Xanthium Strumarium Leaf Haemoparasites and Haematological Study on Prevalence and Monetary Quantitative Real Time RT-PCR

Highlights

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 2 VERSION 1.0

© 2001-2016 by Global Journal of Medical Research, USA
John A. Hamilton,"Drew" Jr.,
Ph.D., Professor, Management
Computer Science and Software Engineering
Director, Information Assurance Laboratory
Auburn University

Dr. Wenying Feng
Professor, Department of Computing & Information Systems
Department of Mathematics
Trent University, Peterborough,
ON Canada K9J 7B8

Dr. Thomas Wischgoll
Computer Science and Engineering,
Wright State University, Dayton, Ohio
B.S., M.S., Ph.D.
(University of Kaiserslautern)

Dr. Osman Balci, Professor
Department of Computer Science
Virginia Tech, Virginia University
Ph.D. and M.S. Syracuse University, Syracuse, New York
M.S. and B.S. Bogazici University, Istanbul, Turkey

Dr. Abdurrahman Arslanyilmaz
Computer Science & Information Systems Department
Youngstown State University
Ph.D., Texas A&M University
University of Missouri, Columbia
Gazi University, Turkey

Yogita Bajpai
M.Sc. (Computer Science), FICCT
U.S.A. Email:
yogita@computerresearch.org

Dr. T. David A. Forbes
Associate Professor and Range Nutritionist
Ph.D. Edinburgh University - Animal Nutrition
M.S. Aberdeen University - Animal Nutrition
B.A. University of Dublin - Zoology

Dr. Xiaohong He
Professor of International Business
University of Quinnipiac
BS, Jilin Institute of Technology; MA, MS, PhD., (University of Texas-Dallas)

Burcin Becerik-Gerber
University of Southern California
Ph.D. in Civil Engineering
DDes from Harvard University
M.S. from University of California, Berkeley & Istanbul University
Dr. Bart Lambrecht  
Director of Research in Accounting and  
Finance Professor of Finance  
Lancaster University Management School  
BA (Antwerp); MPhil, MA, PhD  
(Cambridge)

Dr. Söhnke M. Bartram  
Department of Accounting and  
Finance Lancaster University Management  
School Ph.D. (WHU Koblenz)  
MBA/BBA (University of Saarbrücken)

Dr. Carlos García Pont  
Associate Professor of Marketing  
IESE Business School, University of  
Navarra  
Doctor of Philosophy (Management),  
Massachusetts Institute of Technology  
(MIT)  
Master in Business Administration, IESE,  
University of Navarra  
Degree in Industrial Engineering,  
Universitat Politècnica de Catalunya

Dr. Miguel Angel Ariño  
Professor of Decision Sciences  
IESE Business School  
Barcelona, Spain (Universidad de Navarra)  
CEIBS (China Europe International Business  
School).  
Beijing, Shanghai and Shenzhen  
Ph.D. in Mathematics  
University of Barcelona  
BA in Mathematics (Licenciatura)  
University of Barcelona

Dr. Fotini Labropulu  
Mathematics - Luther College  
University of Regina Ph.D., M.Sc. in  
Mathematics  
B.A. (Honors) in Mathematics  
University of Windso

Philip G. Moscoso  
Technology and Operations Management  
IESE Business School, University of Navarra  
Ph.D in Industrial Engineering and  
Management, ETH Zurich  
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Lynn Lim  
Reader in Business and Marketing  
Roehampton University, London  
BCom, PGDip, MBA (Distinction), PhD,  
FHEA

Dr. Sanjay Dixit, M.D.  
Director, EP Laboratories, Philadelphia VA  
Medical Center  
Cardiovascular Medicine - Cardiac  
Arrhythmia  
Univ of Penn School of Medicine

Dr. Mihaly Mezei  
ASSOCIATE PROFESSOR  
Department of Structural and Chemical  
Biology, Mount Sinai School of Medical  
Center  
Ph.D., Evtos Lornd University  
Postdoctoral Training,  
New York University

Dr. Han-Xiang Deng  
MD., Ph.D  
Associate Professor and Research  
Department Division of Neuromuscular  
Medicine  
Davee Department of Neurology and Clinical  
Neuroscience Northwestern University  
Feinberg School of Medicine
Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo, School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric Sciences, Purdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, Taiwan
University Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsinchu, Taiwan.
Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator, Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh
**President Editor (HON.)**

Dr. George Perry, *(Neuroscientist)*  
Dean and Professor, College of Sciences  
Denham Harman Research Award (American Aging Association)  
ISI Highly Cited Researcher, Iberoamerican Molecular Biology Organization  
AAAS Fellow, Correspondent Member of Spanish Royal Academy of Sciences  
University of Texas at San Antonio  
Postdoctoral Fellow (Department of Cell Biology)  
Baylor College of Medicine  
Houston, Texas, United States

**Chief Author (HON.)**

Dr. R.K. Dixit  
M.Sc., Ph.D., FICCT  
Chief Author, India  
Email: authorind@computerresearch.org

**Dean & Editor-in-Chief (HON.)**

Vivek Dubey *(HON.)*  
MS (Industrial Engineering),  
MS (Mechanical Engineering)  
University of Wisconsin, FICCT  
Editor-in-Chief, USA  
editorusa@computerresearch.org  

Sangita Dixit  
M.Sc., FICCT  
Dean & Chancellor (Asia Pacific)  
deanind@computerresearch.org  

Suyash Dixit  
(B.E., Computer Science Engineering), FICCTT  
President, Web Administration and Development, CEO at IOSRD  
COO at GAOR & OSS

Er. Suyog Dixit  
(M. Tech), BE (HONS. in CSE), FICCT  
SAP Certified Consultant  
CEO at IOSRD, GAOR & OSS  
Technical Dean, Global Journals Inc. (US)  
Website: www.suyogdixit.com  
Email: suyog@suyogdixit.com

Pritesh Rajvaidya  
(MS) Computer Science Department  
California State University  
BE (Computer Science), FICCT  
Technical Dean, USA  
Email: pritesh@computerresearch.org

Luis Galárraga  
JIResearch Project Leader  
Saarbrücken, Germany
Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Comparative Detection of Foot-and-Mouth Disease Virus by the two Commonly used Assays of NSP ELISA and RT-PCR in Uganda with Quantitative Real Time RT-PCR on Field Samples. 1-10
2. Haemoparasites and Haematological Parameters of the One Humped Camel (Camelus Dromedarius) Slaughtered in Maiduguri Abattoir, Nigeria. 11-16

v. Fellows
vi. Auxiliary Memberships
vii. Process of Submission of Research Paper
viii. Preferred Author Guidelines
ix. Index
Comparative Detection of Foot-and-Mouth Disease Virus by the two Commonly used Assays of NSP ELISA and RT-PCR in Uganda with Quantitative Real Time RT-PCR on Field Samples

By Hussein Kafeero Mukasa, Frank Norbert Mwiine, David Kalenzi Atuhaire, Sylvester Ochwo & Ann Nanteza

Abstract- Foot-and-mouth disease (FMD) is a viral disease of Ungulates; both Artiodactyla and Perissodactyla. The mortality rates are low in adult animals but it affects milk yield and international trade. In endemic countries, diagnosis can be based on clinical signs. But these are shared by other vesicular diseases, so a laboratory is needed to confirm the disease. In Uganda the commonly used assays for the laboratory diagnosis of FMD are NSP ELISA and RT-PCR. Serology using ELISA techniques may fail to distinguish between vaccinated and new infection so compromising its sensitivity. The gel passed PCR is involves a lot of advance sample treatment increasing errors due to carry over which also compromises its sensitivity. This work reports comparative the detection of foot-and-mouth virus by NSP ELISA and RT-PCR with real time PCR which was taken as the gold standard. The assays were compared in terms of sensitivity, specificity and disease prevalence and likelihood ratios. A total of 176 cattle were used from which samples that included epithelial tissues (17.05%) and oral swabs (84.09%) were collected from outbreak cases in Eastern Districts of Mbale and Budaka.

Keywords: NSP-ELISA, RT-PCR, sensitivity, specificity, real time PCR, focal screening.

GJMR-G Classification: NLMC Code: WC900

Strictly as per the compliance and regulations of:

© 2016, Hussein Kafeero Mukasa, Frank Norbert Mwiine, David Kalenzi Atuhaire, Sylvester Ochwo & Ann Nanteza. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Comparative Detection of Foot-and-Mouth Disease Virus by the two Commonly used Assays of NSP ELISA and RT-PCR in Uganda with Quantitative Real Time RT-PCR on Field Samples

Hussein Kafeero Mukasa a, Frank Norbert Mwiine a, David Kalenzi Atuhaire p, Sylvester Ochwo o & Ann Nanteza y

Abstract- Foot-and-mouth disease (FMD) is a viral disease of Ungulates; both Artiodactyla and Perissodactyla. The mortality rates are low in adult animals but it affects milk yield and international trade. In endemic countries, diagnosis can be based on clinical signs. But these are shared by other vesicular diseases, so a laboratory is needed to confirm the disease. In Uganda the commonly used assays for the laboratory diagnosis of FMD are NSP ELISA and RT-PCR. Serology using ELISA techniques may fail to distinguish between vaccinated and new infection so compromising its sensitivity. The gel passed PCR is involves a lot of advance sample treatment increasing errors due to carry over which also compromises its sensitivity. This work reports comparative the detection of foot-and-mouth virus by NSP ELISA and RT-PCR with real time PCR which was taken as the gold standard. The assays were compared in terms of sensitivity, specificity and disease prevalence and likelihood ratios. A total of 176 cattle were used from which samples that included epithelial tissues (17.05%) and oral swabs (84.09%) were collected from outbreak cases in Eastern Districts of Mbale and Budaka. These were used for molecular assays of real time PCR and Conventional PCR using primers and probes targeting the 3D P1 gene. The corresponding sera from all the 176 cattle (100%) were used for NSP ELISA using the Prio CHECK®FMDV NSELISA kit. The sensitivities and specificities of conventional PCR and NSP ELISA were compared with real-time PCR taken as the gold standard. The RT PCR and NSP ELISA had sensitivities of 100.00% (95% CI=96.77% - 100.00%) and 37.50% (95% CI=89.76% - 97.67%) respectively. However, NSP ELISA was more specific than with a RT PCR with sensitivities of 95.83% (95% CI=89.67% - 98.85%) and 94.67% (95%CI=89.76% - 97.67%) respectively. The kappa value for diagnostic agreement between real time PCR and RT-PCR was 0.84 (95% CI = 0.733 – 0.947) at a standard error (SE) of 0.055 showing a very good agreement while that for the agreement between real time PCR and NSP ELISA was 0.35 (95% CI=0.231 – 0.496%) at anastandard error (SE) of 0.061 showing a fair agreement. The RT-PCR assay was more sensitive than NSP-ELISA and can be recommended for genotyping and confirmation of FMD in national reference laboratories while NSP ELISA be used for routine screening.

Keywords: NSP-ELISA, RT-PCR, sensitivity, specificity, real time PCR, focal screening.

I. Introduction

Foot-and-mouth disease (FMD) is a devastating viral disease effecting cloven hoofed animals including cattle, pigs, sheep, and goats. The burden of the disease is manifested through reduced productivity and limitation of international trade in live animals and their product causing serious economic losses (Syed & Graham, 2013). It is a highly contagious, trans-boundary, acute, vesicular disease of cloven-hoofed animals including those in the wild (Anderson, Anderson, Doughty, & Drevmo, 1975). The causal agent of FMD is called foot-and-mouth disease virus (FMDV). It is a small, non-enveloped, single stranded RNA virus 8.5 kb long with a positive polarity surrounded with icosahedral capsid symmetry belonging to the genus Aphthovirus of the Picornaviridae family (Boothroyd et al., 1981). It has seven serotypes A, O, C, Asia 1 and the Southern African territories (SAT) 1-3 of which all have occurred in most East African countries (Vosloo, Bastos, Sangare, Hargreaves, & Thomson, 2002) except Asia 1 (Rweyemamu, 1982). Studies have shown that the predominant FMDV serotypes in Uganda are O and SAT-2 (Balinda et al., 2010). Other serotypes reported include SAT-1 and SAT-3 (Vosloo et al., 2002), serotype C was last recorded in the early 1971 (Vosloo et al., 2002).

The disease is characterized by short lasting fever, epithelial lesions on the tongue, dental pad and inner mouth area leading to excessive salivation and drooling and lesions on the feet causing lameness
During the foot-and-mouth disease 2014/2015 outbreak in our country.

b) Study design

A cross-sectional study was carried out following reports of foot-and-mouth disease outbreaks in Mbale district, Bungokho County and in Budaka district, Kamonkoli County as described in our previous study (Kafeero et al., 2016). Purposive sampling was done based on animals having clinical symptoms like oral lesions, history of infection but having healing lesions and any other asymptomatic cattle in the same farm/kraal or grazing with the symptomatic cattle as reported by the Sub-count Veterinary Officer and or the farmers. The inclusion criteria were cattle with clinical symptoms and the asymptomatic ones in the same farm while exclusion criteria were cattle in farms without any clinical signs or history of clinical signs. All farmers in the villages where sampling was done keep few cattle on average 3-4 animals per household and on zero grazing basis, transmission of the virus was assumed to be low between kraals/farms.

c) Sample size determination

The desired confidence interval for sensitivity estimates was 95% (width of 0.05). The specificity of NSP ELISA in previous studies by Diego, Brocchi, Mackay, & De Simone, 1997 was in the range 99%. This was consistent with the studies by Minga et al., 2015 which gave a diagnostic specificity of 99.4% and a diagnostic sensitivity of 64.00%. Sample size at the required absolute precision level for sensitivity was calculated by applying Buderer’s formula (Buderer, 1996). For sample size calculation, an estimate of specificity of 95% and a precision of 5% within the 95% confidence level was considered. In addition, a prevalence of 50% as recommended in outbreak cases was used (Buderer, 1996). From this a total of 176 cattle were used from which 176 sera were obtained for NSP ELISA test. 176 tissues/swabs were obtained for nucleic acid tests of real time RT-PCR as the gold standard (Office International des Epizooties (OIE), 2008) and gel based PCR. The sensitivity, specificity, likelihood ratios and disease prevalence values of the two assays relative to the real time PCR as the OIE recommended gold standard (Office International des Epizooties (OIE), 2008) were established.

d) Sample collection

Samples were collected from Mbale and Budaka Districts of Eastern Uganda during the 2014-2015 foot-and-mouth disease outbreak in Uganda as previously described in our study (Kafeero et al., 2016). Briefly, samples were collected from cattle with clinical signs, those which had healing lesions in the mouth, dental pad or on the feet and the asymptomatic animals in same kraals from the same farmer. Three types of samples were collected from animals; epithelial tissues...
(ETs), oral swabs (OSs) and blood. The ETs were obtained from animals with vesicles in the mouth, feet or teats. The OSs were obtained from animals with no clinical signs but sharing the same kraal with those having clinical signs. Blood was obtained from all the study animals from which serum (S) sample was also obtained. Exclusion criterion involved cattle from kraals with no any animal having clinical signs. These were taken as the non-cases. After the identification of the animal as a case, it was restrained and blood was collected from either the caudal vein or the jugular vein into red top vacutainers by a trained technician using disposable vacutainer needles and given a field identification number. Blood was left to stand at the ambient temperature for serum to separate out and the red blood cells to sediment to the bottom of the tube and later separated in the evening of each day and aliquoted into cryovials then kept on ice. Epithelial tissues and swabs were collected in the cryovials containing virus transport medium PBS/Glycerol, given a field identification number and kept in liquid nitrogen. The date of sample collection, district, county, sub-county, parish, GPS number, type of sample collected as well as the presence of clinical signs were all recorded in the field book. All samples were transported to the virology laboratory, College of Veterinary Medicine Animal Resources and Bio security, Makerere University. The tissues/ swabs were kept at -80°C while the serum was kept at -20°C pending further use.

A total of 176 cattle were used in this study. From all animals (n=176), blood to be used for obtaining serum (100%) was obtained. From 30 animals (n=30) epithelial tissues (17.05%) were obtained. From 148 animals (n=146) oral swabs (82.95%) were obtained (Table 1). Serum was used for serological test using the NSP ELISA while swabs and epithelial tissues were used for molecular assays of real-time PCR and conventional PCR.

Table 1: Total number of samples and sample type collected.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of Sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>176 (95%)</td>
</tr>
<tr>
<td>Epithelial Tissues</td>
<td>30 (8.5%)</td>
</tr>
<tr>
<td>Oral Swabs</td>
<td>146 (41.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>352 (100%)</td>
</tr>
</tbody>
</table>

All the epithelial tissue, ET (n=30) and oral swabs from the dental pads, OS (n=146) were used for molecular diagnosis while all the sera samples (n=176) were used for serological tests using the NSP ELISA.

e) The RNA extraction

Total RNA was extracted from 140 µl original epithelial tissue/ swab suspension using Qiagen RNA extraction kit following the manufactures instructions as described in our previous study (Kafeero et al., 2016). Briefly, 140 µl of original epithelial tissue/ swab suspension was added to 560µl Buffer AVL- carrier RNA in the microcentrifuge tube, vortexed for 15 sec to mix and then incubated at room temperature (25°C) for 10 minutes. The tube was briefly centrifuged to remove drops from the inside of the lid, then 560µl of ethanol (96%) was added to the sample and mixed by pulse-vortexing for 15 seconds followed by brief centrifuging to remove drops from the inside lid. Then 630µl of the solution were applied to the QiAmp Mini column in a 2ml collection tube and centrifuged at 6000xg (8000rpm) for 1minute and the filtrate discarded. This procedure was performed twice. Then 500µl of Buffer AW1 was added and centrifuged again at 6000x (8000 rpm) for 1 minute. The filtrate was discarded and the column was placed in a fresh 2ml collection tube. Then 500µl of buffer AW2 were added to the column then centrifuged at 20,000 X g (14,000 rpm) for 3 min and the filtrate was discarded. Then 65 µl of Buffer AVE was added to the column, equilibrated at room temperature for 1 minute then centrifuged at 6000 X g (8000 rpm) for 1 min. The RNA samples were stored at -80°C until required for RT-LAMP and conventional RT-PCR.

f) The cDNA synthesis

This was synthesized using the Invitrogen superscript First-Strand cDNA synthesis kit following the manufacturer’s instructions as described in our previous study (Kafeero et al., 2016). Briefly 2µl of 10X RNA primer mix, 0.8µl of 25X dNTPs, 2 µl of 10X RT buffer, 1µl of RNase inhibitor, 3.2µl of RNase free water and 1 µl of Superscript III Reverse Transcriptase to a 0.5 ml microcentrifuge tube to a total volume of 10 µl. The mixture was vortexed briefly to mix then placed on ice. Then 10µl of RNA sample were dispensed to the reaction tube to make up the total reaction volume of 20µl. The mixture was incubated in a thermal cycler at 42°C for 2 hours followed by termination of the reaction at 80°C for 15minutes. The mixture was chilled at 4°C for 30 minutes then transferred to ice and 1 µl of RNase H added followed by incubation at 37°C for 20minutes to degrade the RNA template leaving only a single stranded DNA product. The cDNA was stored at -80°C until required for PCR and LAMP (Kafeero et al., 2016).

g) Real time RT-PCR reaction

In this study, the primers and probe previously described by Callahan et.al (2002) that detect the 3D RNA polymerase encoding gene were used as described in our earlier study (Kafeero et al., 2016). Forward Primer: 5’-ACTGGGTTTTACAA CCT GTGA-3’
Reverse Primer: 5’-GCG AGT CCT GCCACGGA-3’
3D
Probes: (5'-FAM-TCC TTT GCA CGC CGT GGG AC-TAMRA-3'). This probe has been labeled with 6-FAM at the 5' end and the quencher tetracyanomethylrhodamine (TAMRA) at the 3' end in Real-time RT-PCR reaction detects the 3Dpolymerase gene sequence in all the FMDV serotypes.

The rRT-PCR reaction was performed on one-step procedure combined with reverse transcription and Real-time assay. Therefore Real-time assay was carried out by SuperScript III/Platinum Taq one-step rRT-PCR kit (Invitrogen). The composition of the 25 µl reaction/Master Mix for the One-Step rRT-PCR included the following: 12.5 µl 2x-reaction buffer, 2.0 µl (10 pmol/µl) of each of the forward and reverse primer, 1.5 µl (1.5 µl) of the probe, 5.0 µl extracted RNA, 0.5 µl Superscript 111 RT/Platinum Taq mix, 1.5 µl of molecular grade H2O. The amplification was done at the following temperature cycle: Reverse transcription (one cycle), 48 °C for 30 minutes, the initial denaturing (one cycle), 95 °C for 10 minutes; then 40 cycles consisting of 30 seconds at 95°C for 15 seconds and 60°C for 1 minute and 72°C for 30 seconds. Negative and positive controls were included in each run. PCR amplification was carried out in the thermal cycler Rotor- Gene Q (Qiagen, German).

h) The PCR reaction

The PCR was carried out as previously described by (Moniwa, Clavijo, Li, Collignon, 2007) using primers designed to target the 3D polymerase encoding gene; forward primer: 5'-CACCTCCACATGGA TTATGGAAGCT-3' and the reverse primer: 5'-ACATCT GAGGGATTATGCGTCAC-3'; Gene bank accession number JF749843 that amplified the 260 bp fragment of the highly conserved RNA polymerase (3D) gene of FMDV. Briefly, the 25 µl reaction mixture composed of 12.5 µl 2X TaqMan Universal Master Mix, 1 µl of each of the forward primers and reverse primers, 5.5 µl of PCR grade water and 5 µl of cDNA template. Negative control (nuclease free water) and positive control (field isolate) were included in each run. The reactions were carried out in an HBA Cycler machine (Mj Research Inc. USA). The following conditions: 95°C for 10 min for Taqman polymerase activation, 95°C for 15 sec for denaturation, 58°C for 30 sec annealing, 72°C extension. These three steps were repeated for 35 cycles and a subsequent hold temperature of 12°C was used.

i) NSP ELISA assay

All sera were screened for antibodies against FMDV nonstructural proteins using Prio CHECK®FMDV NS kit (PriomicsLelystad B.V, The Netherlands). The Prio CHECK®FMDV NS kit is a blocking ELISA that detects antibodies against non-structural 3ABC protein of FMDV of all the seven serotypes. The test plates are coated with 3ABC specific monoclonal antibody (mAb) followed by incubation with antigen (3ABC protein). Hence test plates of the kit contain FMDV NS antigen captured by the coated mAb. The Prio CHECK®FMDV NS kit detects FMDV infected animals independent of the serotype that has caused the infection and independent of the fact that the animal is vaccinated or not.

Standard protocols and procedures were followed according to manufacturer’s instructions. Briefly, 80 µl of ELISA buffer were dispensed to all wells, 20µl of Negative Control to wells A1 and B1, 20µl of Positive Control to wells C1 and D1, 20µl of Positive Control to wells E1 and F1 and 20µl of test samples to the remaining wells. Test Plate was sealed using the enclosed plate sealers and shaken gently then incubated overnight (16hours) at room temperature (25°C).The Test Plate were emptied after the incubation period and washed 6 times with 250µl washing solution (200x) made to a working solution (1x) with de-mineralized water using a micro plate washer (Mrc scientific, Marty Enterprises ltd, Nairobi, Kenya). 100 µl of diluted conjugate was dispensed to all wells and incubated at room temperature for 60minutes at room temperature (25°C).The Test Plates were emptied after the incubation period and washed 6 times with 250µl washing solution using the plate washer as previously described. Then100 µl of Chromogen; tetra methyl benzidine (TMB) Substrate were dispensed to each of the wells and incubated for 20 minutes at room temperature (25°C) .Then 100µl of Stop Solution was dispensed to the all wells.

j) Measurement of the optical density (OD) of the samples

The optical densities (OD) of the wells at 450nm were measured within 15minutes after colour development stopped using Multiskan Ascent spectrophotometer (Thermo lab systems OY UK). The mean OD 450 value of wells A1 and B1 (OD450 max) for negative control was calculated as:

\[
\frac{(ODA_1 + ODB_1)}{2} = OD_{450\max}
\]
The OD 450 values of all samples were expressed as percentage inhibition (PI) relative to the OD450 max.

\[ PI = 100 - \left( \frac{OD_{450 \text{ test sample}}}{OD_{450 \text{ max}}} \right) \times 100 \]

PI < 50% was interpreted as negative while PI ≥ 50% was positive.

k) Detection of amplification products
   i. Real time reverse transcription polymerase chain reaction (rRT-PCR)
      The PCR amplification was carried out in the thermal cycler Rotor- Gene Q (Qiagen, Germany). The successfully amplified target gave an amplification curve and the cycle threshold, Ct at which the target amplicon was initially detected above the background fluorescent levels as determined by the instrument software noted. Each rRT-PCR was performed minimally in duplicate and the mean Ct value with standard deviation reported.
The Ct values in the range $\geq 40.0$ indicated a negative sample and Ct values $< 40$ indicated positive sample (Figs 2 & 3). In all cases, the positive control gave the minimum Ct value and the negative control gave no Ct.

**Figure 3**: Representative real time results with Ct values $\leq 40$ showing positive animals for FMDV

**ii. Reverse transcription polymerase chain reaction (RT-PCR)**

The $2 \mu l$ of the reaction mixture was electrophoresed on a $2\%$ agarose gel electrophoresis after ethidium bromide staining under UV light using a $\phi X174$ marker (Amersham Biosciences, UK) to determine the size of the PCR product.

**Figure 4**: Conventional PCR representative gel for FMD virus detection

A $2\%$ agarose gel electrophoresis of PCR products: Lane M, DNA 2000bp marker (Invitrogen), lane P is for positive control (field isolate), lane N is negative control (nuclease free water), lanes 1-9 FMDV samples. Lanes 1, 3, 4, 5 and 8 are positive samples while lanes 2, 6, 7 and 9 are negative samples.

Positive samples and the positive control gave bands corresponding to the 260bp (Fig. 4) as determined from the marker since it is the size of the 3D pol gene. Negative samples gave no bands.

**i) Data analysis**

Every sample was tested twice by each of the methods and in case of a disagreement, the test was repeated for all the three assays to come up with the final result. Sensitivity and specificity of each test was then determined as percentages with $95\%$ confidence intervals (CIs). The two tests were then each compared to the reference test/gold standard (rRT-PCR) using Fisher’s exact test. The sensitivities and specificities of each test compared to the gold standard were determined. Kappa values to assess the level of test agreement were also determined. All analyses were done at $95\%$ CI.
III. Results

Table 2: Summarizes results of two molecular assays of conventional PCR and real time PCR and, the NSP ELISA assay using the Prio CHECK®FMDV NS kit for all the 176 cattle samples.

<table>
<thead>
<tr>
<th></th>
<th>NSP ELISA</th>
<th>Conventional PCR</th>
<th>Real Time PCR</th>
<th>Number of Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Positive Positive</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Positive Negative</td>
<td>00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Negative Negative Negative</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Negative Negative Positive</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Negative Positive</td>
<td>06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Negative Positive</td>
<td>00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Negative Positive</td>
<td>02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Positive Positive</td>
<td>02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A total of 24 of the 176 cattle tested positive by all the three assays of conventional PCR, real time quantitative PCR and NSP ELISA. A total of 92 cattle tested negative for all the three assays. Real time quantitative PCR identified 34 animals as being positive with FMDV RNA.

The NSP ELISA assay identified 80 out of the 176 animals as positive of which only 30 animals were also positive by the gold standard and 50 negative by the gold standard (Tables 2,3 and Figs 1, 2, 3) giving a diagnostic sensitivity of 37.50% (95% CI=26.92% - 49.04%) and a specificity of 95.83% (95% CI= 89.67% - 98.88%). The RT-PCR assay also identified 24 animals as positive out of the 34 animals identified as positive by real time PCR and missed out 8 animals (Tables 2,3 and Fig. 4 ) giving a diagnostic sensitivity of 100% (95% CI = 86.77% - 100.00%) and a specificity of 94.67% (95% CI = 89.76% - 97.67%). These results for both assays NSP ELISA and RT-PCR were statistically significant (P< 0.0001) when analyzed by Fisher’s exact test.

Table 3: Sensitivity and specificity of conventional PCR and one NSP ELISA assays for identification of foot-and-mouth disease virus in serum and swab/ tissue samples from cattle in Budaka and Mbale districts of Eastern Uganda.

<table>
<thead>
<tr>
<th>Diagnostic Assay</th>
<th>Medium Sensitivity</th>
<th>95% Confidence interval</th>
<th>Medium Specificity</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>NSP ELISA</td>
<td>37.50%</td>
<td>26.92%</td>
<td>49.04%</td>
<td>95.83%</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>100.00%</td>
<td>86.77%</td>
<td>100.00%</td>
<td>94.67%</td>
</tr>
</tbody>
</table>

The study cattle FMDV prevalence (Table. 4) was estimated at 45.45% (95%CI=37.95% - 53.12%) by NSP ELISA and 14.77% (95%CI=9.88%-20.89%). The corresponding medium Positive likelihood ration (Table. 4) was 9.00 with a 95% credible interval of 3.31 to 30 for NSP ELISA and 18.75 for RT-PCR at a 95% credible interval of 9.55 to 36.80. Both likelihood ratios show that the test result is associated with the disease with RT-PCR showing a twice chance of post test probability of the disease (Table.4). The kappa value for agreement between RT-PCR and gold standard, test real time PCR was 0.84 (95% CI=0.733 – 0.947) at a standard error (SE) of kappa of 0.055 showing a very good agreement between the two assays. On the other hand the kappa value for agreement between NSP ELISA and real time PCR assay was 0.35 (95% CI = 0.231 – 0.469) at a standard error (SE) of kappa of 0.061 showing a fair agreement.

Table 4: Disease prevalence and positive likelihood ratio of conventional PCR and NSP ELISA assays for foot-and-mouth disease virus in cattle from Budaka and Mbale districts of Eastern Uganda.

<table>
<thead>
<tr>
<th>Diagnostic Assay</th>
<th>Medium Disease Prevalence</th>
<th>95% Confidence interval</th>
<th>Medium Positive likelihood ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>NSP ELISA</td>
<td>45.45%</td>
<td>37.95%</td>
<td>53.12%</td>
<td>9.00</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>14.77%</td>
<td>9.88%</td>
<td>20.89%</td>
<td>18.75</td>
</tr>
</tbody>
</table>

IV. Discussion

The aim of this study was to compare the sensitivity and the specificity of the NSP ELISA and conventional PCR which are the commonly used assays in the detection of FMD virus in Uganda (Mwiine et al., 2010, Kasambula, 2011) using real time PCR as the gold standard (Office International des Epizooties (OIE), 2008). Previous studies by Saliki, 2000 have shown that
disease recognition is essential for any disease control program. This is again paramount in the control of FMD due to the several serotypes and topotypes causing clinically indistinguishable disease (Vosloo et al., 2002).

In the present study, the results of RT-PCR and NSP ELISA were compared with real time PCR as the gold standard. The ELISA results indicated more infected animals than all the three assays on samples from the same animals. It is noted that 24 (13.64%) of the 176 cattle examined were positive on all the three techniques. However, ELISA positive were 80 (45.46%) and ELISA negative were 96 (54.54%) (Table 2, Fig.1) whereas the RT-PCR positive 26 (14.77%) and RT-PCR negative were 150 cattle (85.23%) (Table 2, Fig.3). This gave FMD virus NSP ELISA sensitivity of 37.50% and specificity of 95.83% as well as the FMD virus RT-PCR sensitivity of 100% and a specificity of 94.67%. The FMD virus NSP ELISA sensitivity in the current study was lower than the sensitivity in the earlier study by Minga et al. (2015) which gave a sensitivity of 64.00%. However, the specificity in our study was almost consistent with that identified by Minga et al., (2015) which gave a specificity of 99.40%. On the other hand, the FMD virus RT-PCR gave a specificity and a sensitivity of 100.00% and 94.67% respectively consistent with the earlier findings by Moniwa M, Clavijo A, Li M, Collignon B, (2007).

The high ELISA positive in this study is not surprising since it has been explained in earlier studies by Alexandersen et al., (2003). Initial virus multiplication occurs in the vesicular epithelium and mucosal swabs in the five days after infection. Later the antibodies remain in plasma for several weeks, or months sampling could have been done in this time when the antibodies have remained in the plasma. Secondly, the high false positives by antigen ELISA assay been explained in earlier studies by Ma et al., (2011). According to their work on overview of ELISA techniques for FMD diagnosis, "no single ELISA technique can differentiate infected from vaccinated animals with confidence. This is aggravated by the use of non-purified vaccines in Eastern Africa which elicit antibodies against NSPs increasing chances of false positive (Ayebazibwe, Mwine, Balinda, Jornehoj, & Alexandersen, 2012). In addition antibodies against NSPs do not appear until 8-9 days after infection (Lu et al., 2007) increasing chances of false negative. Consequently to be effective, NSP ELISA should be used for sera sampled in late subacute or even under chronic or persistent FMDV infection. Fortunately or unfortunately the antibodies against NSP persist for long post infection and therefore NSP ELISA cannot be used with absolute confidence to differentiate new and previous infection (Sørensen et al., 1998). This is consistent with the findings of the current study. This posits a challenge for FMD diagnosis in our country where NSP ELISA is the most commonly used assay for routine detection of FMD in cattle and other domestic ungulates (Namatovu et al., 2013) due to its simplicity. Conventional PCR though it has demonstrated higher sensitivity and specificity compared to NSP FMD virus ELISA both in earlier studies by Moniwa M, Clavijo A, Li M, Collignon B, (2007) and in our study. However in our country, the RT-PCR for foot-and-mouth disease is restricted to research institutions but in national reference laboratories NSP ELISA is the most commonly used as underlined in the previous study by Namatovu et al., (2013).

V. Conclusions and Recommendations

Our study compared the sensitivity and specificity of the two commonly used assays of NSP ELISA and gel based PCR for the detection of FMD in our country using real time PCR as the gold standard. The NSP ELISA assay has demonstrated a high false positive rate compared to gel based PCR using real time PCR which is recommended as the gold standard in countries whose biosafety levels do not permit them to perform virus isolation including Uganda. The conventional PCR demonstrated a higher sensitivity and specificity as compared to NSP ELISA but it uses sophisticated equipment and requires special training of the laboratory staff, its use for routine screening is not practical. So in Uganda, focal screening of FMD is based on NSP ELISA nearly in all regional and national reference labs due to its simplicity and its ability to screen large volumes of samples. This puts FMD diagnosis in our country in an empirical dilemma yet FMD is a highly contagious disease and its management is contingent upon accurate and timely diagnosis. The high frequency of the misclassification of cattle when using NSP ELISA suggest that FMD prevalence estimates based on NSP ELISA may be inflated, therefore confirmation by nucleic acid techniques should be the priority in national referral laboratories. We recommend the use of RT-PCR in the national reference laboratories for foot-and-mouth disease virus for confirmation, genotyping and to justify fresh infection, otherwise the NSP ELISA can be used for routine screening. We further recommend that more studies be done using large samples to improve on the accuracy of the findings. The scope of the sample types can also be extended to oral pharyngeal fluids in asymptomatic animals. Finally we recommend that vaccine strains should be matched with field strains and purified vaccines should be used to reduce on the false positive rates and hence more reliable results.

Conflict of interest

We declare that we have no competing interests in regards to the authorship of this article or its publication.

VI. Acknowledgement

This work was in part supported by the United States Department of Agriculture (USDA) especially field work during sample collection through a grant to
associate professor Frank Norbert Mwiine. The authors are greatly indebted to their contributions.

The authors also thank the Molecular Biology Laboratory staff who helped during sample processing and data analysis. The authors wish to acknowledge the farmers for their cooperation and understanding during sample collection as well as the district veterinary officers and the local leaders who gave them permission for community entry. The authors strongly acknowledge the tireless efforts of the field team who restrained the animals in a scorching tropical sun.

References Références Referencias


Comparative Detection of Foot-and-Mouth Disease Virus by the two Commonly used Assays of NSP ELISA and RT-PCR in Uganda with Quantitative Real Time RT-PCR on Field Samples


Haemoparasites and Haematological Parameters of the One Humped Camel (*Camelus Dromedarius*) Slaughtered in Maiduguri Abattoir, Nigeria

By Egbe-Nwiyi, T.N.C, Paul, B. T. & Muhammed, Y. Y.

*University of Maiduguri*

**Abstract** - Haemoparasitic diseases account for substantial losses in terms of decreased working capacity, growth and productivity of camels. A survey of the one humped camel (*Camelus dromedarius*) slaughtered in Maiduguri was conducted from January to June, 2016 to determine the prevalence of haemoparasites and their effects on some haematological parameters. Blood samples were randomly collected from 209 camels at the point of slaughter and subjected to standard haematological procedures to determine the white blood cell count (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Blood films and Giemsa stained thin smears were prepared on clean glass slides and examined for the presence of haemoparasites. Haemoparasites were identified microscopically to generic level based on morphological features. A total prevalence of 12.6% was recorded for Anaplasma (37.7%), Trypanosoma (33.3%) and Babesia (22.2%), in addition to microfilariae of *Dipetalonema* species (7.5%).

**Keywords**: camels, haemoparasites, haematological parameters, maiduguri, prevalence.

**GJMR-G Classification**: NLMC Code: WA 360
Haemoparasites and Haematological Parameters of the One Humped Camel (Camelus Dromedarius) Slaughtered in Maiduguri Abattoir, Nigeria

Egbe-Nwiyi, T.N.C a, Paul, B. T. a & Muhammed, Y. Y. a

Abstract - Haemoparasitic diseases account for substantial losses in terms of decreased working capacity, growth and productivity of camels. A survey of the one humped camel (Camelus dromedarius) slaughtered in Maiduguri was conducted from January to June, 2016 to determine the prevalence of haemoparasites and their effects on some haematological parameters. Blood samples were randomly collected from 209 camels at the point of slaughter and subjected to standard haematological procedures to determine the white blood cell count (WBC), packed cell volume (PCV), heamoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC). Blood films and Giemsa stained thin smears were prepared on clean glass slides and examined for the presence of haemoparasites. Haemoparasites were identified microscopically to generic level based on morphological features. A total prevalence of 12.6% was recorded for Anaplasma (37.7%), Trypanosoma (33.3%) and Babesia (22.2%), in addition to microfilariae of Dipetalonema species (7.5%). The haematological parameters of infected and uninfected camels were within normal range but, there was a significant difference (p<0.05) in Mean±SE RBC counts of infected and uninfected camels, while thee Mean±SE PCV, Hb, WBC, MCV, MCH and MCHC were comparable (p>0.05). We report the first occurrence of Dipetalonema species in one humped camel in Maiduguri. The role of camels as carriers or reservoir of haemoparasites for other species of domestic animals in Maiduguri is suspected. Routine screening, treatment including preventive chemoprophylaxis and vector control is recommended. There is also need for molecular studies to identify various species of haemoparasite circulating in trade camels in Maiduguri.

Keywords: camels, haemoparasites, haematological parameters, maiduguri, prevalence.

I. INTRODUCTION

Nigeria has an estimated 87,000 camels of which 30.9% are found in Borno state (FDLPCS). Camels are highly adapted to the semi-arid environments and are confined to the northern borders of Sokoto and Borno states in Nigeria (Schwartz and Dioli, 1992; Blench, 1999). They contribute significantly to the food security of the nomadic pastoral households (El-Naya and Barghash, 2016) and economy of northern Nigeria (FDLPCS, 1992), in addition to their work ability, environmental conservation and the provision of meat and milk (Chafe et al., 2003). Despite their role as a member of the food producing family of livestock, camels have for a long time remained the most neglected animal in the field of scientific research. Furthermore, camels are hardy animals that have a strong adaptation to the harsh weather conditions of arid regions because of their unique physiological characteristics (Karimi et al., 2014).

Camels are known to suffer from various types of parasitic diseases which are major constraint in improvement of camel health (Parsani et al., 2008). Haemoparasitic diseases such as Anasplasmosis, Babesiosis, Trypanosomosis, Theileriosis and Dipetalonemia have adverse effects on the health, growth, productivity and working capacity of camels (Ahmad et al., 2004). Various species of haemoparasites have been reported in camels in Nigeria (Egbe-Nwiyi and Chaudhry, 1994; Bamaiyi et al., 2011) and elsewhere (Abdelrahim et al., 2009; Swelum et al., 2014; Faham et al., 2015; El-Naya and Barghash, 2016). Among haemoparasitic diseases of camels, trypanosomosis also known as “sura” is one of the important and serious disease caused by Trypanosoma evansi (Solsby, 1982). It is mechanically transmitted non-cyclically by haematophagous flies such as Tabanus, Stomoxys and Hippoboscid, which are common in Africa, Nigeria inclusive (Agbede, 2013; Eyob and Matios, 2013). Trypanosomosis in camels usually occurs in chronic form but may be acute when the animal is under stress (Parsani et al., 2008). In the acute form, clinically affected camels show fever, anorexia, marked...
generalized oedema, deteriorate rapidly and die while in the chronic form, there is intermittent high fever, progressive loss of body weight, marked generalized muscular atrophy and occasionally abdominal oedema (Eyob and Matios, 2013). Piroplasmosis due to tick-borne Anaplasma, Babesia and Theileria species have also been reported in Camels in Nigeria (Bamaiyi et al., 2011) and elsewhere (Swelum et al., 2014; El-Naya and Barghash, 2016).

Extra intestinal filarid nematodes like Onchocerca, which produces microfilaria have been reported in camels (Parsani et al., 2008). Onchocerca fasciata produces subcutaneous nodules on the head and neck regions while Dipetalonema evansi occurs in blood vessels in the spermatic cord, pulmonary arterial tree, right auricle, lymph nodes and mesentery. The microfilaria is sheathed and found in the blood circulation. Basic diagnosis of haemoparasism relies on clinical symptoms, haematological evaluations and microscopic examinations of blood film or blood smear (Soulsby, 1982).

There has been a steady increase in the number of camels slaughtered for human consumption, as an alternative to goat, sheep and cattle meat in Maiduguri. The increased demand on camel meat is also accompanied by a corresponding rise in prevalence of haemoparasites among them (Egbe-Nwiyi and Chaudhry, 1994; Bamaiyi et al., 2011). It is against this background that this study was conducted to ascertain the prevalence rate of haemoparasites in slaughtered camels so as to design a better preventive and chemotherapeutic approach that could fit into policy formulation in the region.

II. Materials and Methods

a) Study Area and Population

Maiduguri is located in the North east arid zone of Nigeria between Latitude 11° N and Longitude 13° E, and shares international boundaries with Republics of Niger and Chad in the north and Cameroon in the east. It is characterized by a long period of dry season which lasts from October to May and a short period of rainfall from June to September (Hess et al., 1995). The State derives great economic activity from its rich livestock and fishery products (NPC, 2006). Camels are important trade livestock in Maiduguri and also used for meat and milk in addition to their use as portage animals in rural localities. The camels used for this study were trade stock presented for slaughter at the Maiduguri abattoir. The sex were differentiated based on appearance of external genitals while aging was based on rostral dentition as described by Bello et al. (2013). Thus, camels <5 years were categorized as young while older (>5 years) ones were regarded as adults.

b) Study Design and Sample Collection

A cross-sectional study was conducted from January to June, 2016, to investigate the occurrence of haemoparasites and associated changes in some haematological parameters of slaughtered camels. A total of 209 camels were randomly selected at the point of slaughter, and the age and sex of each sampled animal were observed and recorded appropriately. 10ml of blood was collected into two labelled bottles containing sodium EDTA, by jugular venipuncture at the point of slaughter. The samples were submitted to the Veterinary Parasitology and Pathology Laboratories for parasitological and haematological examinations, respectively.

c) Parasitological Examinations

Blood smears were prepared from fresh whole blood on microscope glass slides (75mm by 25mm), air dried, fixed in methanol and stained with Giemsa’s stain while blood films were prepared to examine trypanosomes and microfilaria according to Soulsby (1982). Haemoparasites were identified by direct microscopic examination using X40 and X100 oil immersion objectives of a compound microscope (Olympus, USA), based on morphologic keys described by Soulsby (1982).

d) Haematological Examinations

The blood samples were analyzed for hemoglobin (Hb) by acid hematin (Sahli’s) method, packed cell volume (PCV) by microhaematocrit, and total red blood cell (RBC) and total white blood cell (WBC) counts by Neubauer hemocytometer (Brar et al., 2000). The erythrocyte indices (mean corpuscular volume, MCV; Mean Corpuscular Hemoglobin, MCH; and Mean Corpuscular Hemoglobin Concentration, MCHC) were calculated using standard formula (Jain, 1998).

e) Statistical Analysis

Prevalence was calculated as P (%) = d/n where P= prevalence, d= number infected and n= number examined (Thrusfield, 2005), and the 95% confidence intervals on prevalence was calculated using Vassar Stats® statistical computation web site. The student t-test was used to compare the haematological parameters of infected and uninfected camels and p<0.05 was considered significant.

III. Results

Overall prevalence of haemoparasites and their 95% confidence intervals (CI) in the one humped camel (Camelus dromedarius) slaughtered in Maiduguri is presented in Table 1. Out of 209 blood films and smears examined, 27 (12.4%) were positive for various types of haemoparasites. Young (19.4%) and male (20.8%) camels had insignificantly higher (p>0.05) prevalence than the adult (11.8%) and female (11.9%) counterparts.
The 4 types of haemoparasites identified in blood films and stained blood smears of the one humped camels (Camelus dromedarius) slaughtered in Maiduguri is shown in Table 2. A total of 3 genera of haemoprototiza including Anaplasma (37.7%), Babesia (22.2%) and Trypanosoma (33.3%), in addition to microfilariae of nematode, Dipetalonema (7.5%) were detected.

Mean values of haematological parameters of the infected and uninfected one humped camel (Camelus dromedarius) slaughtered in Maiduguri is shown in Figure 1. All the haematological parameters of the infected and uninfected camels examined in this study were within range of normal values. However, the mean values of RBC in the infected and uninfected camels examined in this study were significantly different (p<0.05) but the mean values of PCV, Hb, WBC, MCV, MCH and MCHC of infected and uninfected slaughtered camels were comparable (p>0.05).

### IV. Discussion

The prevalence of haemoparasites in slaughtered trade camels in Maiduguri has progressively increased in the last two decades. Egbe-Nwiyi and Chaudhry (1994) reported 2.5% prevalence, Bamaiyi et al. (2011) reported 5.7% prevalence while the current study recorded an overall prevalence of 12.9%. The observed increase in prevalence of haemoparasites in this locality could be attributed to preponderance of arthropod vectors due to favorable micro climatic conditions in the region (Biu and Konto, 2011). Also, previous reports on prevalence of haemoparasites in other species of domestic animals in different parts of the country suggests that haemoparasitism is endemic in Nigeria. Biu et al. (2005) reported an overall prevalence of 17.3% in cattle from Maiduguri. Ameen et al. (2008) reported a total prevalence of 4.1% in ruminants from Oyo state. Okeiyeo et al. (2008) reported a total prevalence of 13% for various species of haemoparasites in pastoral sheep from Kaduna state. Shamaki et al. (2009) reported a prevalence of 9.1% for Trypanosoma species in cattle from Gombe state. Furthermore, Ademola and Onyiche (2013) reported a prevalence of 5% in ruminants from Ilbadan. These reports further validate our findings and suggest that the various species of haemoparasites constantly circulates among different species of domesticated and semi-domesticated animals in Nigeria, with some semi-domesticated species probably serving as permanent reservoir of infection. The role of arthropod vectors in transmission of haemoparasites has been described (Soulsby, 1982; Urquhart et al., 1996), and the transhumant conditions under which camels are traditionally raised in the tropics exposes them to the arthropod vectors of haemoparasites.

The higher prevalence of haemoparasites recorded in younger camels in this study agrees with previous report by El-Naga and Barghesh (2016). Similarly, Ademola and Onyiche (2013) also reported an inverse age related decrease in prevalence of haemoparasites in slaughtered animals in Nigeria. The higher prevalence of haemoparasites recorded in male than female camels in this study is in agreement with Ahmed and Bringa (2014) but disagrees with El-Naga and Barghesh (2016) who reported a higher prevalence of haemoparasites in female than male camels. Also, Shamaki et al. (2009) reported a higher prevalence of haemoparasites in female donkeys, sheep and cattle than their male counterparts. Generally, male animals under the extensive system of management in which camels are traditionally raised have high natural tendencies of acquiring diseases than the females because they tend to move about in search of mates for courtship and breeding purposes.

All the 3 genera of haemoprototiza identified in this study were previously reported in camels (Egbe-Nwiyi and Chaudhry, 1994; Bamaiyi et al. 2011) and domestic animals in Nigeria (Abenga et al., 2004; Biu et al., 2005; Kamani et al., 2010; Ademola and Onyiche, 2013; Okorafor and Nzeako, 2014; Qadeer et al., 2015) and elsewhere in the world (Soulsby, 1982; Alonso et al. 1992). The high frequency of Anaplasma species in this study may be due to the abundance of suitable environmental conditions that favours multiplication and survival of the arthropod vectors (Soulsby, 1982; Shah-Fischer and Say, 1989). Similarly, the high prevalence of Trypanosomes in the present study may be linked to the abundance of biting flies such as Stomoxys, Tabanus and Hippoboschids in the region (Agbede, 2013), and the transhumant conditions under which camels are reared may increase their exposure to the arthropod vectors. Previously, few cases of trypanosomosis have been reported in camels from Maiduguri (Egbe-Nwiyi and Chaudhry, 1994). These occurrences were linked to the movement of camels through tsetse infested to tsetse free zone as they travel down towards the northern limit of tsetse distribution in Borno state. Moreover, mechanical vectors such as biting flies which have been incriminated in transmission of trypanosomosis in tsetse free zones (Soulsby, 1982) are abundant in Maiduguri and environs, and could play a significant role in transmission. The occurrence of Dipetalonema species Microfilaria in camels in this study never reported in Maiduguri and the low prevalence rate indicate that filariid nematodes are erratic in the geographical region due to unavailability of suitable ecological conditions for propagation of Simulium species which serve as their natural vector (Soulsby, 1982). Moreover, Mosquitoes are known to play a significant role in transmission of microfilaria (Soulsby, 1982).
The mean values of RBC, PCV, Hb, WBC, MCV and MCH were within normal range of values in desert camels (Farooq et al., 2011) but the MCHC was below normal range. Moreover, mean values of most haematological parameters of infected and uninfected slaughtered camels examined in this study were comparable (p > 0.05). The absence of anemia, which is a reliable indicator of severity in haemoparasitic infections (Adejinmi et al., 2004) may be due to the fact that infected camels were probably carriers with latent infection. In the presence of favourable immunity and good nutrition, there may be adequate compensatory haematopoietic response in the course of most haemoparasitic infections, which could mask the initial anemia, hence the observed normal heamogram in this study. The significantly (p < 0.05) higher mean RBC counts observed in infected camels than uninfected ones may be explained on the basis of active haematological response to the presence of haemoparasites, which usually occurs in the course of natural infections (Soulsby, 1982).

V. Conclusion

This study reports endemic proportion of haemoparasites and the first occurrence of microfilaria of Dipetalonema species in one humped camel in Maiduguri. The results obtained from this study also indicate that camels in Maiduguri may harbor subclinical infections involving various genera of haemoparasites. The role of camels as carriers and or reservoirs for other species of domestic animals is suspected since infection is not associated with significant changes in haematological parameters.

VI. Recommendation

We recommended the need for further studies using molecular methods to elucidate the various species of haemoparasites circulating in camels within the region. Also trade camels coming to Maiduguri for slaughter or other purposes should be screened for and be treated against haemoparasites. There is an immediate need to educate camel herders in this locality on preventive chemoprophylaxis and vector control using effective insecticides, acaricides and environmental management as well as chemotherapeutic control measures.

VII. Acknowledgements

The authors are grateful to Mallam Ismaila Gadaka in the Department of Veterinary Pathology and Mallam Ya’uba Mohammed in the Department of Veterinary Microbiology and Parasitology, both in the University of Maiduguri, for their Technical support in the laboratory analysis of the samples.

Conflict of Interest

The authors did not declare any conflict of interest concerning this work.

References Références Referencias


Table 1: Overall Prevalence of Haemoparasites and their 95% CI in the one Humped Camel (*Camelus dromedarius*) Slaughtered in Maiduguri Abattoir.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. Examined</th>
<th>No. Positive (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>31</td>
<td>6 (19.4)</td>
<td>0.09</td>
</tr>
<tr>
<td>Adult</td>
<td>178</td>
<td>21 (11.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>5 (20.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>Female</td>
<td>185</td>
<td>22 (11.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>209</td>
<td>27 (12.9)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

CI = 95% confidence interval on prevalence, L = lower limit, U = upper limit

Table 2: Types of Haemoparasites identified in the one Humped Camel (*Camelus dromedarius*) Slaughtered in Maiduguri Abattoir.

<table>
<thead>
<tr>
<th>Haemoparasites</th>
<th>No. Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma</td>
<td>10 (37.0)</td>
</tr>
<tr>
<td>Babesia</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Microfilaria</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Trypanosoma</td>
<td>9 (33.3)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (12.9)</td>
</tr>
</tbody>
</table>

Figure 1: Mean values of Some Haematological Parameters of Infected and Uninfected one Humped Camel (*Camelus dromedarius*) Slaughtered in Maiduguri Abattoir.
Study on Prevalence and Monetary Loss Attributed to Hydatidosis in Cattle Slaughtered at Jimma Municipal Abattoir, Southwestern Ethiopia

By Ayub Temam, Benti Deresa & Mukarim Abdurahaman

Jimma University

Abstract- A cross-sectional study was conducted on bovine hydatidosis from November 2015 to June 2016 with the objectives of investigating its prevalence and Monetary loss in cattle slaughtered in Jimma municipality abattoir. Routine ante mortem and post-mortem inspection was performed on a total of 400 selected slaughtered cattle. Infection organs 223 cattle positive, 200 (89.7%) had cysts more in lungs, 20 (8.9%) in liver, 1 (0.45%) in kidney, 1(0.45%) in spleen, whereas, the rest of 1(0.45%) in heart infections involved organs. A significant association was observed (P<0.05) between the disease positivity and age groups, body condition. It was concluded that these zonotic cestodes deserve due attention to safeguard public health and that further studies are needed on epidemiology and public health importance of Echinococcus granulosus in the study area.

Keywords: bovine, hydatidosis, prevalence, monetary loss jimma, abattoir.

GJMR-G Classification : NLMC Code: WC 900
Study on Prevalence and Monetary Loss Attributed to Hydatidosis in Cattle Slaughtered at Jimma Municipal Abattoir, Southwestern Ethiopia

Ayub Temam §, Benti Deresa § & Mukarim Abdurahaman §

Abstract: A cross-sectional study was conducted on bovine hydatidosis from November 2015 to June 2016 with the objectives of investigating its prevalence and Monetary loss in cattle slaughtered in Jimma municipality abattoir. Routine ante mortem and post-mortem inspection was performed on a total of 400 selected slaughtered cattle. Infection organs 223 cattle positive, 200 (99.7%) had cysts more in lungs, 20 (8.9%) in liver, 1 (0.45%) in kidney, 1 (0.45%) in spleen, whereas, the rest of 1 (0.45%) in heart infections involved organs. A significant association was observed (P<0.05) between the disease positivity and age groups, body condition. It was concluded that these zoonotic cestodes deserve due attention to safeguard public health and that further studies are needed on epidemiology and public health importance of Echinococcus granulosus in the study area.

Keywords: bovine, hydatidosis, prevalence, monetary loss, jimma, abattoir.

I. Introduction

Hydatidosis caused by the larval stage (metacestode) of Echinococcus granulosus is the most widespread parasitic zoonoses (Ibrahim, 2010; Getaw et al., 2010). Dogs are the usual definitive hosts while a large number of mammalian species are intermediate hosts, including domestic ungulates and man. It is a familiar with many different countries (cosmopolitan) zoonotic infection (Azlaf and Dakkak, 2006).

Despite the large efforts that have been put into the research and control of echinococcosis, it still remains a disease of worldwide significance. In some areas of the world, Cystic echinococcosis caused by E. granulosus is a re-emerging disease in places where it was previously at low levels (Urquhart et al., 1996; Kebede et al., 2009a).

Echinococcus granulosus infection is endemic in East and South Africa, Central and South America, South Eastern and Central Europe, Middle East, Russia and China. The highest incidence is reported mainly from sheep and cattle rearing areas (Arene, 1995). The disease is most important in livestock production which is based mainly on extensive grazing system. Several reports from different parts of Ethiopia indicate that hydatid cyst is prevalent in livestock population of the country (Jobre et al., 1996; Kebede et al., 2010).

According to Abebe and Yilma (2011) a prevalence of 72.4%, 37.72%, 33.78% and 13.7% in cattle slaughtered in Asella, Adama, Gonder, and Dire Dawa was documented respectively indicating its importance in the livestock industry. Its distribution is higher in developing countries especially in rural communities where there is close contact between dogs (definitive host) and various domestic animals intermediate hosts (Eckert and Deplazes, 2004). By affecting many animal species, intermediate animal hosts and humans, hydatid cyst causes tremendous economic losses worldwide and specially in those areas where the parasite is endemic (Urquhart et al., 1996).

Knowledge about the prevalence of the diseases together with associated risk factors as part of the epidemiology of the disease is crucial for any attempt of prevention and control of the disease in question. Moreover, determination of the economic significance of the disease is important for decision making, planning, and implementation of local control strategies. The present study were, therefore, conducted in the area with objective of determining the prevalence of Hydatidosis, its associated risk factor in cattle slaughtered at Jimma municipal abattoir and to estimate the economic significance of the disease in cattle.

II. Material and Method

a) Study area

The study was conducted in Jimma town which is located at about 352km south west of Addis Ababa. The area receives a bimodal rain fall with an average annual rain fall of 1530mm. The long rainy season occur during the months of June to September while the short rainy season occurs during the months of March to May. The climatic condition of the town is “Weynadega” and the town is located at an altitude of 1915masl. The annual maximum and minimum temperature ranges from 24-30°C and 7-14°C respectively. According to the statistical data obtained (CSA, 2009), Jimma district has a livestock population of 2,016,823 cattle, 288,411 goats, 942,908 sheep and 74574 horses, 49,489 donkey, 28,371 mules and 1,139,735 poultry.
b) Study animals

The study animals were local breeds of cattle coming from different wereda of the Jimma zone to Jimma municipal abattoir. Only male cattle and sheep were slaughtered, but majority of animals slaughtered in the abattoir were male cattle. The majority of slaughter animals came from seven weredas, this are Agaro, Asandabo, Bilida, Dedo, Jimma, Sarbo and seka. The body condition score was classified into poor, medium and good (fat), (Nicholson and Butter worth, 1986). The age was determined by dentition formula according to the method described by De Lahunuta and Habel (1986), and animals categorized into three age groups (< or = 5, 5 – 8, and > 8 years).

c) Sample size and sampling method

The study animals were selected from the slaughter line using simple random sampling technique. The required sample size was determined based on prevalence of 61% (Koskei, 1998) using the formula given by (Thrusfield, 2005). The study considered 95% confidence interval and 5% precision level. Accordingly a total of 384 animals were calculated, but to increase precision, the number of examine animals were reached to 400. For this study sex, age, origin and body condition of animals were considered as risk factors.

d) Study Design

A cross sectional study was conducted from November 2015 to June, 2016 by collecting data on events associated with hydatidosis in cattle slaughtered in Jimma municipal abattoir. This study was conducted to determine update information on the prevalence and economic impact on bovine hydatidosis at Jimma municipal abattoir. (Two slaughtering days per week) visits were made to abattoir.

e) Study Methodology

i. Ante mortem inspections

Pre-slaughter examinations of cattle were conducted in the lairage in order to determine the sex, age, body condition and origin of animals. Identification number was given for each animal to examine after evisceration. During ante-mortem examination, animals were clinically examined for any sign of illness while standing and moving according to (Urquhart et al., 1996). And following the judgments passed by (FAO, 1994).

ii. Post mortem inspection

During post mortem examination organs especially liver, lung, spleen, kidney and heart as a whole were systematically inspected for the presence of hydatid cyst by applying the routine meat inspection procedure of primary examination followed by secondary examination. The primary examination involves visualizations and palpation of organs, were as secondary examination involves further systemic incision of each organs into pieces and whenever evidence of hydatid cyst was found, it was classified as live or calcified and the cyst distribution into organs was recorded.

iii. Examination of cysts for fertility and viability

Based on the presence or absence of brood capsules containing protoscolices in hydatid fluid, cysts were identified and classified as fertile and infertile according to the method described by Macpherson (1985). Individual hydatid cysts were carefully incised and examined for protoscolices, which resembled white dots on the germinal epithelium; such cysts were characterized as fertile cysts.

Fertile cysts were subjected to viability test. A drop of the sediment containing the protoscolices were placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movements with 40x objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices in hydatid fluid on microscope slide with the principle that viable protoscolices should completely or partially exude the dye while the dead ones absorb it (Macpherson et al., 1985). Furthermore, infertile cysts were further classified as sterile or calcified. Sterile hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in their content. Typical calcified cysts produce a gritty-sound heard at incision (Soulby, 1982).

iv. Determination of Monetary losses due to hydatid cyst

An attempt was made to estimate the annual economic losses from hydatidosis in cattle taking into account losses from cost of organ condemnation and from carcass weight. The retail market price of average size offal (lung, liver, kidney, heart and spleen) and the cost of one kg beef were obtained from information gathered from local butchers. Annual economic loss due to organ condemnation was determined by considering annual slaughter rate of cattle and prevalence of hydatidosis per organ and an estimated 5% carcass weight loss (Getaw et al., 2010) was considered. Average carcass weight of Ethiopian local breed cattle is estimated as 108 kg (Negassa et al., 2010). The total economic loss was calculated as the summation of cost of offal condemned plus the cost of carcass weight losses (Kebede et al., 2009a; Getaw et al., 2010).

\[
LOC = (NAS \times ph \times plu \times cplu) + (NAS \times ph \times phr \times cphr) + (NAS \times ph \times pli \times cpli) + (NAS \times ph \times psp \times cpsp) + (NAS \times ph \times pkiid \times cpkid); \\
\]

Where NAS =Average number of cattle slaughtered annually
Loc-loss of organs condemned
Ph-prevalence rate of hydatidosis
Plu-percent involvement of lung
Cplu-current mean retail price of lung
Phr- percent involvement of heart  
Cphr- current mean retail price of heart  
Pli- percent involvement of liver  
Cpli - current mean retail price of liver  
Psp- percent involvement of spleen  
Cpsp - current mean retail price of spleen  
Pkid- percent involvement of kidney  
Pkid - current mean retail price of kidney  
N: B-All prices are determined from the price at Jimma town.

Total economic loss was evaluated by considering both loss from organ condemnation and loss from carcass weight loss. Total loss = direct loss (loss from organ condemnation) + indirect loss (loss from carcass weight loss).

Data Analysis and Management

The data obtained was coded in Microsoft excel sheet 2007 and subjected to descriptive statistics and chi-square in order to assess the magnitude of the difference of comparable variables using SPSS version 20.0 software. Statistically significant association between variables is considered to exist if the p-value is less than 0.05.

III. RESULTS

a) Prevalence and Risk Factors

i. Age group

Out of the total 400 heads of cattle slaughtered and examined, 218 (54.5%) were infected with hydatid cyst, more cysts involving different visceral organs (lung and liver). Rate of infection in different age groups (<5 and, 5-8 and >8 years) was assessed and described in (Table 1). Prevalence in age groups have shown as statistically highly significant variation (P<0.05, \( \chi^2 = 16.615 \)) with young group having higher infections.

ii. Body condition score

Prevalence was also assessed in terms of body condition score (Table 2). It was found that cattle having poor body condition had the highest prevalence (74%) followed by medium (46.6%) and good (52.5%). There was highly significant difference revealed between body condition scores (\( P<0.05, \chi^2=28.332 \)) with poor animals groups having higher infections.

iii. Origin of animals

Prevalence of Hydatidosis in cattle slaughtered at Jimma Municipal abattoir in origin of animals at Bilida (61%) was higher infected but, at Sarbo 43.5% was less infected (Table 3).

b) Cyst Distribution

Overall distribution of cysts in different organs of cattle slaughtered at Jimma Municipal abattoir was described (Table 4). Of the 223 cattle positive, 200 (89.7%) had cysts merely in lungs, 20 (8.9%) in liver, 1 (0.45%) in kidney, 1(0.45%) in spleen, whereas, the rest of 1(0.45%) in heart infections involved organs.

c) Characters of hydatid cyst in different organs

Out of 98 organ infected by cysts to tested for fertility, 50(17.3%) cysts of lung, 45(54.87%) cyst of liver, 1(100%) cysts of kidney,1(100%) cysts of spleen, and 1(100%) cysts of heart origins had protoscolices detected and hence, fertile. Out of the total cyst counts, 98(26.2%) cyst counts are fertile, 216(57.8%) are sterile and 60(16%) calcified. Fertility status of cysts from different organs has shown, but the cysts of lung origin being highly fertile (Table 5).

d) Estimated Monetary loss incurred by hydatidosis

Due to aesthetic value and to break the life cycle of the Echinococcus parasites infected organs are condemned. A total of lung, liver, kidney, spleen and heart were condemned due to hydatidosis with an economic loss of 89249.2ETB, 22312.3 ETB, 676.89 ETB, 225.63ETB and 676.89 ETB respectively. The direct and indirect economic loss was about 133140.91 ETB and 3249072 ETB respectively. The total annual financial loss due to bovine hydatidosis was estimated to be 3362212.9 ETB, (Table 6).

Table 1: Prevalence of hydatidosis in different age groups of cattle slaughtered at Jimma Municipal abattoir

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number of cattle examined</th>
<th>Infected</th>
<th>Infected Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (&lt; 5years (young))</td>
<td>38</td>
<td>23</td>
<td>62.2%</td>
</tr>
<tr>
<td>Group 2 (5-8 years (adult))</td>
<td>303</td>
<td>159</td>
<td>52.3%</td>
</tr>
<tr>
<td>Group3 (&gt;8years (old))</td>
<td>39</td>
<td>36</td>
<td>61%</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>218</td>
<td>54.5%</td>
</tr>
</tbody>
</table>

\( \chi^2 = 16.615, P = 0.034 \)
Table 2: Prevalence of Hydatidosis in cattle slaughtered at Jimma Municipal abattoir on body condition basis

<table>
<thead>
<tr>
<th>Body condition score</th>
<th>Animals Examined</th>
<th>Infected</th>
<th>Prevalence</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>77</td>
<td>57</td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>146</td>
<td>68</td>
<td>46.6%</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>177</td>
<td>93</td>
<td>52.5%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>218</td>
<td>54.5%</td>
<td></td>
</tr>
</tbody>
</table>

χ² = 28.332, P = 0.00

Table 3: Prevalence of Hydatidosis in cattle slaughtered at Jimma Municipal abattoir on origin of animals

<table>
<thead>
<tr>
<th>Origin of animals</th>
<th>Number of examined</th>
<th>Number of infected</th>
<th>Total % of infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaro</td>
<td>48</td>
<td>28</td>
<td>58%</td>
</tr>
<tr>
<td>Asandabo</td>
<td>45</td>
<td>26</td>
<td>57%</td>
</tr>
<tr>
<td>Bilida</td>
<td>94</td>
<td>58</td>
<td>61%</td>
</tr>
<tr>
<td>Dedo</td>
<td>59</td>
<td>30</td>
<td>50%</td>
</tr>
<tr>
<td>Jimma</td>
<td>48</td>
<td>25</td>
<td>52%</td>
</tr>
<tr>
<td>Sarbo</td>
<td>79</td>
<td>35</td>
<td>43.5%</td>
</tr>
<tr>
<td>Seka</td>
<td>28</td>
<td>16</td>
<td>56.5%</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>218</td>
<td>54.5%</td>
</tr>
</tbody>
</table>

χ² = 64.742, p = 0.00

Table 4: Distribution of Hydatid cysts in different organs of positive cattle at Jimma Municipal abattoir

<table>
<thead>
<tr>
<th>Organs affected</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung only</td>
<td>200</td>
<td>89.7%</td>
</tr>
<tr>
<td>Liver only</td>
<td>20</td>
<td>8.9%</td>
</tr>
<tr>
<td>Kidney only</td>
<td>1</td>
<td>0.45%</td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
<td>0.45%</td>
</tr>
<tr>
<td>Heart</td>
<td>1</td>
<td>0.45%</td>
</tr>
<tr>
<td>Total</td>
<td>223</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 5: Fertility, sterility and viable of cysts collected from different organs of cattle slaughtered at Jimma Municipal abattoir

<table>
<thead>
<tr>
<th>Organ</th>
<th>Fertile cyst (%)</th>
<th>Sterile cyst (%)</th>
<th>Calcified (%)</th>
<th>Total cyst counts%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>50(17.3)</td>
<td>180(62.28)</td>
<td>59 (20.4)</td>
<td>289(77.27)</td>
</tr>
<tr>
<td>Liver</td>
<td>45(54.87)</td>
<td>36(43.9)</td>
<td>1(1.2)</td>
<td>82(21.9)</td>
</tr>
<tr>
<td>Kidney</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
<td>1(0.26)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
<td>1(0.26)</td>
</tr>
<tr>
<td>Heart</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
<td>1(0.26)</td>
</tr>
<tr>
<td>Total</td>
<td>98(20)</td>
<td>216(57.75)</td>
<td>60(16)</td>
<td>374(100)</td>
</tr>
</tbody>
</table>

Table 6: Direct economic losses associated with CE in infected cattle in Jimma municipal abattoir. organs condemned and their price in ETB during study period

<table>
<thead>
<tr>
<th>Organs</th>
<th>No.of organs condemned</th>
<th>% of condemned</th>
<th>price per organs</th>
<th>Total price in ETB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>200</td>
<td>89</td>
<td>20</td>
<td>4000</td>
</tr>
<tr>
<td>Liver</td>
<td>20</td>
<td>8.9</td>
<td>50</td>
<td>1000</td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td>0.45</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
<td>0.45</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Heart</td>
<td>1</td>
<td>0.45</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>223</td>
<td>100</td>
<td>140</td>
<td>5070</td>
</tr>
</tbody>
</table>

Direct economic loss from loss of organs condemned=NAS X ph [(plu x cplu) +(pli x cpli) +(psp x cpsp) +(phr x cphr) + (pkid x cpkid)].

NAS=Number of animals slaughtered annually in Jimma municipal abattoir were=9600
In the present study the prevalence of Bovine hydatidosis in Jimma Municipal abattoir was found to be 54.5% which was comparable with the results of other works conducted, this study was much higher compared to the prevalence reported at Jimma 31.44% (Tolossa et al., 2009) and 22.4% (Moges, 2003), Konso 22.57% (Fikre, 1994), Adigrat 20.3% (Kebede, et al., 2009b) and Nekemte 31.19% (Feyissa, 1987). Much lower prevalence was also reported by Kebede, 2009b (7.5%) in Shire and Tsehaye, 1995 (7.2%) in Debre Birhan and also high in Asella 61.0% (Koskei, 1998), 62.96% around Bale Robe (Woubet, 1988), and 59.9% Bahirdar (Nebiyou, 1990).

The present prevalence rate was high (54.5%). This might be due to the abundance and frequent contact between the intermediate and infected final hosts. It could also be associated to slaughtering of aged cattle which have had considerable chance of exposure to the parasitic ova, backyard slaughtering of small ruminants and provision of infected offal’s to pet animals around homesteads. Moreover, poor public awareness about the disease and presence of few slaughter houses could have contributed to such a higher prevalence rate.

Generally, variation among the prevalence of hydatidosis at different geographical location could be associated to the strain difference of *Echinococcus granulosus* that exist in different geographical locations (McManus, 2006). Additionally variation could be with age factors of the animals and other factors like difference in culture, socio-economical activities and attitudes to dogs and their population. Similar to the present finding, it was reported that cystic Echinococcosis infection was higher for older animals (Azlaf & Dakkak, 2006; Faysa et al., 2010). Animals with more than eight years of age were found to be highly infected that stastically significant (P value < 0.05). This could be mainly due to the fact that aged animals have longer exposure time to *Echinococcus granulosus* eggs. In addition, older animals might have weaker immunity to combat against infection (Himonas, 1987). This finding is similar to the finding of Fikre Lobago (1994), Hagos Yihdego (1997), Umur (2003), Azlaf and Dakkak (2006) and (Esatgil and Tuzer, 2007).

The prevalence of hydatidosis by origin of slaughtered cattle was assessed and statistically significant difference (P value < 0.05) was found indicating that geographical regions play an important role in distribution of the cysts. This could be due to the difference in the socio-economic status and animal husbandry practices of community in all areas from where animals were brought for slaughter and frequent contact of animals with infected definite host.

The prevalence of hydatidosis among different organs involved in harboring of the cyst showed that lung was found to be the most commonly affected organ (50%) followed by liver (43%) and this was equivalent with Bizuwork (2013), 50.5% for lung and 40.6% for liver and also similar result of 54.5% and 43.5% was reported by Debas and Ibrahim (2013) on lung and liver respectively. This finding was higher than finding of Abunna et al., (2012) who reports 12.5% and 4.25% prevalence for lung and liver respectively while 92.7% in lung and 53.2% in liver which is higher than this study was also reported by Abera et al., (2013). In this study number of cysts collected from lung 200(89.7%) was greater than that collected from liver 61(8.9%) and that of spleen, heart and kidney in which 1(0.45%) was recorded. Comparable results were reported by (Alemu and Yitagele, 2013), 47.04% and 44.2% for lung and liver respectively and 9.41% for spleen, heart and kidney. This might be due to the fact that cattle are slaughtered at older age, during the time the liver capillaries are dilated and most oncospheres directly pass to the lung; additionally, it is possible for the hexacanth embryo to enter the lymphatic circulation.
and be carried via the thoracic duct to the heart and lungs in such a way that the lungs may be infected before or instead of liver (Arene, 1985).

Additionally, the lung and liver which are most commonly infected organs, this could be due to the fact that lungs and livers possess the first great capillaries of sites encountered by migrating Echinococcus onchosphere (hexacanth embryo) which adopt the portal vein route the first large capillaries encountered by migrating blood borne onchospheres and primarily negotiate pulmonary and hepatic filtering system sequentially before any other organ is involved. However, development of hydatid cysts occur occasionally in other organs like spleen, kidney and heart and other organs and tissues when onchosphers escaped into general systemic circulation (Urquhart et al., 1987).

Lung harbored highest number of calcified cysts (20.4%) followed by liver (1.2%). This finding is comparable with finding of Mesele et al. (2013) 60.2% , 22.6% and 4.3% respectively for lung, liver and spleen and higher than the report of Bizuwork et al. (2013) with the prevalence’s of 36.8% for lung, 14.6% for liver and 0% for spleen. This can be due to the host defence mechanisms of killing more efficiently the parasitic larvae at the early stage of development (Himonas, 1987).

The percentage of fertile cysts in this study was 26.2%. This is higher compared to the fertility rate of 26.9%, 24.4% and 19.3% reported by Faye et al. (2010), Solomon (2011) and Zelalem (2008) respectively from different parts of Ethiopia. But the present study was quite lower compared to the 96.9% reported from South Africa (Arene, 1985). Yet much lower fertility record such as 1.76%, 9.85% and 6.2% were reported in cattle from Wolayita Soddo (Nigatu, et al., 2009), Nekemt (Bersissa, 1994) and Bahir Dar (Nebiyou,1990) respectively. The variation in fertility rates among different species and in different geographical Zones could be due to differences in strain of Echinococcus granulosus (McManus, 2006). Strain of the parasite and the host can modify the infective pattern of the parasite (Gemmell et al., 2002).

Comparison of fertile cyst from different organs was found to be lower for lung (17.3%) than liver (54.87%). This finding was agreement with finding of (Debele et al., 2014) 66.7 and 40.7% for lung and liver respectively and the present finding was higher than Debas and Ibrahim (2013) 25% and 7.5% for lung and liver respectively.

Out of a total 400 cattle carcasses, 218 (54.5%) were infected with hydatid cysts. Of these cysts 98 (20%) fertile, 216 (57.75%) sterile and 60 (16%) were calcified. Higher infected when compared with (Tolossa et al., 2009) in Jimma out of a total 512 cattle, 161 (31.44%) were infected with hydatid cysts. a total of 1171 hydatid cysts being collected from the infected animals. Of these cysts, 223 (19.4%) were fertile, 505 (43.13%) were sterile and 349 (29.80%) calcified. These indicate that cattle are an important intermediate host for the perpetuation of the life cycle of the parasite in Jimma and its surroundings.

The annual economic loss incurred by hydatidosis was calculated to be 3362212.9 ETB. The result was relatively comparable with the report of (Zelalem, 2008) 5,544,591.74 ETB in Addis Ababa abattoir and lower than that of the Terefe et al. (2012) 19,847,704.5ETB at Addis Ababa abattoir enterprise and higher than that of Belina et al. (2015) and Zewdu et al. (2010) with annual economic loss of 841,419.3 and 160,032.23 respectively. The economic losses was different from the reports of others studies in the country which may be due to the variation in prevalence of the disease and mean annual number of cattle slaughtered in different Abattoirs and variation in retail market price of organs (Polydorus, 1981).

As described above Hydatid disease is generally considered to be a rural disease because of its way of transmission cycle, which involves domestic herbivorous animals (cattle, sheep, pigs and so on) and dogs. However, it is possible that urban residents may have been in contact with Echinococcus granulosus eggs, in this matter backyard slaughtering and inappropriate disposal affected organs plays major role for the continuity of parasite life cycle.

V. Conclusion

The overall prevalence observed in the study indicated relatively high and an important zoonotic disease in the area and this could be due to several factors of which keeping dogs in close association with cattle. Hydatidosis also causes substantial visible and invisible economic losses in cattle of the study area as a result of condemnation of edible offal and carcass weight loss. The most preferred predilection sites of hydatid cyst in cattle like liver, kidney, heart and lungs and condemnations of these important organs having a single or multiple hydatid foci is really a huge loss. From the result obtained in the present study and considering the reality in Jimma municipal abattoir and its surrounding, it is mandatory for launching a control program proper disposal of affected offal’s freely for dogs and wild canids (the usual practice in the community) should be stopped and all the condemned organs should be either buried or incinerated. Moreover, further studies are needed on genotyping, epidemiology and public health importance of Echinococcus granulosus in the study area.

VI. Acknowledgments

The authors acknowledge Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) for financing the project in the period from November 2016 to June 2016.
References Références Referencias

47. Trop., 113: 26-33.
FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (FARSM)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards “FARSM” title to individuals. The ‘FARSM’ title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

The “FARSM” is a dignified title which is accorded to a person’s name viz. Dr. John E. HallPh.D., FARSS or William Walldroff, M.S., FARSM.

FARSM accrediting is an honor. It authenticates your research activities. After recognition as FARSM, you can add ‘FARSM’ title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSM designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.
The FARSM can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSM, you will be given a renowned, secure and free professional email address with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.

The FARSM will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSM member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSM, you may send us a scanned copy of all of you credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSM members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSM member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.
The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

The FARSM member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

The 'MARSM' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The “MARSM” is a dignified ornament which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., MARSM or William Walldroff, M.S., MARSM.

MARSM accrediting is an honor. It authenticates your research activities. After becoming MARSM, you can add 'MARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefits can be availed by you only for next three years from the date of certification.

MARSM designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSM, you will be given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.

© Copyright by Global Journals Inc. (US) | Guidelines Handbook
We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.
Auxiliary Memberships

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as “Institutional Fellow of Open Association of Research Society” (IFOARS).

The “FARSC” is a dignified title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as “Institutional Board of Open Association of Research Society”-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA)

The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of “Open Association of Research Society, U.S.A (OARS)” so that proper amendment can take place for the benefit of entire research community.

We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.
We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as “Institutional Fellow” and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf. The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals: Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.
In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10% discount if one is co-author or main author among group of authors.

- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3 yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA).
- This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in-depth understanding of the application of suitable techniques to a particular area of research practice.

**Note:**

In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.

In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.

In case of “Difference of Opinion [if any]” among the Board members, our decision will be final and binding to everyone.
The Area or field of specialization may or may not be of any category as mentioned in ‘Scope of Journal’ menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as ‘Knowledge Abstract’ at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of ‘Author Guideline’ Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC, *.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission. Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click ‘Submit Manuscript’. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not convenient, and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.
PREFERRED AUTHOR GUIDELINES

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, “Abstract” word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.

Author Guidelines:

1. General,
2. Ethical Guidelines,
3. Submission of Manuscripts,
4. Manuscript’s Category,
5. Structure and Format of Manuscript,
6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global
Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission.

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.
2) Drafting the paper and revising it critically regarding important academic content.
3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board’s decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author’s responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author’s responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.
To avoid postal delays, all transactions are preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT’S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5. STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also. Preparation of research paper or how to write research paper, are major hurdles, while writing a manuscript. The research articles and research letters should be fewer than three thousand words, the structure of original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a) Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, “Abstract” (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper’s subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.
The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

**Format**

*Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.*

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10⁻³ m³, or 4 mm somewhat than 4 × 10⁻³ m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

**Structure**

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

*Abstract, used in Original Papers and Reviews:*

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art. A few tips for deciding as strategically as possible about keyword search:
• One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.

• It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.

• One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author’s name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.
Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded (Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors’ final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher’s terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org.
Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. **Choosing the topic**: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourse to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be “Yes” then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. **Evaluators are human**: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. **Think Like Evaluators**: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. **Make blueprints of paper**: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. **Ask your Guides**: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can’t clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. **Use of computer is recommended**: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. **Use right software**: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. **Use the Internet for help**: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. **Use and get big pictures**: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. **Bookmarks are useful**: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. **Revise what you wrote**: When you write anything, always read it, summarize it and then finalize it.
12. **Make all efforts**: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. **Have backups**: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. **Produce good diagrams of your own**: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating “hotchpotch.” So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. **Use of direct quotes**: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. **Use proper verb tense**: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. **Never use online paper**: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. **Pick a good study spot**: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. **Know what you know**: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. **Use good quality grammar**: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straightforward. put together a neat summary.

21. **Arrangement of information**: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. **Never start in last minute**: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. **Multitasking in research is not good**: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. **Never copy others’ work**: Never copy others’ work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. **Take proper rest and food**: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. **Go for seminars**: Attend seminars if the topic is relevant to your research area. Utilize all your resources.
27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren’t essential and shouldn’t be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

XVII
Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

- Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

- Use standard writing style including articles ("a", "the," etc.)
- Keep on paying attention on the research topic of the paper
- Use paragraphs to split each significant point (excluding for the abstract)
- Align the primary line of each section
- Present your points in sound order
- Use present tense to report well accepted
- Use past tense to describe specific results
- Shun familiar wording, don’t address the reviewer directly, and don’t use slang, slang language, or superlatives
- Shun use of extra pictures - include only those figures essential to presenting results

**Title Page:**

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address(es) of all authors.
Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript—must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The Introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook
principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:
- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:
- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that’s all.

Approach:
- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer’s interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from
- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently. You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.
Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report.
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts.
- Despite of position, each figure must be numbered one after the other and complete with subtitle.
- In spite of position, each table must be titled, numbered one after the other and complete with heading.
- All figure and table must be adequately complete that it could situate on its own, divide from text.

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information.
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook
The Administration Rules

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

**Segment Draft and Final Research Paper:** You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else’s analysis.

- Do not give permission to anyone else to “PROOFREAD” your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.

- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.
Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

<table>
<thead>
<tr>
<th>Topics</th>
<th>Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-B</td>
</tr>
<tr>
<td></td>
<td>C-D</td>
</tr>
<tr>
<td></td>
<td>E-F</td>
</tr>
<tr>
<td>Abstract</td>
<td>Clear and concise with appropriate content, Correct format. 200 words or below</td>
</tr>
<tr>
<td></td>
<td>Above 200 words</td>
</tr>
<tr>
<td>Introduction</td>
<td>Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited</td>
</tr>
<tr>
<td>Methods and Procedures</td>
<td>Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads</td>
</tr>
<tr>
<td>Result</td>
<td>Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake</td>
</tr>
<tr>
<td>Discussion</td>
<td>Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph, reference cited</td>
</tr>
<tr>
<td>References</td>
<td>Complete and correct format, well organized</td>
</tr>
</tbody>
</table>
# Index

## A

- Artiodactyla · 1
- Aspergillus · 16

## B

- Bahirdar · 2
- Benzidine · 4
- Bizuwork · 2, 3

## C

- Capsulatum · 12
- Cestodes · 1, 4
- Chromogen · 4
- Collignon · 4, 8, 9, 10

## D

- Dodecandra · 13, 17
- Dromedarius · 18

## E

- Echinococcus · 1, 3, 2, 3, 4

## F

- Farciminosum · 12

## H

- Hippoboscid · 19
- Hydatidosis · 1, 2, 3, 2, 3, 4, 5

## I

- Icosahedral · 1

## N

- Nekemte · 2, 3, 4
- Neubauer · 19

## P

- Perissodactyla · 1
- Picornaviridae · 1

## S

- Slaughtered · 18, 21, 23, 24, 1, 3, 4, 5
- Strumarium · 12

## T

- Tetramethylrhodamine · 4
- Trypanosoma · 18, 19, 20, 22, 23
- Trypanosomosis · 18, 21, 22

## Y

- Yitagele · 2, 3