluated by agar diffusion method and the primary screening revealed that the AgNPs suppress the growth of Gram positive and Gram negative bacteria at different concentrations (40, 60, 80, and 100) mg/mL; among the bacteria, *Staphylococcus aureus, Klebsiella pneumonia*, *Bacillus subtilis* and *Escherichia coli.* The results revealed that the growth of bacteria suppressed by the AgNPs and comparatively revealed better activity at concentration 100%.

This experiment showed the antibacterial effect of silver nanoparticles against some microbes. Silver nanoparticles have an antibacterial effect and the zone of inhibition was observed in figure (2). The following tables and figure show the size of zone of inhibition for the different concentration used.

Table 3: Antimicrobial pattern of AgNPs on *Staphylococcus spp.*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean± SE
1	100%	25	19	22	21	20.7 ± 0.9
2	80%	25	18	20	19	19 ±0.6
3	60%	25	17	18	16	17 ±0.6
4	40%	25	16	14	14	14.7 ±0.7

Conc. = Concentration, (%) = Percentage, (mm) = millimeter zone of inhibition, (SE) = Standard Error

Table 4: Antimicrobial pattern of AgNPs on *Bacillus subtilis*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean ± SE
1	100%	40	40	41	37	39 ± 1.2
2	80%	40	39	37	38	38 ± 0.6
3	60%	40	36	34	35	35 ± 0.6
4	40%	40	34	32	33	33 ± 0.6

Conc.= Concentration, (%) = Percentage, mm= millimeter zone of inhibition, SE = Standard error

DISCUSSION

Green synthesis of nanoparticles is advanced than alternative strategies because of its simple, comparatively consistent, and cost-efficient and sometimes leads to additional stable materials (Kalaiarasi *et al*., 2010). Plants have been used for the synthesis of nanoparticles were coated by the plant extract that has medical advantages and may be used as drug and cosmetic applications (Mallikarjunaa *et al*., 2011).

Aqueous seed extract of Fenugreek acts as a reducing agent (Thombre *et al*., 2013) that reduces metallic silver to Nano silver and hence the color modification was obtained. It's standard that silver nanoparticles exhibit red pink colourize solution due to excitation of surface Plasmon vibrations in silver nanoparticles. Ag^{+2} ions of silver nitrate are found to be reduced to Ag atoms. The synthesized nanoparticles were characterized using SEM and XRD spectroscopy analysis.

The spectrum of the sample was obtained for wavelength range in-between 1nm- 100nm. The λ max of the nanoparticles was observed at 75 nm. This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by silver nanoparticles. The silver nanoparticles oscillate when exposed to electromagnetic radiation and this oscillation gives a typical peak value (Smitha *et al*., 2008). The SEM images of the nanoparticles represents the topography of the particles is shown in images figure (1). The results obtained in this study is near to that found by (Jing *et al*., 2009) who mentioned that, the size of silver nanoparticles synthesized by green synthesis was estimated to be around 20- 50 nm.

The results obtained during this study and conferred in tables 1, 2, 3, 4 and figure 2 demonstrate that AgNPs at completely different concentrations were quite effective against test organisms. This means that the extract of seeds with silver nitrate contains substances, which will inhibit the expansion of some microorganisms. This analysis conducted on gram positive and gram-negative bacterium and also the results seem that enormous zone of inhibition and these finding more accept as true by alternative analysis groups, (Kong and Jang 2008) wherever it had been verified that silver nanoparticles exert identical result on Gram positive and Gram-negative strains.

Our result at identical line with that found by (Sung and lee, 2010) who obtained that, phytochemical constituents of the medicinal plants, principally concerned within the alteration of the gram positive and gram-negative microorganism cell walls by neutering the membrane. The phytochemical within the seeds reduce the silver salts and not solely manufacture silver nanoparticles however additionally stabilize it by capping the nanoparticles with the plant peptides. The antimicrobial activity of the nanoparticles is high increased because of the presence of plant proteins and phytochemical. (Rajesh and Neelu, 2015). The mode of action of silver nanoparticles is analogous to it of silver ions, which complex with electron donor groups containing sulfur, oxygen or nitrogen atoms that are normally present as thiols or phosphates on amino acids and nucleic acids (McDonnell 2007).

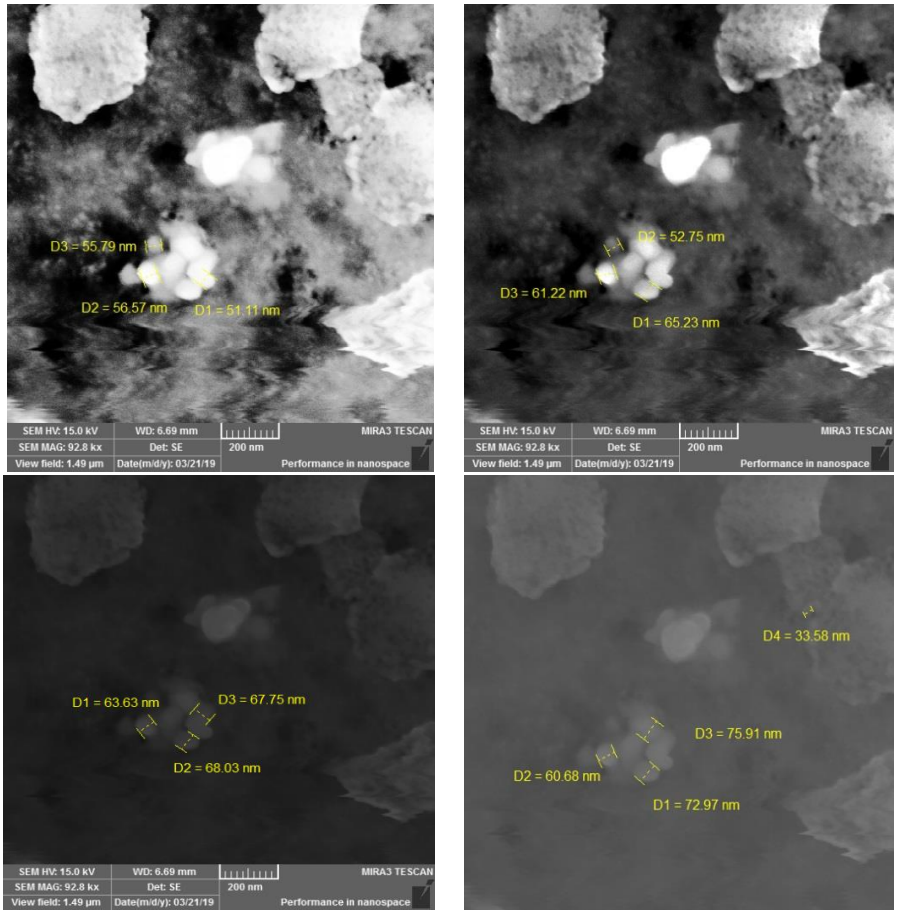

Figure 1: SEM images of silver nanoparticles produced by Fenugreek seeds extract

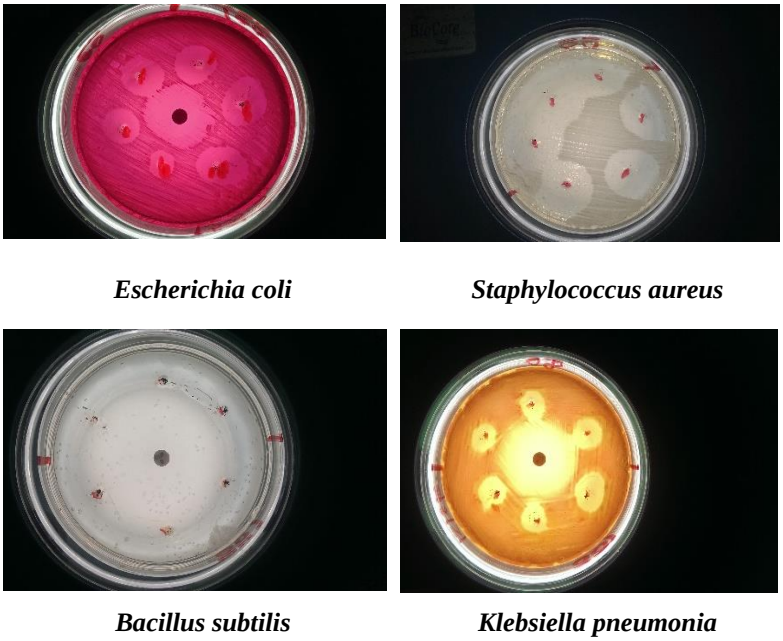

Figure 2: Zone of growth inhibition of the AgNPs against tested bacteria species

Like silver nanoparticles, silver ions additionally exert their activity through a broad vary of mechanisms, together with denaturing the 30s ribosome subunit, suppressing the expression of enzymes and proteins essential to adenosine triphosphate production. Inhibiting metabolic process enzymes thereby causing the assembly of reactive oxygen species (Yamanaka *et al*., 2005), binding and dimerizing ribonucleic acid RNA and deoxyribonucleic acid (DNA) (Rai *et al*. 2009). Consequently, silver nanoparticles got to reach the cytomembrane to realize an antibacterial drug result. Indeed, silver nanoparticles attach to the surface of the cell membrane and disturb it perform, penetrate bacterium, and unharness silver destabilizing and disrupting the outer cell membrane (Lok *et al*., 2006).

CONCLUSION

The present work reassessed that, green synthetic method was a low-cost approach and capable of synthesizing silver nanoparticles at room temperature. The size and structure of obtained silver nanoparticles were characterized by SEM and XRD. The stability and biocompatibility of the silver nanoparticles synthesized using biological protocols was found to be extremely high than the chemically synthesized silver nanoparticles. In addition, silver nanoparticles are broad-spectrum antibacterial agents. Since plants are widely distributed, readily available and at the same time safe to handle, there will be safe to use keeping food from animal origin, a lot to do to develop this methodology of synthesis inspired by several conventional ideas

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RESEARCH ARTICLE

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The effect of chemical intervention on chicken microbial quality in Khartoum State, Sudan

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KEYWORDS

Poultry
Slaughterhouses
Chemical interventions
Cross contamination

ABSTRACT

This descriptive and experimental study was conducted between June and September 2020 in six poultry slaughterhouses in Khartoum State Sudan. The objectives of this study were to evaluate the effect of chemical interventions on reduction of poultry carcasses bacterial load and to assess the management measures adopted in poultry processing plants and their effects on reducing bacterial load. Slaughterhouses were classified into three groups according to the chemical interventions used. Both checklist and swab samples were applied to assess the status of the prerequisite programs (PRPs), activities during operational processes (OP) and total bacterial count (TBC). The samples were taken directly from the processing line from three steps: after final wash, after drying and after freezing. According to the checklist assessment, the study revealed that the slaughterhouses used sodium chloride failed to comply with the acceptable limit and scored 69.1%. The mean of TBC after final wash was found 183±19.6 CFU, after chemical intervention 123±24 CFU, after freezing 133.8±30 CFU with statistical significant difference 0.020 with $p \leq 0.05$. Whereas, slaughterhouses used acetic acid failed to comply with the acceptable limit and scored 73%, the mean of TBC after final wash was found 260.8±18.8 CFU, after chemical intervention 158.4±34 and after freezing 299±1 CFU with statistical significant difference 0.001, with $p \leq 0.05$.Also, slaughterhouses used hydrogen peroxide failed to comply with the acceptable limit and scored 61.9% and the mean of TBC after final wash was found 247.2±29CFU, after chemical intervention 10±10 CFU, after freezing 115.6±21 CFU, with highly statistical significant difference 0.000, with $p \leq 0.05$. This study concluded that the chemical interventions had reduced the bacterial load but the slaughterhouses need more management to minimize contamination in the final product.

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INTRODUCTION

The highest number of cases of food borne pathogens such as Campylobacter and Salmonella reported in European Union were largely associated with fresh poultry meat and eggs and pork (EFSA, 2011). In one hand, the decontamination of poultry carcasses is gaining increased interest, especially because poultry is implicated as a risk factor in human campylobacteriosis (Chen *et al.*, 2012).

On the other hand, operational process steps in modern poultry industry are designed to reduce bacterial contamination and extends shelf life.

In industrial poultry processing, hot water immersion is used to facilitate feather withdraw but it also reduces bacterial load accompanying that process step. Therefore, poultry carcasses should be quickly chilled by reducing their temperature from approximately 40 to 4 °C (James *et al.*, 2006). Other operational process steps such as washing and sanitizing procedures have generally proven effective for reducing overall bacterial populations as well as numbers of specific bacterial pathogens on meat (Sofos, 1994).

Chemical interventions usually applied to inhibit microorganisms because of their ability to disrupt cellular membranes or other cellular constituents and interrupt physiological processes (Loretz *et al.*, 2010).

Chemical interventions primarily comprised organic acids, chlorine-based treatments, or phosphate-based treatments. Acetic acid, acidified sodium chlorite, and trisodium phosphate can reduce bacterial load in the range from 1.0 to 2.2 orders of magnitude (Loretz *et al.*, 2010). Microbial reductions obtained for inoculated bacteria, including aerobic bacteria, non-pathogenic E. coli, E. coli O157:H7 and Salmonella spp., varied between 0.7 log and 4.9 logs (Loretz *et al.*, 2010). The reduction of bacterial load in meat carcasses by applying hydrogen peroxide in poultry chiller water was also reported by Midgley and Small (2006).

The objectives of this study were to evaluate the effect of chemical interventions on reduction of poultry carcasses bacterial load and to assess the management measures adopted in poultry processing plants in the study area and their effect on reducing bacterial load.

MATERIALS AND METHODS

Study area and design

This descriptive and experimental study was conducted between June and September 2020 in six poultry slaughterhouses in Khartoum State, Sudan. The slaughterhouses were categorized into three groups according to the type of chemical intervention currently used. Group (1) used sodium chloride with an addition rate of 0.15 g/l, group (2) used acetic acid with an addition rate of 0.2 ml/l, and group (3) used hydrogen peroxide with an addition rate of 0.05 ml/l.

Each chemical intervention was investigated in 2 slaughterhouses. Both checklist and microbiological tests were used in this evaluation.

Data collection

The assessment of the status of some of the prerequisite programs (PRPs) and other related activities during operational processing (OP) that could directly affect meat safety in slaughterhouses were investigated using a checklist.

Checklist design

A structured checklist was designed with three sections. Section one comprised good manufacturing practices (GMPs) and weighed 30%, section two comprised good hygiene practices (GHPs) and weighed 30% and section three comprised operational processing (OPs) and weighed 40%.

The first section GMP consisted of building standards, separation between clean and dirty area, water proof flooring, system of ventilation, and compliance of equipment with international standards. The second section was GHP contained application of hygiene policy procedures, cleaning and waste management system, and the third section consisted of activities related to operational processing (OP) that could directly affect the safety of meat such as defeathering temperature and washing after evisceration, changing of immersion water and addition of ice in chiller tank, chemical interventions used and rate of addition, workers' movement in the slaughterhouse and their protective equipment.

Sampling

Ninety samples were collected using sterile swabs from six slaughterhouses with the purpose of evaluating total bacterial count (TBC). For each chemical intervention in each slaughterhouse a total of 15 swab samples were taken directly from the processing line from three process steps: after final washing, after chemical intervention and after freezing of the final product. The collected swabs were then aseptically transferred into sterile containers, and then kept in an icebox and transported at 4 ºC to the laboratory of the College of Veterinary Medicine, University of Bahri.

Procedure for evaluation of bacterial load

Total bacterial count was used as described by Marshall (1992). The TBC was calculated by using original dilution and then added to 9 ml normal saline for the first dilution, then 1 ml was taken from first dilution and added to 9 ml normal saline for the second dilution. Then 1 ml of the fifth dilution that prepared previously was taken and added to labeled petri dish with about 20 ml plate count agar and spread to facilitate absorption and incubated for 24 hours at 37 ºC. Bacterial colonies were counted and documented for analysis.

TBC means were compared to the Sudanese Standards and Metrology Organization (SSMO) where the acceptable total bacterial load in whole poultry carcass is supposed to be 3×105 CFU by Aeropic Plate Count (APC) test.

Statistical analysis

The obtained data were coded and analyzed by using statistical packaging for the social sciences SPSS/PC software program, version 21 for windows. Data were analyzed for descriptive statistical analysis and ANOVA test.

RESULTS

The results of this study showed that the mean TBC in the final product in all investigated broiler meat in the slaughterhouses had exceeded the acceptable limits set by SSMO.

The mean of TBC in the three process steps was found 183± 19.6 CFU/ml after final wash, 123±24.2 CFU/ml after chemical intervention (sodium chloride) and 133.83±0.7 CFU/ml after freezing with significant differences (0.02) between the three process steps with $p \leq 0.05$ (Table 1).

Table 1: Average of total bacterial count in the three process steps in the slaughterhouses using sodium chloride.

Process step	Mean	SD	SE	Sig
1- After final wash	183.667	76.199	19.674	0.02
2- After chemical intervention	123.000	94.101	24.297	
3- After freezing	133.800	119.196	30.776	

SD= Standard deviation; SE= Standard Error, Sig = Significance

Table 2: Average of total bacterial count in the three process steps in the slaughterhouses using acetic acid.

Process step	Mean	SD	SE	Sig
1- After final wash	260.900	59.748	18.894	0.001
2- After chemical intervention	158.400	109.389	34.593	
3- After freezing	299.000	3.162	1.000	

SD= Standard deviation; SE= Standard Error, Sig = Significance

The mean of TBC in the three process steps was 260.9±18.8 CFU/ml after final wash, 158.4±34.5 CFU/ml after chemical intervention (acetic acid) and 299.0±1.00 CFU/ml after freezing of the final product. The results show significant differences (0.001) between the three process steps with $p \leq 0.05$ (Table 2)

The mean of TBC in the three process steps was found 247.2±29.4 CFU/ml after final wash, 10±10.0 CFU/ml after chemical intervention (hydrogen peroxide) and 115.6±21.0 CFU/ml after freezing. There were significant differences (0.00) between the three process steps, with $p \leq 0.05$ (Table 3).

Table 3: Average of total bacterial count in the three process steps in the slaughterhouses using hydrogen peroxide.

Process step	Mean	SD	SE	Sig
1- After final wash	247.200	65.7856	29.420	0.000
2- After chemical intervention	10.000	22.3618	10.000	
3- After freezing	115.600	47.1310	21.077	

SD= Standard deviation; SE= Standard Error, Sig = Significance

Table 4: Assessment of PRPs and OP for the investigation of slaughterhouses

Chemical intervention	Assessment of the checklist sections			
	GMP	GHP	OP	Total
1- Sodium chloride	13.90%	22.00%	33.10%	69.20%
2- Acetic acid	26.30%	15.20%	31.50%	73.00%
3- Hydrogen peroxide	17.80	18.70%	25.00%	61.50%

PRPs= Prerequisite programs, OP= Operational processes

Table 5: Assessment of PRPs and OPs of slaughterhouses and average of TBC (±SE) in the three stages of slaughter processing

Chemical intervention	PRPs and OP assessment		The mean of TBC (CFU/ml)		
			Process steps		
	Acceptable	Unacceptable	Final wash	Chemical intervention	Freezing
1-Sodium chloride	39.80%	61.20%	183.6 ±19	123±24	133.8 ±30
2- Acetic acid	27.00%	73.00%	260.9±19	158.4±34	299±1
3- Hydrogen peroxide	38.50%	61.50%	247.2±29	10±10	115.6±21

PRPs= Prerequisite programs, SE= Standard error of mean, OP= Operational processes, TBC= Total bacterial count, CFU= Colony forming unit

The assessment of the status of PRPs and their effects on operational processing in terms of bacterial load is shown in Table (5). The PRPs assessment though found unacceptable with regard to the three chemical interventions, yet their effect on bacterial load showed a decrease in TBC after final wash step and an increase after freezing process step.

DISCUSSION

The objectives of this study were to evaluate the effect of chemical interventions on reduction of poultry meat bacterial load and to assess the management measures adopted in poultry processing plants and their effects on reducing bacterial load.

Microorganisms are considered good indicators of the hygienic conditions present during food manufacturing process (Hoffmann *et al.*, 2004). For example, high coliform counts indicate post-processing contamination and/or unsuitable sanitization (Kottwitz *et al.*, 2010).

This study investigates the use of different chemical interventions such as sodium chloride, acetic acid and hydrogen peroxide that were added to the pre-chilling and chilling water tanks. It was revealed that all chemical interventions used had significantly reduced the TBC of the product. This will eventually improve the quality and shelf life of product. This is also stated by Guastalli *et al.* (2016) who reported that chemicals were added to the pre-chilling and chilling water tanks with the purpose of minimizing carcass bacterial load.

In the present study chemical intervention using sodium chloride after the final wash reduced TBC, though TBC increased after freezing, with significant differences (0.02) between the three process steps, with $p \leq 0.05$. The increase of TBC observed after freezing may be due to inadequate sanitation in this process step.

This study also shows that chemical intervention using acetic acid after the final wash reduced TBC, despite that the TBC

increased after freezing, with significant differences (0.001) between the three process steps, with $p \leq 0.05$. Dickson and Anderson (1992) attributed the reduction of bacterial load when using acetic, lactic and citric acids to the fact that these acids have bactericidal effects.

The increase in TBC after freezing may be attributed to inadequate sanitation in this process step. Moreover, EFSA (2011) stated that acetic acid, which is a natural component of vinegar, is not expected to raise any safety concern (Midgley and Small, 2006).

Research studies indicating the efficacy of acetic acid in reducing bacterial load in meat were also conducted by Loretz *et al.* (2010) who evaluated acetic acid on inoculated beef carcass surfaces under laboratory conditions. They found microbial reductions obtained for inoculated bacteria, (including aerobic bacteria, nonpathogenic *E. coli*, *E. coli* O157:H7, and *Salmonella* spp.) varied between 0.7 log and 4.9 logs.

This study also shows that chemical intervention using hydrogen peroxide after the final wash reduced TBC despite its increase after freezing with significant differences (0.000) between the three process steps, with $p \leq 0.05$. The increase in TBC after freezing may be due to inadequate sanitation and the probable cross contamination that may occur in this process step. This finding was in line with those published by Gorman *et al.* (1995) who stated that decontamination can be accomplished with the use of hydrogen peroxide which inactivates microorganisms by acting as an oxidant. Similar finding was reported by Reagan *et al.* (1996) who studied some procedures that included hydrogen peroxide (5%) as intervention treatment on beef carcasses and revealed that hydrogen peroxide reduced aerobic plate counts by 1.14 log cfu/cm². The reduction of bacterial load in meat carcasses by applying hydrogen peroxide in poultry chiller water was also reported by Midgley and Small (2006**)** who found that hydrogen peroxide as a bactericide reduced aerobic organisms by 95–99.5% with 6,600 ppm or higher and *E. coli* by 97–99.9% with 5,300 ppm or higher.

It worth noting in this study that the high microbial load in the final product after the freezing step may be due to inadequate hygiene activities which applied in slaughterhouses such as cross contamination from the hands of workers. In addition, the researchers observed poor sanitation and cleaning and disinfection of freezers and tools. This complied with that stated by Sheridan *et al.* (1992) who identified personal equipment, such as knives, mesh gloves, and aprons as reservoirs of bacteria in the abattoir.

Assessment of the status of PRPs and their effects on operational processing in terms of bacterial load in the present study revealed low acceptable scores. Despite this nonconforming PRPs, the three process steps showed a decrease in TBC in chemical intervention process steps. These findings proved the significance of chemical intervention in reducing bacterial load in meat carcasses. The studies of Loretz *et al.* (2010) and Dickson and Anderson (1992) also attributed the decrease in TBC by applying chemical intervention to the fact that using sanitizing agents have generally proven effective for reducing overall bacterial populations as well as numbers of specific bacterial pathogens on meat.

CONCULISION AND RECOMUNDITIONS

This study concluded that using chemical intervention after the final wash reduced TBC in broiler carcasses and that there was an increase in TBC after freezing process step. To avoid high bacterial count in the final product, good hygiene measures must be applied.

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RESEARCH ARTICLE

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Blood parasites in dairy cattle in Al Nohud and Al Obied cities in West and North Kordufan States, Sudan

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KEYWORDS

Anaplasma
Babesia
Blood
Cattle
Parasites
Theileria,

ABSTRACT

This study was conducted during the year 2019. It was aiming at investigating the prevalence of blood parasites in cattle in Al Obied and Al Nohud cities in North and West Kordufan respectively. Sixty-seven blood samples were collected from each city. These samples were subjected for parasitological examination using thin blood smears which stained and microscopically examined. Blood samples collected from Al Obied city; the prevalence of protozoan parasites was 52.2%. The prevalence of *Theileria spp.*, *Babesia spp.*, *Anaplasma spp.* were 14.9%, 25.4%, 11.9%, and 2.9% respectively. Mixed protozoan parasitic infestations represented 3.0% in Al Obied city. The mixed protozoan parasitic infestations were (*Theileria spp.* and *Babesia spp.*) and (*Babesia spp.* and *Anaplasma spp.*). Samples collected from Al Nohud city; the prevalence of blood parasites was 28.4%. The prevalence of *Theileria spp.* was 17.9%, *Babesia spp.* 3.0%, *Anaplasma spp.* 7.5%. The mixed infestation was 1.5%. The mixed protozoan parasitic infestation was 1.5%. The mixed protozoan parasitic infestation was *Anaplasma spp.* and *Theileria spp.*

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INTRODUCTION

Heamoparasites are the main livestock production constraints all over the world causing serious economic losses. In case of infection with these blood parasites up to 75% erythrocytes may be destroyed, leading to severe anemia. Heamoparasites are the main livestock production constraints all over the world. Ticks are regarded as important external parasites of animals' especially in tropical and sub-tropical zones where they transmit most of the serious diseases, among which the majority are blood parasites and *Rickettsia*. Blood parasites are generally transmitted by arthropods either mechanically or biologically (Hartelt, 2004).

Blood parasite infections in cattle are primarily caused by the protozoans such as *Babesia spp.*, Trypanosoma *spp.*, *Anaplasma spp.* and *Theileria spp*. (Abdus, 1989; Abdel Rahman, 2007). These protozoans are transmitted by arthropod vectors such as ticks and flies. Common ticks that can transmit these are Dermatocentor *spp.*, Hyalomma *spp.*, Boophilus *spp.* or Rhipicephalus *spp*. Flies such as Tabanas and Stomoxys are also commonly found (Cheah *et al*., 1999), thus vector control is an important activity in a farm to reduce the morbidity and mortality caused by these infections. Piroplasmosis is highly fatal disease and has serious economic impact on livestock. This disease is caused by protozoan parasites belonging to the family Babesiidae and family Theileriidae of suborder Piroplasmidae. Babesiosis and Theileriosis are of the most important and serious blood parasitic diseases affecting animals in the area (Radwan and El Kelesh 2009; Mervat and Ola, 2010; Ica *et al*., 2007). Babesiosis is caused by intraerythrocytic protozoan parasites of the genus *Babesia*. Transmitted by ticks, Babesiosis affects a wide range of domestic and wild animals and occasionally people (Ali, 2005). Although the major economic impact of Babesiosis is on the cattle industry, infections in other domestic animals, assume varying degrees of importance throughout the world (Kuttler, 2018; Ali, 2005). Two important species in cattle are *B. bigemina* and *B. bovis*, which are widespread in tropical and subtropical areas. The main vectors of *B. bigemina* and *B. bovis* are Rhipicephalus *spp.* and Boophilus *spp.* ticks (Abo Sakaya, 2009; Mullen and Durden, 2009).

Anaplasmosis, formerly known as gall sickness, is a disease of ruminants caused by a rickettsial parasite, family *Anaplasma*taceae, and genus *Anaplasma* the microorganism is gram-negative (Hartelt *et al.*, 2004) and infects red blood cells. Cattle, sheep, goats, buffalo, and some wild ruminants can be infected with erythrocytic *Anaplasma*. Anaplasmosis occurs in tropical and subtropical regions worldwide. It is transmitted by natural means through a number of haematophagous species of ticks. Anaplasmosis can also be transmitted by the use of surgical, dehorning, castration, and tattoo instruments and hypodermic needles that are not disinfected between uses (Capucille, 2011). Two important species infect cattle, *Anaplasma marginale* found worldwide. *Anaplasma centrale* is found mainly in South America, Africa and the Middle East (Boes and Durham, 2017).

Theileriosis is the name given to infections caused by several species of *Theileria*, of the several *Theileria* species infecting domestic ruminants, the two most economically important are *T. parva* and *T. annulata* (Morrison and McKeever, 2006; Abd El Raof *et al*., 2000). The distribution of *T. parva* is limited to eastern, central and southern Africa where it is predominantly transmitted by the tick *Rhipicephalus appendiculatus. Theileria annulata*, transmitted by several species of Hyalomma *spp*. ticks, it is more widely distributed, extending from northern Sudan and the Mediterranean countries to the Middle East, India, southern Asia and China (Abdul Manan *et al*., 2007; Peter *et al*., 2017).

This study was aiming to investigating the prevalence of blood parasites in cattle in Al Obied and Al Nohud cities in North and West Kordufan States.

MATERIALS AND METHODS

Samples

A total of 134 blood samples were collected from dairy cattle in Al Obied and Al Nohud cities in North and West Kordufan States (67 samples for each).

The Blood samples were collected in the morning from the jugular veins using vacutainers with Ethylene Diamine Tetra Acetic acid (EDTA). The samples were labeled with animal

number, placed in an ice box at 4°C and transported as soon as possible to the laboratory before processing for parasitological examinations.

Parasitological examination (Thin blood film)

Blood smears were prepared according to Soulsby (1982).

RESULTS

Prevalence of blood protozoans parasites in Al Obied city

Out of 67 blood samples collected from dairy cattle in Al Obied city, 35 (52.2%) samples were positive for blood parasites (Figures 1, 5, 6, 7). The prevalence of *Theileria spp.*, *Babesia spp.* and *Anaplasma spp.* was 14.9%, 25.4% and 11.9% respectively (Table 1). Mixed protozoans infestations represented 2 (3.0%). The mixed protozoans infestations were (*Theileria spp.* and *Babesia spp.*) and (*Babesia spp.* and *Anaplasma spp.*) (Figure 2).

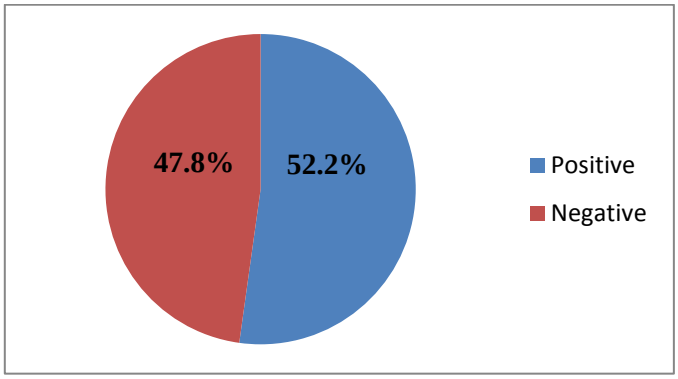

Figure 1: Positive and negative blood samples collected from Al Obied city for detection of blood parasites

Table 1: Number and percentage of blood parasites detected in blood samples collected from Al Obied city.

Blood parasite	Number	Percentage
Theileria spp.	10	14.9%
Babesia spp.	17	25.4%
Anaplasma spp.	8	11.9%
Negative samples	32	47.8%
Total	**67**	**100%**

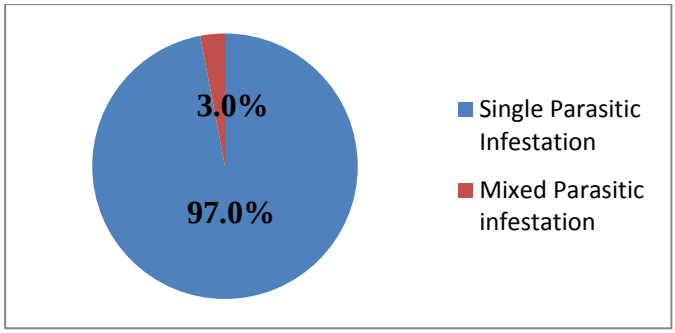

Figure 2: Single and mixed protozoan infestations detected in blood samples collected from Al Obied city.

Prevalence of blood protozoans parasites in Al Nohud city

Out of 67 blood samples collected from dairy cattle in Al Nohud city, 19 (28.4%) samples were positive for blood parasites (Figure 3, 5, 6, 7). The prevalence of *Theileria spp.*, *Babesia spp.* and *Anaplasma spp.* was 17.9%, 3.0% and 7.5% respectively (Table 2). Mixed protozoans infestation represented 1 (1.5%) (Figure 4). The mixed protozoans parasites infestation was *Theileria spp.* and *Anaplasma spp.*

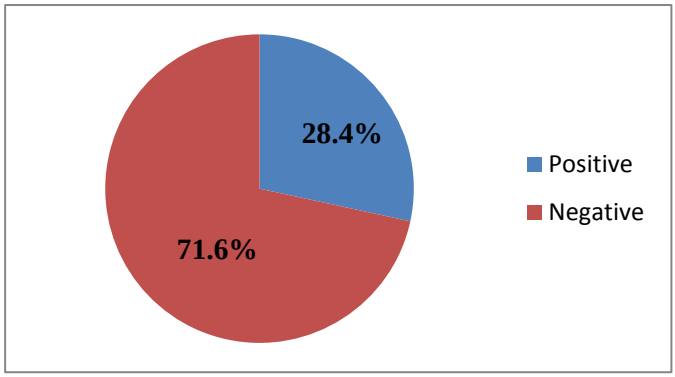

Figure 3: Positive and negative blood samples collected from Al Nohud city for detection of blood parasites.

Table (2): Number and percentage of blood parasites detected in blood samples collected from Al Nohud city.

Blood parasite	Number	Percentage
Theileria spp.	12	17.9%
Babesia spp.	2	3.0%
Anaplasma spp.	5	7.5%
Negative samples	48	71.6%
Total	**67**	**100%**

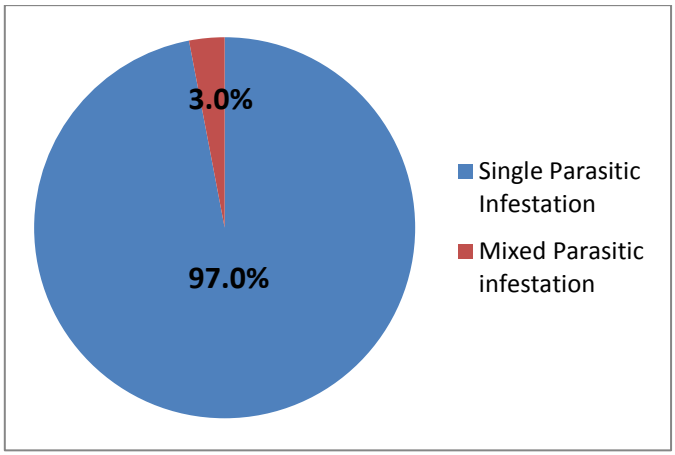

Figure 4: Single and mixed protozoan parasites infestations detected in blood samples collected from Al Nohud city.

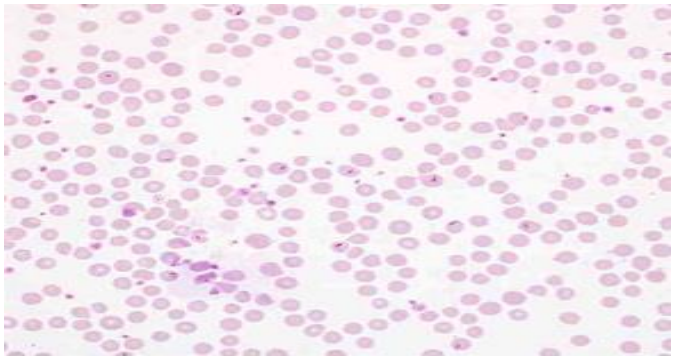

Figure 5: Thin blood smear stained with Giemsa stain and positive for *Theileria spp.*

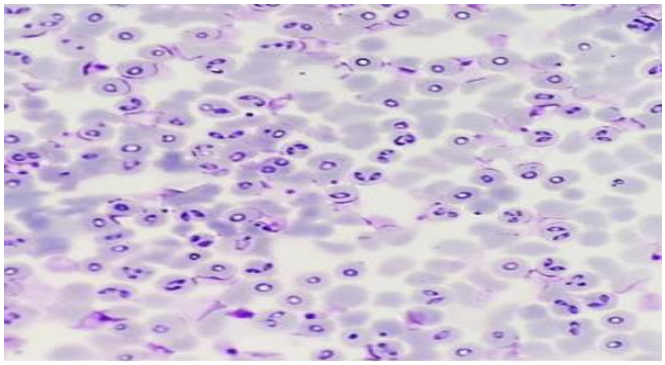

Figure 6: Thin blood smear stained with Giemsa stain and positive for *Babesia spp.*

DISCUSSION

In this study the prevalence of blood protozoans parasites in Al Obied city was 52.2% and 28.4% in Al Nohud city. In Al Obied city the prevalence of *Theileria spp.*, *Babesia spp.* and *Anaplasma spp.* was 14.9%, 25.4% and 11.9% respectively. In Al Nohud city the prevalence of *Theileria spp.*, *Babesia spp.* and *Anaplasma spp.* was 17.9%, 3.0% and 7.5% respectively.

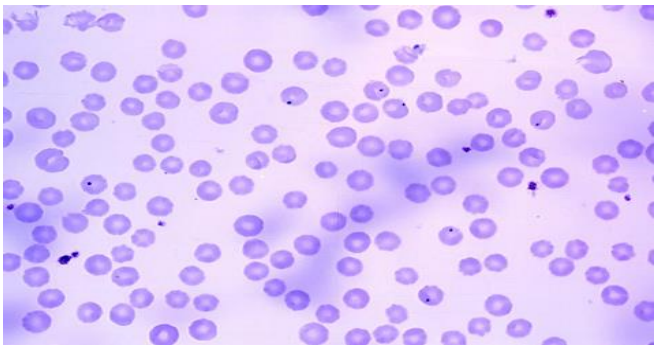

Figure 7: Thin blood smear stained with Giemsa stain and positive for *Anaplasma spp.*

In Pakistan Khan *et al*. (2004) reported the prevalence of blood protozoans parasites to be 27.7%. Ibrahim *et al.* (2010) reported the prevalence of 33.3% for *Theileria spp.*, 28.2% for *Anaplasma spp.* and 5.1% for *Babesia spp.* in two farms in Bhari locality. A prevalence rate of 0.12% of Babesiosis was reported in indigenous cattle in Sagadi area (Abdalla, 1984) during a field survey on Tick Borne Diseases (TBDs) in the Blue Nile and the White Nile regions. Abo Sakaya (2009) reported Babesiosis as one of the diseases which causes high motility of exotic heifers imported to El Gezira State. *B. bigemina* was the causative agent of the nine cattle cases of red water disease reported in Kassala region (Mohamed and Yagoub, 1990). In Khartoum State, Anaplasmosis was occasionally diagnosed, but babesiosis was not encountered in blood smears from cattels surveyed in Soba, Shambat and Hillat Hamad areas (Mohammed Safieldin *et al*., 2011). Zein El aabdeen (1995) was not able to diagnose any case of Babesiosis in cattle and sheep slaughtered in Khartoum State. However, 16 (10.8%) and 39(50%) positive cases of bovine Babesiosis were parasitologically and serologically detected, respectively (Sulieman, 2004). In Khartoum State, Mohammed Safieldin *et al.* (2011) mentioned that the prevalence of blood parasites was 8, 5%. The prevalence of *Theileria species* infection was found to be 7, 5.25 and 6.32% for dry cool, dry hot and wet hot season, respectively. While the prevalence of *Babesia* species infection was only recorded in the dry cool season as (1%). There was no effect ($\chi 2 = 0.6$, $p > 0.05$) of the season on the occurrence of blood parasites. Strong association (t-test= –43.6, $p < 0.05$) was found between the presence of

blood parasites and milk yield. Idriss *et al*. (2012) reported the prevalence of 5.9% and 5.2% for *Theileria* and *Babesia* in Abyei area in South Sudan respectively.

CONCLUSION AND RECOMMENDATIONS

The current study revealed that the prevalence of *Theileria spp.*, *Babesia spp.* and *Anaplasma spp.* in Al Obied city were 14.9%, 25.4% and 11.9% respectively. In Al Nohud city the prevalence of *Theileria spp.*, *Babesia spp.* and *Anaplasma spp.* was 17.9%, 3.0% and 7.5% respectively.

Education or extension program must be done for animals' owners in order to increase their awareness about blood parasitic disease, their transmission, treatment and control. Enough water and feeds in the areas of livestock must be provided during the dry period of the summer. Marketing and transporting the livestock prior to slaughter and export problems must be solved. Good infrastructure such as research, extension, roads, education and health services must be provided.

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RESEARCH ARTICLE

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Anti-inflammatory effects of ethanolic extract of *Amaranthus viridis* against carrageenan induced paw oedema in Albino Rats

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KEYWORDS

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Albino Rats

ABSTRACT

The anti-inflammatory effects of ethanolic crude extracts of *Amaranthus viridis* whole plant was investigated in carrageenan induced rat paw oedema. Albino Rats were classified into four groups, each of 5. Group 1 received (0.1 ml of 1% W/V carrageenan in 0.9 ml normal saline in a dose of 2 ml/kg body weight) at sub-plantar region and served as negative control , group 2 received indomethacin (10 mg/kg bwt) intraperitoneally and served as standard reference (positive control) , group 3 and group 4 received crude ethanolic extract intraperitoneally at 250 mg/kg and 500 mg/kg bwt respectively. The oedema was quantified by measuring the hind paws thickness at 0, 1, 2 and 3 hour after the carrageenan injection. Administration of ethanolic extract (250 mg/kg and 500 mg/kg) significantly reduced the oedema thickness in a time and dose dependent manner. The inhibition percentage of inflammation was 40.30 % and 70.4 % at dose of 250 and 500 mg/kg respectively. The ethanolic extract at dose of 500 mg/kg shows a potent activity at the last hour of following up. The present study concluded that *Amaranthus viridis* extract displays remarkable anti-inflammatory activity and recommended for the possible use as anti-inflammatory remedy.

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INTRODUCTION

The plant kingdom is known to provide a rich source of botanical anti-inflammatories (Nadkarni, 1954). A number of medicinal plants have been used to treat inflammations in man and animals (Nadkarni, 1954). Numerous natural products have been tested as various therapeutics (Sannigrahi *et al.*, 2010).

Some medicinal plant considered as potential source of anti-microbial agents. Seventy-six extracts of thirty-one Sudanese medicinal plants belonging to twenty-one families were investigated for their anti-bacterial activity against four bacteria by (El Tohami *et al.*, 1997). Out of the seventy-six extracts tested, sixty-four exhibited inhibitory effects against at least one of the tested micro-organisms. Of these, seven plants showed significant activity against the four tested organisms, namely *Bacillus subtilis*, *Staphylococcus auerus*, *Escherichia coli* and *Pseudomonas aueroginosa*.

Acute inflammation is a rapid, short–lived physiological response, characterized by accumulation of fluid, plasma proteins and the leukocytes which release a large number of soluble mediators which modulate and maintain the inflammation (Rosenberg and Gallin, 1999).

Carrageenan induced rat paw oedema has been used for assessment of the anti-inflammatory activity of many plant extracts and essential oils (Hajhashemi *et al.*, 2003; Khalil *et al.*, 2006; Orhan *et al., 2006*) .

The *Amaranthus viridis* plant locally called Lissan elTair Kabir, is a member of the Amaranthaceae family, has been used in traditional medicine in many parts of the world. Traditionally is an edible plant which is grown in all regions of India, stem used as antidote for snake bites (Obi *et al.*, 2006). Leaves used for scorpion stings, constipation, inflammation, eczema, bronchitis, anemia, leprosy, an infusion of powdered used for stomach problems (Sena, 1998). Seeds also used in pregnant women to lessen labor pain. Infusion of plant has been used as a diuretic and anti-inflammatory agent of the urinary tract, venereal diseases, vermifuge, anti-emetic and laxative (Quershi *et al.*, 2008). Poultice and boils of leaves are used for abscesses and skin cleansing, anti-diabetic (Kesari *et al.*, 2005), anti-histaminic (Yamamura *et al.*, 1998) and anticarcinogenic (Yen *et al.*, 2001). In Sudan, the plant is used in Western Darfur and Wad Madani as anti-helminthic and a fodder for grazing animals. The present study aimed to investigate the anti-inflammatory effects of ethanolic extract of *Amaranthus viridis* in carrageenan induced rat paw oedema model.

MATERIALS AND METHODS

Plant material

Amaranthus viridis plant was obtained from Nile river banks (November 2012) in Khartoum, Sudan. The plant dried under sun-rays. The sample was kept in the Department of Pharmacology and Toxicology at the Medicinal and Aromatic Plants Research Institute (MAPRI) – National Center for Researches (Khartoum). Plant was authenticated by Dr.Haider AbdelGader (MAPRI) .

Preparation of extract

The plant dried under sun-rays, after complete dryness removed for extraction. Specific weight of the plant sample (260 gram) was soaked in 2500 ml of 80 % ethanol for about 3 days with daily filtration and evaporation of the solvent under reduced pressure using rotary evaporator apparatus. Final extract residues allowed to air in petri-dishes till complete dryness (Harborne, 1984).

All drugs used were of the highest commercially available purity. Indomethacin (powerful anti-inflammatory, time dependent, non-selective inhibitor for cycloxygenase enzymes), carrageenan (standard inflammatory inducer) and normal saline were purchased from Sigma – Aldrich, Germany.

Experimental animals

Albino rats were obtained from the Faculty of Pharmacy, University of Khartoum. No informed consent was obtained.

Experimental design

Carrageenan-induced rat paw oedema has been used for assessment of the anti-inflammatory activity of many plant extracts. The method used was carried out according to Winter *et al.* (1962). Twenty Albino rats were housed within the premises of the Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, with feed and water provided *ad libitum*. The rats were allotted into four groups each of 5 rats.

Group 1: received the vehicle carrageenan (2 ml/kg) at sub-plantar region and served as negative control.

Group 2: received indomethacin (10 mg/kg) intraperitoneally, and served as standard reference and act as positive control.

Group 3: received the crude ethanolic extract intraperitoneally at 250 mg/kg.

Group 4: received the crude ethanolic extract intraperitoneally at 500 mg/kg.

One hour following the previously mentioned treatments, paw swelling was induced by sub-plantar injection of 0.1 ml of 1 % w/v carrageenan in normal saline 0.9 ml into the right hind paw of all groups. The oedema was quantified by measuring the hind paw thickness at 0, 1, 2 and 3 hours after injection, with a micrometer screw gauge. The increase with linear diameter of the right hind paws were taken as an indication of the paw oedema. The percentage inhibition of the inflammation was calculated from the formula described by (Hajhashemi *et al.*, 2003).

$$Percent\ inhibition = D_0 - D_T / D_0 \times 100$$

D_0 is the the average inflammation (hind paw oedema) of the control group of rats at a given time.

Dt is the average inflammation of the drug treated (that is, reference indomethacin or extracts) in rats.

Statistical analysis

The collected data were statistically analyzed using Mann-Whitney test. P value < 0.05 was considered statistically significant.

RESULTS

The results of the effects of *Amaranthus viridis* plant extracts and indomethacin on oedema in carrageenan model are summarized in Table 1.

Negative inhibition percentage (-0.51 %) in the table at 250 mg/kg, means there was no reduction for oedema at Hour 1 according to the equation described earlier. The crude ethanolic extract of *Amaranthus viridis (*250 mg/kg) when compared with the control and the reference drug, produced significant inhibition ($P \leq 0.05$) in rat paw oedema of 40.34% at 3 hours post treatment. The crude ethanolic extract of *Amaranthus viridis (*500 mg/kg) when compared with the control and the reference drug, produced significant inhibition ($P \leq 0.05$) in rat paw oedema of 70.45 % at 3 hours post treatment.

DISCUSSION

The present study was conducted to investigate the possible anti-inflammatory effects of Amaranthus viridis extract in carrageenan model. Paw swelling is one of the major factors in assessing the degree of inflammation and efficacy of the tested drugs (Begum and Sadique, 1988) and (Mizushima *et al.*, 1972). The crude ethanolic extract of Amaranthus viridis at two doses level were investigated for their anti-inflammatory activity. The two doses significantly inhibited or decreased oedema in a time and dose dependent manner and the maximum inhibition percentage were recorded with the dose of 500 mg/kg as 70.45% at the end of the follow up period.

The current study results might be considered as first report for the anti-inflammatory activity by both dose levels of ethanolic extract at that time of Amaranthus viridis whole plant. Phytochemical screening of the medicinal plants showed good anti-inflammatory activity as it may contain secondary metabolites like alkaloids, triterpenoids, polyphenolics and flavonoids. These classes of plant, which possess secondary metabolites, are considered the sources of chemicals, which are responsible for wide therapeutic activities of several plants (Debella, 2002). Phytochemical investigation on Amaranthus viridis yielded several classes of secondary metabolites such as flavonoids, steroids, saponins and phenolic compounds many of which express biological activities (Mayer *et al.*, 1982; Emam, 1999; Khan *et al.*, 1982). These compounds are known to be potent cyclo-oxygenase-1 (COX-1) inhibitors, through their binding nature with proteins. (Reddy and Reddy, 2009). Since the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agent acting by the mediators of acute inflammation. (Sawadogo *et al.*, 2006). The extract may have exhibited its anti-inflammatory actions by means of either inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin act by the reduction of sensitization of pain receptors caused by prostaglandins at the

Table 1: Effects of treatment of crude ethanolic extract of *Amaranthus viridis* on oedema in carrageenan model.

Group	Hour 1	Hour 2	Hour 3
Control negative (Carrageenan)	1.95	1.89	1.76
2 mg/kg B.Wt			
Control positive (Indomethacin)	1.16 ± 0.37	0.47 ± 0.24	0.10 ± 0.06
10 mg/kg B.Wt	(40.51 %)	(75.13%)	(94.32%)
Ethanolic extract of *Amaranthus viridis*	1.96 ± 0.11	1.66 ± 0.35	1.05± 0.32
250 mg/kg	(-0.51 %)	(12.17%)	(40.34%)
P *value*	0.009*	0.009*	0.009*
Ethanolic extract of *Amaranthus viridis*	1.25± 0.41	1.10 ± 0.28	0.52± 0.22
500 mg/kg	(35.89%)	(41.79%)	(70.45%)
P value	0.117	0.009*	0.012*

B.Wt: Body weight
Data were expressed as mean ± SD and percentage
*P < 0.05 was considered statistically significant

inflammation site (Dhara *et al.*, 2000). The different triterpenoids, polyphenolics and other chemical constituents of the plant extract may be involved in the observed anti-inflammatory effects of the plant extract and may be having actions similar to NSAIDs.

CONCLUSION

The present study concluded that the ethanolic extract of Amaranthus viridis possessed potent anti-inflammatory activity at 250 and 500 mg/kg.

Recommendation: The present study recommended the possible use of Amaranthus viridis as a remedy for treatment of inflammation.

Further studies are needed to investigate the phytochemical/s responsible for the anti-inflammatory effect and toxicological studies are needed to evaluate the safety of the plant constituents in the different animal species.

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RESEARCH ARTICLE

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Bacterial load of water used for livestock's operations in Al Obied city of North Kordofan State, Sudan

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KEYWORDS

Bacterial load
Coliform
Dairy farm
Water

ABSTRACT

This study was conducted in Al Obied city in North Kordofan State in order to evaluate the bacterial load in water for livestock operations, based on bacteriological examinations and viable counts. A total of 40 water samples were obtained (10 from dairy farms, 14 from animal's markets and 16 from slaughterhouses). All samples were cultured on Blood Agar and MacConkey for bacterial isolation and on nutrient agar for viable counts. The result of bacterial viable count of water samples collected from different sources was high and ranging between $7X10^8$ and $36X10^8$ CFU/ml. Application of analysis statistic using one way ANOVA for bacterial count in water collected from different sources, revealed that there was statistical significance (p. value= 0.000, p-value< 0.05) for water samples collected from different sites. Bacterial isolates from dairy farms comprised 10 isolates including 3 *Escherichia coli* (25.1%), 2 *Klebsiella pneumoniae* (16.7%), 2 *Pseudomonas aerogenosa* (16.7%), 1 *Staphylococcus aureus* (8.3%), 1 *Streptococcus uberis* (8.3%), 1 *Bacillus subtilis* (8.3%), 1 *Micrococcus variens* (8.3%) and 1 *Micrococcus luteus* (8.3%). Gram negative Bacterial isolates represented the higher percentage (58.5%) of the total bacterial isolates from dairy farms. Bacteria isolated from animals' markets were 5 *E. coli* (31.3%), 4 *Ps. aerogenosa* (25.0%), 4 *S. aureus* (25.0%), 2 *Str.uberis* (12.5%), 1 *B. subtilis* (6.2%). Gram negative Bacteria represented the higher percentage (56.3%) of the total bacteria isolated from animals' markets. Bacteria isolated from slaughterhouses were 5 *S. aureus* (31.3%), 4 *Ps. aerogenosa* (25.0%), 3 *Str. uberis* (18.7%), 2 *M. variens* (12.5%),2 *M. luteus* (12.5%). Gram positive Bacteria represented the higher percentage (75.0%) of the total bacteria isolated from slaughterhouses.

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INTRODUCTION

Animals and humans can survive days, weeks or months without food, but only about four days without water (WHO, 2008). Water, although an absolute necessity for life can be a carrier of many diseases. Water can be hard or soft, natural or modified, bottled or tap, carbonated or still (Kendall, 1992). Good quality water is odorless, colorless, tasteless and free from fecal pollution and chemicals in harmful amounts. The World Health Organization (WHO) has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water, or unavailability of water (WHO, 2008). Microorganisms enter into drinking water via humans and animals' intestinal secretions in areas where sanitation conditions are poor or absent. When found in drinking water, microorganisms constitute a real indication that it should not be used for human consumption if these contaminants are found in excess of the maximum permissible level (1×10^2 CFU ml-1) (WHO, 2008). Bacterial contamination can get into groundwater by many ways; wild and domestic animals, birds and dairy farms wastes situated in a watershed area or within the hydrological catchments of groundwater. However, these have been found to be a pathogenic contamination source of drinking water (Obiri and Jones, 2001). Also, the presence of campylobacter in waters within agricultural areas is a real evidence of environmental contamination by sewage effluent coming from agricultural areas (Obiri and Jones, 2001). Biomass that resulted from degradable materials are deposited into drinking water distribution pipes and accumulates biofilms which accelerate the growth of microorganisms and protect them against disinfection agents (Lewis, 2001). A major type of bacteria in polluted water is coliform bacteria. The most important species of the group includes *E. coli, klebsiella spp. and Enterobacter spp.* (Lewis, 2001). Non-coliform bacteria are also common in polluted water and include *Streptococcus, Proteus and Pseudomonas spp. Bifidobacteria* are one of the most common bacterial types found in the intestines of humans and other animals and may be used as indicators for human fecal pollution (Bonjoch *et al.*, 2004). In Sudan Aliaa (2005) isolated coliforms from drinking water samples collected from Khartoum State. Amna and Atif (2014) isolated *Micrococcus spp., Pseudomonas spp., Bacillus spp., Staphylococcus spp., Corynobacterium spp., Aeromonas spp., Actenobacillus spp., Moraxella spp. and Flavibacterium spp*. Regarding bacterial counts, application of analytical statistic using one-way analysis of variance (ANOVA) revealed that there was no statistical significance (F-value = 0.198, p-value > 0.05) for bacterial counts of the drinking water in Khartoum State (Amna and Atif, 2014).

This study was aiming at evaluating the bacterial load of water in livestock operations in Al Obied city in North Kordofan State.

MATERIALS AND METHODS

Source of samples

A total of 40 water samples were collected from different sources (tap and tank water) in Al Obied city, North Kordofan state during the last 6 months of the year 2019.

Sampling procedure

Fifty milliliters of tap water were collected in glass bottles. Collection was done as follows:

(1) The outside nozzle of the tap was cleaned carefully.

(2) The tap was turned on full, and the water was allowed to run to waste for 1 minute.

(3) The sample bottle was then filled from the gentle flow of water.

(4) Contamination was avoided by not allowing any surface to touch the screw thread of the bottle neck or the inside of the cap.

(5) The cap of the bottle was then replaced.

Fifty milliliters of tank water were collected in glass bottles. Collection was done as follows:

(1) The cap was removed and the mouth of the bottle was faced up.

(2) The bottle was pushed forward horizontally until it was filled.

The samples from taps and tanks were labeled with a sample code number, and transported at 4°C to the laboratory of college of Veterinary Medicine, University of Bahri.

Bacterial viable count

According to Quinn et al. (2011), tenfold dilutions of a bacterial suspension was made before conducting a viable count to find the number of bacteria/ml in the original sample. Sample was thoroughly mixed before sampling and a separate pipette was used for each transfer step. Serial dilutions of water samples were made. Spread plate method was followed and an inoculum of 0.1 ml of each dilution was placed on the surface of an agar plate. The inoculum was spread rapidly over the entire agar surface. Inoculated plates were left to dry and then incubated for 24–48 hours at 37C. The total colony count per milliliter of water was calculated by multiplication of the number of colonies counted by dilution marked. After incubation, plates inoculated with a sample dilution yielding between 30 at 300 colonies are read; the colony count should be an average of the two or four plates inoculated with the selected dilution.

Isolation, identification and characterization of bacterial isolates

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full of the enriched broth streaked onto blood agar, MacConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37^{o}C for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in carbohydrates media and for sediment formation. One half colony from each plate was used for performing gram staining. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4^{o}C.

Biological and biochemical identification of the bacteria

The purified isolates were identified as previously described by Smith et al. (1986) and Barrow and Feltham (2004). The identification includes: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation fermentation test, motility test, glucose breakdown test and fermentation of carbohydrates).

RESULTS

Bacterial viable count of water samples collected from dairy farms in Al Obied city

All of the 10 water samples collected from dairy farms had a bacterial viable count ranging between 20X10^{8} and 36X10^{8} CFU/ml (Table 1).

Table 1: Bacterial viable counts of water samples collected from dairy farms in Al Obied city.

Sample number	CFU/ml	Sample number	CFU/ ml
1	35X10^{8}	6	35X108
2	34X10^{8}	7	35X10^{8}
3	35X10^{8}	8	35X10^{8}
4	28X10^{8}	9	28X10^{8}
5	36X10^{8}	10	20X10^{8}

Bacterial viable count of water samples collected from animals' markets in Al Obied city

All of the 14 water samples collected from animals' markets had a bacterial viable count ranging between 7X10^{8} and 35X10^{8} CFU/ml (Table 2).

Bacterial viable count of water samples collected from slaughterhouses in Al Obied city

All of the 16 water samples collected from slaughterhouses had a bacterial viable count ranging between 8X10^{8} and 35X10^{8} CFU/ml (Table 3).

Comparison between bacterial viable counts of water from different sources

Application of analytical statistic using one-way ANOVA revealed that there was statistical significant (p-value= 0.000, p-value< 0.05) for bacterial viable counts of water collected from different sites.

Table 2: Bacterial viable counts of water samples collected from animals' markets in Al Obied city.

Sample number	CFU/ml	Sample number	CFU/ml
1	18X108	8	13X108
2	19X108	9	20X108
3	23X108	10	11X108
4	20X108	11	7X108
5	23X108	12	10X108
6	13X108	13	9X108
7	35X108	14	8X108

Table 3: Bacterial viable counts of water samples collected from slaughterhouses in Al Obied city.

Sample number	CFU/ml	Sample number	CFU/ml
1	$28X10^8$	9	$34X10^8$
2	$25X10^8$	10	$35X10^8$
3	$29X10^8$	11	$20X10^8$
4	$28X10^8$	12	$16X10^8$
5	$33X10^8$	13	$24X10^8$
6	$19X10^8$	14	$9X10^8$
7	$18X10^8$	15	$8X10^8$
8	$20X10^8$	16	$10X10^8$

Bacteria isolated from water samples collected from dairy farms in Al Obied city

In this investigation a total of 12 bacterial isolates were obtained from 10 water samples. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 3 *E. coli* (25.1%), 2 *Klebsiella pneumoniae* (16.7%), 2 *Pseudomonas aerogenosa* (16.7%), 1 *Staphylococcus aureus* (8.3%), 1 *Streptococcus uberis* (8.3%), 1 *Bacillus subtilis* (8.3%), 1 *Micrococcus variens* (8.3%),1 *Micrococcus luteus* (8.3%) (Table 5).

Bacteria isolated from water samples collected from animals' markets in Al Obied city

In this investigation a total of 16 bacterial isolates were obtained from 14 water samples. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 5 *E. coli* (31.3%), 4*Ps. aerogenosa* (25.0%), 4*S. aureus* (25.0%), 2*Str.uberis* (12.5%), 1 *B. subtilis* (6.2%) (Table 6).

Bacteria isolated from water samples collected from slaughterhouses in Al Obied city

In this investigation a total of 16 bacterial isolates were obtained from 16 water samples. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 4) the identified bacteria were: 5*S. aureus* (31.3%), 4*Ps. aerogenosa* (25.0%), 3*Str.uberis* (18.7%), 2*M. variens* (12.5%),2*M. luteus* (12.5%) (Table 7).

DISCUSSION

Microbiological quality analysis of drinking water in dairy farms, animal markets and slaughter houses is of paramount concern because of the possible risk to health caused by bacteria in drinking water. Monitoring and assessment of quality of drinking water is primarily a health-based activity which helps to protect public health Lack of basic knowledge affects clearly the quality of water and this could strongly result in water borne disease (Al Beeli, 2006).

In this study the bacterial viable count of water samples collected from dairy farms, animal markets and slaughterhouses in Al Obied city, was high and ranging between $7X10^8$ and $36X10^8$ CFU/ml. According to WHO (2008) microorganisms should not exceed the maximum permissible level (1×10^2 CFU ml^{-1}) in water used for consumption. Also, clean water is important for animals' health and many pathogenic bacteria can be transmitted through water (peter *et al.*, 2017). Amna and Atif (2014) mentioned that the total viable count for bacteria showed that water samples collected from Khartoum State were found most loaded; this may be logical because troughs are exposed to contamination

Table 4: Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***E. coli***	***S. aureus***	***Ps. aerogenosa***	***K. pneumoniae***
Aerobic growth	+	+	+	+
Colonies on MacConkey	Bright Pink	Pink	Bright Pink	Pink
Haemolysis on blood agar	+	+	+	-
Gram reaction	-	+	-	-
Shape	Rods	Cocci	Rods	Rods
Spore	-	-	-	-
Motility	+	-	+	-
Catalase	+	+	+	+
Oxidase	-	-	+	-
Indole	+	-	-	+
Methyl red	+	+	-	-
VP	-	-	-	-
Citrate	-	-	+	+
H_2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	-	+
Lactose	+	+	-	+
Coaggulase	-	+	-	+

Table 4 (continued): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***Str. uberis***	***M. luteus***	***M. variens***	***B. subtilis***
Aerobic growth	+	+	+	+
Colonies on MacConkey	Pink	Pink	Pink	Pink
Haemolysis on blood agar	-	+	-	+
Gram reaction	+	+	+	+
Shape	Cocci	Cocci	Cocci	Rods
Spore	-	-	-	+
Motility	-	-	-	+
Catalase	-	+	+	+
Oxidase	-	+	+	-
Indole	-	-	-	-
Methyl red	-	-	-	-
VP	+	-	-	-
Citrate	-	-	-	-
H_2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	+
Coaggulase	-	-	-	-

from many sources like cattle while drinking, animal faeces, air, dust and feed stuffs, similarly from bacterial contamination and bad storage of water.

In this study different bacterial species (*E. coli*, *K. pneumoniae*, *Ps. Aerogenosa*, *S. aureus*, *M. variens*, *M. luteus*, *Str. uberis* and *B. subtilis*), were isolated from water samples collected from different sources in Al Obied city. Amna and Atif (2014) reported that *Micrococcus spp*., *Pseudomonas spp.*, and Bacillus spp. were dominant in water samples collected from dairy farms in Khartoum State. Sanaa and Rawda (2009) isolated faecal coliform (*E. coli*), coliform group (*Klebsiella spp., Citrobacter spp., Enterobacter spp.*), some pathogenic and potential pathogenic bacteria (*S. aureus*, Salmonella spp., *Yersienia enteriocolitica*, *Proteus spp*., *Bacillus spp.* and *Pseudomonas aeruginosa*). Isolation of pathogenic bacteria is of highly importance and indicated that the water is unsafe.

In this study coliforms were predominant (51.5% and 56.3% in dairy farms and animal markets respectively) bacteria compared to gram positive bacteria. Also, in this study faecal coliorm (*E. coli*) represented the predominant bacteria (25.1% and 31.3% in dairy farms and animal markets respectively). Sara *et al*. (2016) reported that most water samples obtained from Khartoum state showed the presence of total coliform, and fecal coliform. In addition, Amira (2014) reported that water samples collected from Wadmedani were highly contaminated with total coliform and fecal coliform compared to Khartoum drinking water samples. In relevance to our findings, Al-Beeli (2006) reported that *E. coli* is the predominant bacteria (18%) isolated from water samples collected from dairy farms in Eastern and Southern Sudan. Also Mohamed *et al*. (2013) reported high levels of total coliform in water samples collected from dairy farms in West Kordofan. Workers in dairy farms, animal markets and slaughterhouses in Al Obied city are at risk of infection with bacterial diseases transmitted by water because of their bad practices as washing their hands with animals' water. Also, meat consumers will be at risk after washing carcasses with unsafe water.

Table 5: Total number and percentage of bacteria isolated from water samples collected from dairy farms in Al Obied city.

Isolated bacteria	No. / %
E. coli	3 (25.1%)
K. pneumoniae	2 (16.7%)
Ps. aerogenosa	1 (5.6%)
Str. uberis	2 (16.7%)
S. aureus	1 (8.3%)
M. luteus	1 (8.3%)
B. subtilis	1 (8.3%)
Total	**12 (100%)**

Table 6: Total number and percentage of bacteria isolated from water samples collected from animals' markets in Al Obied city.

Isolated bacteria	No. / %
E. coli	5 (31.3.1%)
Ps. aerogenosa	4 (25.0%)
Str. uberis	2 (12.5%)
S. aureus	1 (6.2%)
B. subtilis	1 (6.2%)
Total	**16 (100%)**

Table 7: Total number and percentage of bacteria isolated from water samples collected from slaughterhouses in Al Obied city.

Isolated bacteria	No. / %
S. aureus	5 (31.3.0%)
Ps. aerogenosa	4 (25.0%)
Str. uberis	3 (18.7%)
M. variens	2 (12.5%)
M. luteus	2 (12.5%)
Total	**16 (100%)**

CONCLUSION AND RECOMMENDATIONS

Regarding bacterial count, the results showed high level of contamination of all water samples collected from dairy farms, animals' markets and slaughterhouses in Al Obied City. Application of analysis statistic using one-way ANOVA

revealed that there was statistical significant (p.value = 0.000, p-value< 0.05) for water sample collected from different sites. Coliforms (*K. pneumoniae*, *Ps. Aerogenosa*) and faecal coliorm (*E. coli*) were predominant bacterial isolates.

Microbiological analysis of water for total bacteria and coliform is necessary to determine sanitary quality. The possible consequence is of such severity than its control which is always very important and should never be compromised.

Water analysis for the detection of faecal pollution should be prompted to determine the level of faecal pollution in ground water resources whenever water is intended for animal and human use.

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RESEARCH ARTICLE

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Comparative studies on two types of chilling methods in poultry slaughterhouses in Khartoum State, Sudan

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KEYWORDS

Poultry slaughterhouses
Chilling methods
Process steps
Bacterial contamination

ABSTRACT

The study aimed to evaluate the effect of chilling methods and their related process steps (scalding, de-feathering, evisceration, and chilling) on chicken bacterial load in poultry processing plants in Khartoum State during the period from November 2019 to May 2020. Immersion and air chilling methods were both investigated in eight poultry slaughterhouses, four slaughterhouses for each method. Both checklist and microbiological tests were used in the evaluation. A total of 160 samples were collected from 32 carcasses, four carcasses were taken after each process step, from five parts of broiler carcasses (wings, legs, thigh, breast, and backbone) and all these samples were examined bacteriologically. The samples were subjected to the total bacterial count and isolation of *Enterobacter*iacea and *E. coli*. The result revealed that the mean bacterial load count of legs samples was highly significantly greater in emersion method (2.1±.05) compared to air chilling method (.7±.1), p=0.00 Also, the result indicates highly significant differences between the two chilling methods with regard to *E. coli+Salomnella* and *E. coli+Enterobacter* (0.00), *E. coli+Shigella* (0.001), *E. coli+Citrobacter* (0.00). When comparing the chilling methods with their respective process steps, scalding process showed no significant difference between the two chilling methods. Concerning scalding water change during each shift, all slaughterhouses (100%) were not changing scalding water. Washing process step revealed no significant differences between the two chilling methods in terms of in and out washing of carcasses, P.214 and .500, respectively. For chiller temperature all plants using air chilling practiced optimal temperature (4°C or less), while three quarters (75%) of plants using immersion chilling exceeded the acceptable limit but there were no significant differences between the two chilling methods, with p>0.071. In conclusion the microbial contamination of poultry meat in slaughterhouses in Khartoum State was higher in emersion chilling method compared to air chilling method.

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INTRODUCTION

The legal frame of food safety in Sudan started with the Public Health Act (1939) which deals with food hygiene issues. Poultry industry is growing very fast since the last ten years in Sudan. This rapid growth involved diversified stakeholders in poultry production without having proper training and knowledge in hygienic practices, and food safety system include hazard analysis and critical control point (HACCP) (Mustafa *et al*., 2016).

Mustafa *et al*. (2016) reviewed research studies conducted in Khartoum State. These reflected that the situation of microbial and chemical contamination in poultry and red meat in the slaughterhouses and factories of Khartoum State was higher than the level recommended by international regulatory bodies. This was attributed to the absence of implementation of Good Manufacturing Practices and Good Hygiene Practices. This was also evidenced by Munir *et al*. (2014) who investigated the bacterial contaminants of poultry meat and poultry products in Khartoum State and revealed that *E. coli* represented the highest contaminant with prevalence of 34.6% followed by Proteus spp. 32.2%, then Citrobacter spp. 13.5% and Salmonella spp. 10.4%.

Contamination of broiler meat with Salmonella spp., *Escherichia coli* and Staphylococcus spp. was evident in many studies. Ahmed (2014) assessed the measures of poultry meat safety in one of the Khartoum State slaughterhouses with the aim to detect the bacterial load and types of bacteria on the carcasses of broiler by applying the principles of HACCP. The contaminating bacteria isolated in the study were *E. coli*, *Staphylococcus spp.* and Salmonella spp. organisms. Contamination of broiler meat with Salmonella spp. and *Escherichia coli* were predominant in slaughterhouse processing as reported by Omer *et al*. (2015) who studied poultry meat carcasses at an automatic slaughterhouse in Khartoum State.

Enterobacteriaceae is a useful indicator of hygiene and post processing contamination of heat-processed foods. This family has been used as indicators of food quality and also for food safety. Enterobacteriaceae counts are an effective method to assess environments, such as post process food contact surfaces and help to quickly determine potential sources of contamination (Halkman *et al*., 2014). To this end, quality assurance programs in slaughterhouses are applied to ensure the safety of meat for human consumption (Govender, 2014). The objective of this study was to evaluate the effect of chilling method and its related process steps on chicken bacterial load in poultry processing plants in Khartoum State.

MATERIALS AND METHODS

Study Area

This study was conducted in the three localities of Khartoum State; Khartoum, Omdurman and Bahri.

Study design

A cross sectional and analytical study that lasted for 28 weeks from November 2019 to May 2020 was carried out in eight poultry slaughterhouses. Of these, seven were commercial poultry companies and one was a commercial slaughterhouse serving small poultry farms.

Chilling methods and related process steps

Air chilling and immersion chilling were the two investigated chilling methods, whereas the related process steps were a) scalding (scald temp and turbulence used; scald time per seconds); b) De-feathering (time sprayed with water -seconds); c) Evisceration (viscera mechanically opened; chickens hanged on legs till the end of evisceration; the technique for viscera suction); d) inside and outside washing (water temp.-washing duration).

Two slaughterhouses in Khartoum locality used air chilling method, four slaughterhouses in Omdurman locality used both air chilling method (two slaughterhouses) and immersion chilling method (two slaughterhouses) and two slaughterhouses in Bahri locality used immersion chilling method (one of which was commercial serving small poultry farms). Both checklist and microbiological tests were used in the evaluation.

Collection of Samples and sample Size

Simple random swab sampling of 160 swab samples were collected from the investigated poultry slaughterhouses.

Type of Samples

All samples were taken from 32 carcasses. Twenty swab samples were collected from 4 carcasses in each slaughterhouse from 5 parts: leg, thigh, breast, wing and backbone in 4 different process steps: after scalding, after de-feathering, after evisceration and after chilling.

Sample collection and transport

The samples were taken directly during the processing in the investigated slaughterhouses which operated in the morning shift except one (the commercial slaughterhouse) operated at night.

In the slaughterhouse, an average of 11,000 birds were killed per day during one shift either in early morning or in the evening. Each day, five parts of carcasses were randomly collected before they entered the chilling system and after entering in four different process steps: scalding, defeathering, and in and out carcass washing and chilling.

Samples were packed in sterile polyethylene bags and transported to the laboratory of the College of Veterinary Medicine at University of Bahri using an insulated ice box contained an ice pack at 4°C.

Bacterial Count

The total bacterial count is one of the key indicators in the field of hygiene management. Standard plate or viable count a sample is diluted in a serious of dilution blanks, then the dilutions plated from 5th serial dilution onto MacConkey media for Enterobacteriaceae.

Microbiological analysis

Aerobic colony count (ACC), Enterobacteriaceae and coliforms using ISO 4833 and *E. coli* using ISO 16649-1, ISO 16649- 2 (2008) method were performed.

Statistical Analysis

The collected data were coded and analyzed using Statistical Packaging for the Social Sciences (SPSS/PC version 21 for windows). Data were analyzed for descriptive statistical analysis to test for significant differences ($P < 0.05$) between the different chilling methods and the related process steps.

RESULTS

The mean bacterial load count of legs samples was highly significantly greater in emersion method (2.1±.05) compared to air chilling method (.7±.1), p=.000. Also, the mean bacterial count of breast (1.8±.08 vs. 1.6±.2), thigh (1.9±.1vs. 1.8±.1), backbone (1.8±.08 vs. 1.5±.2) and wings (1.6±.1 vs. 1.5±.1) was not significantly greater in emersion method compared to air chilling method, respectively, p> 0.05 (Table 1).

Table 1: Mean of bacterial load count Log 10 (CFU/ml) X 10^5 in MacConkey Agar (MCA) media for enterobacteriaceae from different carcass sites with different chilling methods

Chilling method		Sample location				
	Statistic	legs	Breast	Thigh	Backbone	Wings
Emersion	Mean± SE	2.1±.05	1.8±.08	1.9±.1	1.8±.08	1.6±.1
	Minimum	1.7	1.3	1.2	1.2	1.0
	Maximum	2.4	2.5	2.8	2.3	2.4
Air chilling	Mean± SE	0.7±.1	1.6±.2	1.8±.1	1.5±.2	1.5±.1
	Minimum	0.3	0.00	1.00	0.00	0.7
	Maximum	2.4	2.5	2.5	2.5	2.5
Total	Mean± SE	1.4± .1	1.7±.1	1.8±.1	1.7±.1	1.6±.1
	Minimum	0.3	0.00	1.00	0.00	0.7
	Maximum	2.4	2.5	2.8	2.5	2.5
ANOVA (P-value)		0.00*	0.445	0.814	0.084	0.681

SE = Standard error of means

*P-value considered significant at less than 0.05 levels

Table (2) represents the incidences and microbial counts for enterobacteriaceae and *E. coli* in emersion and air chilling methods using Ethylene Methylene Blue (EMB). The result indicates highly significant differences between the two chilling methods with regard to *Enterobacter and Citrobacter* (.017), *Shigella* (.004), *Klebseilla* (0.001), *E.coli+Salomnella and E.coli+Enterobacter* (0.00), *E.coli+Shigella* (0.001), *E.coli+Citrobacter* (0.00), *and Enterobacter+ Citrobacter* (.041).

This study also investigated the two chilling methods with their related process steps using a checklist. Concerning scalding water change during each shift, all slaughterhouses (100%) were not changing scalding water during the shift. Also, no significant difference was found between scalding water change during the shift and all chickens immersed together (Table 3).

Table (4) shows the efficacy of defeathering machines to keep chickens not to fall down, proper cleaning of defeathering fingers and chicken pass with feather return to machine for more trimming in the two methods. Nonetheless, no significant differences were found between the two methods, with p> 0.214.

Table (5) shows that no significant difference was found between the two chilling methods in terms of opening viscera mechanically, chicken hanged on legs until evisceration process step finished, technique for viscera suction and in and out washing machine for carcasses. No significant differences were found between the two methods, with p> 0 .214.

Table (6) revealed no significant difference between the two chilling methods in terms of in and out washing of carcass and availability of potable water free from salt with p .214 and .500, respectively.

The study revealed that sodium chloride was used in three quarters 3 (75%) of the slaughterhouses using immersion chilling method and one quarter used citric acid, while half (2) (50%) of the slaughterhouses using air chilling method used sodium chloride. There was no significant difference between the two chilling methods, with p>.500.

The study also revealed that only half of the slaughterhouses using immersion chilling didn't change water of chillers during shifts. There was significant difference between the two chilling methods with p> .018.

Table 2: Incidences and microbial counts for enterobacteriaceae and *E. coli* in emersion and air chilling methods using EMB

Type of bacteria	Chilling method	Mean	SE	Sig.
E.coli	Emmersion	0.163	0.042	0.75
	Air chilling	0.147	0.029	
	Total	0.152	0.024	
Enterobacter	Emersion	0.088	0.032	0.017*
	Air chilling	0.020	0.012	
	Total	0.043	0.012	
Citrobacter	Emersion	0.100	0.034	0.017*
	Air chilling	0.027	0.013	
	Total	0.052	0.015	
Shigella	Emersion	0.075	0.030	0.004*
	Air chilling	0.007	0.007	
	Total	0.030	0.011	
Klebseilla	Emersion	0.075	0.030	0.001*
	Air chilling	0.000	0.000	
	Total	0.026	0.011	
Salmonella	Emersion	0.150	.0402	0.522
	Air chilling	0.120	.0266	
	Total	0.130	0.022	
E.coli+Salomnella	Emersion	0.188	0.044	0.000*
	Air chilling	0.000	0.000	
	Total	0.065	0.016	
E.coli+Enterobacter	Emersion	0.213	0.046	0.000*
	Air chilling	0.013	0.009	
	Total	0.083	0.018	
E.coli+Shigella	Emersion	0.076	0.030	0.001*
	Air chilling	0.000	0.000	
	Total	0.026	0.011	
E.coli+Citrobacter	Emersion	0.088	0.032	0.00*
	Air chilling	0.000	0.000	
	Total	0.030	0.011	
Enterobacter +Citrobacter	Emersion	0.075	0.030	0.04*
	Air chilling	0.020	.0115	
	Total	0.039	.0128	
Enterobacter +Citrobacter+shigella	Emersion	0.038	0.021	0.09
E.coli+Salomnella +Enterobacter	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
Enterobacter +salmonella	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
E.coli+Enterobacter +Citrobacter	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
E.coli+klebsiella +Citrobacter	Emersion	0.013	0.013	0.17
	Air chilling	0.000	0.000	
	Total	0.004	0.004	
E.coli+kebseilla	Emersion	0.025	0.018	0.052
	Air chilling	0.000	0.000	
	Total	0.009	.0061	
Salmonella+Citrobacter	Emersion	0.000	0.000	0.47
	Air chilling	0.007	0.007	
	Total	0.004	0.004	

*P-value considered significant at less than 0.05 level

EMB= Ethylene methylene blue, SE= Standard error of means

Table 3: Scalding processes with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Scalding water change during shift	Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	Not computed
	No	4 (100%)	4 (100%)	8 (100%)	
Total		4 (100%)	4 (100%)	8 (100%)	
All chickens are immersed together in hard scalding	Yes	2 (50%)	0 (0.0%)	2 (25%)	0.214
	No	2 (50%)	4 (100%)	6 (75%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 4: Defeathering procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-value
		Immersion chilling	Air chilling		
The efficacy of defeathering machines to keep chickens not to fall down; Proper cleaning of de-feathering machine rubber fingers	Yes	2 (50%)	4 (100%)	6 (75%)	0.214
	No	2 (50%)	0 (0.0%)	2 (25%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Are chickens pass with feather return to machine for more trimming?	Yes	2 (50%)	0 (0.0%)	2 (25%)	0.214
	No	2 (50%)	4 (100%)	6 (75%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 5: Evisceration procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Is viscera mechanically opened? Are chickens hanged on legs till the end of evisceration? Is there any technique for viscera suction?	Yes	2 (50%)	4 (100%)	6 (75%)	0.214
	No	2 (50%)	0 (0.0%)	2 (25%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 6: Washing procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Is there in and out washing machine for washing carcasses?	Yes	2 (50%)	4 (100%)	6 (75%)	0.214
	No	2 (50%)	0 (0.0%)	2 (25%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Is potable water free from salt available?	Yes	1 (25%)	2 (50%)	3 (37.5%)	0.500
	No	3 (75%)	2 (50%)	5 (62.5%)	
Total		4 (100%)	4 (100%)	8 (100%)	

Table 7: Chilling procedures with different chilling methods

Parameter	Response	Chilling method		Total	P-Value
		Immersion chilling	Air chilling		
Chicken internal breast temperature before entering chiller 40°C	Yes	4(100%)	4(100%)	8(100%)	0.214
	No	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Chiller temperature more than 4°C reach 11°C	Yes	3 (75%)	0 (0.0%)	3 (37.5%)	0.071
	No	1 (25%)	4(100%)	5 (62.5%)	
Total		4 (100%)	4 (100%)	8 (100%)	
Water chiller is changed during shift	Yes	2 (50%)	0 (0.0%)	2 (25%)	0.018*
	No	2 (50%)	0 (0.0%)	2 (25%)	
	NA	0 (0.0%)	4 (100%)	4 (50%)	
Total		4 (100%)	4 (100%)	8 (100%)	

*P-value considered significant at less than 0.05 level

DISCUSSION

This study aimed to evaluate the effect of chilling method and its related process steps on chicken bacterial load in poultry processing plants in Khartoum State.

In this study the mean total values of the standard plate count were lower than values obtained by Abdalla *et al*. (2013) who recorded 8.16±0.11 log10 CFU/ml for legs, 8.68±0.25 log10 CFU/ml for back, 9.18±0.13 log10 CFU/ml and for the breast. The reason for these lower bacterial findings was that this study expressed values of bacterial contamination after emersion or air chilling which logically preceded by lower microbial values.

Similarly, higher results also were obtained by Abdalla *et al*. (2013) who reported that the TVC revealed the highest contamination level of the backs recorded after de-feathering was 9.99±0.01 log10 CFU/ml, while the highest contamination level of the breasts after chilling and packing was 1.86±0.01 log10 CFU/ml and the highest contamination level of the legs after scalding was 9.96 ±0.01log10 CFU/ml.

Also, higher results were revealed by Kabour (2011) who reported mean TVCs 7.69±2.6 in legs, 7.49±1.6 in backs and 8.38±2.1 in breasts after defeathering. But the mean TVCs obtained from chicken carcasses after spray wash and after chilling and packing were lower than those reported in this study.

In this study the results of the effect of chilling methods on microbiological quality of meat revealed that the mean bacterial load count of legs samples was significantly greater in emersion method compared to air chilling method (p-value =.000). This might be due to poor cleaning and disinfection of rails and equipment, poor handling and poor health status of workers as observed by the researcher.

The study conducted by Geornaras *et al*. (1997) reported that contamination may occur due to bacterial contamination associated with water from the scald tank and from rubber fingers at the exit of defeathering machine.

This study revealed that scalding water was not changed during working shift in the two chilling methods. The effect of not changing scalding water during working shift in this study may pose hazards on the safety of broiler meat. This finding is in line with Anand *et al*. (1989) who stated that spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin.

Defeathering process step is considered vital as birds arriving to the poultry slaughterhouse for processing are generally highly contaminated with bacteria, especially with potential human pathogenic bacteria, such as Coliform and Salmonella (Göksoy *et al.,* 2004).

In this study all plants used air chilling had defeathering machines efficient enough to keep chickens not to fall down, while only half of the plants used immersion chilling were efficient. These findings were found true in the bacteriological

analysis in this study where bacterial load count was significantly greater in emersion method compared to air chilling method specifically in legs samples and not significantly greater in emersion method in breast, thigh, backbone and wings samples compared to air chilling method.

This study showed that mechanical opening of the viscera was practiced in all plants that used air chilling, while only half of the plants used immersion chilling practiced it. This was evident in that bacterial load count was significantly greater in emersion method compared to air chilling method in terms of dealing with viscera. This finding is in line with that recorded by Hinton *et al*. (2000) who reported that broiler carcasses can be contaminated by bacteria when contact with ingesta and feces during evisceration.

This study revealed that all operations using air chilling method had in and out washing machines while only 50% of those using immersion method had such a system. Mead (2004) reported the importance of in and out washing machines of broiler carcasses in reducing TVCs and coliform bacteria counts.

Department of Agriculture, Food Safety and Inspection Service, demands the chilling of carcasses below 4.4 °C until 4 hours postmortem (Savell *et al*., 2005).

This study displays the effect of chilling process step on reducing carcass temperature measured at internal breast from 40°C to 4°C. All slaughterhouses using air chilling method practiced optimal temperature. There was no significant difference between the two chilling methods, with $p>0.071$.

These findings were in line to that reported by James *et al*. (2006) who stated that in industrial processing of poultry, immediately after hot water scalding and the further steps poultry carcasses have to be chilled to reduce their temperature from approximately 40 to 4 °C, which contributes to ensure safe products.

The efficacy of air chilling and drying in this study is supported by the microbial load of chicken which was significantly lower (p-value =.000) than the immersion method.

On the other hand, several authors have postulated that surface drying during air chilling reduces water activity, retards bacterial growth, and causes enough injury to pathogenic bacteria to reduce recovery (Huezo *et al*., 2007).

This study revealed that only half of the slaughterhouses using immersion chilling didn't change water of chillers during shifts and thereby practice cleaning and disinfection. This might be one of the reasons why in this study bacterial load in immersion method was higher than air chilling method as birds are usually soaked in a large pool of water with tons of other chickens and their bacteria. This is also evidenced by that stated by Mead *et al*. (2010) who reported that during slaughter, the chilling of poultry carcasses is one of the main points for potential contamination, requiring constant monitoring.

The addition of chemicals in immersion method during chilling resulted in the low microbial load in this study. This is also in line to that recorded by Ismail *et al*. (2001) who stated that the attempts to apply chemicals to reduce the microbial contamination of poultry carcasses such as acetic acid (2.5%), Trisodium phosphate (8%) and/or sodium hypochlorite (800 pm) had resulted in significant reduction in the number of microorganisms.

CONCLUSION AND RECOMMENDATIONS

The study concluded that the situation of microbial contamination of poultry meat in all investigated slaughterhouses in Khartoum State was in the level recommended by International regulatory bodies. It is recommended that HACCP prerequisites programs should be well maintained in all poultry slaughterhouses.

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RESEARCH ARTICLE

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Aerobic bacteria associated with calf pneumonia in dairy farms in Bahri Locality, Sudan

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KEYWORDS

Bahri Locality
Calf pneumonia
Dairy calves

ABSTRACT

This study was aiming at: 1. Isolation and identification of aerobic bacteria implicated in dairy calf pneumonia in Bahri locality of Khartoum State (Sudan). 2. General evaluation of the housing condition, type and hygiene level and diseases of dairy calves. Eighty naso-pharyngeal swab samples were collected from untreated dairy calves showing typical signs of pneumonia. The swab samples were bacteriologically examined and 83 bacterial isolates were found. The identified bacteria were: 15 *Staphylococcus aureus* (18.2%), 7 *Staphylococcus epidermidis* (8.4%), 4 *Staphylococcus chromogenes* (4.8%), 9 *Streptococcus pneumoniae* (10.8%), 5 *Streptococcus uberis* (6.0%), 7 *Klebsiella pneumoniae* (8.4%), 13 *Escherichia coli* (15.7%), 11 *Pseudomonas aerogenosa* (13.3%), 4 *Bacillus subtilis* (4.8%), 5 *Micrococcus variens* (6.0%) and 3 *Micrococcus luteus* (3.6%). Gram positive Bacteria represented the higher percentage (59.0%) compared to Gram negative bacteria which represented 41.0% of the total bacteria isolated from nasal swabs. Staphylococci represented the predominant bacteria (31.4%) isolated from naso-pharyngeal swabs compared to other bacteria Streptococci (16.8%), *E. coli* (15.7%), *Ps. aerogenosa* (13.3%), Micrococci (9.6%) *K. pneumoniae* (8.4%) and *B. subtilis* (4.8%). Results of the questionnaire survey in Bahri locality showed that calf pneumonia is one of the main health problems in calves with a prevalence of 45.5%. We conclude the high prevalence of calf pneumonia among dairy calves in Bahri Locality. We recommend feeding colostrum during the first day of birth. Other management factors should not be underestimated.

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INTRODUCTION

Calf diseases that cause morbidity and mortality are the results of complex interaction of the management practices and environment, infectious agents and the calf itself (Wudu *et al*., 2008). Common causes of calf diseases and deaths are diarrhoea, pneumonia, joint problems, umbilical diseases, trauma, congenital abnormalities, nutritional deficiencies, dystocia and other infections (Svensson *et al*., 2003; Singla *et al*., 2013). Calf losses were significantly reduced by introducing new techniques of management including on-time colostrum feeding, housing, feeding and nutrition (Razzaque *et al*., 2009). Pneumonia is a cause of major economic loss for the cattle industry, associated with decreased production, higher levels of mortality and increased veterinary and labour costs. The long-term impact can be equally, if not more, damaging. The fibrosis and loss of functional lung capacity in animals that recover from pneumonia has a negative impact on daily live weight gains. For the beef producer this means a longer finishing time, whilst for those rearing dairy replacements, it means an increase in the age at first calving and the subsequent negative effects this has on production and reproductive performance (Tim, 2007). Pneumonia is inflammation of the pulmonary parenchyma, usually accompanied by inflammation of the bronchioles and often by pleuritis. It is manifested clinically by an increase in the respiratory rate, changes in the depth and character of respirations, coughing, abnormal breath sounds on auscultation, and, in most bacterial pneumonias, evidence of toxemia (Radostits *et al.,* 2007). Pneumonia may be associated with viruses, mycoplasmas, bacteria, or a combination of all three; fungi; metazoan parasites; and physical and chemical agents. The main bacterial causes of pneumonia include: *Pasteurella haemolytica, Pasteurella multocida* with or without parainfluenza-3 virus, *Histophilus somnus,* bovine respiratory syncytial virus, bovine herpesvirus 1, parainfluenza-3, adenovirus-1, -2, and -3, rhinovirus, reovirus and *Chlamydia spp*., *Mycoplasma spp*., *Actinomyce arcanobacterium* or *Corynebacterium pyogenes, Streptococcus spp*., *Bedsonia sp*., and *Actinobacillus actinoides, Klebsiella pneumoniae, Fusobacterium necrophorus, Trueperella pyogenes, Parachlamydia acanthamoebae* and *Mortierella wolfii* (Peter *et al,* 2017). Zulfekar and Shirin (2012) reported that the most frequent bacteria isolated from cases of pneumonia were *Staphylococcus spp.* and *Pasteurella haemolytica*. Francis and Ameh, (2015) isolated *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pneumonia, Proteus vulgaris, Pasteurella multocida, Escherichia coli, Corynebacterium spp, Salmonella spp*, and *Enterobacter spp* from cases of pneumonia in Nigeria.

In Sudan Eltigany and Elayis (2021) mentioned that the prevalence of calf pneumonia in Bahri Locality was 8.9%. Abdullatief *et al.,* (2014) reported that pneumonia is the most important cause of calf mortality beside diarrhoea in dairy farms at Khartoum State. Different managemental and environmental factors were reported to affect significantly, calf morbidity and mortality, these include: colostral feeding, housing, calving assistance, production system, herd size, season and hygiene of micro- environment (Shiferaw *et al*., 2002).

This study was aiming at the isolation and identification of aerobic bacteria implicated in pneumonia of dairy calves in Bahri locality, Sudan.

MATERIALS AND METHODS

Source of samples

A total of 80 naso-pharyngeal swabs were collected from untreated dairy calves in Bahri locality showing typical signs of pneumonia during the year 2020.

Sampling procedure

Questionnaires were filled then swabs samples were collected from nostrils of calves showing typical signs of pneumonia and didn't receive any treatment. The collected samples were put in an ice box containing ice and transported to the laboratory of college of Veterinary Medicine University of Bahri. The swabs were placed in tubes containing nutrient broth, incubated at 37C^{o} and examined on the next day. The next day the swabs were removed from the incubator and then cultured, characterized and identified.

Isolation, identification and characterization of bacterial isolates

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full of the enriched broth was streaked onto blood agar, Mac-Conkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37ºC for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in CHO media and for sediment formation. One half colony from each plate was used for performing gram staining. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4ºC.

Biological and biochemical identification of the bacteria

The purified isolates were identified as described by Smith *et al.* (1986) and Barrow and Feltham (2004). The identification included: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation fermentation test, motility test, glucose breakdown test and fermentation of carbohydrates).

Questionnaire survey of dairy farms in Bahri locality

A questionnaire on 11 dairy farms in Bahri locality was conducted before collection of samples. The questionnaire included information about housing type, housing condition, common diseases, availability of veterinary services, calves' health, previous cases of pneumonia and morbidity, mortality and treatment of pneumonia.

RESULTS

Questionnaire survey of dairy farms in Bahri locality

Table 1 shows the analysis of the questionnaire of 11 dairy farms in Bahri Locality illustrated that: 100% of the housing systems were loose corral, 81.8% of the stall surfaces were clay and 18.2% uses concrete surfaces. The general evaluation of the housing condition was good for 54.5% of the farms and poor for the rest. Seventy-two point two of the farms were suffering from Tick-borne diseases and other infections and 45.5% were suffering from mastitis. Veterinary services were available in 90.2% of the farms and hygienic level was poor in 72.2% of the farms and was good in the rest. Calves' health records were available in 36.4% of the farms. Calves diseases in the farms were diarrhoea in 72.7% of the farms, Pneumonia in 54.5% and Tick-borne disease in 45.5%. Colostrum was not presented during first hours of birth in 72.7% of the farms. Vaccination system was not adopted in 72.7% of the farms. Concerning calves' pneumonia 100% of the farms experienced previous cases of the disease. All owners considered that the three first weeks of calves' age are the most hazardous in cases of calves' pneumonia and the risk decreases in older ages. All owners confessed losses of calves due to calf pneumonia and that they adopted different treatment trials of the disease. The majority (54.5%) of the owners used Penicillin for treatment of calf pneumonia and 36.4% used Tetracycline and Enrofloxacin.

Bacteria isolated from nasal swabs collected from Bahri locality

In this investigation a total of 83 bacterial isolates were obtained from 80 naso-pharyngeal swab samples collected from pneumonic calves in Bahri locality. According to the cultural characteristics, bacterial morphology and biochemical reactions results shown in Table (2). The identified bacteria were: 15 *S. aureus* (18.2%), 7 *S. epidermidis* (8.4%), 4 *S. chromogenes* (4.8%), 9 *Str. Pneumoniae* (10.8%), 5 *Str. uberis* (6.0%), 7 *K. pneumoniae* (8.4%), 13 *E. coli* (15.7%), 11 *Ps. aerogenosa* (13.3%), 4 *B. subtilis* (4.8%), 5 *M. variens* (6.0%)

Table 1: Summary of the questionnaire survey of 11dairy farms in different areas in Bahri locality

Unit	Frequency (%)	Unit	Frequency (%)
Housing		**Calves' health**	
Housing type		*Records*	
-Free stall	0 (0%)	-Yes	4 (36.4%)
-Loose corral	11 (100%)	-No	7 (63.6%)
-Stanchion	0 (0%)	*Diseases*	
Stallsurface		-Diarrhoea	8 (72.7%)
-Concrete	2 (18.2%)	-Pneumonia	6 (54.5%)
-Clay	9 (81.8%)	- Tick-borne diseases	5 (45.5%)
-Sand	0 (0%)	*Colostrum during first hours of birth*	
Housing condition		-Yes	3 (27.3%)
-Excellent	0 (0%)	-No	8 (72.7%)
-Good	6 (54.5%)	*Vaccination system*	
-Poor	5 (45.5%)	-Yes	3 (27.3%)
Common diseases		-No	8 (72.7%)
-Mastitis	5 (45.5%)	*Previous cases of pneumonia*	
-Tick-borne diseases	8 (72.7%)	-Yes	11 (100%)
-Other diseases	8 (72.7%)	-No	0 (0%)
Veterinary services		*Population at risk*	
-Yes	10 (90.9%)	-One week old	5 (45.5%)
-No	1 (9.1%)	-Two weeks old	8 (72.7%)
Hygiene Level		-Three weeks old	11 (100%)
-Excellent	0 (0%)	-One month old	10 (90.9%)
-Good	3 (27.3%)	-More than one month old	9 (81.8%)
-Poor	8 (72.7%)	*Losses due to pneumonia per year*	
		-Yes	11 (100%)
		-No	0 (0%)
		Treatment of calf pneumonia	
		-Penicillin	6 (54.5%)
		-Tetracycline	4 (36.4%)
		-Enrofloxacin	4 (36.4%)

and 3 *M. luteus* (3.6%) (Table 3). Gram positive Bacteria represented the higher percentage (59.0%) compared to gram negative bacteria which represented 41.0% of the total bacteria isolated from nasal swabs. Staphylococci represented the predominant bacteria (31.4%) isolated from nasal swabs compared to other bacteria Streptococci (16.8%), *E. coli* (15.7%), *Ps. aerogenosa* (13.3%), Micrococci (9.6%) *K. pneumoniae* (8.4%) and *B. subtilis* (4.8%) (Figure 1).

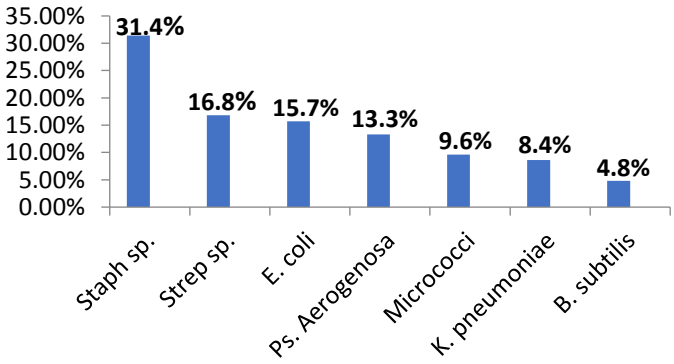

Figure 1: Bacteria isolated from pneumonic dairy calves' naso-pharyngeal swabs collected from Bahri locality.

Table 2: Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***E. coli***	***S. aureus***	**Ps. *aerogenosa***	***K. pneumoniae***
Aerobic growth	+	+	+	+
Colonies on MacConkey	Bright Pink	Pink	Bright Pink	Pink
Haemolysis on blood agar	+	+	+	-
Gram reaction	-	+	-	-
Shape	Rods	Cocci	Rods	Rods
Spore	-	-	-	-
Motility	+	-	+	-
Catalase	+	+	+	+
Oxidase	-	-	+	-
Indole	+	-	-	+
Methyl red	+	+	-	-
VP	-	-	-	-
Citrate	-	-	+	+
H_2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	-	+
Lactose	+	+	-	+
Coaggulase	-	+	-	-

Table 2 (continued): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***Str. uberis***	***M. luteus***	***M. variens***	***B. subtilis***
Aerobic growth	+	+	+	+
Colonies on MacConkey	Pink	Pink	Pink	Pink
Haemolysis on blood agar	-	+	-	+
Gram reaction	+	+	+	+
Shape	Cocci	Cocci	Cocci	Rods
Spore	-	-	-	-
Motility	-	-	-	+
Catalase	-	+	+	+
Oxidase	-	+	+	-
Indole	-	-	-	-
Methyl red	-	-	-	-
VP	+	-	-	-
Citrate	-	-	-	-
H2S	-	-	-	-
O/F	+	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	+
Coaggulase	-	-	-	-

Table 2 (continued): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	***Str. pneumoniae***	***S. epidermidis***	***S. chromogenes***
Aerobic growth	+	+	+
Colonies on MacConkey	Pink	Pink	Pink
Haemolysis on blood agar	+	-	-
Gram reaction	+	+	+
Shape	Cocci	Cocci	Cocci
Spore	-	-	-
Motility	-	-	-
Catalase	-	+	+
Oxidase	-	-	-
Indole	+	-	-
Methyl red	-	-	-
VP	-	-	-
Citrate	+	-	-
H2S	-	-	-
O/F	+	+	+
Glucose	+	+	+
Lactose	+	-	+
Coaggulase	-	-	-

Table 3: Bacteria isolated from pneumonic dairy calves' naso-pharyngeal swab samples collected from Bahri Locality.

Bacterial species	Number	Percentage
S. aureus	15	18.2%
S. epidermidis	7	8.4%
S. chromogenes	4	4.8%
Str. Pneumoniae	9	10.8%
Str. uberis	5	6.0%
M. luteus	3	3.6%
M. variens	5	6.0%
B. subtilis	4	4.8%
E. coli	13	15.7%
Ps. aerogenosa	11	13.3%
K. pneumoniae	7	8.4%
Total	**83**	**100%**

DISCUSSION

Calf pneumonia is multifactorial disease caused by environmental factors (crowding, humidity, temperature, air quality, stress) and infectious agents (Peter *et al*, 2017).

In this study which lasted for 6 months, 11 dairy farms in Bahri locality of Khartoum State were investigated for the problem of calf pneumonia.

According to the questionnaire survey of dairy farms, the general evaluation of the housing condition was poor for 45.5% of the farms, 81.8% of the stall surfaces were clay and the hygiene level was poor in 72.7% of the farms. According to Radostits *et al.* (2000) finding, these factors increase the incidence of any disease, especially calf pneumonia. Matching with Svensson *et al*. (2003) who found that diarrhea is one of the most common diseases reported in calves up to three months old. The present survey results proved that the main health problem in calves was calf diarrhoea (72.7%). According to Peter *et al*. (2017), several owners' practices may help to decrease health problems among herds, for example the availability of the veterinary services, adoption of vaccination program and colostrum giving to the calves during first hours of birth. In this study all owners considered that the two first weeks of calf's age are the most hazardous and the risk decreases with old ages, and this is in accordance with the findings of Curtis *et al*. (1988). The analysis of the data on treatments adopted to the affected calves in the areas of the study showed different drugs with different percentages of adoption: Penicillin (54.5%), Tetracycline (36.4%) and Enrofloxacin (35.8%), These treatment strategies were also recommended by Peter *et al*. (2017) with different routes of administration of drugs.

In this investigation a total of 83 bacterial isolates were obtained from 80 nasal swab samples collected from pneumonic calves in Bahri locality. The identified bacteria were: 15 *S. aureus* (18.2%), 7 *S. epidermidis* (8.4%), 4 *S. chromogenes* (4.8%), 9 *Str. Pneumoniae* (10.8%), 5 *Str. uberis* (6.0%), 7 *K. pneumoniae* (8.4%), 13 *E. coli* (15.7%), 11 *Ps. aerogenosa* (13.3%), 4 *B. subtilis* (4.8%), 5 *M. variens* (6.0%) and 3 *M. luteus* (3.6%). Zulfekar Ali and Shirin Sultana (2012) also isolated Staphylococcus spp. and *Pasteurella haemolytica* from cases of calf pneumonia. Francis and Ameh (2015), Benesi *et al*. (2010), Hartel *et al*. (2004), Autio *et al*. (2007), Angen *et al*.(2009) and Oliveira *et al*. (2016) isolated *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pneumonia, Proteus vulgaris, Pasteurella multocida, Escherichia coli,* Corynebacterium spp., Salmonella spp, and Enterobacter spp. from cases of calf pneumonia in Nigeria. Nicholas *et al.* (2003) reported that facultative anaerobes; *Mannheimia* (*Pasteurella*) *haemolytica*, *Pasteurella moltocida*, *Haemophilus somnus*, *Arcanobacter pyogenes*, *Pseudomona saeruginosa*, *Escherichia coli*, *Actinobacillus pleuropneumoniae*, *streptococcus spp., staphylococcus species, Moraxella spp, salmonella spp.* are the most common bacteria involved in pneumonia.

Dwight *et al*. (2004) reported that the majority of pathogens of the respiratory tract of cattle belong to the *Streptococcus spp.*. Isam Eldeen (2003) isolated *Streptococcus* species from cattle in Khartoum State that suffered from pneumonia.

Klebsiella pneumonia represented 8.4% of the isolated bacteria, Carter (1986) reported that *Klebsiella* spp. can be found

associated with some diseases as secondary invaders but may also act as primary aetiological agents of diseases.

Pseudomonas aeruginosa represented 13.3% of the isolated bacteria, Carter (1986) reported that *Pseudomonas aeruginosa* isolated frequently from wound infections in a number of domestic animals.

CONCLUSION AND RECOMMENDATIONS

According to the results of Questionnaire survey in Bahri locality of Khartoum State, calf pneumonia is one of the main health problems in calves with the prevalence of 45.5%. The mortality rate among pneumonic calves is higher during second and third weeks of age. Staphylococci represented the predominant bacteria isolated from nasal swabs of pneumonic calves compared to other bacteria such as Streptococci, *E. coli*, *Ps. aerogenosa*, Micrococci, *K. pneumoniae* and *B. subtilis*.

Further studies should include a survey of more animals in different farms and the significance of bacteria in calf diarrhoea. Further studies should be carried out to investigate the predisposing factors related to the incidence of neonatal calf pneumonia and to identify different causes of calf diarrhoea. Pregnant cows should be isolated within the last two weeks before calving. Moreover, feeding colostrum during the first day is strongly advised. Other management factors should not be underestimated.

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