

Journal of Veterinary Medicine and Animal Health

Volume 6 Number 3 March, 2014



*Academic
Journals*

ABOUT JVMAH

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

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

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

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jvmah@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Veterinary Medicine and Animal Health (JVMAH) will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Fuqiang Li PhD

Division of Cardiology
Department of Medicine
Cedars-Sinai Medical Center
8700 Beverly Blvd
CA 90048
USA

Dr. Lachhman Das Singla

Department of Veterinary Parasitology
College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University
Ludhiana-141004
Punjab
India

Dr. Viktor Jurkovich

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

Dr. Sathurkulasingam Reuben Shanthikumar

606 Alvarado Avenue
Apt # 64, Davis, CA 95616
USA

Dr. Adeolu Alex Adedapo

Department of Veterinary Physiology
Biochemistry and Pharmacology
University of Ibadan
Nigeria

Prof. Anca Mihaly Cozmuta

Faculty of Sciences
North University of Baia Mare
Romania, Victoriei Str. 76 A, Baia Mare
Romania

Dr. Ramasamy Harikrishnan

Faculty of Marine Science
College of Ocean Sciences
Jeju National University
Jeju city
Jeju 690 756
South Korea

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JPP to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The **Title** should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The **Abstract** should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included

in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Ansell J, Hirsh J, Poller L (2004). The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic. Therapy. 126:204-233

Ansell JE, Buttaro ML, Thomas VO (1997). Consensus guidelines for coordinated outpatient oral anti coagulation therapy management. Ann. Pharmacother. 31:604-615

Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

Furmaga EM (1993). Pharmacist management of a hyperlipidemia clinic. Am. J. Hosp. Pharm. 50: 91-95

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences:

(1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a \$550 handling fee. Publication of an article in the Journal of Veterinary Medicine and Animal Health (JVMAH) is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Copyright: © 2014, Academic Journals.

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JVMAH, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.

ARTICLES

Research Articles

- Heat stress amelioration and production performance in layers supplemented with herbal liquid anti-stressor product** 69
N. V. Jadhav, B. Awati, S. Kulkarni, P. G. Waghmare, M. D. Suranagi, K. Ravikanth, M. Dandale and Shivi Maini
- Effects of transportation positions and orientations on muscular damage of goats transported by road for 12 h during the hot-dry conditions** 75
Ndazo Salka Minka and Joseph Olusegun Ayo
- A three year follow-up study on the occurrence of bovine ehrlichiosis (cowdriosis) at Gondar University dairy farm** 83
Achene Melaku, Basaznew Bogale, Mersha Chanie, Wassie Molla and Gizat Almaw
- Cloacal fecal carriage and occurrence of antibiotic resistant Escherichia coli in chicken grown with and without antibiotic supplemented feed** 91
Nwankwo Chinwe, Ayogu Thomas, Iroha Ifeanyichukwu, Ejikeugwu Chika, Nwakaeze Emmanuel, Oji Anthonia and Ilang Donatus
- Bacteriological evaluation of the drinking water quality in dairy farms in Khartoum state, Sudan** 95
Amna Mustafa Musa and Atif Elamin Abdelgadir

Full Length Research Paper

Heat stress amelioration and production performance in layers supplemented with herbal liquid anti-stressor product

N. V. Jadhav¹, B. Awati², S. Kulkarni³, P. G. Waghmare⁴, M. D. Suranagi⁵,
K. Ravikanth⁶, M. Dandale⁶ and Shivi Maini^{6*}

¹Department of Livestock Production Management, Veterinary College, Bidar, Karnataka, India.

²Department of Veterinary Microbiology, Veterinary College, Bidar, Karnataka, India.

³Department of Veterinary Biochemistry, Veterinary College, Bidar, Karnataka, India.

⁴Department of L. P. M., Veterinary College, Bidar, Karnataka, India.

⁵Veterinary College, Bidar, Karnataka, India.

⁶R&D Center, Ayurvet Ltd, HP, India.

Accepted 19 September, 2013

As the body temperature of birds rise, feed consumption, growth rate, feed efficiency, survivability, egg production and egg quality tends to decline. In order to address the problem of heat stress, an experiment was conducted to determine the effect of AV/LAP/19 liquid (Supplied by M/s Ayurvet Ltd. Baddi, HP, India) containing natural vitamin C with bioflavonoids and selenium, and synthetic ascorbic-acid (vitamin C) supplementation on the performance of commercial layers. 120 birds, 55 weeks of age were divided into 4 equal groups of 30 birds each. They were exposed to a heat stress of $39\pm 8^{\circ}\text{C}$ temperature and temperature humidity index (THI) of 81.33 ± 1.20 . All bird groups were offered basal diet deficient in vitamin C. Negative effect of high ambient temperature and relative humidity was evidenced from the high serum cortisol level (mg/L) (3.42 ± 0.19 to 3.67 ± 0.20) in all groups. It was significantly optimized and lowered down in all treated groups. Concomitantly, increased alkaline phosphatase and alkaline transaminase levels were also lowered in treated birds. The total protein, albumin and globulin were having numerically higher levels in treated birds. It was concluded that the herbal liquid AV/LAP/19 at both dosages ameliorated the heat stress in layer birds as well as resulted in better feed efficiency and immunomodulation. However, significantly better egg production (%) was recorded in the birds supplemented with AV/LAP/19.

Key words: AV/LAP/19, heat stress, herbal products, egg production, vitamin C.

INTRODUCTION

Heat stress remains a perpetual challenge for the poultry enterprises in tropical climate of India. It results from a negative balance between the net amount of energy flowing to its surrounding environment and the amount of heat energy produced by the layers (Ajakaiye et al., 2011). High temperatures, especially when coupled with high humidity, impose severe stress on layer birds and

lead to reduced performance (Ajakaiye et al., 2011). High environmental temperatures stimulate the hypothalamo hypophyseal-adrenocortical axis which increases corticosteroid secretion in response to stress (Ramnath et al., 2008). Higher levels of circulating corticosteroids have a catabolic effect through increase in the free radicals by altering oxidative metabolism, causing impairment of cellular

*Corresponding author. E-mail: shivi@ayurvet.in.

functions and thus damage to the cell membrane, muscle wasting and retarded growth (Sujatha et al., 2010). During the periods of heat stress, most of the production energy is diverted to thermoregulatory adaptations which results in oxidative stress induced immunosuppression, predisposing birds to various infectious diseases and high mortality rates (Maini et al., 2007). In laying hens, heat stress suppresses body weight, egg production, egg weight, shell quality and is generally accompanied by suppression of feed intake (Mashaly et al., 2004). Therefore, it remains a prime issue to re-evaluate the poultry management in hot weather, so that heat stress is minimized. There are various strategies to minimize heat stress in layer hens either by changing their environmental condition or by modification of their diets (Ajakaiye et al., 2010). Nutritional strategy being more viable during heat period is based on diet balancing in order to cover the needs of stressed birds for amino acids (protein), energy and electrolytes (Balnave, 2004; Dagher, 2009). The body requirement of ascorbic acid during heat stress in poultry is greater than the amount synthesized by normal tissues and its administration to broilers during heat stress has been shown to be beneficial to the body (Balogun et al., 1996). Non enzymatic antioxidants vitamin C and vitamin E are used and are being explored extensively in the poultry diet, because of their antioxidant effect in the neutralization of the free radicals generated during heat stress (MUSA-Azara et al., 2012; Ajakaiye et al., 2010, 2011; Ramnath et al., 2008; Bell and Marion, 1990; Orban et al., 1993; Zapata and Gernat, 1995).

In the past few decades, a number of Ayurvedic herbal preparations have been extensively used in poultry industry (Ramnath et al., 2008). Polyherbal products containing different immunomodulator (*Withania somnifera*), antistressor (*Phyllanthus emblica*, *Mangifera indica*) and adaptogenic (*Ocimum sanctum*, *W. somnifera*) herbs have been used to protect tissues from superoxide radicals and enhance cell survival by stimulating antioxidative enzymatic systems (Sujatha et al., 2010). The objective of this study was to determine the comparative beneficial effects of dietary incorporation of feed additive-synthetic vitamin C and polyherbal liquid supplement AV/LAP/19 (M/s Ayurved Ltd. Baddi, HP, India) containing natural vitamin C with bioflavonoids and selenium, on feed consumption, egg production, weight and mortality in laying hens exposed to a chronic heat stress.

MATERIALS AND METHODS

The experiment was conducted at the Department of Livestock Production Management, College of Veterinary Sciences, Bidar, Karnataka State, India. Due approval for conducting the experiment was taken from 'Committee for the Purpose and Control and Supervisions on Experimental Animals' (CPCSEA), India. The experimental setup was arranged during the extreme hot summer to early monsoon period (May 2012 to July 2012), where mean

temperature-humidity index was 81.33 ± 1.20 . c was calculated as per the formula proposed by Kelly and Bond (1971):

$$\text{Temperature humidity index (THI)} = (\text{Tdb}) - (0.55 - 0.55 \text{ RH}) \times (\text{Tdb} - 58)$$

where Tdb is dry bulb temperature (°F) and RH is relative humidity expressed as fraction of 1.

Bidar is located at 17.9°N 77.55°E with an average elevation of 615 m from seashore. The climate of this place is characterized by general dryness throughout, where summer is the driest part of the year. May is observed as the hottest month with average daily maximum temperature of 38.8°C and the relative humidity in the afternoon is between 30 and 40%.

Experimental design

Total of one hundred and twenty (n=120) commercial layers of 55-week old age were reared in cages. The layers were divided into 4 groups, namely T0, T1, T2 and T3 with 3 replicates in each group comprising of 10 birds per replicate. The separation of groups was vertically arranged in 2 tier reverse cages to nullify tier effect. All the groups were managed under identical management and environmental conditions except the nutritional treatment given to them. All the birds were exposed to environmental and physiological stress. Commercial layer mash without vitamin C was fed *ad libitum* to all the groups except group T1 where vitamin C at 100 g/tonne of feed was supplemented. Groups T2 and T3 were supplemented with liquid AV/LAP/19 through water for 7 weeks at 3 and 5 ml/100 birds/day, respectively. Thus supplementation of herbal product AV/LAP/19 and synthetic Vitamin C in treatment groups was immediately commenced from 56th week and continued up to 62nd week. The assessment of performance continued for another 5 weeks, that is, up to 67th week. Group T0 was not given any treatment and served as control group in the study. Growth attributes in terms of daily/weekly egg production, egg weight, hen-day egg production (%), and weekly feed consumption were calculated.

Laboratory analysis

In order to determine the level of heat stress and relevant treatment efficacy, biochemical and hormonal estimation were carried out on 56, 60 and 67th week. Blood from individual birds was drawn and serum separated to estimate the serum cortisol measured by radio immune assay (RIA). Total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels were determined by using Autospan clinical chemistry diagnostic kits (SPAN diagnostics Ltd, Surat, India). The hemagglutination inhibition (HI) titers were estimated as per the method of Allan and Gouch (1974). The data from the study was pooled and was subjected to suitable statistical analysis as described by Snedecor and Cochran (1994). Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Record of temperature was maintained on daily basis where mean maximum daily temperature of $39 \pm 8^\circ\text{C}$ was recorded throughout the experiment. The combination of daily temperature of $39 \pm 8^\circ\text{C}$ and THI of 81.33 ± 1.20 being above the threshold established for poultry (Tao and Xin, 2003; Karaman et al., 2007) indicates that the layer birds

were subjected to heat stress. All respective birds groups except control were treated with synthetic vitamin C and natural vitamin C with bioflavonoids and selenium containing liquid AV/LAP/19 effect as the following.

Growth performance

Environmental stress to layers resulted in declined growth performance in terms of average body weights, total body weight gain, weekly egg production percent, feed efficiency and egg weights. Results have been summarized in Tables 1 and 2.

Body weight

Polyherbal liquid supplement AV/LAP/19 at 3 and 5 ml/100 birds/day, respectively in group T2 and T3 had numerically higher body weight (g) (1609.42 and 1608.28) than that of control group T0 (1512.71) as well as synthetic vitamin C supplemented group T1 (1518.86). Supplementation of synthetic as well as herbal antistressor vitamin C has been scientifically well proven to increase body weight gain as well as improve growth and performance of birds during summer (Sahin et al., 2003; Sapkota et al., 2006; Maini et al., 2007; Sujatha et al., 2010).

Egg production

Exposure of hens to high temperatures resulted in a significant decrease in egg production and quality. The mean egg production (%) recorded for the present experiment was significantly ($P < 0.05$) higher for the liquid AV/LAP/19 supplemented group T2 (82.21) and T3 (79.30) followed by synthetic vitamin C supplemented group T1 (78.84). Lowest egg production was noticed in untreated control group T0 (76.36). The vitamin C supplementation in layer birds improved ($P < 0.05$) the mean egg production, egg weight and egg shell thickness (Khan and Sardar, 2005), egg length, egg width, albumen weight, albumen height and yolk height were obtained for birds given vitamin C supplementation (MUSA-Azara et al., 2012).

Egg weight

The mean egg weight of layer birds in groups T2 and T3 (59.10 and 58.59) was improved. However, synthetic vitamin C supplementation in group T1 (56.82) could not mark its influence over the egg weight as mean egg weight in the untreated control group T0 (55.02) did not differ significantly from it. Thus herbal liquid antistressor formulation AV/LAP/19 does not only ameliorated the

heat stress in group T2 and group T3, but also helped the treated birds to achieve the higher production performance.

Feed efficiency

Higher growth and production performance resulted in better feed efficiency in AV/LAP/19 supplemented group T2 (1.79) and T3 (1.84). However, the feed efficiency in vitamin C supplemented group T1 (1.86) differed non-significantly from untreated control group T0 (1.92). The average feed consumption of layers was improved ($P < 0.05$) with supplementation of vitamin C (Khan and Sardar, 2005).

Biochemical analysis

The results of biochemical estimations (60 and 67th week, 56th week-base reading) are tabulated in Table 3. Before treatment in all birds, higher levels of serum cortisol (mg/L) content was found indicating the effect of high temperature and relative humidity. High ambient temperature induces production and release of corticosteroids (Siegal, 1980), exerts catabolic effects (mobilization of proteins and lipids) through muscle wasting and reduces growth rate (Sujatha et al., 2010; Odedra et al., 1983; Hayashi et al., 1994). Similar results were obtained in the present study, where serum cortisol levels (Table 3) were significantly ($P \leq 0.05$) higher in the control as compared to the treatment groups. After treatment serum cortisol level (mg/L) significantly reduced in all treated groups T1, T2 and T3 (1.97, 1.06, 1.09) as compared to control T0 (3.50). However, liquid AV/LAP/19 supplementation at both dose levels (3 and 5 ml) had even lower concentration ($P < 0.05$) of serum cortisol to those of birds offered synthetic vitamin C. The lowered concentration of cortisol signified the amelioration of heat stress in treated birds. The findings of the present experiment are in corroboration with that of Sujatha et al. (2010) where polyherbal premix (Stresroak at 1 kg/tonne of feed) was used to minimize heat stress in broilers during summer months. The amelioration action was also noted for SGPT (IU/l) and SGOT (IU/l) where AV/LAP/19 supplementation significantly improved and optimized their levels as compared to control group T0 as well as synthetic vitamin C supplemented group T1. Similarly, concentrations of blood enzymes (alkaline phosphatase (ALP), SGPT and SGOT) were lowered ($P < 0.01$) with vitamin C supplementation in all layer birds (Khan and Sardar, 2005). Similarly, decreased serum enzymes levels after vitamin C supplementation was also reported by Chakraborty and Sadhu (1983) and Takeda and Hara (1985).

The total protein, albumin and globulin levels (Table 3) were having numerically higher levels in treated birds;

Table 1. Effect of AV/LAP/19 and synthetic vitamin C anti-stressor on body weights and total weight in laying hens (g).

Age (weeks)	Group			
	T0	T1	T2	T3
56	1442	1431	1491	1496
57	1473	1439	1542	1540
59	1490	1492	1579	1586
61	1506	1525	1612	1617
63	1517	1554	1639	1646
65	1553	1581	1688	1671
67	1608	1610	1715	1702
Total weight gain	166	179	224	206
Change over control	-	13	58	40
Mean	1512.71	1518.86	1609.42	1608.28
Standard error (SE)	22.10027	25.93897	30.37442	27.59461

Results non-significant.

Table 2. Effect of feeding herbal and synthetic antistressor on weekly egg production (%) and average egg weight (g) in laying hens.

Attribute	Group			
	T0	T1	T2	T3
Mean egg production (%)	76.36 ^a	78.84 ^a	82.21 ^b	79.30 ^b
Standard error (SE)	1.20	0.97	1.71	1.50
Mean egg weight (g)	55.02 ^a	56.82 ^a	59.10 ^c	58.59 ^{bc}
Standard error (SE)	0.473036	0.319525	0.524982	0.398569
Feed efficiency	1.92 ^a	1.86 ^a	1.79 ^b	1.84 ^a
Standard error (SE)	0.027121	0.036957	0.029244	0.037257

The values bearing minimum one common superscript in a row do not differ significantly.

however, their levels did not differ significantly in control as well as treated birds.

Immunomodulation

The results on immuno-modulatory investigations

are depicted in Table 4. The observations and analysis of antibody titers against Ranikhet disease (RD) in 59th, 63rd and 67th week revealed cognizable enhancement in antibody titers of herbal product treated layers (1:256) in comparison with non-treated as well as layers supported

supported with vitamin C (1:64, 1:128).

Conclusion

Heat stress is a major welfare problem in the

Table 3. Effect of feeding synthetic vitamin C and herbal antistressor on biochemical parameters in ameliorating heat stress in layers.

Parameter	Control		Synthetic vitamin C		AV/LAP/19 (3 ml/100 birds/day)		AV/LAP/19 (5 ml/100 birds/day)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Cortisol (mg/ml)	3.64 ^a ±0.15	4.21 ^a ±0.13	3.50 ^a ±0.17	1.97 ^b ±0.23	3.67 ^a ±0.20	1.06 ^d ±0.05	3.42 ^a ±0.19	1.09 ^d ±0.05
Total protein (g/dl)	5.27±0.15	5.16±0.13	5.30±0.14	5.43±0.13	5.47±0.18	5.28±0.11	5.26±0.17	5.26±0.11
Albumin (g/dl)	2.41±0.08	2.21±0.09	2.34±0.08	2.38±0.09	2.59±0.12	2.48±0.07	2.45±0.13	2.40±0.07
Globulin (g/dl)	2.86±0.09	2.95±0.07	2.96±0.11	3.05±0.06	2.89±0.08	2.79±0.08	2.81±0.07	2.86±0.07
SGPT (IU/L)	58.13 ^a ±1.70	59.83 ^a ±1.69	57.53 ^a ±2.18	46.07 ^b ±1.94	59.33 ^a ±1.69	38.80 ^b ±0.87	58.73 ^a ±2.36	38.70 ^b ±0.72
SGOT (IU/L)	61.93 ^a ±1.85	63.07 ^a ±1.11	59.73 ^a ±2.24	43.90 ^b ±1.94	58.80 ^a ±1.48	40.10 ^b ±0.75	58.07 ^a ±1.63	40.40 ^b ±0.75

Mean values with different superscripts in a row differ significantly (P<0.05).

Table 4. Antibody titre levels against RD by using HI tests in layers supplemented synthetic and herbal anti-stressor products vs. untreated control.

S/N	Sampling week	Sample no.	HI titre			
			T ₀	T ₁	T ₂	T ₃
1	59	1	1:32	1:32	1:128	1:128
		2	1:64	1:64	1:256	1:128
		3	1:32	1:64	1:128	1:256
		4	1:64	1:128	1:256	1:128
		5	1:32	1:32	1:128	1:128
2	63	1	1::64	1:128	1:256	1:256
		2	1:128	1:256	1:256	1:256
		3	1:64	1:128	1:256	1:128
		4	1:128	1:128	1:256	1:256
		5	1:128	1:128	1:256	1:256
3	67	1	1:32	1:64	1:128	1:128
		2	1:64	1:128	1:256	1:128
		3	1:64	1:64	1:128	1:128
		4	1:32	1:128	1:256	1:128
		5	1:32	1:64	1:256	1:256

poultry industry leading to huge economic losses every year, because of mortality and decreased production. Dietary supplementation of synthetic vitamin C and herbal liquid AV/LAP/19 containing

natural vitamin C with bioflavonoids and selenium ameliorated the heat stress by optimizing serum cortisol, SGPT and SGOT levels. A non-significantly different positive impact of supplementing

AV/LAP/19 at two different dose rates was reflected from improved total body weight gain, feed efficiency and egg weight in layers as well as immunopotential when compared with non

production (%) was recorded in the birds of group T2 supplemented with AV/LAP/19 at 3 ml/100 birds/day followed by T3 supplemented with AV/LAP/19 at 5 ml/100 birds/day as compared to untreated control group. Thus it may be recommended to use herbal liquid formulation to curb the losses incurred from heat stress.

ACKNOWLEDGEMENT

The authors are thankful to the administration of College providing infrastructure and necessary facilities to conduct the research.

REFERENCES

- Ajakaiye JJ, Perez-Bello A, Mollineda-Trujillo A (2010). Impact of Vitamins C and E Dietary Supplementation on Leukocyte Profile of Layer Hens Exposed to High Ambient Temperature and Humidity. *Acta. Vet. Brno.* 79:377-383.
- Ajakaiye JJ, Perez-Bello A, Mollineda TA (2011). Impact of heat stress on egg quality in layer hens supplemented with l-ascorbic acid and dl-tocopherol acetate. *Vet. Arch.* 81 (1):119-132.
- Allan WH, Gouch RE (1974). A standard Haemagglutination inhibition for ND (I). A comparison of macro and micro-methods. *Vet. Rec.* 15:120-123.
- Balnave D (2004). Challenges of accurately defining the nutrient requirements of heat-stressed poultry. In: *World's Poultry Science Association Invited Lecture.* *Poult. Sci.* 83:5-14.
- Balogun TF, Afolayan SB, Njoku FC, Dafwang II (1996). Effect of Ascorbic Acid and Acetylsalicylic Acid supplementation of broiler diets on performance during the hot season. *Samaru J. Agric. Educ.* 5:80-86.
- Bell DE, Marion JE (1990). Vitamin C in laying hen diets. *Poult. Sci.* 69:1900-1904.
- Chakraborty AK, Sadhu DP (1983). Effect of acute heat stress and its modifications by adrenaline and adrenolytic drugs in pigeons. *Indian J. Anim. Sci.* 53:575-578.
- Daghir NJ (2009). Nutritional strategies to reduce heat stress in broilers and broiler breeders. *Loh. Inform.* 44:6-15.
- Hayashi K, Nagai Y, Ohtsuka A, Tomita Y (1994). Effects of dietary corticosterone and trilostane on growth and skeletal muscle protein turnover in broiler cockerels. *Brit. Poult. Sci.* 35:789-798.
- Karaman S, Tarhan S, Ergunes G (2007). Analysis of indoor climatic data to assess the heat stress of laying hens. *Int. J. Nat. Engl. Sci. (Suppl II):*65-68.
- Kelly CF, Bond TE (1971). *Bioclimatic factors and their measurement.* National Academy of Science, Washington D.C.
- Khan SH, Sardar R (2005). Effect of vitamin C supplementation on the performance of desi, Faoumi and commercial white leghorn chicken exposed to heat stress. *Pak. Vet. J.* 25(4):136-166.
- Musa-Azara SI, Ogah DM, Hassan DI, Yakubu A (2012). Effects of vitamin C supplementation on egg quality characteristics of Harco black layers during the hot season. *Egypt Poult. Sci.* 32(III):607-611.
- Maini S, Rastogi SK, Korde JP, Madan AK, Shukla SK (2007). Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. *J. Poult. Sci.* 44:339-347.
- Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO, Patterson PH (2004). Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poult. Sci.* 83:889-894.
- Odedra BR, Bates PC, Millward DJ. (1983). Time course of effect of doses of corticosterone on protein turnover in rat skeletal muscle and liver. *J. Biochem.* 214:617-627.
- Orban JI, Roland DA, Cummins K, Lovell RT (1993). Influence of large doses of ascorbic acid on performance, plasma calcium, bone characteristics and egg shell quality in broilers and Leghorn hens. *Poult. Sci.* 72:691-700.
- Sahin K, Onderci M, Sahin N, Gursu MF, Kucuk O. (2003). Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. *J. Nutr.* 133:1882-1888.
- Sapkota D, Islam R, Upadhyaya TN (2006). Dietary supplementation of *Emblica officinalis* for amelioration of experimental aflatoxicosis in commercial broilers. *Anim. Nutr. Feed Tech.* 6(1):65-71.
- Siegal HS (1980). Physiological stress in birds. *Biosci.* 30:529.
- Snedecor GW, Cochran WG (1994). *Statistical methods* 8th Ed., Oxford and IBH Publishing Company, New Delhi.
- Sujatha V, Korde JP, Rastogi SK, Maini S, Ravikanth K, Rekhe DS (2010). Amelioration of heat stress induced disturbances of the antioxidant defense system in broilers. *J. Vet. Med. Anim. Health.* 2(3): 18-28.
- Takeda Y, Hara M (1985). Significance of ferrous ion and ascorbic acid cycle. *J. Biol. Chem.* 214:657.
- Tao X, Xin H (2003). Temperature-humidity-velocity-index for market size broilers. *Proceedings of the 2003 ASAE Annual International Meeting Nevada, USA.* pp. 034-037.
- Zapata LF, Gernat AG (1995). The effect of four levels of ascorbic acid and two levels of calcium on egg shell quality of forced molted White Leghorn hens. *Poult. Sci.* 74:1049-1052.

Full Length Research Paper

Effects of transportation positions and orientations on muscular damage of goats transported by road for 12 h during the hot-dry conditions

Ndazo Salka Minka^{1*} and Joseph Olusegun Ayo²

¹College of Agriculture and Animal Science Kaduna, Division of Agricultural Colleges, P. M. B. 2134, Ahmadu Bello University, Mando-Kaduna, Nigeria.

²Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Accepted 17 January, 2014

The influence of road transportation position and orientation on muscular damage was evaluated in 30 goats. The animals were divided into two groups: 20 goats transported by road for 12-h during hot-dry conditions (TG) and 10 non-transported sedentary goats (SG). During the 12-h transportation period, the goats spent 7 h standing, of which 40.0% of the goats spent 4.5 ± 0.7 h standing in perpendicular direction, 30.0% spent 1.5 ± 0.5 h in parallel direction, while 20.0 and 10% spent 0.5 ± 0.1 h each in opposite and diagonal directions, respectively. Of the 5 h spent by the goats lying down, 33.6% of the goats spent 2.5 ± 0.7 and 1.5 ± 0.5 h each lying down in opposite and diagonal directions, respectively. Goats that adopted standing or lying down position in perpendicular and opposite directions during the transportation period had predominantly lower values of creatine phosphate kinase, alanine aminotransferase, aspartate aminotransferase, glucose and urea, which suggested less muscular damage and best transportation position. Lying down in opposite or diagonal orientations produced the highest ($P < 0.05$) activities of blood enzymes, and glucose and urea concentrations, which indicated high level of muscular damage and worst position. In conclusion, lying down or standing in opposite or diagonal orientations may have adverse effects on the welfare, health, productivity and meat quality of goats transported by road.

Key words: Goats, muscular damage, position and orientation, serum enzymes, stress, road transportation.

INTRODUCTION

Road transportation of animals is an inevitable husbandry practice. It is important and yet a critical period in animal production. Every year, several millions of goats, particularly the Red Sokoto goats are transported from the Northern Nigeria and neighbouring Northern countries like Niger and Chad to the Southern and other parts of

Nigeria, and to countries like Benin and Togo for the purpose of slaughter for meat, skin, breeding or further production (Minka and Ayo, 2010, 2012). Such transportation covers thousands of kilometers, lasting 2 to 4 days on the road. Few studies have investigated whether long-distance road transportation of goats under

*Corresponding author. E-mail: mnsalka@yahoo.com. Tel: 23436527538.

adverse climatic conditions may compromise the well-being, and consequently, meat quality of goats (Kannan et al., 2000; Minka and Ayo, 2012). Besides, the thermal aspects of the environment during the three (wet, hot-dry and harmattan) seasons in the Savannah zones of Nigeria are stressful. This is especially so during the hot-dry and harmattan seasons when the ambient temperature and relative humidity fluctuate between 20.0 to 39.4°C and 65 to 75%, respectively during the hot-dry and 12.4 to 29.3°C and 18 to 22%, respectively during the harmattan seasons (Igono et al., 1982; Minka and Ayo, 2010).

The Red Sokoto goat (RSG) or Maradi breed is characterized by a uniformly dark-red coat colour, short and horizontal ears and horns in both sexes. The males are heavier and usually weigh an average of 27 kg, while females weigh about 25 kg. The breed is extremely resistant to harsh environmental conditions, which has contributed to its great population density in the West and North Africa sub-regions. The RSGs serve as a good source of meat and high-quality skin used in leather industries, and income to the small scale farmer. They are highly productive and have a good food conversion ratio (Aganga et al., 1986; Ayo et al., 1998).

One important indicator of stress during road transportation of animals is the change in behaviour, which shows that some aspect of transportation procedure is aversive (Broom, 2000). Some behavioural changes may be signs of distress (Ayo et al., 2002, 2006; Young et al., 2012). Thus, the understanding of the behaviour of goats during transportation may provide relevant information on how transportation stress factors affect their well-being, and consequently, the meat/milk quality.

Previous investigations have estimated the effects of road transportation on different blood parameters, useful as marker of welfare condition in ruminants (Giannetto et al., 2011; Piccione et al., 2012, 2013). Blood enzymes such as creatine phosphate kinase (CPK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and metabolites have been used also as reliable physiological indices of muscle tissue injury or damage in humans and animals subjected to different stressors, including transportation stress (Ekiz et al., 2012; Cafazzo et al., 2012).

Generally, several studies have been conducted on the effect of road transportation on muscle enzymes and metabolites in cattle (Cafazzo et al., 2012; Averos et al., 2008), sheep (Galipalli et al., 2004; Ekiz et al., 2012, 2013; Zhong et al., 2012), goats (Kannan et al., 2000, 2002; Galipalli et al., 2004) and roe deer (Montane et al., 2002). Few studies have been carried out on the best travel position or orientation that is less stressful for horses (Kay and Hall, 2009; Padalino et al., 2012; Tateo et al., 2012) and cattle (Cafezzo et al., 2012; Earley et al., 2012). However, the best travel position and the effects

of travel positions and orientations on serum biochemical activities of goats, transported by road have not been elucidated. Such information, if available, may enhance the health, welfare status, and consequently, meat quality of goats subjected to long-distance road transportation.

The aim of the present study was to investigate the best travel position and orientation for goats by assessing the effects of standing and lying down positions and orientations on muscular damage following long-duration transportation of goats during the hot-dry climatic conditions.

MATERIALS AND METHODS

Study area and environmental conditions

The experiment was performed at the Livestock Farm of the College of Agriculture and Animal Science, Ahmadu Bello University, Kaduna, located in the Northern Guinea Savannah zone of Nigeria during the hot-dry period of the month of April. Transportation for 12 h was conducted from Kaduna (11° 10' N, 07° 38' E) to Makera (12° 31' N, 06° 11' E), Nigeria; and from Makera back to Kaduna, covering a total distance of about 600 km. The ambient temperature (AT) and relative humidity (RH) were recorded at the study site using a wet- and dry-bulb thermometer (DTH 1, Clarke Int., Epping, Essex, UK) at 07:00, 13:00 and 18:00 h daily for 7 consecutive days before and after the transportation. The AT and RH were also recorded hourly inside the vehicle during the 12-h transportation period. After the transportation, the goats were returned to the same pen and managed as it was done before the transportation.

Animals and management

The study was approved by the Postgraduate Research Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria and conducted according to international guidelines on animal ethics (Gavinelli and Simonin, 2003). Thirty Red Sokoto goats of both sexes (aged 3 years old and weighing between 23 and 25 kg) were used. The goats were kept at night in a standard goat pen at a density rate of 1 goat/m² (Kannan et al., 2002). The top half of the wall of the pen was open, provided with a wire mesh for adequate ventilation. The roof of the pen was made of aluminum with a wooden ceiling, while the floor was concrete. The goats were not restrained inside the pen. Each day, the goats were grazed on a natural pasture, from 09:00 to 18:00. Water was available *ad libitum*. On transportation day, the goats were divided into two groups: 20 transported goats (TG) and 10 non-transported sedentary goats (SG). The SG were deprived of food and water for the entire period of the transportation.

Loading, stocking density and transportation

The loading and transportation of the goats were carried out humanely in accordance with the EU legislation governing transport of live animals, Directives 19/628 EEC amended by Directives 95/29/EEC (Earley et al., 2012). Briefly, the floor of the vehicle was covered with wood shavings with a thick rubber mat placed on top. The loading of the goats was done individually by two persons. In a

relatively calm condition, one person easily caught one goat randomly at a time and calmly lifted it up by supporting the goat with two hands from the abdomen. The goat was then handed to the other person, already inside the vehicle. The goats were stocked at a rate of 0.3 m² per animal (Kannan et al., 2000; Minka and Ayo, 2012) in a standard articulated Bedford, open pickup van, popularly used in the Guinea Savannah zone of West Africa for transportation of small ruminants. The journey started around 08:00 h and was terminated at 20:00 h. The vehicle used for the journey traveled with an average speed of 50 km/h and was driven by two licensed drivers in a similar pattern (that is not exceeding the average speed and by gentle negotiation of corner and application of breaks) on the same type of road. During the journey, the vehicle had four stop-overs; at veterinary and police control posts, and each stop-over time took about 10 to 15 min. In adherence to EU legislation on transport of live animals, the goats were rested for 1 h after an 8-h transportation period (Gavinelli and Simonin, 2003). During the resting period, the vehicle was parked in a shade to avoid direct effect of heat from sun. The goats were not fed or watered during the resting period (Richardson, 2002; Minka and Ayo, 2012).

Behavioural measurements

The behavioural events of the goats were recorded visually as earlier described (Altmann, 1974; Das et al., 2001) with little modification in timing, and using a digital camera (Sony, Minato-Tokyo, Japan) mounted at an angle in each truck to complement any visual lapses. Briefly, four trained observers recorded the behaviours; each observer stood at each of the four corners of the truck. During 12 h of transportation, each observer recorded behaviours of 5 goats per person on specially designed cards. The observers recorded standing and lying down positions and orientations of the goats after loading, before the start of driving and during the 12-h transportation period. The observers scanned each individual current activity (standing or lying down in perpendicular, parallel, opposite or diagonal orientations) at each 10 min interval, giving a period of 30 min of direct observation made for every 1 h. The percentage number of goats and the amount or percentage of time that individuals devoted to various activities (standing and lying down positions and orientations) were noted. The times spent in different positions and orientations were estimated for every 10 min using the 'instantaneous' sampling method described by Altmann (1974). Similarly, the percentage number and time the SG spent in standing and lying down positions were also assessed by two observers during the 12-h fasting period.

Blood analysis

A week before transportation (at 8:00 h and 20:00 h), before loading at 8:00 h and at the end of the journey, immediately after unloading, at 20:00 h, blood samples were collected from each goat in both groups by jugular venepuncture using #21 scalp vein set (Jiangsu Kanghua Medical Equipment Co. Ltd., Kiangsu, China) into non-heparinized test tubes. The blood samples were centrifuged at 1500 g × 10 min after clotting at room temperature (about 22 to 26.7°C). The plasma obtained was immediately analysed for the assessment of activities of ALT, AST and CPK using an automated analyzer (COBAS MIRA, Roche, Nutley, NJ, USA), while glucose and urea were assessed using an autoanalyzer (YSI 2300 STAT Plus, Yellow Springs, OH) at the Clinical Pathology Laboratory, Ahmadu Bello University, Zaria, Nigeria.

Statistical analysis

The AT and RH values recorded during the journey and in the goat's pen were compared using Student's *t*-test. The differences among standing or lying down positions and orientations (perpendicular, parallel, opposite and diagonal) of the animals during transportation were analyzed using Chi-square. The Wilcoxon-signed rank test was used to compare the hourly rates of behavioural categories observed at each sampling time, and the behaviour of animals under the different conditions (before, during and after the journey). Blood parameters were analysed using repeated-measures analyses of variance according to the procedure of the Generalized Linear Model of SAS (2006). Independent variables were the positions and orientations, time of observations and the interactions between these variables. Tukey's *post-hoc* test was used for statistical multiple comparisons, taking $P < 0.05$ to be significant.

RESULTS

Environmental conditions

The environmental conditions recorded inside the vehicles during the transportation periods and in the pen are shown in Figure 1. Before and after transportation, the AT and RH values fluctuated between 26.2 to 37.7°C and 50.0 to 80.0%, respectively; while during transportation, the AT and RH inside the vehicle fluctuated between the values of 28.2 to 40.1°C and 58 to 79%, respectively. The AT values recorded inside the vehicle, especially during the afternoon hours, were significantly ($P < 0.05$) higher than those recorded in the pen where the SG were kept, and also higher than ($P < 0.05$) the corresponding values recorded before and after transportation. There was no significant difference in the RH value recorded inside the vehicle and in the goats' pen (Figure 1).

Behaviour of goats

The first 30 min to one hour of the journey was characterized by frequent changes in standing positions, with parallel orientation taking precedence (not shown in Figure or Table). However, as the journey progressed, the behaviour of the goats became stable with little alteration when the vehicle negotiated bends or applied breaks. The hourly fluctuation in standing behaviour of the TG and SG are shown in Figure 2. The first half of the transportation period was characterized by standing behaviour. The time spent by TG in standing position during the 12-h transportation period was significantly ($P < 0.05$) higher than that spent in standing position by the SG. Table 1 shows the time spent and percentage number of goats that exhibited different positions and orientations during the transportation period. Of the 12-h

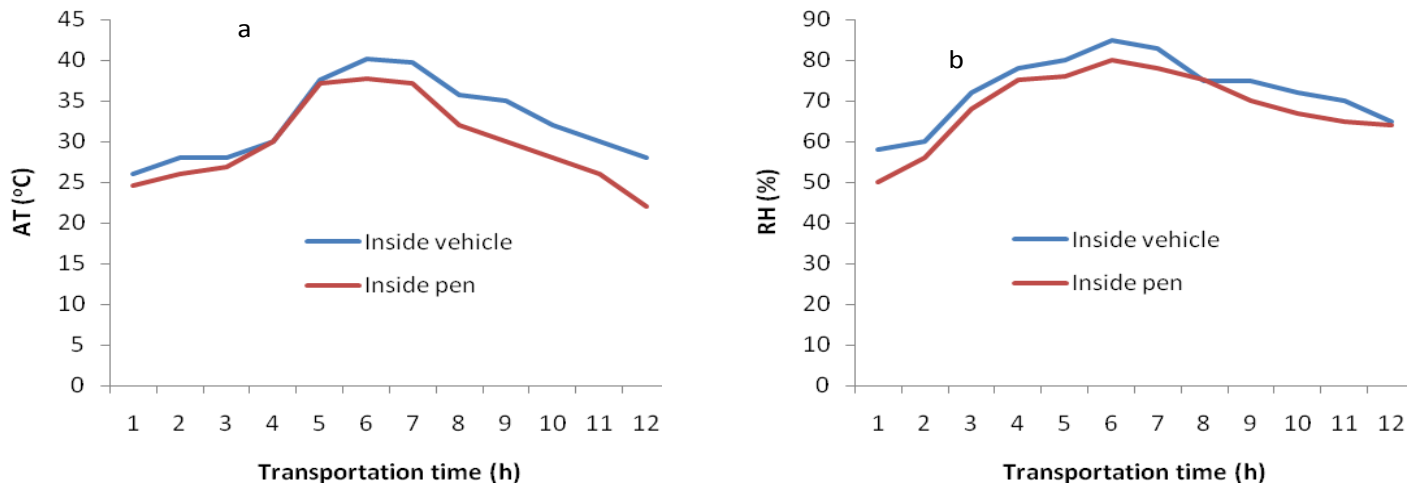


Figure 1. (a) AT (°C) and (b) RH (%) fluctuations recorded inside the vehicle and pen during the 12 h of road transportation.

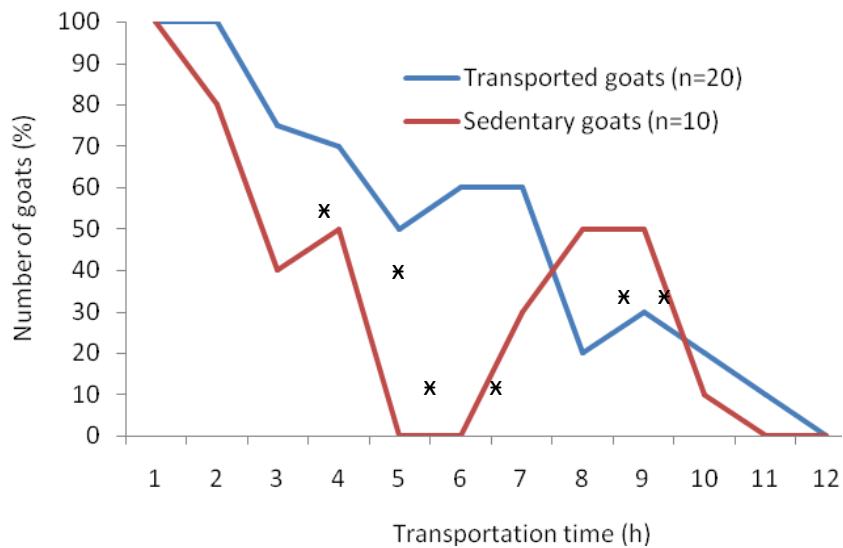


Figure 2. Changes in standing behaviour of transported and sedentary goats during 12 h of road transportation period. *P < 0.05 vs. transported.

transportation period, the goats spent 7 h standing, of which 40.0% of the goats spent 4.5 ± 0.7 h standing in perpendicular direction, 30.0% spent 1.5 ± 0.5 h in parallel direction, while 20.0 and 10% spent 0.5 ± 0.1 h each in opposite and diagonal directions, respectively. Of the 5 h spent by the goats lying down, 33.3% of the goats spent 2.5 ± 0.7 and 1.5 ± 0.5 h each lying down in opposite and diagonal directions, respectively (Table 1).

Plasma enzyme activities and metabolites

The concentrations of plasma biochemical activities and metabolites obtained in SG were not different from the base-line values. Immediately after transportation, mean activities of CPK, AST, ALT, and glucose and urea concentrations recorded in TG were elevated ($P < 0.05$) over base-line, and over the values recorded in SG (Table 2).

Table 1. Mean time spent and percent number of transported goats ($n = 20$) that exhibited different positions and orientations during 12 h of road transportation.

Orientation	Position			
	Standing	Lying down	Standing	Lying down
	Mean time (h)		Percent (%) number of goats	
Parallel	1.5±0.5 ^{ax}	0.5±0.9 ^{bx}	30 ^{ax}	16.6 ^{bx}
Perpendicular	4.5±0.7 ^{ay}	0.5±1.6 ^{bx}	40 ^{ay}	16.6 ^{bx}
Opposite	0.5±0.1 ^{az}	2.5±0.7 ^{by}	20 ^{az}	33.3 ^{by}
Diagonal	0.5±0.1 ^{az}	1.5±0.5 ^{bz}	10 ^{az}	33.3 ^{by}
Total	7.0	5.0	100	100

^{ab} = Values under each separate heading with different superscript letters along the same row are significantly different ($P < 0.05$). ^{xyz} = Values under each separate heading with different superscript letters along the same column are significantly different ($P < 0.05$).

Table 2. Effects of 12 h road transportation and food deprivation on serum biochemical activities of transported ($n = 20$) and fasted sedentary goats ($n = 10$).

Variable	Group	Measurement time			Significance
		Base-line	Pre-transportation	Post-transportation	
CPK (IU/L)	TG	87.6±2.7	86.8±5.5	97.1±4.5 ^b	*
	SG	86.6±2.5	84.5±3.5	88.7±5.5 ^a	ns
	Significance	ns	ns	*	-
AST (IU/L)	TG	36.8±3.1	38.9±4.5	54.7±6.1	*
	SG	38.7±2.2	38.7±2.5	40.2±1.2	Ns
	Significance	ns	ns	*	-
ALT (IU/L)	TG	58.2±4.0	60.1±1.8	86.8±6.2	*
	SG	58.8±5.0	58.8±1.8	60.0±3.8	ns
	Significance	ns	ns	*	-
Glucose (mg/dl)	TG	185.7±8.4	184.0±8.9	234.5±9.4	*
	SG	187.2±11.5	184.0±10.2	190.2±8.2	ns
	Significance	ns	ns	*	-
Urea (mmol/l)	TG	3.4±0.1	3.2±0.5	3.0±0.7	ns
	SG	3.5±0.2	4.0±0.7	3.5±1.5	ns
	Significance	ns	ns	ns	-

CPK = creatine phosphate kinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase. ns = $P > 0.05$; * = $P < 0.05$.

The increase in plasma biochemical activities and metabolite concentrations was lower in TG that spent more time standing and lying down perpendicular to the direction of the vehicle movement, compared to values obtained in goats that adopted other positions (Table 3). Goats that spent more time standing and lying down in opposite or diagonal direction had predominantly higher values in all the enzymes and metabolites studied compared to those that stood or lay down in perpendicular or parallel orientations (Table 3).

DISCUSSION

The AT and RH (which are measures for temperature humidity index, THI) values obtained inside the vehicle during the transportation, especially during the hot afternoon hours of the day, may induce heat stress because the values were outside the reference thermo-neutral zone of 22 to 35°C and 58 to 65%, respectively, established for goats in the tropics (Igono et al., 1982; Richardson, 2002). Such increase in environmental

Table 3. Effects of standing and lying down positions and orientations on serum biochemical activities of goats (n = 20) transported for 12 h during the hot-dry conditions.

Variable/position	Orientation			
	Perpendicular	Parallel	Diagonal	Opposite
CPK (U/l)				
Standing	91.2±2.5 ^{ax}	94.5±5.5 ^{ax}	97.6±2.5 ^{ax}	98.5±4.8 ^{ax}
Lying down	92.1±4.5 ^{ax}	99.6±4.7 ^{ax}	101.4±8.2 ^{ax}	102.5±7.9 ^{ax}
AST (U/l)				
Standing	40.2±2.8 ^{ax}	50.4±1.5 ^{ay}	58.1±*5.0 ^{ay}	58.2±4.0 ^{ay}
Lying down	42.5±3.5 ^{ax}	55.6±2.0 ^{ay}	65.4±5.2 ^{ay}	67.5±5.0 ^{ay}
ALT (U/l)				
Standing	74.6±2.7 ^{ax}	82.1±4.7 ^{ax}	90.2±3.0 ^{ay}	90.8±7.5 ^{ay}
Lying down	79.2±3.5 ^{bx}	89.4±5.5 ^{by}	95.6±5.5 ^{ay}	95.2±6.8 ^{ay}
Glucose (mg/dl)				
Standing	198.4±12.5 ^{ax}	200.5±15.3 ^{ax}	245.1±10.5 ^{ay}	260.2±10.8 ^{ay}
Lying down	211.5±10.5 ^{bx}	227.4±11.5 ^{bx}	245.4±12.5 ^{ay}	270.0±15.5 ^{ay}
Urea (mmol/l)				
Standing	3.5±0.5 ^{ax}	3.8±0.7 ^{ax}	4.2±0.5 ^{ax}	4.2±0.6 ^{ax}
Lying down	3.5±0.5 ^{ax}	3.9±0.2 ^{ax}	4.4±0.2 ^{ay}	4.5±0.8 ^{ay}

CPK = creatine phosphate kinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase. ^{ab} = Values under each separate heading with different superscript letters along the same row are significantly different (P<0.05). ^{xy} = Values under each separate heading with different superscript letters along the same column are significantly different (P<0.05).

temperature is characteristic of the hot-dry season in the zone of study and has been shown to induce oxidative stress, which consequently reduces the coping ability of the animals (Minka and Ayo, 2012). The unfavourable environmental conditions acting on the animals in this study have been considered a risk factor for animal welfare (Minka and Ayo, 2012), which often results in their death or compulsory slaughter (Ibironke et al., 2010; Minka and Ayo, 2012). Thus, management factors toward alleviation of the adverse effect of environmental conditions on transported vehicles should be employed, especially if the animals are to be transported during the hot afternoon hours of the day. The standing position observed in the majority of the goats suggested that the goats preferred standing position during transportation period and only lay down when exhausted and fatigued, which set in after 7 to 8 h of transportation. This finding was supported by the results obtained in Indian goats transported for 50 min by Das et al. (2001).

The results of standing orientations during the transportation showed that the majority of the goats that exhibited standing positions spent more time in perpendicular orientation to the direction of the vehicle movement. The

present result disagrees with the finding of standing orientation parallel to the direction of vehicle movement reported in Indian goats transported by road for only 50 min (Das et al., 2001). The difference in the orientation may be due to variation in the journey duration. Similar result on standing position parallel to the vehicle as reported by Das et al. (2001) was observed during the first hour of transportation in the present study. However, as the journey progressed the goats changed their positions from parallel to perpendicular. In transported cattle, the predominant standing orientations observed were parallel or perpendicular to the direction of movement (Eicher and Morrow-Tesch, 2000; Eicher, 2001; Nanni et al., 2003).

In transported horses, the most preferred and less stressful standing orientation was opposite to the direction of vehicle movement (Padalino et al., 2012), which is in contrast to the present result. Cafazzo et al. (2012) showed that provision of adequate space for animals during transportation makes the choice of a particular standing orientation unnecessary. The reason for the choice of standing in perpendicular orientation by the goats in the present study may be due to increased demand in oxygen as the journey progressed. Thus, the

goats apparently adopted this orientation so that their nostrils will be exposed to more air as they inclined their heads in perpendicular direction. This may require further investigation. The observation that the TGs spent more time in standing position during transportation than the time spent by the SG suggested that the transportation affected standing stereotype of the goats.

The increase in the biochemical activities of AST, ALT and CPK observed over transportation suggested that transportation increased muscle cell permeability and injury, which resulted in the leakage of the enzymes. The result showed that transportation may cause muscle break-down and bruising, similar to those obtained in transported cattle (Averos et al., 2008; Cafazzo et al., 2012), sheep (Zhong et al., 2011; Ekiz et al., 2012, 2013), goats (Kannan et al., 2000; Galipalli et al., 2004) and roe deer (Montane et al., 2002). The increase in plasma glucose during the post-transportation period may be due to increase in glycogenolysis, stimulated by increased secretions of catecholamine and glucocorticoid hormones, which are under the control of the sympathetic nervous system (Ekiz et al., 2013). Thus, during the stressful transportation period, the sympathetic nervous system was apparently activated to trigger the secretion of hormones, responsible for the stimulation of glycogenolysis for more production of glucose from the liver and muscles into the systemic circulation (Rajion et al., 2001; Kannan et al., 2002; Averos et al., 2008). The increase ($P < 0.05$) in plasma urea indicated an increase in protein break-down, apparently due to excess fatigue, increase in cortisol concentration and prolong food deprivation, which cause break-down (catabolism) of some proteins and nucleic acids in muscles during stressful transportation conditions (Kannan et al., 2002; Ekiz et al., 2013).

The lower values of enzymatic activities, though not statistically significant, recorded in goats that spent more time in standing position, as compared to those that lay down, demonstrated that these goats were less stressed, and thus, suffered less muscular damage. It is expected that longer duration of standing should produce greater adverse effect on muscles tissues of the goats, but surprisingly, this was not so. The result suggested that standing and maintenance of balance inside transportation vehicle may not be a major problem for goats, apparently due to their light live-weight. Furthermore, the fact that the goats were reared under the extensive management system used to grazing in different terrains, climbing of shrubs and wall fences, apparently, facilitated their ability to maintain stable and long duration of standing position, with little stress during the transportation. Such adaptational abilities were reported in transported cattle and goats reared under the extensive management system (Kannan et al., 2002; Minka and Ayo, 2012). The significant decline in standing position observed from the 8th h of the transportation showed that journeys exceeding

exceeding 8-h in goats were stressful and resulted in muscular fatigue, as evidenced by the large number of goats that lay down from the 8th h of the transportation period.

Furthermore, the fact that the physiological indices of muscular damage recorded in the TG after the transportation were above the values recorded in SG, strongly suggested that the increase in the values was mainly due to transportation conditions, rather than food and water deprivation. The insignificant increase in enzyme activities and glucose and urea concentrations recorded in SG after the 12 h of food and water deprivation supported the findings of Schoen (1968) and Aganga et al. (1986), who showed that food and water deprivation in goats, for up to 72 h had little or no effect on their basic physiological parameters.

The lower activities of enzymes, and concentrations of glucose and urea obtained in goats that spent more time standing or lying down in perpendicular orientation demonstrated that those goats suffered less stress as compared to their counterparts goats that spent time standing in other different orientations. The result showed that perpendicular orientation was the most preferred standing orientation during long duration of road transportation of goats, reared under the extensive management system under hot-climatic conditions, while the worst lying down orientation was opposite or diagonal to the direction of travel. This was evidenced by higher physiological indices of stress recorded in goats that adopted lying down position in opposite or diagonal orientations. The result of the present study, for the first time, demonstrated the adverse effects of standing and lying down positions and orientations on activities of plasma enzymes, concentrations of glucose and urea of goats, subjected to long-distance road transportation under hot-climatic conditions. Therefore, management strategies towards alleviation of road transportation stress in goats' should include vehicle modification that would allow the animals to stand or lay perpendicularly to the direction of vehicle movement.

Conclusions

Standing or lying down perpendicularly during road transportation of goats induced less muscular damage, while lying down in opposite or diagonal direction to the vehicle movement induced greater muscular damage, which may adversely affect the welfare of the goats. Perpendicular orientation may be the best travel position for goats transported by road under adverse environmental conditions.

ACKNOWLEDGEMENTS

The authors are grateful to the Laboratory staff of the

College of Agriculture and Animal Science, Ahmadu Bello University, Mando-Kaduna, Nigeria for their technical assistance.

REFERENCES

- Aganga AA, Umunna NN, Okoh PN, Oyedipe EO (1986). Water metabolism of ruminants: A review. *J. Anim. Prod. Res.* 6:171-181.
- Altmann J (1974). Observational study of behaviour: sampling methods. *Behaviour* 48:227-265.
- Averos A, Martin S, Riu M, Serratos J, Gosalvez LF (2008). Stress response of extensively reared young bulls being transported to growing-finishing farm under Spanish summer commercial conditions. *Livest. Sci.* 119:174-182.
- Ayo JO, Oladele SB, Fayomi A, Jumbo SD, Hambolu JO (1998). Body temperature, respiration and heart rates in the Red Sokoto goat during the harmattan season. *Bull. Anim. Health. Prod. Afr.* 46:161-166.
- Ayo JO, Oladele SB, Fayomi A (2002). Behavioural reactions of cattle to stress situations: A review. *J. Agric. Technol.* 8:15-20.
- Ayo JO, Minka NS, Mamman M (2006). Excitability scores of goats administered ascorbic acid and transported during hot-dry conditions. *J. Vet. Sci.* 7:127-131.
- Broom DM (2000). Welfare assessment and problem areas during handling and transport. In: Grandin T (Ed.) 'Livestock Handling and Transport'. CAB International: Wallingford. pp. 43-61.
- Cafazzo S, Magnania D, Calàa P, Razzuolub E, Gerardic G, Bernardinic D, Amadorib M, Costaa LN (2012). Effect of short road journeys on behaviour and some blood variables related to welfare in young bulls. *Appl. Anim. Behav. Sci.* 139:26-34.
- Das KS, Srivastava BB, Das N (2001). Standing orientation and behaviour of goats during short-haul road transportation. *Small Rumin. Res.* 41:91-94.
- Earley B, Murray M, Prendiville DJ, Pintado B, Borque C, Canali E (2012). The effect of transport by road and sea on physiology, immunity and behavior of beef cattle. *Res. Vet. Sci.* 92:531-541.
- Eicher SD (2001). Transportation of cattle in the dairy industry: Current research and future directions. *J. Dairy Sci.* 84:E19-E23.
- Eicher SD, Morrow-Tesch JL (2000). Behaviour following subcutaneous electrolyte treatment in transported calves. In: Proc. 34th Inter. Conf. Inter. Soc. Appl. Ethol. (Flori-anopolis: Brazil)
- Ekiz B, Ergül Ekiz E, Yalcintan H, Kocak O, Yilmaz A, Gunes H (2012). The effects of transport stress on certain welfare parameters and behaviours in Red Karaman, Imroz, Sakiz and Karakul rams. *J. Fac. Vet. Med. Istanbul Univ.* 38:15-28.
- Ekiz B, Ergül Ekiz E, Yalcintan H, Yilmaz A, Kocak O, Gunes H (2013). Effect of ram-ewe mixed transportation on certain welfare parameters in Red Karaman and Imroz sheep. *J. Fac. Vet. Med. Istanbul Univ.* 39(2):155-167.
- Galipalli S, Gadiyaram KM, Kouakou B, Terril TH, Kannan G (2004). Physiological responses to pre-slaughter transportation stress in Tasco-supplemented goats. *S/Afr. J. Anim. Sci.* 34:198-200.
- Gavinelli A, Simonin D (2003). The transport of animals in the European Union: The legislation, its enforcements and future evolutions. *Vet. Res. Comm.* 27:529-534.
- Giannetto C, Fazio F, Casella S, Marafioti S, Giudice E, Piccione G (2011). Acute phase protein response during road transportation and lairage at a slaughterhouse in feedlot beef cattle. *J. Vet. Med. Sci.* 73 (11):1531-1534.
- Ibironke AA, McCrindle CME, Adejuwon TA, Cadmus SIB (2010). Losses associated with mortality of cattle and camels during transportation to Oko-Oba abattoir, Lagos State, Nigeria. *Eur. J. Translat. Myol-Basic Appl. Myol.* 1(1 and 2):13-16.
- Igono MO, Molokwu ECI, Aliu YO (1982). Body temperature responses of Savannah Brown goats to the harmattan and hot-dry season. *Int. J. Biometeorol.* 26:225-230.
- Kannan G, Terrill TH, Kouakou B, Gazal OS, Gelaye S, Amoah EA, Samake S (2000). Transportation of goats: Effects on physiological stress responses and liveweight loss. *J. Anim. Sci.* 78:1450-457.
- Kannan G, Terrill TH, Kouakou B, Gelaye S, Amoah EA (2002). Simulated preslaughter holding and isolation effects on stress responses and liveweight shrinkage in meat goats. *J. Anim. Sci.* 80:1771-1780.
- Kay R, Hall C (2009). The use of a mirror reduces isolation stress in horses being transported by trailer. *Appl. Anim. Behav. Sci.* 116:237-243.
- Minka NS, Ayo JO (2010). Physiological responses of food animals to road transportation stress. *Afr. J. Biotechnol.* 9(40):6601-6613.
- Minka NS, Ayo JO (2012). Assessment of thermal load on transported goats administered with ascorbic acid during the hot-dry conditions. *Int. J. Biometeorol.* 56:333-341.
- Montane J, Marco I, Lopez-Olvera J, Manteca X, Lavin S (2002). Transport stress in roe deer (*Capreolus capreolus*): Effect of the short acting antipsychotic. *Anim. Welfare* 11:295-303.
- Nanni Costa L, Lo Fiego DP, Cassanelli MG, Tassone F, Russo V (2003). Effect of journey time and environmental condition on bull behaviour and beef quality during road transport in Northern Italy. *Dtsch. Tierarztl. Wochenschr.* 110:107-110.
- Padalino B, Maggolino A, Boccaccio M, Tateo A (2012). Effects of different positions during transport on physiological and behavioural changes of horses. *J. Vet. Behav: Clin. Applic. Res.* 7(3):135-141.
- Piccione G, Casella S, Giannetto C, Giudice E, Fazio F (2012). Utility of acute phase proteins as biomarkers of sport stress in ewes. *Small Rumin. Res.* 107:167-171.
- Piccione G, Casella S, Giannetto C, Bazzano M, Giudice E, Fazio F (2013). Oxidative stress associated with road transportation in ewes. *Small Rumin. Res.* 112:235-238.
- Rajion MA, Soat IM, Zulkifli I, Goh YM (2001). The effects of road transportation on some physiological stress measures in goats. *Asian-Aust. J. Anim. Sci.* 9:1250-1252.
- Richardson C (2002). Lowering stress in transported goats. Ontario Ministry of Agriculture and Food-Livestock Technology Branch, Northern Ontario Regional Office.
- Schoen A (1968). Studies on the water balance of the East African goats. *East Afr. Agric. For. J.* 34:256-262.
- Tateo A, Padalino B, Boccaccio M, Maggolino A, Centoducati P (2012). Transport stress in horses: Effects of two different distances. *J. Vet. Behav: Clin. Appl. Res.* 7:33-42.
- Young T, Creighton E, Smith T, Hosie C (2012). A novel scale of behavioural indicators of stress for use with domestic horses. *Appl. Anim. Behav. Sci.* 140:33-43.
- Zhong RZ, Liu HW, Zhou DW, Sun HX, Zhao SC (2011). The effects of road transportation on physiological responses and meat quality in sheep differing in age. *J. Anim. Sci.* 89:3742-3751.

Full Length Research Paper

A three year follow-up study on the occurrence of bovine ehrlichiosis (cowdriosis) at Gondar University dairy farm

Achenef Melaku^{1*}, Basaznew Bogale², Mersha Chanie², Wassie Molla² and Gizat Almaw³

¹Veterinary Pharmacy and Biomedical Sciences Department, Faculty of Veterinary Medicine, University of Gondar, Ethiopia.

²Veterinary Paraclinical Studies Unit, Faculty of Veterinary Medicine, University of Gondar, Ethiopia.

³National Animal Disease diagnostic and Investigation Centre, Sebeta, Ethiopia.

Accepted 18 December, 2013

Bovine ehrlichiosis (cowdriosis) is an acute, fatal, non-contagious, infectious, tick-borne rickettsial disease of ruminants, caused by *Ehrlichia ruminantium* and transmitted by *Amblyomma* ticks. During the 3 years period, 37 cases were examined at the University of Gondar dairy farms with diagnosis based on clinical, post-mortem and squash smear preparations. The age of animals which showed clinical signs ranged from two months to 3.5 years, with an average of 1.27 years. Most cases were aged less than one year. Cases were recorded throughout the year; however, it was more prevalent in the rainy season than others. Of the 37 cases, 26 (70.27%) were female and 11 (29.73%) were male. Clinical signs observed include a body temperature of 37 to 41.5°C, reduced or no food consumption, depression, conjunctival congestion, grunting, shivering, head pressing, excessive chewing, salivation and self inflicted trauma. The most commonly observed findings at post-mortem examination were hydro-pericardium, peticheal or generalized haemorrhages in the peritoneum and other organs. The economic loss to the farm, during the period of study was 7884.67 USD (142,924.20 Birr {Ethiopian Currency}). The disease should be included in the differential diagnosis of febrile conditions, so that the appropriate treatment and control measures can be implemented, as early as possible.

Key words: Longitudinal study, cowdriosis, dairy farm, clinical observations, post-mortem, squash smear examination.

INTRODUCTION

Bovine ehrlichiosis (also known as heartwater or cowdriosis) is an infectious and tick-borne disease of ruminants caused by the rickettsial organism, *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) and transmitted by ticks of the genus *Amblyomma*, particularly *Amblyomma variegatum* and *Amblyomma habraeum* which are widespread in Ethiopia (Mekonnen et al., 2001;

Tessema and Gashaw, 2010), including Gondar (Miruts, 2010). The disease is endemic in sub-Saharan African countries (Deem et al., 1996a; McMillan and Meltzer, 1996; Radostits et al., 2007), and it has a serious negative impact on livestock productivity, with high morbidity and mortality rates (up to 90%) in susceptible ruminants. European breeds are generally more susceptible than

*Corresponding author. E-mail: achenefmela@yahoo.com

more susceptible than indigenous African breeds (OIE, 2011).

Although bovine ehrlichiosis is recognized as one of the most devastating livestock diseases; the exact epidemiology is poorly understood in Ethiopia or other African countries. Many of the published studies on the disease are often for only short periods, without detailed follow up, which precludes any extensive evaluation of the risk factors, which might facilitate better understanding of the disease and its control. This study set out to evaluate the disease in the Gondar University dairy farm, including clinical observations, post-mortem and squash smear examination and resultant economic losses.

MATERIALS AND METHODS

Methodology

The study was conducted by included clinical examination, diagnosis based on squash smear preparations and post-mortem evaluation. Sick animals were thoroughly examined and clinical observations recorded on a standard template. Post-mortem examination was conducted and gross lesions were recorded for all animals which died. Brain squash smears were prepared for diagnostic confirmation.

Study area

The study was conducted at University of Gondar, dairy farm, North-Western Ethiopia, from June, 2009 to May, 2012. The farm is located in the university compound, 740 km from Addis Ababa (the capital) at latitude, longitude and altitude of 12.3 to 13.8 North, 35.3 to 35.7° East and 2200 m above sea level, respectively. The average annual rainfall may reach up to 1772 mmHg. The annual mean minimum and maximum temperature of the area varies between 12.3 to 17.7 and 22 to 30°C, respectively. The area has two seasons, the rainy season from June to September, and the dry season from October to May when there is low and erratic rainfall (MoA, 2003).

Study population

The farm, which was established in May, 2009 comprises approximately 80 cross breed cows (Holestin Friesian × local Zebu), a dynamic population, where some cows were culled due to poor reproductive performance, infectious diseases and malness and calves were born during the study period. The herd is kept for milk production to generate income and for teaching purposes. Except one teaser bull, and those male calves born during the study period, all animals studied were female. The system of husbandry was semi-intensive, where animals were allowed access to grazing and supplied with brewery by-products and hay, typically late in the afternoon and evening, when they are housed in well constructed shade. All sick animals were examined clinically and treatment was given based on the diagnosis. Acaricides were applied when the majority of animals were infested with ticks.

Data analysis

Preliminary data was entered into Microsoft Excel. The average incidence was calculated by adding the number of cases in three years and divides it by three, or the number of cases divided by the

total population in the specified period (Thrusfield, 2005). The economic loss induced by cowdriosis was based on the formula given by Singh and Prasad (2008). It included losses due to mortality, reduced milk yield, carcass meat (body weight) and the cost of acaricide and antibiotic treatment.

Direct losses from mortality

This was calculated as the product of the number of animals which died (D) due to the disease and probable average value (P) of the animal: $A = D \times P$.

Losses in milk yield

For the proportion of cows in milk in a year, the losses were expressed in terms of the reduction in milk yield, based on the market price of milk. When a cow died as a result of the disease, the loss was calculated based on the percentage of its lactation lost. Double counting or costing was avoided.

The overall cost of losses due to reduced milk production was calculated using the formula:

$$M_L = (S - D) \times P_i \times L \times Z \times P_M$$

Where M_L is the milk losses in Birr (Ethiopian currency), S is the number of sick animals, D is the number of dead animals, P_i is the proportion of animals in milk, L is the proportion of lactation lost, Z is the annual average milk yield per milk cows, P_M is the price of milk (per kg)

Estimation of losses in body weight

The body weight loss in non-milking animals was estimated by the formula:

$$B_L = (S - D) (1 - P_i) \times W_L \times W_A \times P_W$$

B_L is the body weight losses in Birr, S is the number of sick animals, D is the number of dead animals, P_i is the proportion of animals not in milk, W_L is the proportion of body weight loss, B_A is the average body weight, P_M is the price of meat (per kg).

Cost of treatment

In order to estimate the cost of treatment, records of acaricide and antibiotics used were added up and multiplied by the average price of drugs.

RESULTS

Incidence

Thirty seven cattle, from a total of 80 animals observed for a period of three years, developed bovine ehrlichiosis, with an average incidence of 12.33 cases/annum (3.05 standard deviation (SD)) or 15% of the herd.

The number of cases was higher during the first year of the study and showed a decrease in the subsequent 2 years (Figure 1).

Risk factors

Age, season and sex were considered as factors that

Table 1. Temperature response of case of bovine ehrlichiosis and survival rate.

Body temperature (°C)	Number of cases		Mortality		Recovery	
	No.	%	No.	%	No.	%
≥39.5	21	56.76	3	14.29	18	80
38-39.5	7	18.92	2	28.57	5	60
<38	9	24.32	4	44.44	5	33
Total	37	-	9	24.32	28	75.68

Total fatality rate (9/37) = 24.32 or 8.1%/annum.

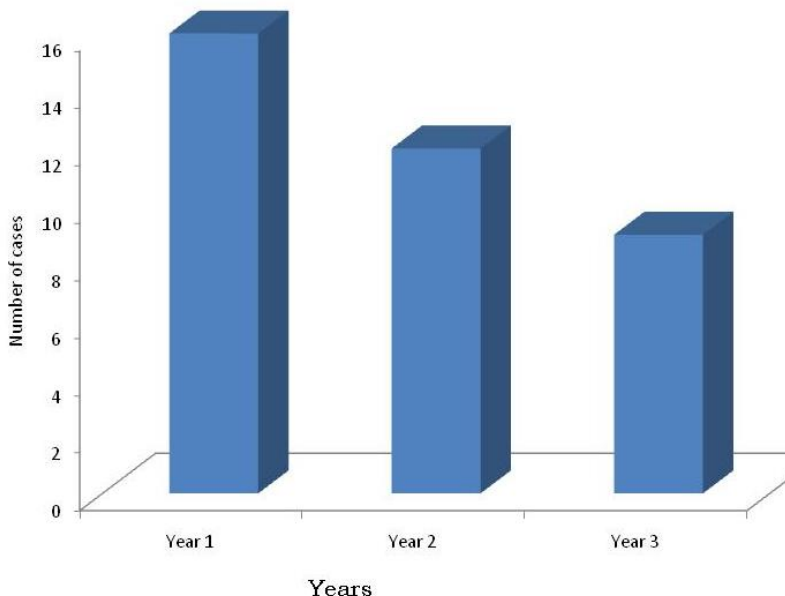


Figure 1. Number of cases in first, second and third year.

may contribute for the occurrence of the disease. The age of animals which showed clinical signs ranged from two months to 3.5 years, with an average of 1.27 years. Most cases were of age less than one year (Figure 2). Cases were recorded throughout the year; however, it was more prevalent in the rainy season than others (Figure 3). Of the 37 cases, 26 (70.27%) were female and 11 (29.73%) were male.

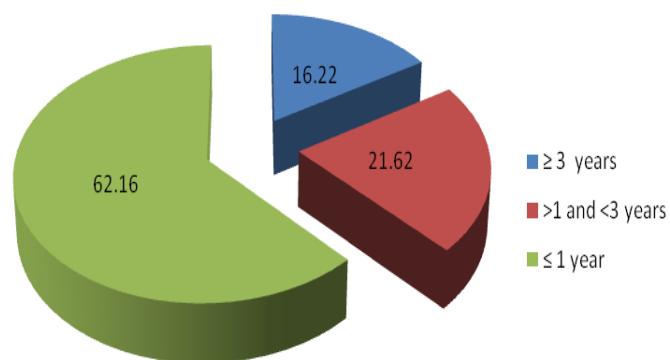


Figure 2. Age categories of cases of bovine ehrlichiosis.

Clinical signs

A variety of clinical signs were observed during the study period as explained thus.

Altered body temperature

A change in body temperature was recorded, ranging from 37 to 41.5°C, with an average of 39.41°C, (median 38.20°C; mode 41°C). The recovery rate tended to be better if treatment commenced, while an animal was febrile (Table 1).

In-appetence

Reduced feed consumption or complete cessation of eating was observed in most cases, when most animals stopped eating grass, but would drink small amounts of milk.

Depression

Affected animals became isolated from the herd, and distant from the feeding and watering area.

Conjunctiva congestion

Conjunctival congestion was commonly observed in most of the cases of bovine ehrlichiosis (Figure 4).

Respiratory distress

Respiratory distresses like, grunting was observed in most of the cases, particularly when the animals are in a recumbent position.

Head pressing

Head pressing was observed in severe advanced cases, either against the wall or the ground (Figure 5).

Frequent chewing

Frequent chewing movement and attempts to eat non edible materials such as soil or sand were also observed.

Salivation

Excessive salivation was also observed in 12 (32.43%) cases (Figure 6).

Trauma

Traumatic injuries as a result of frequent falling were observed in some cases (Figure 7).

Post-mortem examination

Seven out of 9 animals which died were autopsied. A number of findings were recorded including hydropericardium (Figure 8), peticheal or generalized haemorrhages in the peritoneum and several organs (abomasum, heart and kidney). The gallbladder was also enlarged with excessive thick bile (Figure 9).

Economic losses

The economic losses due to the disease are associated with deaths, the cost of acaricides and antibiotics and loss of milk yield (Table 2).

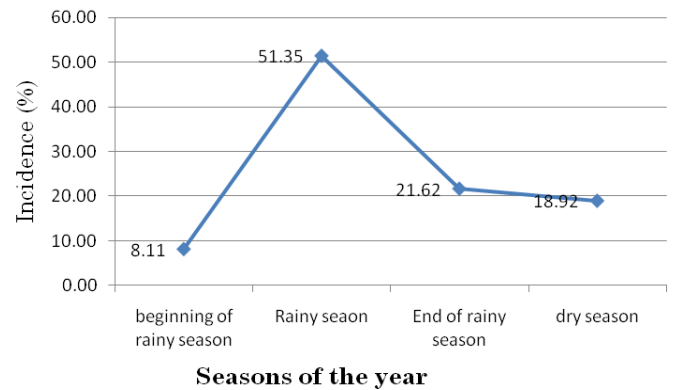


Figure 3. Bovine ehrlichiosis cases in different seasons of the year.



Figure 4. Animals showing congestion of the conjunctiva.



Figure 5. Head pressing; Sign of a case of bovine ehrlichiosis.

Table 2. Summary of the economic losses due to bovine ehrlichiosis in the three years study period.

Parameter	Estimated cost lost (Birr)	Estimated cost lost (USD)	Remark
Mortality	36,000.00	2000.00	-
Acaricide cost	1029.60	57.20	-
Cost of treatment	980.00	54.44	-
Cost due to loss of milk	15,256.22	847.57	-
Costs related with meat loss	87,178.38	4843.24	-
Management cost	1480.00	82.22	-
Total	141,924.20	7884.67	-

**Figure 6.** Animals showing salivation.**Figure 7.** Traumatic lesion induced on the poll.

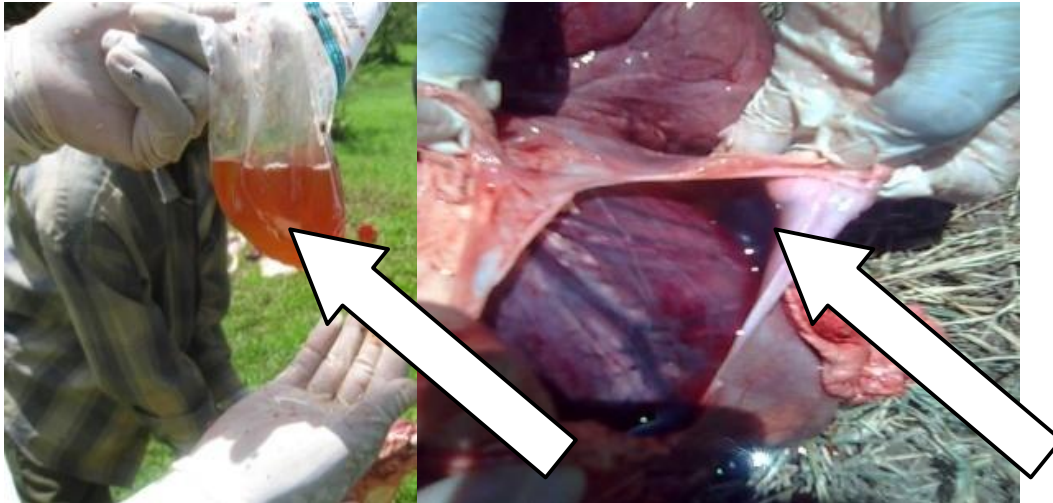


Figure 8. Fluid collected from pericardium and fluid around the heart (Hydropericardium).

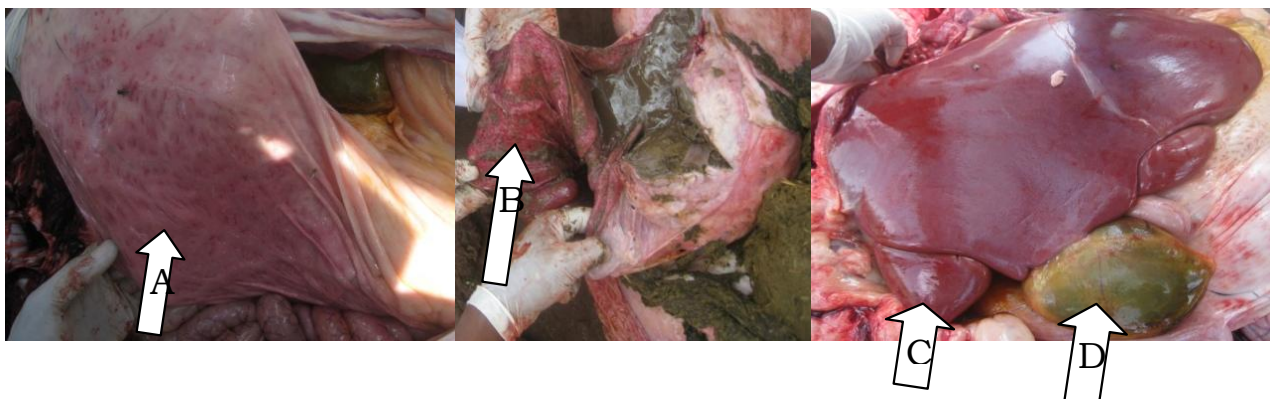


Figure 9. Haemorrhage on the peritoneum (A), and abomasum (B), enlarged liver (C) and gall bladder (D).

Mortality

There were 9 deaths 9 × 4,000 Birr (animal’s value) 36,000 Birr (2000.00 United States Dollar (USD)) was lost due to mortality.

Tick control and cost of acaricides

The acaricides were applied using a hand knap sac sprayer. The animals were sprayed on average once every two weeks. The frequency was high during the rainy season when the tick challenge increased. Three full (20 ml) knap sac sprayers were enough for one round of spraying, totalling 60 ml to spray the herd. In the study period, 780 rounds of spraying took place, totalling 4680 ml of acaricide. The average price of the acaricides was 0.22 Birr/ml. The approximate cost of acaricide utilisation was 1029.6 Birr (57.2 USD).

Treatment costs

For the treatment of these cases of cowdriosis, a number of different drugs were used, most commonly oxytetracycline 10%. Twenty eight vials were used, at 25 Birr/vial, a total cost of 700.00 Birr (38.88 USD).

Oxytetracycline 20% was used for more susceptible animals as prophylaxis during peak outbreaks. For this purpose, 7 vials were used, a total cost of 280 Birr (15.56 USD).

Milk losses

The milk loss was calculated using the formula: $M_L = (S - D) \times P_l \times L \times Z \times P_M$

where M_L is the milk losses in Birr, S is the number of

sick animals = 37, D is the number of dead animals = 9, P_i is the proportion of animals in milk = 5/37, L is the proportion of lactation lost = 15%, Z is the annual average milk yield per milk cows = 6 Lx360 days, P_M is the price of milk (per kg) = 8.00

Hence, the total loss due to reduced milk yield was 15,256.22 Birr (847.47 USD).

Costs related to meat loss (body weight)

The body weight loss in non-milking animals was estimated using the formula:

$$B_L = (S - D) (1 - P_i) \times W_L \times W_A \times P_W$$

where B_L is the body weight losses in Birr, S is the number of sick animals = 37, D is the number of dead animals = 9, P_i is the proportion of animals in milk = 5/37, W_L is the proportion of body weight loss = 10%, W_A is the average body weight = 300 kg, P_M is the price of meat (per kg) 80 Birr.

Hence, the total loss due to reduced body weight was 87,178.38 Birr (4,843.24 USD). The cost of increased management and professional advice was approximately 40 Birr/sick animal. $37 \times 40 = 1480.00$ (82.22 USD).

DISCUSSION

This study has demonstrated an annual incidence of 12.33 animals/annum (15% of the herd) succumbing to cowdriosis in one susceptible dairy herd, where there was clinical monitoring and treatment, with implementation of vector control measures. The numbers of cases were higher during the first year of the study period and show slight decrement in the following years. This may be related to the restocking process which took place during the beginning of the study period, as restocking of animals into endemic areas is known to increase the incidence of bovine ehrlichiosis with mortality (Hanks and Lopes Pereira, 1998), particularly where the restocked animals are from more susceptible breeds. The annual fatality rate in this study was 8.1% of affected animals. In a similar study conducted in Arsi, Ethiopia the mortality rate was 15.71% (Obsa and Zerihun, 1993). Heartwater accounted for 51% of all mortalities on farms in Zimbabwe (Meltzer et al., 1996). Such differences may be related to breed, the severity of the tick challenge, the vector control programme and the timing of treatment. The increased incidence in females reflects the relatively low number of male animals on the farm.

The youngest animals affected were 2 months old. An age-dependent resistance has long been recognized and young animals which are believed to have innate resistance (Radostits et al., 2007). Colostrum-derived antibodies to *Cowdria* have been detected in sera from calves up to four weeks old (ILRAD, 1991). The reduced

susceptibility of young calves may also reflect reduced exposure to ticks of indoor calves.

In addition to tick borne disease, vertical transmission of *C. ruminantium* from cows to their calves (Deem et al., 1996b) has been demonstrated, suggesting that at an early age calves may have two sources of infection, either from the dam or tick infestation. It has also been observed that in calves, seroprevalence rose steadily with age up to a maximum of 73% in endemic areas (Koney et al., 2004).

In this study, no animal was re-infected or exhibited clinical signs after recovery, which concurs with the observation that cattle recovering from the disease are immune for 6 months to 4 years (Radostits et al., 2007). A long period of antibody persistence has been detected in animals after clinical disease, which may prevent reoccurrence of the disease (ILRAD, 1991).

In this study, cases were recorded throughout the year, but more frequently in the rainy season. This may be related to the seasonal occurrence of *Amblyomma* vectors, reported by a number of different authors (Gebre et al., 2001; Tesema and Gashaw, 2010; Koney et al., 2004; Kivaria et al., 2012; Bekker et al., 2001). The characteristic clinical signs of heartwater, cowdriosis (Radostits et al., 2007) were recorded in this study. The temperature elevation declines as the disease progresses which results in a more unfavourable prognosis.

Bovine ehrlichiosis can induce significant economic losses. In this study, approximately 141,924.20 Birr (7884.67 USD) was lost during the three year period. This is an approximate estimation as the price of animals has since increased. Mukhebi et al. (1999), estimated total annual losses of Z\$ 61.3 million (US\$ 5.6 million) in Zimbabwe, the most significant portion attributed to the costs of acaricides (76%), followed by milk loss (18%) and the cost of antibiotics (5%).

In conclusion, bovine ehrlichiosis is prevalent in the Gondar University dairy farm and can be detected throughout the year, but more often in the rainy season. The typical clinical signs and post-mortem lesions were observed and the economic loss was significant. The disease should be suspected if any unrecognised febrile condition occurs, particularly in young stock. Various tick control strategies need to be implemented. Any evidence of clinical disease needs to be reported without delay, so that intravenous treatment can be commenced immediately, at high doses. Two or three vials of short acting (10%) Oxytetracycline needs to be made available on the farm for a quick response. To determine the prevalence of the disease, other diagnostic techniques, including serology should be implemented, and care should be taken when introducing new stock, especially if it is from susceptible breeds.

REFERENCES

Bekker CJ, Vink D, Pereira CM, Wapenaar W, Langa A, Jongejan F

- (2001). Heartwater (*Cowdria ruminantium* Infection) as a Cause of Post-restocking Mortality of Goats in Mozambique. *Clin. Diagn. Lab. Immunol.* 8(4):843-846.
- Deem SL, Norval RA, Yonow T, Peter TE, Mahan SM, BurrIDGE MJ (1996a). The Epidemiology of *Heartwater*: Establishment and Maintenance of Endemic Stability. *Parasit. Today* 12:402-405.
- Deem SL, Norval RA, Donachie PL, Mahan SM (1996b). Demonstration of vertical transmission of *Cowdria ruminantium*, the causative agent of heartwater, from cows to their calves. *Vet. Parasitol.* 61:119-132.
- Gebre S, Nigist M, Kassa B (2001). Seasonal variation of ticks on calves at Sebeta in western Shewa Zone. *Ethiop. Vet. J.* 7:17-30.
- Hanks J, Lopes Pereira CM (1998). The development of a restocking decision support aid for Mozambique. Technical consultation RLA AGDX/ AGA 11. Department of Agriculture, Reading, United Kingdom.
- ILRAD (1991). Annual Scientific Report, International Laboratory for Research on Animal Diseases. P.O. Box 30709, Nairobi, Kenya.
- Kivaria FM, Kapaga AM, Mbassa GK, Mtui PF, Wani RJ (2012). Epidemiological perspectives of ticks and tick-borne diseases in South Sudan: Cross-sectional survey results. *Onder. J. Vet. Res.* 79(1):1-10.
- Koney EB, Dogbey O, Walker AR, Bell-Sakyi L (2004). *Ehrlichia ruminantium* seroprevalence in domestic ruminants in Ghana. II. Point prevalence survey. *Vet. Microbio.* 103:183-193.
- McMillan DE, Meltzer MI (1996). Vector-Borne Disease Control in sub-Saharan Africa: A Necessary but Partial Vision of Development. *World Dev.* 24(3):569-588.
- Mekonnen S, Hussein I, Bedane B (2001). The distribution of ixodid ticks in central Ethiopia. *Onder. J. Vet. Res.* 68 (4):243-251.
- Meltzer MI, Perry BD, Donachie PL (1996). Mortality percentages related to heartwater and economic impact of heartwater disease on large-scale commercial farms in Zimbabwe. *Prev. Vet. Med.* 26:187-199.
- Miruts G (2010). The Distribution and Identification of Hard Tick Species on Cattle in and Around Gondar. DVM thesis, Faculty of Veterinary Medicine, University of Gondar, Ethiopia.
- MoA (2003). Ministry of Agriculture, Annual report, Addis Ababa, Ethiopia
- Mukhebi AW, Chamboko T, O'Callaghan CJ, Peter TF, Kruska RL, Medley GF, Mahan SM, Perry BD (1999). An assessment of the economic impact of Heartwater (*Cowdria ruminantium* infection) and its control in Zimbabwe. *Prev. Vet. Med.* 39:173-189.
- Obsa T, Zerihun A (1993). Incidence of Bovine Cowdriosis at Asela Livestock Research Farm. Proceedings of the Fourth National Livestock Improvement Conference, Institute of Agricultural Research, Addis Ababa (Ethiopia). Addis Ababa (Ethiopia): IAR, p. 293-297.
- OIE (2011). World organization for animal diseases. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2. 27 Available at: http://www.oie.int/leng/normes/mmanual/A_00046.htm.
- Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007). *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats.* 10th ed. Saunders Elsevier, London. pp. 1462-1464.
- Singh B, Prasad S (2008). Modelling of Economic Losses due to Some Important Diseases in Goats in India. *Agri. Econ. Res. Rev.* 21:297-302.
- Tessema T, Gashaw A (2010). Prevalence of ticks on local and crossbred cattle in and around Asella town, Southeast Ethiopia. *Ethiop. Vet. J.* 14(2):79-89.
- Thrusfield M (2005). *Veterinary Epidemiology.* 3rd ed. Blackwell Science Ltd, London.

Full Length Research Paper

Cloacal faecal carriage and occurrence of antibiotic resistant *Escherichia coli* in chicken grown with and without antibiotic supplemented feed

Nwankwo Chinwe¹, Ayogu Thomas⁴, Iroha Ifeanyichukwu¹, Ejikeugwu Chika^{2*}, Nwakaeze Emmanuel¹, Oji Anthonia¹ and ILang Donatus³

¹Department of Applied Microbiology, Ebonyi State University, P.M.B 053, Abakaliki, Nigeria.

²Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, P.M.B 5025, Awka, Nigeria.

³Department of Microbiology, Federal University Ndufu-Alike, Ikwo, Nigeria.

⁴Department of Food Sciences and Technology, Institute of Management and Technology Enugu state, Nigeria.

Accepted 31 January, 2014

Drug resistant *Escherichia coli* persist in the intestinal flora of poultry birds, and these serve as route via which they can be transmitted directly to humans, thus contributing to the already growing crisis of antibiotic resistance. The purpose of this study was to determine the cloacal faecal carriage and occurrence of antibiotic resistant *E. coli* isolates from chicken fed with and without antibiotic supplemented feeds. Cloacal faecal swabs (n = 200) were aseptically obtained from two poultry farms in Abakaliki metropolis, Ebonyi state of Nigeria, and these were inoculated on MacConkey and cystine-lactose-electrolyte-deficient (CLED) media and incubated at 37°C for 18 to 24 h. Suspected colonies of *E. coli* growing on the agar media were subcultured, purified and further characterized using standard microbiology techniques. Antibigram was investigated using the Kirby-Bauer disk diffusion method as per the clinical laboratory standards institute (CLSI) criteria. A total of 45 *E. coli* was isolated from the 200 cloacal faecal swab samples used for this study. Overall, 28% of *E. coli* were isolated from chicken fed with feed supplemented with antibiotics while only 17% of *E. coli* was isolated from chicken that received feed without antibiotics supplements. All the *E. coli* isolates showed varying rates of resistance and susceptibility to the tested antibiotics. Our results strongly reveal the occurrence of antibiotic resistant *E. coli* from chicken fed with and without antibiotic supplemented feeds. It is very critical that the continuous use of antibiotics in poultry production be strictly monitored, controlled and discouraged in order to contain the emergence and spread of antibiotic resistant bacteria through poultry production.

Key words: Resistance, *Escherichia coli*, poultry, veterinary, Nigeria.

INTRODUCTION

The usage of antimicrobial agents including antibiotics for either clinical or non-clinical reasons is amongst the singular purpose there is for the growing global increase in the emergence and spread of antibiotic resistance genes in pathogenic bacteria. Antibiotics have been continuously

used for different veterinary and agricultural purposes including animal husbandry and poultry production where the feeds of poultry birds are constituted with antibiotics (Witte, 1998; Chah and Nweze, 2001). Apart from fighting infection and controlling the population of bacteria, the

*Corresponding author. E-mail: ejikeugwu_chika@yahoo.com. Tel: +2348097684562.

controlling the population of bacteria, the antibiotics are also used as growth promoters in the birds. This scenario allows for the selection of resistance strains of pathogenic and non-pathogenic bacteria including *Escherichia coli* and other bacteria exposed to the antibiotics in the intestinal flora of the birds, and such practices has the potential to increase the frequency of resistant bacteria in the poultry birds (Pidcock, 1996; Al Ghamdi et al., 1999; Bisht et al., 2009). Though a natural phenomenon of bacterial genetics and evolution, antibiotic resistance builds up following every usage of antibiotics (whether rational or irrational) including the acquisition of resistance genes from other bacteria in the environment.

The discovery of antibiotics by Alexander Fleming in the early 1920s was one of the most remarkable breakthroughs in the field of medicine; owing to the fact that humanity was saved and is still being saved by these agents from the untoward and killing prowess of pathogenic bacteria (Fernandes, 2006; Jayaramah, 2009; Sundsfjord et al., 2004). But this very significant discovery (that is, the discovery of antibiotics referred to as "magic bullets") however, was inundated by the emergence of resistant strains of bacteria that can thrive even in the face of an antimicrobial onslaught. Antimicrobial resistance limits the life span of a drug, thus making it difficult and even more expensive to treat an infection. Antibiotics have been used and are currently used in the compounding of the feeds of birds and other poultry activities in many parts of the world including Nigeria (Chah and Nweze, 2001; Oyinloye and Ezekiel, 2011; Miranda et al., 2008). Such practices portend danger for public health (human and animal health inclusive) because of the likelihood of the development and transmission of resistant strains of bacteria from poultry birds to humans either directly or through consumption of poultry products.

In this study, the cloacal fecal carriage and frequency of antibiotic resistant *E. coli* from chicken fed with and without antibiotic supplemented feeds was investigated to ascertain the fecal carriage of these pathogens in chicken from two poultry farms in Abakaliki metropolis, Ebonyi state of Nigeria.

MATERIALS AND METHODS

Study area and collection of cloacal fecal swab samples

This research was carried out in the Microbiology Department of Ebonyi state university, Abakaliki, Nigeria in line with ethical consideration of the 2004 Declaration of the World Medical Association (WMA) in Helsinki regarding principles guiding experiments that involves human and non-human subjects (WMA, 2004). A total of two hundred (200) birds from two poultry farms were included in this study. One of the poultry farm used antibiotic supplemented feed in the growth of their birds while the other used non-antibiotics supplemented feed. However, the type and name of antibiotic used for compounding the feed was not made known by the manufacturer of the feed. In each, cloacal fecal samples were randomly taken from 20 days old and 40 days old chicks in two

different batches designated flock A (chicken fed with antibiotic supplemented feed) and flock B (chicken fed with feed without antibiotic supplements), and these were transported to the laboratory in normal saline tubes and stored at 4°C until use.

Isolation and identification

Cloacal fecal swabs were inoculated on MacConkey and cystein lactose electrolyte deficient (CLED) agar plates (Oxoid UK) and incubated at 37°C for 18 to 24 h. *E. coli* grows on MacConkey and CLED medium as smooth pink colonies and yellow colonies, respectively. Only these colonies were counted and further analyzed after 18 to 24 h incubation at 37°C. Suspected colonies of *E. coli* was grown on Mueller-Hinton (MH) agar (Oxoid UK) plates after series of subculturing on MacConkey and CLED agar plates. The isolates were cultured on nutrient agar plates (Oxoid UK) and characterized by Gram staining, triple sugar iron agar (TSIA), indole test and citrate test (Cheesbrough, 2000).

Antibiogram

Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method as per the CLSI criteria, formerly National Committee for Clinical Laboratory Standards (NCCLS) (CLSI, 2012) on Mueller-Hinton agar plates using single antibiotic disks of chloramphenicol (10 µg), tetracycline (30 µg), sulphamethoxazole/trimethoprim (15 µg), ciprofloxacin (5µg), gentamicin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), amoxicillin/clavulanic acid (30 µg), and ampicillin (10 µg). These antibiotics were selected and used for this study because they (including some related antibiotics) have been used regularly in poultry production and for other agricultural purposes either on the prescription of a veterinary doctor or on self-medication. The plates were incubated at 37°C for 18 to 24 h, and the inhibition zone diameters (IZDs) measured with a meter rule and recorded as recommended by the CLSI (CLSI, 2012).

RESULTS

Overall, 200 cloacal fecal samples (100 samples from each poultry farm) were examined microbiologically for the cloacal fecal carriage of *E. coli*. A total of 45 *E. coli* isolates was isolated from the 200 cloacal fecal swab samples employed in this study. Cloacal fecal samples from chicken fed with feeds supplemented with antibiotics (flock A) showed a 14% carriage of *E. coli* in their faeces while chicken fed with feeds without any antibiotics supplements (flock B) showed a total of 8.5% *E. coli* carriage in their fecal samples (Table 1). The antibiotics used to supplement the feed that was given to flock A chicken was not documented on the bag of the feed but it was carefully recorded by the manufacturer that the feed was compounded with antibiotics that could serve as growth promoters as well as control bacterial population in the poultry birds. However, the restriction of the use of antibiotics in the production of poultry birds is not yet restricted by the Nigerian government, though the practice has been greatly discouraged by concerned authorities. The results of antimicrobial susceptibility of the *E. coli* isolates to some selected antibiotics are shown in Tables 2 and 3. Of the two batches of chicken populations included

Table 1. Percentage frequency of *Escherichia coli* isolation from the chicken.

Age of chicken (day old)	% Chicken given feed supplemented with antibiotics (Flock A: n=100)	% Chicken given feed without antibiotics supplement (Flock B: n=100)
20	9	13
40	19	4
Total	28	17

Table 2. Results of susceptibility of *Escherichia coli* isolated from chicken grown with antibiotics supplemented feed.

Susceptibility pattern	CHL	TET	SXT	CIP	GEN	CTX	CAZ	CFO	AMC	AMP
Susceptible (n)	6	10	14	13	6	14	4	12	16	6
Resistant (n)	20	14	13	14	18	13	14	16	10	19
Intermediate (n)	2	4	1	1	4	1	10	0	2	3

n = number of isolates, CHL=chloramphenicol, AMP=ampicillin, TET= tetracycline, SXT=sulphamethoxazole/trimethoprim, CIP=ciprofloxacin, GEN=gentamicin, CTX=cefotaxime, CAZ=ceftazidime, CFO=cefoxitin, AMC=amoxicillin/clavulanic acid.

Table 3. Results of susceptibility of *Escherichia coli* isolated from chicken grown with feed without antibiotics supplement.

Susceptibility pattern	CHL	TET	SXT	CIP	GEN	CTX	CAZ	CFO	AMC	AMP
Susceptible (n)	15	16	14	11	13	12	13	15	17	17
Resistant (n)	1	1	1	5	2	3	1	1	0	0
Intermediate (n)	1	0	2	1	2	2	3	1	0	0

n=number of isolates, CHL=chloramphenicol, AMP=ampicillin, TET= tetracycline, SXT=sulphamethoxazole/trimethoprim, CIP=ciprofloxacin, GEN=gentamicin, CTX=cefotaxime, CAZ=ceftazidime, CFO=cefoxitin, AMC=amoxicillin/clavulanic acid

in this study (that is, flock A and flock B), the highest degree of resistance of the *E. coli* isolates to the tested antibiotics was detected in chicken fed with feeds supplemented with antibiotics (flock A) (Table 2). Similar tendency of resistance was also detected in chicken fed with feeds without any antibiotic supplements (flock B); however, the trend and frequency of resistance in *E. coli* isolates from flock B chicken is lower than the number and degree of resistance observed in *E. coli* isolates from flock A chicken (Table 3).

DISCUSSION

The resistance of pathogenic bacteria to antibiotics is not a new phenomenon in both the practice of human and veterinary medicine but it is a problem that is becoming more dangerous, and must be contained in order to protect and extend the efficacy and shelf life of available antibiotics. This is very important due to the slow pace in research and development of novel antibiotics and other antimicrobials that can effectively assuage the resistance problem that pathogenic bacteria express *in vivo* against antibiotics. Antibiotics apart from being used for human medicine are also used for other veterinary purposes both for prescription reasons to control bacteria invasion and as growth promoters to increase poultry bird's production.

In this study, the faecal carriage and occurrence of antibiotic resistant *E. coli* was investigated amongst chicken from two poultry farms in Abakaliki metropolis, Ebonyi state of Nigeria. From our study, we discovered that there was a higher percentage of the isolation of *E. coli* (28 %) from flock A chicken than from flock B chicken (17%). A possible reason for the low occurrence of *E. coli* in flock B chickens compared to flock A chickens could be attributed to the retention of resistant bacterial strains in the alimentary canal of the poultry birds and the non-exposure of the birds to initial antibiotic challenge which is one of the prerequisite that allows pathogenic bacteria to develop resistance towards a particular drug via selective pressure.

E. coli strains are routinely found in the gut as part of the indigenous microbiota. However, some strains of *E. coli* have been implicated in a number of resistant infections in humans, and this includes *E. coli* strains that harbour multidrug resistance genes such as extended spectrum beta lactamase (ESBL) enzymes amongst others (Rupp and Paul, 2003). The frequency of faecal carriage of *E. coli* in chicken given feed supplemented with antibiotics in this study is in line with available data that reveal the impact and effect of antibiotics when they are used for non-human purposes such as in the production of livestock. A recent work carried out in Owerri, Nigeria also reported over 40% increase in the isolation of *E. coli* from poultry birds in Owerri metropolis,

Imo state of Nigeria (Duru et al., 2013).

In Saudi Arabia, antibiotic resistant *E. coli* has been isolated from chicken including patients and poultry workers (Al Ghamdi et al., 1999). Antimicrobial resistance is a serious global health problem that knows no border, and that strikes at the core of infectious disease control. It has the potential to halt, and possibly even to roll back some of the many progresses achieved in the medical sciences as is related to antimicrobial chemotherapy. Overcrowding, poor poultry sanitation, and over usage of antibiotics in the production of poultry birds are some of the factors contributing to the upward trend of antimicrobial resistance development and spread in bacteria emanating from poultry farms. Antibiotics are infrequently used as a prophylactic measure as well as a growth promoting agent in the rearing of poultry birds. This gives room for high antibiotic selection and resistance development amongst bacterial population.

In this study, the antimicrobial susceptibility of the *E. coli* isolates from cloacal fecal sample swabs of chicken fed feeds with and without antibiotic supplements was investigated against a battery of 10 selected antibiotics. Higher degree of antibiotic resistance was detected in *E. coli* isolates from cloacal fecal swab samples of chicken fed with feed supplemented with antibiotics (flock A). However, a lesser degree and number of *E. coli* isolates from cloacal fecal swab samples of chicken fed with feeds without antibiotic supplements (flock B) was resistant to the tested antibiotics. Generally, a higher occurrence of resistance was found in cloacal fecal samples of flock A chicken compared with those from flock B chicken. Resistance of *E. coli* isolates from poultry origin to some conventional antibiotics has been reported both within and outside Nigeria (Zhang et al., 2010; Duru et al., 2013; Gray et al., 2004). Antibiotic resistant *E. coli* may persist in the intestinal tract of these poultry birds for a long period of time with or without the use of antibiotics, and these can serve as route via which they can be transmitted to human population directly or through consumption. The continuous usage of antibiotics outside the health system, especially in veterinary and livestock purposes still continues in Nigeria and other parts of the emerging economies. For us not to go back to the pre-antibiotic era when they were barely no conventional antibiotics as we now have to treat many bacterial related diseases, it is very important that urgent and consolidated efforts are put in place and sustained in order to abate the problem of antibiotic resistance.

Conclusion

Conclusively, our results confirm the fecal carriage of antibiotic resistant *E. coli* in poultry birds reared in Abakaliki metropolis, Ebonyi state of Nigeria, making it the first presumptive study to be conducted on the matter in this part of Nigeria. Co-operation between human, animal

health and scientists in the agriculture profession is very important in containing antibiotic resistance in poultry farms since the use of antibiotics in food animal production also contributes immensely to the increased drug resistance that we now face in the world today.

REFERENCES

- Al Ghamdi MS, El Morsy F, Al Mustafa ZH, Al Ramadhan M, Hanif M (1999). Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. *Trop. Med. Inter. Health* 4:278-283.
- Bisht R, Katiyar A, Singh R, Mittal P (2009). Antibiotic Resistance – A Global Issue of Concern. *Asian J. Pharm. Clin. Res.* 2 (2):34-39.
- Chah KF, Nweze NE (2001). Antibiotic use in poultry production in Nsukka, Southeast Nigeria. *Proc. Nig. Soc. Anim. Prod.* 26:69-72.
- Cheesbrough M (2002). *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge University Press. pp. 54-59.
- Clinical and Laboratory Standards Institute (Formerly National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Susceptibility Testing; 15th Informational Supplement 2012; (M100-S15).
- Duru C, Nwanegbo E, Adikwu M, Ejiogwu C, Esimone C (2013). Extended-Spectrum Beta-Lactamase-Producing *Escherichia Coli* Strains of Poultry Origin in Owerri, Nigeria. *World J. Med. Sci.* 8 (4):349-354.
- Fernandes P (2006). Antibacterial discovery – the failure of success? *Nature Biotechnol.* 24(12):1.
- Gray JT, Hungerford LL, Fedorka-Cray PJ (2004). Extended spectrum-cephalosporin resistance in *Salmonella* enteric isolates of animal origin. *Antimicrob. Agents Chemother.* 48:3179–81.
- Jayaramah R (2009). Antibiotic Resistance: an overview of mechanisms and a paradigm shift. *Curr. Sci.*, 96(11):1475-1484.
- Lapara TM, Firl SJ, Onan LJ, Ghosh S, Yan T, Sadowsky MJ (2006). Municipal Waste – Water Treatment: A Novel Opportunity to show the Proliferation of Antibiotic – Resistant Bacteria? Fall 18-23.
- Miranda JM, Franco CM, Vazquez BI, Fente CA, Barros-Velazquez J, Cepeda A (2008). Evolution of resistance of poultry intestinal *Escherichia coli* during three commonly used antimicrobial therapeutic treatments in poultry. *Poult. Sci.* 87(8):164-1648.
- Oyinloye JMA, Ezekiel CN (2011). Extended-Spectrum Beta-Lactamase (ESBL) – Producing Multidrug Resistant *Enterobacteriaceae* from Commercial Poultry Feeds in Nigeria. *Annals Biol. Res.* 2(2):250-254.
- Piddock LJV (1996). Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? *J. Antimicrob. Chemother.* 38:1–3.
- Rupp ME, Paul D (2003). Extended Spectrum β -Lactamase (ESBL) Producing *Enterobacteriaceae*. *Drugs*, 63(4)353-356.
- Sundsford A, Simonsen GS, Haldorsen BC, Haaheim H, Hjelmvoll S, Witte W (1998). Medical consequences of antibiotic use in agriculture. *Sci.* 279:996-997.
- World Medical Association (WMA) Declaration of Helsinki. Ethical Principles for Medical Research involving Human Subjects. Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004. 9.10.2004.
- Zhang CH, Liu YL, Wang JH (2010). Detection of ESBLs and Antimicrobial susceptibility of *Escherichia coli* isolated in Henan, China. *J. Anim. Vet. Adv.* 9(15):2030-2034.

Full Length Research Paper

Bacteriological evaluation of the drinking water quality in dairy farms in Khartoum state, Sudan

Amna Mustafa Musa¹ and Atif Elamin Abdelgadir^{2*}¹Ministry of Animal Resources and Fisheries, Khartoum, Sudan.²Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

Accepted 12 February, 2014

This study was conducted in Khartoum State in order to evaluate the quality of the drinking water in dairy farms based on bacteriological examinations and viable counts. A total of 39 water samples were obtained from dairy farms (13 in Khartoum, 13 in Omdurman and 13 in Khartoum North). All samples were cultured on Blood Agar and MacConkey for bacterial isolation and on nutrient agar for viable counts. The main result revealed that 39 bacterial isolates were detected in drinking water of the dairy farms in Khartoum state. *Micrococcus* spp., *Pseudomonas* spp., and *Bacillus* spp. were dominant in Khartoum, giving a percentage of 7.69 (n=3) for each. *Staphylococcus* spp. and *Corynebacterium* spp. were also observed in the samples of dairy farms in Khartoum (5.12%) (n=2) for each. The bacteria isolated from dairy farms in Omdurman were *Micrococcus* spp. and *Aeromonas* spp. (5.12%) (n=2) for each. In the same site, *Staphylococcus* spp., *Actenobacillus* spp., *Moraxella* spp. and *Flavibacterium* spp. were also detected with percentage of 2.56% and frequency of one for each. The most frequent isolate in Khartoum North was *Micrococcus* spp. (17.95%) (n=7) followed by *Aeromonas* spp. (10.26%) (n=4) and *Staphylococcs* spp. (5.12%) (n=2). Regarding bacterial counts, the results have shown high level of contamination of drinking water for all dairy farms in Khartoum State. The results were inter-operated, depending on international critical level (cut-off point) (100 CFU ml⁻¹). For instance, high mean of bacterial counts 6.44×10^8 was observed in dairy farm in Omdurman, followed by Khartoum and Khartoum North, with mean bacterial counts of 4.93×10^8 and 3.81×10^8 , respectively. 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 of dairy farms in different sites of Khartoum state.

Key words: Bacterial counts, bacterial isolation, drinking water, Khartoum State, Sudan.

INTRODUCTION

Water is the most important nutrient for dairy cattle but its importance has been commonly forgotten in dairy systems. It is required for all of life's processes; transport of nutrients and other compounds to and from cells, digestion and metabolism of nutrients, elimination of waste materials (urine, faeces, respiration, and excesses heat from the body), maintenance of a proper fluid and

ion balance in the body and provision of fluid environment for the developing fetus.

An adequate supply of clean and fresh drinking water is widely considered essential for optimal cow health and maximum milk production. Microorganisms enter into drinking water via humans and animals' intestinal secretions in areas where sanitation conditions are poor or absent.

*Corresponding author. E-mail: atifvet@yahoo.com.

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) (World Health Organization (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 (Gleeson and Gray, 1997; 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).

Long storage of good-quality drinking water is a main factor of faecal coliforms contamination through faecal contaminated hands or utensils. In addition, coverless public reservoir contributes to pathogenic accession especially from birds feces. Contamination by microorganisms can occur through improperly installed or/and through undetected leaks in the water pipe system (Gleeson and Gray, 1997).

It has been reported that contamination with faecal coliforms may be caused by infiltration of pollutants in the recharge area of the springs (Daghray, 2009). The presence of microorganisms does not necessarily indicate that drinking water poses a health risk. The important consideration is the kind of microorganisms that are present.

Objectives

1. To evaluate the bacteriological quality of the drinking water in dairy farms in Khartoum state using colony count.
2. To isolate the most common bacteria in the drinking water in dairy farms in Khartoum state.

MATERIALS AND METHODS

The study area

The study was conducted in Khartoum State which is situated in Northern Sudan between latitude 15° 38' N and longitude 32° 26' E. The total area extends over approximately 21,000 square kilometer. The climate of Khartoum is an arid type which is characterized by a wide range in daily and seasonal temperatures. During cool season, between December and February, the weather is cool and dry, with minimum daily temperature of 24°C. The season is characterized by low humidity. A hot dry weather prevails between

March and October, where a temperature of 45°C may occur during the day. The maximum rainfall is during the period from mid July to September; in this season there is an increase in relative humidity, with a maximum of 68% in August. It is more convenient to divide the year into a cool dry season, hot dry season and hot wet season.

Sampling methods

Non-probability sampling method was employed as described by (Thrusfield, 2007). This means not all farms in Khartoum state had the same chance to be selected but only 39 farms were selected based on willingness and support of the owners.

Samples collection

A total of 39 water samples (13 from Khartoum, 13 from Omdurman, 13 from Khartoum North) were collected and all samples taken from troughs. All samples were taken by sterile 10 ml syringe and all precautions were taken in order to prevent accidental contamination of the water during its transportation in laboratory and put in sterile closed glass bottles (previously sterilized in autoclave at 120°C under 15 lb atmospheric pressure for 15 min).

Solid culture media

Nutrient agar

This media contained peptone (5 g) lab-lemco powder (1 g), yeast extract (2 g), sodium chloride (5 g) and agar No. 3 (15 g). The medium was prepared by dissolving 28 g of the dehydrated medium in one liter distilled water, and the pH adjusted to 7.4 then sterilized by autoclaving for 15 min at 121°C. The medium was allowed to cool to 55°C and poured aseptically in 15 to 20 ml amounts into sterile petri dishes (Quinn et al., 2000).

Blood agar

This is one of the enriched media that was composed of blood agar base and defibrinated sheep blood. The blood agar base contained protease peptone (15 g), liver digest (2.5 g), yeast extract (5 g), sodium chloride (5 g) and agar (12 g). It was prepared by dissolving 40 g of the basal medium in one liter of distilled water. The mixture was then boiled until the powder dissolved completely. The solution was autoclaved at 121°C for 15 min, and then cooled to 45 to 50°C. 7% of sterile blood was added with gentle rotation and then the medium was poured into petri dishes (15 to 20 ml) and left to solidify (Quinn et al., 2000).

MacConkey agar

This media contained peptone (20 g), lactose (10 g), bile Salts (1.5 g), sodium chloride (5 g), neutral red (0.03 g), crystal violet (0.001 g) and agar No. 3 (15 g). The medium was prepared by dissolving 52 g in one liter of distilled water by heating. The pH was adjusted to 7.4 and then autoclaved at 121°C for 15 min. Then it was allowed to cool to 55°C and poured gently at 15 ml amount into sterile Petri dishes (Quinn et al., 2000).

Semi- solid media

Hugh and liefsons (O/F) medium

This medium was used to test the ability of the organism to attack dextrose under aerobic and anaerobic conditions. This medium was prepared by dissolving all ingredients in one liter of distilled water by heating in water bath set at 55°C, except bromothyl blue solution which was added after adjustment of the pH to 7.1. Then sterile solution of the appropriate carbon hydrate was added aseptically to give a final concentration of 1% and the medium was sterilized at 115°C for 20 min. A volume of 10 ml of sterile glucose solution was aseptically added to 90 ml of medium, then the medium was mixed and distributed aseptically in 10 ml amounts in sterile test tube. The prepared medium was kept at 4°C until use (Quinn et al., 2000).

Peptone water sugars

The method of preparation depends on the indicator. A total of 900 ml peptone water was added to 10 ml indicator solution (bromocresol purple) and sterilized at 115°C for 20 min. A total of 5 to 10 g of the appropriate sugar was dissolved in 90 ml water and sterilized by filtration, then added to sterile peptone water indicator and distributed into sterile tubes with inverted inner (Durham) tubes and finally steamed for 30 min (Quinn et al., 2000).

Primary culture

Primary culture for all water samples was done onto blood agar and MacConky agar media. Each water sample was centrifuged at 8000 rpm for 5 min and the sediment was cultured, then all Petri dishes were incubated at 37°C for 24 h.

Staining

Smears were prepared by emulsifying part of typical and well isolated colony in a drop of sterile normal saline and spread in a clean slide. The smears were then allowed to dry by air then fixed by gentle flaming. All smears were examined by gram stain.

Microscopic examination

A smear was made from culture and purified colonies, fixed by heating and stained by Gram's method. Then the stained smears were examined microscopically under oil immersion lens. The smears were examined for cell morphology and staining reaction.

Biochemical tests

Oxidase test

The oxidase test was performed by removing a portion of freshly grown colonies with a sterile glass rod and rubbing it on a strip of filter paper which have been impregnated with 1% solution of oxidase reagent . The immediate development of a dark purple colour with 10 s indicated appositve reaction (Quinn et al., 2000).

Catalase test

This test detects the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen. A loopful of grown bacteria was taken from the top of colonies to avoid the nutrient agar medium, and were put in a clean slide and dropped with 3% hydrogen peroxide. Presence of oxygen gas within a few seconds indicated appositve reaction (Quinn et al., 2000).

O/F test

Duplicate tubes were cultured by stabbing with straight wire to one of the tubes. A layer of melted soft paraffin (petrolatum) was added in a depth of about 1 cm, then incubated at 37°C for 24 h and examined (Quinn et al., 2000).

Identification of isolates

Purified isolates from the primary or from sub cultured plates were identified to the genus level according to Barrow and feltham (1993). The identification was based mainly on colony characteristics, staining, motility and biochemical reactions

Bacterial viable count

The bacterial count was done according to Milles and Misra (1938).

Preparation of the dilution

The serial dilution was prepared according to Harrigan and Maccance (1976). A micropipette with sterile tip was held vertically and introduced not more than 3 cm below the surface of the water sample and then 1 ml was taken to the first tube of the dilution (which contain 9 ml sterile normal saline) series without touching the diluting fluid, the tip was discarded and the tube was labeled as the first dilution tube 1/10. A fresh sterile tip was used to mix the content of the first dilution and 1 ml of the first tube was transferred to the second tube of dilution series (which contain 9 ml normal saline) also without touching the diluting fluid. Then the tip was discarded and the tube was labeled as the second dilution tube 1/100. Further dilutions of 1/1000, 1/10000 or 1/100000 were prepared similarly.

Plate count agar

Method of preparation

Twenty three gram medium of plate count agar were dissolved in 1000 ml cold d/w and heated to boiling to dissolve the medium completely. The pH was adjusted to 7.0 ± 0.2 and sterilized in an autoclave at 15 lbs (121 c) pressure for 15 min, which was very hygroscopic. The medium was then stored in a refrigerator.

Colony count

Colonies were counted according to surface colony count method (Milles and Misra, 1938). An average colony count from at least 5 drops of each dilution was obtained, the conversion factor was 50

Table 1. Bacteria isolated from drinking water of dairy farms in Khartoum state.

Isolate	Khartoum Omdurman Khartoum North			Total frequency (%)
	Frequency (%)			
<i>Micrococcus spp.</i>	3 (7.69)	2 (5.12)	7 (17.95)	12 (30.76)
<i>Aeromonas spp.</i>	0	2 (5.12)	4 (10.56)	6 (15.38)
<i>Staphylococcus spp.</i>	2 (5.12)	1 (2.56)	2 (5.12)	5 (12.82)
<i>Pseudomonas spp.</i>	3 (7.69)	0	1 (2.56)	4 (10.26)
<i>Bacillus spp.</i>	3 (7.69)	0	0	3 (7.69)
<i>Corynebacterium spp.</i>	2 (5.12)	0	0	2 (5.12)
<i>Cardiobacterium spp.</i>	0	0	3 (7.69)	3 (7.69)
<i>Actenobacillus spp.</i>	0	1 (2.56)	0	1 (2.56)
<i>Moraxella spp.</i>	0	1 (2.56)	0	1 (2.56)
<i>Flavibacterium spp.</i>	0	1 (2.56)	0	1 (2.56)
<i>Enterobacterium spp.</i>	0	0	1 (2.56)	1 (2.56)
Total	13 (33.33)	8 (20.51)	18 (46.15)	39 (100)

Table 2. Gram +ve and Gram -ve isolated from drinking water of the dairy farms in Khartoum State.

Site	No. of samples examined	Gram +ve Gram -ve	
		Frequency (%)	
Khartoum	13	10 (76.92)	3 (23.07)
Omdurman	13	8 (61.53)	5 (38.46)
Khartoum North	13	3 (23.07)	10 (76.92)
Total	39	21 (53.48)	18 (46.15)

to obtain figure for the bacteria /ml in the original sample. The formula used for counting was:

The total number of bacteria = The average of colonies count × dilution factor × 50

Statistical analysis

Statistical package for social sciences (SPSS) version 17.5 was used for data analysis. Descriptive statistic such as frequency and percentage was used for bacterial isolates. While mean, standard error and 95% confidence interval were recorded for bacterial count of drinking water of dairy farms in Khartoum state. Analytical statistic using one way ANOVA was used in order to know the significant difference regarding bacterial count of drinking water of the dairy farms in different sites of Khartoum state. Determination of contamination of the drinking water was done according to the critical level (100 CFU/ml) (Enviro labs Ltd, 2011).

RESULTS

This study was conducted in Khartoum State in order to evaluate the quality of the drinking water in dairy farms based on bacteriological examinations and viable counts.

A total of 39 water samples were obtained from dairy farms (13 in Khartoum, 13 in Omdurman and 13 in Khartoum North). The main result revealed that 39 bacterial isolates were detected in drinking water of the dairy farms in Khartoum state. *Micrococcus spp.*, *Pseudomonas spp.*, and *Bacillus spp.* were dominant in Khartoum, giving a percentage of 7.69% (n=3) for each. *Staphylococcus spp.* and *Corynebacterium spp.* were also observed in the samples of dairy farms in Khartoum (5.12%) (n=2) for each. The bacteria isolated from dairy farms in Omdurman were *Micrococcus spp.* and *Aeromonas spp.* (5.12%) (n=2) for each. In the same site, *Staphylococcus spp.*, *Actenobacillus spp.*, *Moraxella spp.* and *Flavibacterium spp.* were also detected with percentage of 2.56% and frequency of one for each. The most frequent isolate in Khartoum North was *Micrococcus spp.* (17.95%) (n=7) followed by *Aeromonas spp.* (10.26%) (n=4) and *Staphylococcus spp.* (5.12%) (n=2). The rest of the results are presented in Tables 1 and 2.

Regarding bacterial count, the results have shown high level of contamination of drinking water for all dairy farms in Khartoum State. The results were inter-operated based on international critical level (cut-off point) (100 CFU/ml).

Table 3. Descriptive statistic of bacterial count for drinking water of dairy farms in Khartoum state.

Site	No. of samples examined	Mean CFU/ml	Standard error	95% Confidence interval
Khartoum	13	4.93×10^8	1.67×10^8	1.28×10^8 - 8.58×10^8
Omdurman	13	6.44×10^8	4.74×10^8	-3.88×10^8 - 1.68×10^9
Khartoum North	13	3.81×10^8	1.03×10^8	1.56×10^8 - 6.06×10^8
Total	39	5.06×10^8	1.67×10^8	1.67×10^8 - 8.45×10^8

Critical level (cutoff point) = 100 CFU/ml.

Table 4. Analytical statistic of bacterial counts of drinking water of dairy farms in Khartoum State.

Unit	Sum of square	df	Mean Square	F-value	p-value
Between sites	4.51×10^{17}	2	2.26×10^{17}		
Within sites	4.10×10^{19}	36	1.40×10^{18}	0.198	0.821*
Total	4.14×10^{19}	38	-		

*Statistically not significant ($P > 0.05$).

For instance, high mean of bacterial counts (6.44×10^8) was observed in dairy farm in Omdurman followed by Khartoum and Khartoum North with mean bacterial counts of 4.93×10^8 and 3.81×10^8 , respectively (Table 3). Application of analytical statistic using one way ANOVA revealed that there was no statistical significant (F-value = 0.198, p-value > 0.05) for bacterial counts of the drinking water of dairy farms in different sites of Khartoum state. The results are show in Table 4.

DISCUSSION

An adequate supply of good quality water for dairy cattle is extremely important for optimal production. The presence of high viable bacteria in drinking troughs was an indication of the contamination at these sites; this agreed with Jeffrey et al. (2001) who reported that water offered to dairy cattle is often of poor microbiological quality. The extent of bacterial contamination observed in the drinking water troughs may demonstrate animal's daily exposure to bacterial infection from water source. Water sample from direct main source of water supply are completely free from coliform bacteria (El Tom, 1997). So water can be contaminated after being poured in troughs for the following reasons:

1. Bad hygiene measures in the farms.
2. Retention of water for long time in troughs.
3. Water troughs are not cleaned regularly.
4. Disinfectants are not used for washing troughs.

The viable count technique used in this study was Miles-

Misra (1938). This method has advantages of being economical and sensitive, also it requires less laboratory equipments and glass ware compared with other techniques (Quinn, 2000). The total viable count for bacteria showed that water samples were found most loaded; this may be logical because troughs are exposed to contamination from many sources like cattle while drinking, animal faeces, air, dust and feed stuffs, similarly from bacterial contamination and bad storage of water. In contrast, the main sources of water protected from direct contact, surface water usually treated with disinfectants and ground water is expected to contain minimum bacteria unless mixed with human sewage (Alcano, 1997).

As seen from the result, a high percentage of *Micrococcus spp.* and *Aeromonas spp.* was found in Khartoum North which is similar to Shirin (2010). The result could be explained by the fact that storage places in these farms were exposed to contaminated air and dust and may rarely be cleaned. The highest isolated bacteria in all water samples were *Micrococcus spp.* (30.76%), *Aeromonas spp.* (15.38%), *Staphylococcus spp.* (12.82%), *Pseudomonas spp.* (10.26%) and *Bacillus spp.* (7.69%). These genera are pathogenic and might be of importance due to their contribution to water borne infections.

Microbiological quality of drinking water in dairy farms is of paramount concern because of the possible acute risk to health caused by bacteria in drinking water. Therefore, regular monitoring and assessment of drinking water is primarily a health-based activity which helps to protect public health through ensuring provision of quality water. Bad habits, water mishandling and lack of basic

knowledge affects clearly the quality of water in dairy farms; thus physical appearance of water had been clearly affected, and this could strongly result in bad hygiene situation, causing a high level in the incidence of water-borne disease (Al Beeli, 2006).

Conclusion

Water is the most important essential nutrient supplied to dairy cattle, however, at times and in some dairy farms, quality and provision of water may not be optimal to maximize animal performance and health. Hence, water analysis programs are needed at all drinking water sites not only in Khartoum state but all over Sudan to assess the exact magnitude of ground water pollution with faecal matters. Also, the people and animal owners should be alerted of the potential health hazards.

RECOMMENDATIONS

1. 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.
2. 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.

REFERENCES

- Al Beeli AI (2006). Survey of bacterial load of drinking water in eastern and southern Sudan. M.Sc. thesis, University of Khartoum, Sudan.
- Alcano EL (1997). Fund.Microbiol. 4th(ed.). The Benjamic Cuming Publishing Company Inc, Red Wood city.
- Barrow WG, Fethma RK. (1993). Cowan and Steels Manual for the identification of medical bacteria. 3rd (Ed.). Cambridge University press, UK.
- Daghran GA (2009). Water quality study of Wadi ElQilt-West bank-Palestine. Asian J. Earth Sci. 2:28-38.
- El Tom AA (1997). Microbiology of Port- Sudan water supply, M. Sc. Thesis, University of Khartoum, Sudan.
- Enviro Labs Ltd. (2011). ELL holds Hoklas accreditation for testing Hetrotrophic plate count or aerobic plate count in water, waste water, marine water and food samples. China.
- Harrigan WF, Margaret EM (1976). Laboratory method in food and dairy microbiology; Academic press; London, UK.
- Gleeson C, Gray NF (1997). The Coliform Index and Waterborne Disease: Problems of Microbial Drinking Water Assessment. Spon press, London, ISBN: 9780203476888.
- Jeffrey T, Thomas E, Hancock DD (2001). Cattle water troughs as reservoirs of *Eschereichia coli* 0157.Appl. Envir. Microbiol, 67:3053-3057.
- Lewis K (2001). Riddle of biofilm resistance. Antimicro. agents Chermother. 45:999-1007.
- Miles AA, Misra SS (1938). In laboratory methods in food and dairy microbiology 1976. Academic press.31.
- Obiri K, Jones K (2001). The effect of a new sewage treatment plant on faecal indicator numbers, Campylobacters and bathing water compliance in morecamber bay. J. Appl. Microbial. 86:603-614.
- Quinn P J, Carter M E, Markey B, Kand Carter G B (2000). Clinical Veterinary Microbiology. Faculty of Veterinary Medicine, Collage of Dubline; MOSBYintrenational Limeted. Tiland.
- Shirin BA (2010). Types and load of bacteria contaminating drinking water in dairy farms in Khartom North. M. Sc. Thesis., University of Khartoum, Sudan.
- Thrusfield M (2007). Veterinary Epidemiology, 3rd (ed.). Blackwell Sience Ltd. UK.
- WHO (2008). Guidelines for Drinking water- Quality. Incorporating the first and second Addenda volume1: Recommendations. World Health Organization, Geneva, Switzerland.

UPCOMING CONFERENCES

11th International Congress on the Biology of Fish, Edinburgh,
Scotland, 3 Aug 2014

ICBF2014

11th INTERNATIONAL CONGRESS ON THE

BIOLOGY OF FISH

3-7 AUGUST, 2014, HERIOT-WATT UNIVERSITY, EDINBURGH

World Congress on Controversies in Veterinary Medicine (CoVet),
Prague, Czech Republic



CoVet

World Congress on Controversies,
Debates & Consensus in Veterinary Medicine

Prague, Czech Republic
October 23-26, 2014



Conferences and Advert

May 2014

[2nd International Conference on Animal Health Surveillance, La Havana, Cuba](#)

October 2014

World Congress on Controversies in Veterinary Medicine (CoVet), Prague, Czech Republic



Journal of Veterinary Medicine and Animal Health

Related Journals Published by Academic Journals

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

academicJournals