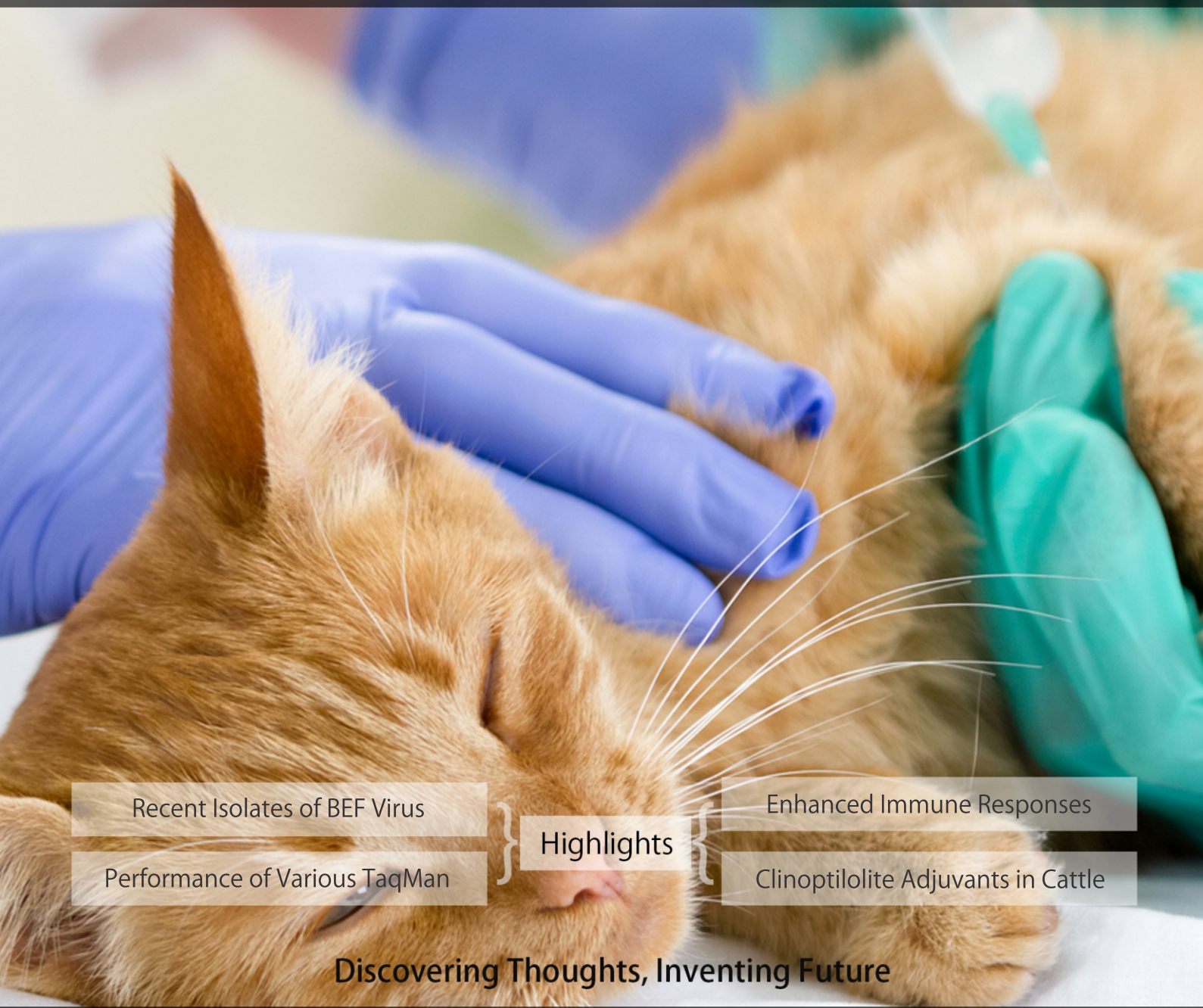


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VOLUME 18 ISSUE 2 (VER. 1.0)

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GLOBAL JOURNAL OF MEDICAL RESEARCH: G  
VETERINARY SCIENCE AND VETERINARY MEDICINE  
Volume 18 Issue 2 Version 1.0 Year 2018  
Type: Double Blind Peer Reviewed International Research Journal  
Publisher: Global Journals  
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Performance of Various TaqMan and SYBRGreen rRT-PCR Methods in Detecting the Egyptian Foot-and-Mouth Disease Viruses

By Hany I Abu-Elnaga, Sonia A Rizk & Akram Z Hegazy  
*Veterinary Serum and Vaccine Research Institute (VSVRI)*

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**Keywords:** *foot-and-mouth disease virus, rRT-PCR, taqman.*

**GJMR-G Classification:** *NLMC Code: WC 900*



PERFORMANCE OF VARIOUS TAQMAN AND SYBRGREEN RT-PCR METHODS IN DETECTING THE EGYPTIAN FOOT-AND-MOUTH DISEASE VIRUSES

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# Performance of Various TaqMan and SYBR Green rRT-PCR Methods in Detecting the Egyptian Foot-and-Mouth Disease Viruses

Hany I Abu-Elnaga <sup>α</sup>, Sonia A Rizk <sup>σ</sup> & Akram Z Hegazy <sup>ρ</sup>

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**Keywords:** *foot-and-mouth disease virus, RRT-PCR, taqman.*

## I. INTRODUCTION

Foot-and-mouth disease virus (FMDV) is an ancient contagious particle as cited by Fracastorius (1478-1553) of Verona in 1514 in his classical treatise on contagions where he pointed out to the virus symptoms in oxen in Italy with pustules in the oral cavity and gradually the disease descended to the shoulders and thence to the feet (Greenwood, 1927). The virus has a linear single-stranded RNA genome. Disease is one of the most infectious diseases known in part due to rapid and massive viral replication in the host and considerable genetic variability (Tam et al., 2009 and Othman et al., 2018).

Developing countries face usually challenge against transboundary disease specially foot-and mouth-disease due to less strict measures implemented at borders, besides less sanitary condition and precaution in the quarantine areas. Thus, rapid differentiation of FMDV is imperative to take effective counter measures to control the spread of the disease. Nowadays, molecular assay, especially, real-time RT-PCR is considered a trend of fast and accurate discrimination of FMDV with reduced post-PCR processing steps.

Numbers of fluorescent and fluorophore formats were performed. Draw back was noticed such as false

Negative results, where it was cited that 5' UTR probe assay greater sensitivity in the detection of type a isolates while 3Dpol target assay greater sensitivity in the detection of FMD SAT isolates (Calhan et al. 2002; Reid et al., 2002; Tam et al., 2009). The false result will give inaccurate data about the disease spreading status, which will result in more animals infected and losses affecting the economic welfare especially of the poor peasants in the low annual income countries that posse's individual animals breeding and not mass herd farms.

Accordingly, the study of Taqman probe as an advanced tool for precise detection of FMDV is an alternative to SYBR Green real-time RT-PCR (RRT-PCR) format in terms of sensitivity and specificity. The fast and accurate discrimination of FMD virus is imperative for proficient containment and eradication of the disease. Therefore, the article was designed for inspection the efficiency of Taqman probe RRT-PCR in the accurate determination of FMDV. Besides, examination of two different RRT-PCR formats depending on fluorophore and fluorescent dye for sensitive and specific detection of the virus.

## II. MATERIALS AND METHODS

### a) *Viruses and samples*

An archived vaccine strains viz. O/EGY/2009 iso1 (propagated on cell culture), A/EGY/2009 iso-Cai (clinical suspension) and SAT2/EGY/H1Ghb/2012, local strains of serotypes O, A and SAT2, respectively (Abu-Elnaga, 2011; EL-She hawy et al., 2011 and 2014) were used. They were exploited as the starting material for RNA extraction to optimize the real-time RT-PCR (RRT-PCR) protocols, as well as reflect the specific ability of the primers and probe to detect different FMDV serotypes found in Egypt. Other FMD viruses representing the former serotypes, previously identified by RT-PCR assay and some of them type confirmed by nucleotide sequencing were used as unknown samples in the validity of the RRT-PCR assays. These FMD viruses were clinical and culture grown isolates, O ( $n=10$ ), A ( $n=4$ ) and SAT2 ( $n=2$ ). Furthermore, Bovine Viral Diarrhea (BVD), belonging to the family *Flaviviridae*, is one of the endemic viruses in Egypt that cause mucosal disease with excessive drool and

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lameness in chronic infection was fortunately obtained and passed versus FMDV designed specific primers and probe.

#### b) RNA Extraction and Analytic Sensitivity

RNA was extracted using the QIAamp® Viral RNA kit (Qiagen, Germany) according to the manufacturer's protocol. Extracted RNAs from three FMDV O were quantitated by ultraviolet (UV) spectrophotometer and used as; in-home Standard (2017 identified virus), Positive control 1 (2012 identified virus) and Positive control 2 (2009 identified virus). Two spectrophotometer instruments were used, one was classic spectrophotometer (Milton Roy 601 Spectronic 335104, USA) and the other was recent spectrophotometer (Nano Drop 2000c Spectrophotometer, Thermo Fisher Scientific, and USA). In the context of the standard, it was diluted seven times in a serial of 10-fold in RNase-free water to obtain the Standard RNA Dilutions (SRD). Archived Stock viruses (SV) RNAs (Azab et al., 2012) were exploited in the current study to evaluate the performance of RRT-PCR assays on RNAs with long storage period. Briefly, ten-fold serial dilutions of stock virus (SV) in minimum essential medium (MEM) with Hank's salts in the range of  $10^{-1}$ - $10^{-8}$  were performed. Each dilution was exposed to RNA isolation procedures to prepare SV RNAs. Analytic sensitivity was applied on the former different RNAs preparation formats termed SRD and SV RNA. Negative controls involved: no template control (NTC) which was RNase free water; negative control 1 that was RNA from healthy BHK cells; and negative control 2 that was RNA from non-infected BHK cells showed contamination.

#### c) Real-time RT-PCR

All the extracted RNAs were tested on a fluorometric thermal cycler (the acquired Corbett Research, Sydney, Australia) using either Quantities Probe RT-PCR Kit or QuantiTect SYBR Green RT-PCR Kit (Qiagen, Germany). The 3D Forward primer: ACTGG-GTTTT-ACAAA-CCTGT-GA; Reverse primer: GCGAG-TCCTG-CCACG-GA and labeled Taqman probe: TCCTT-TGCAC-GCCGT-GGGAC (Callahan et al., 2002 and OIE, 2017) was used in Taqman RRT-PCR method. The Taqman probes were labeled with a 5'-reporter dye, 6-carboxyfluorescein and a 3'-quencher, tetramer thylrhodamine (5'-FAM-3'-TAMRA). The forward/reverse primers were purchased from Bioneer, Korea; while, the Probe was designed by Metabion, Germany. Another primer pair PoR/PoF (Shin et al., 2003), derived from the virus 3D polymerase were used in SYBR Green RRT-PCR runs. PoF (5'-CCT ATG AGA ACA AGC GCA TC -3') and PoR (5'-CAA CTT CTC CTG TAT GGT CC -3') was synthesized by Metabion (Germany) and involved in SYBR Green RRT-PCR protocol. For Fluorogenic RRT-PCR, cycling conditions were: 1 cycle at 50 C for 30

min, 1 cycle at 95 C for 15 min and 45 cycles at 95 C for 15 s and 60 C for 1 min. For the Fluorescence RRT-PCR, the optimized reaction also contained 5% of the reaction volume RNA template and 0.6  $\mu$ M from each primer. The cycling parameters were 50 C for 30 min and 95 C for 15 min; then 45 cycles consisting of 94 C for 30 s, 55 C for 30 s and 72 C for 30 s. At least one of the positive controls and negative controls was involved in every run.

### III. RESULTS

RNA concentration of the standard, its dilutions, and positive controls were determined by spectrophotometer using both two devices, the traditional one depend on dilution 1/100 of the measured RNA samples in 200  $\mu$ l quartz cuvette (Milton Roy 601 Spectronic) and the modern one without any dilution depending on the whole volume of 2  $\mu$ l (Nano Drop 2000c Spectrophotometer). The nucleic acid concentration readings for the Standard, Positive Control 1 and Positive Control 2 were 6, 4.8 and 5.75 ng/ $\mu$ l, respectively; while the dilutions gave minus reading (Table 1).

The specificity of the primers/probe for detection of different Egyptian FMDV serotypes was achieved. Besides, negative controls gave neither threshold cycle ( $C_T$ ) values nor PCR positive band. However, when specificity assay was repeated to confirm the former result, the same conclusion was obtained, but for negative controls, something was changed. In detection limit run, negative controls gave higher  $C_T$  values around and above the cut off ( $\geq 40$ ), in addition to, no or very difficult seen bands on agarose gel electrophoresis (Fig. 1 and 2). Sensitivity assay of the Taqman and SYBR green methods illustrated the range of RNA detection reached six hundred attograms (ag) RNA per microliter ( $\mu$ l) for Taqman, while failed to achieve the previous value in using SYBR Green. Using Taqman Probe, the standard virus and its serially 10-fold dilutions showed a 3.3-3.7 increment increasing of  $C_T$  values between undiluted virus until its  $10^{-5}$  dilution (undiluted & 5 dilution series), whereas, there was a lull in the  $C_T$  values at dilutions  $10^{-6}$  and  $10^{-7}$ . Likewise, using SYBR Green protocol, the standard virus and its serially 10-fold dilutions gave a 3.3-4.4 increment variations of  $C_T$  values between undiluted virus until its  $10^{-5}$  dilution (undiluted & 5 dilution series). Furthermore, dilution  $10^{-6}$  recorded a 2.6 increment from the previous one, while dilution  $10^{-7}$  did not produce  $C_T$  value.

Investigation, the archived stock virus (SV) RNA, fluorogenic and fluorescent signals with positive results were obtained (Fig. 3 and 4). Signals  $C_T$  values were in direct proportion to dilutions from  $10^{-1}$  to  $10^{-6}$  using Taqman method, while from  $10^{-1}$  to  $10^{-5}$  using the SYBR Green assay. Furthermore, the detection limit extended to  $10^{-8}$  for the probe and  $10^{-7}$  for the SYBR methods. Melt curve peak ( $T_m$ ) illustrated the specific amplification

giving the expected peak, affirmed by yielding the fragment size on agarose-based electrophoresis. The negative samples controls did not exhibit the anticipated specific former  $T_m$  of the primers with either no or border  $C_T$  values around the concluded negative cut off, 40.

#### IV. DISCUSSION

Two different operators measured the nucleic acid concentrations using the traditional Milton Roy 601 Spectronic on three successive days, whereas, one operator quantified them using the modern NanoDrop 2000c Spectrophotometer at the same day. The accuracy and sensitivity of the NanoDrop were better; specifically it was read in ISO certified lab. Thus, its read was the dependable in the current article. A point of interest, during evaluation the sensitivity of the former instruments (especially for the NanoDrop) in quantification the standard RNA dilutions, were unreliable read beyond  $1\text{ng}/\mu\text{l}$  and plateau results in dilutions  $10^{-1}$ - $10^{-7}$ . To investigate the reason of limitations of the NanoDrop spectrophotometer to give a trustable reading in the 10-fold serial dilutions, its manual was revised, and its specification illustrated that its detection limit is two  $\text{ng}/\mu\text{L}$  dsDNA.

Taqman probe was specific to FMD viruses as revealed by the test carried versus FMD viruses from each virus serotype found in Egypt. Besides no cross-amplification was occurred with one of FMDV, mucosal disease, bovine viral diarrhea, in Egypt. However, in repeating the sample on the agar gel electrophoresis, a very faint band appeared from Taqman assay as well as an obvious weak band was shown from SYBR Green test. Nevertheless, when analysis the Quantitative values obtained from the former two RRT-PCR methods, the concentration value of BVD RNA from fluorescent emission was  $2.4\text{ fg}/\mu\text{l}$ , while from fluorogenic signal was two  $\text{Fg}/\mu\text{l}$ , in addition to,  $C_T$  values were 37 and 40, respectively. The RRT-PCR value results were somewhat acceptable because it illustrated a bare detection of BVD.

Six-years before the current assay, detection limit of the SYBR Green RRT-PCR PCR for the stored Stock virus (SV) RNA was extended to  $10^{-7}$  dilutions using 20% RNA template/rxn volume (Azab et al., 2012). In context, the current article achieved the previous result for SV RNA detection that extended to dilution  $10^{-7}$  using SYBR methods, while over passed to dilution  $10^{-8}$  using Taqman method, taking in consideration that the RNA template input in this paper was 4x lesser. This result was very satisfactory on a long-term preserved RNA. The stored SV RNA for 6-years in fridge was exploited in the current study to evaluate the performance of a long period storage on RNAs in  $-20\text{ C}$  fridge, which is an economical price household appliance, in comparison to storage at  $-70\text{C}$  using a

relatively expensive price fridge. Fortunately, the following was applied to minimize the degradation of the nucleic acid stored at  $-20\text{ C}$ . Fridge had a well-arranged cabinet to allow empty aeration spaces. The cabinet door cautiously handled with rapid open/close action, usually ranged between 15-60 seconds, to avoid the escape of the chilled air. The temperature usually ranging between  $-18$  to  $-22\text{ C}$ , according to the year season. Also, the former fridge exposed to pause periods ( $\sim 15$  to  $30\text{ min}$ ) of the Freon freezing cycle along the day that downsize the temp to  $-7$  to  $-13\text{ C}$  due to the effect of the pre-programmed switch of built-in timer and not the voluntarily switched thermostat.

Lastly, Taqman based probe/primer overcame SYBR Green- based primer RRT-PCR by 10-fold for detecting less FMDV nucleic acid. In contrary, early lower  $C_T$  values with fewer nucleic acid copies biased to the fluorescent RRT-PCR with 3-4 cycles fewer. The Taqman method had better sensitivity and specificity, while SYBR Green method displayed less detection limit and precision. Higher  $C_T$  values with negative control pose in future to try or search for other probes or another fluorogenic chemistry that might give no or negligible  $C_T$  value with any negative control to improve the performance of detection avoiding any diminished cross amplification that might occur.

#### ACKNOWLEDGMENTS

Prof. Dr. Laila EL-Shehawy, as well as, Science and Technology Development Fund (STDF), [www.STDF.org.egy](http://www.STDF.org.egy), in addition to, the Egyptian Veterinary Serum and Vaccine Research Institute (VSVRI) for funding the research.

*Conflict of Interest:* The authors declare that they have no conflict of interest.

#### REFERENCES RÉFÉRENCES REFERENCIAS

1. Abu-Elnaga HI (2011) Single tube RT-PCR for simultaneous differentiation of foot-and-mouth disease virus O and An isolated in 2009 in Egypt. *Egyptian J. Virol.* 8: 30 – 5.
2. Azab AM, Abu-Elnaga HI, Zakria A (2012) Detection of foot-and-mouth disease virus (FMDV) by RRT-PCR in Egypt. *Arab journal of biotechnology* 15 (1): 61-70.
3. Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM (2002) Use of a portable RRT-PCR assay for rapid detection of FMDV. *J. Am. Vet. Med. Assoc.* 220, 1636-1642.
4. EL-Shehawy L, Abu-Elnaga H, Rizk S, Abd El-Kreem A, Mohamed A, Fawzy H (2014) Molecular Differentiation and Phylogenetic analysis of the Egyptian foot-and-mouth disease virus SAT2. *Arch Virol* 159:437-443.



5. Greenwood M (1927) some epidemiological observations on foot-and-mouth disease, with special reference to the recent experience of Holland. *Journal of Hygiene*, 26: 465-48.
6. Laila EL-Shehawy, Abu-Elnaga H, Talat A, El-Garf E, Zakria A, Azab A (2011) A nucleotide sequencing of foot-and-mouth disease virus Egyptian strains. *Journal of American Science* 7(7): 430-435.
7. OIE (2017) Foot and mouth disease. In: OIE Standards Commission, *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Office International des Épizooties, Paris, France (Chap. 2.1.8).
8. Othman O, Khodary M, El-Deeb A, Hussein H (2018) Five BoLA-DRB3 genotypes detected in Egyptian buffalo infected with Foot and Mouth

- disease virus serotype O. *Journal of Genetic Engineering and Biotechnology* (in press).
9. Reid SM, Ferris NP, Hutchings GH, Zhang Z, Belsham GJ, Alexandersen S (2002) Detection of all seven serotypes of FMDV by real-time, fluorogenic RT-PCR assay. *J. Virol Methods* 105, 67–80.
10. Shin JH, Sohn HJ, Choi KS, Kwon BJ, Choi CU, Kim JH, Hwang EK, Park J H, Kim JY, Choi SH, Kim OK (2003) Identification and isolation of foot-and-mouth disease virus from primary suspect cases in Korea in 2000. *J Vet Med Sci* 65: 1-7.
11. Tam S, Clavijo A, Engelhard EK, Thurmond MC (2009) Fluorescence-based multiplex real-time RT-PCR arrays for the detection and serotype determination of foot-and-mouth disease virus. *J Virol Methods* 161(2):183-91.

Table 1: Spectrophotometer measurement readings

No.	Sample	Milton Roy 601 Spectronic			NanoDrop 2000c Spectrophotometer			
		1 <sup>st</sup> day reading	2 <sup>nd</sup> day reading	3 <sup>rd</sup> day reading	1 <sup>st</sup> reading	2 <sup>nd</sup> reading	3 <sup>rd</sup> reading	Average mean
1	Standard	52	88	20	4.3	4.9	8.8	~6
2	Standard, 1 <sup>st</sup> dilution (10 <sup>-1</sup> )	ND	ND	24	-1.2	-2.1	ND	-1.65
3	Standard, 2 <sup>nd</sup> dilution (10 <sup>-2</sup> )	ND	ND	12	-2.6	ND	ND	-2.6
4	Standard, 3 <sup>rd</sup> dilution (10 <sup>-3</sup> )	ND	ND	44	-2.5	ND	ND	-2.5
5	Standard, 4 <sup>th</sup> dilution (10 <sup>-4</sup> )	ND	ND	12	-3.2	ND	ND	-3.2
6	Standard, 5 <sup>th</sup> dilution (10 <sup>-5</sup> )	ND	ND	4	-3.4	ND	ND	-3.4
7	Standard, 6 <sup>th</sup> dilution (10 <sup>-6</sup> )	ND	ND	4	-3.3	ND	ND	-3.3
8	Standard, 7 <sup>th</sup> dilution (10 <sup>-7</sup> )	ND	ND	12	-3.3	ND	ND	-3.3
9	Positive Control 1	40	156	32	5.4	4.2	ND	~4.8
10	Positive Control 2	108	104	20	5.7	5.8	ND	~5.75

ND=not done, reading measurement were in the unit of ng/μl

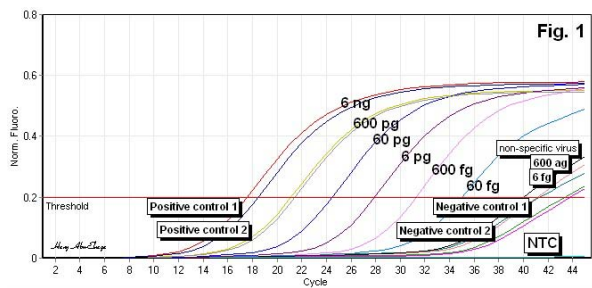


Figure 1A

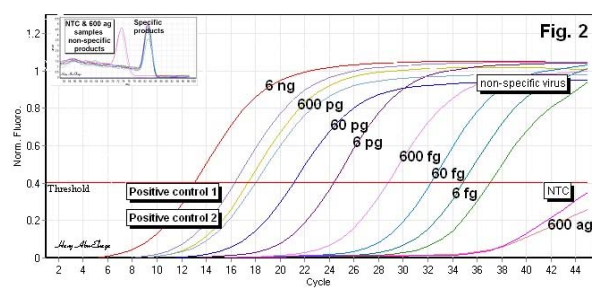


Figure 2A

Figure 1: Performance of Taqman probe RRT-PCR for the detection limit of FMDV O RNA pre-quantified, 6 ng/μl, and serially 10-fold diluted until theoretically reach 6 ag/μl. The former virus was used as standard. Nomenclature on the real-time figure is representing curves either by intersecting the 1st digit (i.e. 6) in the quantity number of the RNA masses or by having an annotation with a black shadow pointed to it. The threshold is the horizontal red line intersecting the curves. (A) Quantitation data of the standard and its dilutions. Two positive controls, two negative controls, no template control (NTC) and non-specific virus (BVDV) were involved in the assay run. Positive CT values were curves peaks above the threshold, while negative values were peaks at the borders or below the threshold (B) Agar-based electrophoresis of the assay run to affirm and investigate the performance of the amplified products by the Taqman probe. M: 100 bp ladder. Positive bands were approximately 107 bp of FMDV 3D gene. Lanes 1-8: The standard dilutions from 6 ng/μl to 6 ag/μl, Lanes 9-10: negative control 1 & 2, Lanes 11: BVDV, Lane 12: NTC, Lanes 13-17: Unknown samples, that were skipped in Quantitation data figure to not hide the illustrated standard curves. Positive controls were not shown.

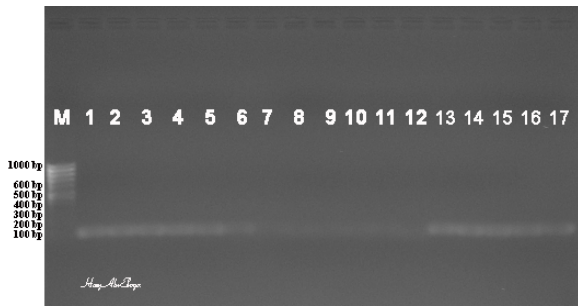


Figure 1B

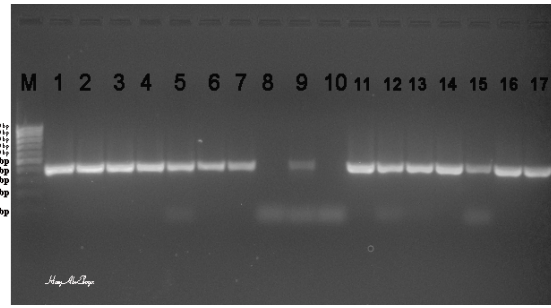


Figure 2B

**Figure 2:** Implementation of SYBR Green RRT-PCR using PoF/PoR primers for minimum detecting of FMDV O RNA pre-quantified, 6 ng/ $\mu$ l, and serially 10-fold diluted until theoretically reach 6 ag/ $\mu$ l. The former virus was used as standard. (A) Graphical representation of real time one-step RT-PCR data of the standard and its dilutions. Two positive controls, no template control (NTC) and non-specific virus (BVDV) were involved in the assay run. Positive CT values were curves peaks above the threshold, while negative values were peaks at the borders or below the threshold (B) Electrophoresis on agarose gel of the RRT-PCR assay samples using SYBR Green. M: 100 bp ladder. Positive bands were approximately 422 bp of FMDV 3D gene. Lanes 1-8: The standard dilutions from 6 ng/ $\mu$ l to 6 ag/ $\mu$ l, Lane 9: BVDV, Lane 10: NTC, Lanes 11-15: Unknown samples, that were skipped in Quantitation data figure to not overlap the standard curves, Lane 16: positive control 2, Lane 17: positive control 1. Melting curve analysis (insets) revealed amplification specificity.

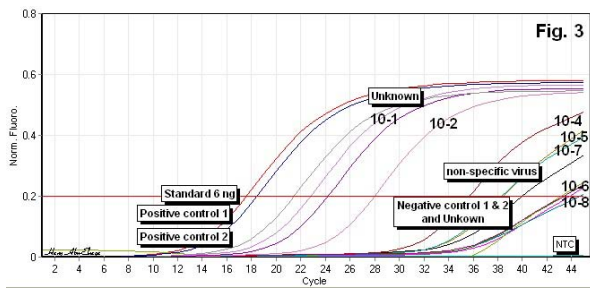


Figure 3A

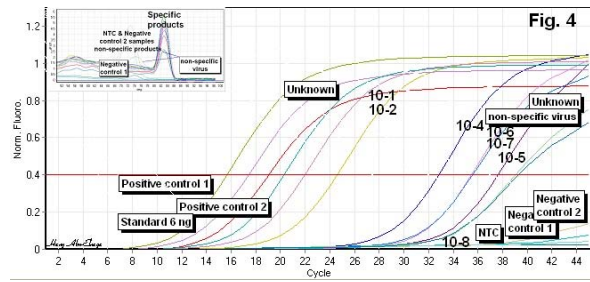


Figure 4A



Figure 3B

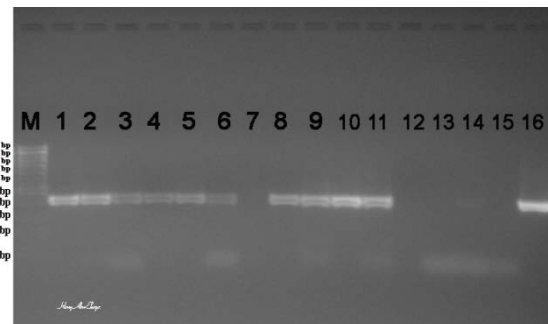


Figure 4B

**Figure 3:** (A) Detection of an archived FMDV stock virus (SV) RNA by Taqman probe RRT-PCR. SV dilutions  $10^{-1}$ - $10^{-8}$ , except  $10^{-3}$ , showed various  $C_T$  values with amplification cycles. Two positive and negative controls, NTC, non-specific virus (BVDV) and unknown samples were involved in the test. (B) Detection of stock virus (SV) RNA with anticipated 422 bp. M: 100 bp ladder. Lanes: 1-7 SV dilutions  $10^{-1}$ - $10^{-8}$ , except the not done dilution  $10^{-3}$ . Lanes 8 & 13 was positive control 1 & 2. Other lanes were unknown samples.

**Figure 4:** (A) Investigation of an archived FMDV stock virus (SV) RNA by SYBR Green RRT-PCR using PoF/PoR primers. SV dilutions  $10^{-1}$ - $10^{-8}$ , except  $10^{-3}$  illustrated variable  $C_T$  values with PCR cycles. Two positive and negative controls, NTC, non-specific virus (BVD) and unknown samples were involved in the assay. Insets show melting curve analysis. (B) Screening of stock virus (SV) RNA with anticipated 422 bp. M: 100 bp ladder. Lanes 1-7: SV dilutions  $10^{-1}$ - $10^{-8}$ , except the missed dilution  $10^{-3}$ . Lane 8: positive control 2. Lanes 9-11, 16: unknown samples. Lanes 12-13: negative control 1 & 2, Lane 14: BVD, Lane 15: NTC



مادختساب يقى قى قح الح لس لس ستملا قرم لبلا لعافت و يسكعلا خس نلا رابتخا نم قفلت تخم قرط اءاأ TaqMan and SYBR green  
ةى علقللا ىمحلل ضرمل ةى رصملا تاسورى فللا ناىب تسلا  
يزاجح اى ركز مرگا ، قزر دمأ اى نوس ، اجنلا وبأ مى هاربأ ىناه  
ةى علقللا ىمحلل ثوحب مسق  
ب.ص-قره اقل-ةى سابعلا -ةى رطى بللا تاح اقللاو لاصلملا ثوحب ده عم 131

### ى برعلا صللملا

نم طقف سىل ، رصم ىف اى دحت لثمى لازى ل ، ةى علقللا ىمحلل ضرم سورى نع فشكلا ةمدقتملا اى جولونكتلا قى ببطت

دى جلا طى طختلل اضى انكلو ، رم تسم لكشب دودحلل رباعلا و روطتملا سورى فلل قى قى دللا و عى رسلا دى دحتلا لجا ثى ح  
نى تقى رط لوانت مت ، ساردلا هذى ىف . ةءافكلا ىلاع حاقل عى نصت ىلع عى وطنت ىتلا ضرمل ىف مكحتلا ةى جى تارتسل  
ادامتعا ىظحلل لس لس ستملا قرم لبلا لعافت Taqman تنس رول فللا و SYBR Green فشكلا لجا نم و يسكعلا خس نلا رابتخل  
ةلى قللا خس نلا نع قى قى دللا Taqman ماظن ىلع عى SYBR Green لقللا فشكلا ىف فاعضأ 10 رادقمب روفورول فللا ةغبص ىلع  
ضمحلا ةفعا ضمل ةى باجى ل تارودلا مىق نإف ، كلذ سىكع ىلع . سورى فلل ىوونلا ضمحلا خس نل نع ةق رط تقوفت . سورى فلل  
هخسن SYBR Green 3 عم - ءاأ ناف ، ارىخا و . اركبم تارود 4 Taqman ةى صوصخو ةى ساسحب مس تا ىف نع فشكلا و ىوونلا  
ةق رط ترهظأ امنى ب ، لصفأ SYBR Green . ةق دللا و فاشتكلا ىف لقا اءح ىللا تزاحنا ةلى قللا



GLOBAL JOURNAL OF MEDICAL RESEARCH: G  
VETERINARY SCIENCE AND VETERINARY MEDICINE  
Volume 18 Issue 2 Version 1.0 Year 2018  
Type: Double Blind Peer Reviewed International Research Journal  
Publisher: Global Journals  
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

## Molecular Characterization of Recent Isolates of BEF Virus in Egypt

By Albehwar, A.M., Wafaa, M. El-Neshwy, Hiam, M. Fakhry & Hemmat, S. El-Emam

*Veterinary Serum and Vaccine Research Institute*

**Abstract-** The present work studied an outbreak of bovine ephemeral fever (BEF) at El-Salhia dairy farm during July and August 2015. The herd was consisting of 1600 previously vaccinated cows. Clinical examination of the herd revealed clinical signs lead to suspect infection with bovine ephemeral fever as fever; hurried respiration; lameness and recumbency in 103 cows. Serum samples, buffy coats and blood plasma samples from the diseased cows were used for identification and characterization of recent isolates of BEFV. The 103 animals were found to have non-protective low titers of BEF antibodies ( $\leq 2-8$ ) as tested by serum neutralization test (SNT). Trials of virus isolation in baby mice brain and BHK21 cell culture from buffy coat and blood plasma samples revealed specific signs in inoculated mice (paralysis of the limbs, nervous symptoms, and death within 3-4 days post inoculation), and specific cytopathic effect in BHK-21 cell culture. Virus identification using virus neutralization tests (VNT) and direct fluorescent antibody technique (FAT) confirmed the presence of BEFV. Also, PCR, sequencing analysis and phylogenetic tree showed that the obtained isolate is closely related to Egypt-2005 strain. These findings indicated that BEF still causes a risk to the cattle industry in Egypt although there is an effective vaccine used against the disease. So much more studies should be conducted on the risk factors of the disease.

**Keywords:** BEFV–isolation, clinical signs, PCR – FAT.

**GJMR-G Classification:** NLMC Code: WA 360



MOLECULAR CHARACTERIZATION OF RECENT ISOLATES OF BEF VIRUS IN EGYPT

*Strictly as per the compliance and regulations of:*



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# Molecular Characterization of Recent Isolates of BEF Virus in Egypt

Albehwar, A.M. <sup>α</sup>, Wafaa, M. El-Neshwy <sup>σ</sup>, Hiam, M. Fakhry <sup>ρ</sup> & Hemmat, S. El-Emam <sup>ω</sup>

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**Keywords:** BEFV-isolation, clinical signs, PCR – FAT.

## I. INTRODUCTION

**B**ovine ephemeral fever (BEF) is an important arthropod-borne viral disease that affects cattle and water buffalo. It is common in tropical and subtropical regions of Africa, Asia, and Australia. It is characterized by sudden onset of fever (40.5-41°C), increase respiratory rate, nasal and ocular discharges. Muscular signs become more evident on the second day. On the third day, the animal begins eating and ruminating, and the febrile reaction disappears (Radostitis et al. 2007).

The disease causes severe economic losses in cattle industry represented in cessation of milk production, reduction in animal condition, immobilization of animals used for draught power, impacting on trade and marketing of live animals (Walker and Klement, 2015) in addition to effect on animal reproductivity through cessation of ovarian activity and abortion (Zaher and Ahmed, 2011).

BEF was first detected in Egypt by Plot in 1895 - It was known as dengue fever of cattle- but the first detailed report about the disease was of an epizootic in 1909 (Ragbagliati, 1924). Subsequent outbreaks were recorded in 1915 and 1919-1920 in addition to Recent Out breaks in 1990-1991, 200-2001, 2004-2005 and 2011 (Davies et al.1992, Daoud et al. 2005, Zaher and Ahmed, 2011).

It is suggested that the disease is transmitted by hematophagous insects, so the pattern of the disease is seasonal outbreaks usually occur from late spring to autumn (Walker, 2005). The insects can be transported by air current from the sub-Saharan Africa to many countries as Egypt, Israel, Syria, Iraq, Turkey, Iran, and KSA. Although some of these countries were endemic, mutation during transmission of the disease by insect might occur (Aziz-Boaron et al. 2012).

The disease is caused by BEFV which is a member of the genus Ephemerovirus, family Rhabdoviridae. BEFV has a (-ve) ss RNA genome and five structural proteins including a nucleoprotein (N), a polymerase-associated protein (P), a matrix protein (M), RNA-dependent RNA polymerase (L) and a surface glycoprotein (G) (Aziz-Boaron et al. 2012). G- protein is the target of virus neutralizing antibodies (Cybinski 1990) and its amplification for virus diagnosis (Zaher and Ahmed 2011).

Trials of diagnosis of BEF begin with the history of the outbreak and clinical examination of the affected animals (Walker et al. 1991). Isolation of the virus on baby mice or cell culture and confirmation of the results with IFAT are accurate and sensitive diagnostic methods of field cases of BEF (Uren et al. 1992)

Although BEFV appears to have a single serotype worldwide, an analysis of some Australian and Chinese isolates has revealed some antigenic variation (Bastawecy et al. 2009). Determining the nature of these variations help in understand the epidemiology of BEFV infection and in the design of broadly protective vaccines.

As indicated by phylogenetic analysis, significant differences have been detected among the field strains circulating in the Middle East and other isolates (Aziz-Boaron et al. 2012).

The present work aimed to determine the molecular characterization of the recent isolate of BEFV with consideration of some risk factors that may affect the epidemiology of the disease.

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## II. MATERIALS AND METHODS

### a) Animals

One hundred and three Holstein Frisian cow (from total 1600 cow) reared at Al- Sahlia dairy farm, Sharkia Governorate, Egypt were clinically examined according to (Rosenberger et al. 1979) during the period of July and August 2015. The animals were previously vaccinated with live BEF vaccine produced by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. The animals received double doses of the vaccine with one month interval and the last dose was conducted in April 2015.

### b) Samples

One hundred and three serum samples were obtained from cattle showing clinical signs suspect infection with BEF. In addition to buffy coat samples and blood plasma samples obtained from diseased cattle during fever.

### c) Bovine ephemeral fever virus (BEFV)

Local bovine ephemeral fever virus (BEFV/Abassia/2000) adapted on BHK<sub>21</sub> cell culture (Azab ET al.2002) was supplied by the Department of Pet Animal Vaccine Research (DPAVR); Veterinary Serum and Vaccine Research Institute (VSVRI) with a titer of 10<sup>6</sup>TCID<sub>50</sub>/ml and used in serum neutralization test and as a positive control in virus identification assays.

### d) BEFV antisera

Anti-BEFV serum and locally prepared anti-BEFV serum conjugated with fluorescein isothiocyanate (FITC) were supplied by DPAVR and used in VNT and direct FAT.

### e) Baby mice

Suckling Albino Swiss baby mice (3-4 days old) supplied by DPAVR were used for trials of BEFV isolation.

### f) Baby hamster kidney cell culture (BHK<sub>21</sub>)

BHK<sub>21</sub> cell culture was supplied by DPAVR and used for virus isolation; virus neutralization (VNT) and serum neutralization tests (SNT).

### g) Serum neutralization test (SNT)

SNT was carried out using the microtiter technique according to (Yoneda et al. 2008) to determine the BEF antibodies in the collected serum samples and the antibody titer was expressed as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID<sub>50</sub> of the virus according to (Singh et al. 1967).

### h) Virus isolation

#### i. In baby mice

Each buffy coat and blood plasma sample was inoculated intracerebrally in each of 5 baby mice with 0.3ml/mouse according to (Hamoda et al. 2002). Inoculated mice were kept under hygienic measures in separate cages with their dams subjecting for daily clinical observations. Healthy baby mice were kept as test control. On the 3<sup>rd</sup> to 4<sup>th</sup> day post inoculation; when affected mice showed specific signs of BEFV infection (nervous signs; limb paralysis and cyanosis followed by death); the brains of dead mice were collected and subjected to other 2 viral passages in baby mice brains. Brain smears were prepared from affected mice and subjected to fluorescent antibody technique for virus identification.

#### ii. In cell culture

Buffy coat samples were inoculated in BHK<sub>21</sub> cell culture for three successive passages according to (Azab et al. 2002) where obtained cytopathic effect was described.

### i) Virus identification

#### i. Virus neutralization test

Samples showing specific signs of BEFV infection in baby mice and CPE in BHK cells were subjected to VNT according to (Soad et al. 2001).

#### ii. Direct fluorescent antibody technique (FAT)

Direct FAT was carried out on BHK<sub>21</sub> cell culture infected with the obtained isolates of BEFV using specific anti-BEFV conjugated with FITC.

### j) Polymerase chain reaction (PCR)

Primers: The used primers for BEFV are demonstrated in table (1).

Table 1: BEFV primers

Primer of bovine ephemeral fever	Sequence
Bef (Forward primer) at position 19 of the G gene,	TTAATACGACTCACTATAGGGAGATTACAATGTTCCGGT GAA
Reverse primer	GGTATCCATGTTCCGGTTAT

#### i. Nucleic acid recognition of virus samples

RT-PCR was used to amplify genome fragment from the prepared samples followed by nucleotide sequencing using BEFV specific primers. These oligos were synthesized by Bio Basic, Canada. Primers of BEF were used to amplify the expected at position 523 according to (Stram et al. 2005).

RNA Extraction was done using QIAamp Viral RNA Mini Kit (QIAGEN, Germany) Cat. No 52904 according to the manufacturer's protocol.

RT-PCR was carried out using One-Step RT-PCR Kit (Qiagen, Germany). The cycling parameters of the reaction conditions were: 95°C for 1 min; then 35 cycles of (94 °C for 45 sec, 56 °C for 45 sec, and 72 °C



for 50 sec) and then a final incubation at 4°C overnight. Amplified products were analyzed on agarose gel. Positive and Negative control samples and DNA ladder were involved in agarose gel electrophoresis.

ii. *Nucleotide sequence and Phylogenetic analysis*

Amplified viral RT-PCR products were sent to MacroGen Lab. (Korea) for DNA sequencing. The sequenced samples represented bovine ephemeral fever (BEFV) isolates. All the received sequence results were aligned with nucleotide sequences database at the National Centre for Biotechnology Information site (NCBI) using Basic Local Alignment Search Tool programs to assert the new sequences BEFV.

Analysis of the sequences identity phylogenetic relationship was performed using the cluster W method.

III. RESULTS

a) *Clinical examination*

The total number of cows at Al-Salhia dairy farm was 1600 cows. Clinical examination of these

animals revealed the appearance of clinical signs leads to suspect infection with BEFV on 103 cows. All diseased animals showed high fever more than 40°C, salivation, respiratory distress, stiffness and some animals showed different degrees of lameness (Figure 1). The total milk production of the farm was decreased from 14,000 kg to 12,400 kg per day during the period of the diseases. According to history there were 12 cows dead along the course of the outbreak in the farm.

Disease complications appeared on six animals as tabulated in the table (2). The six cows showed different forms of recumbency including sternal and lateral recumbency (Figure 2-4). From the six cows there were two cows' revealed severe respiratory signs as much hurried respiration, stretching of the neck and opening mouth (Figure 3), and two cows revealed subcutaneous emphysema (Figure 4).

Table 2: Complications of the BEF

Animal number	Recorded signs			
	Recumbency		Severe resp. distress	Emphysema
	Sternal	Lateral		
1		+ve	+ve	+ve
2		+ve	+ve	
3	+ve			+ve
4	+ve			
5	+ve			
6	+ve			



Fig. 1: Cow showing stiffness and lameness



Fig. 2: Cow showing lateral recumbency with emphysema



Fig. 3: Cow showing lateral recumbency with sever respiratory distress as a disease complication



Fig. 4: Cow showing sternal recumbency with emphysema

It was found that the morbidity rate of the disease in the farm was 6.4% while the mortality rate was 0.8% and the case fatality rate was 10.4%.

It was noticed that the clinical signs of the disease were sever in pregnant heifers than that in adult cows and no clinical signs were observed in calves.

The farm was an open system and suffering from a bad hygienic condition which helps on spreading

of the vector of the disease in addition to the climatic condition when its occurred in the summer season.

b) *Determination of the immune status of examined cattle*

Serum samples obtained from diseased animals and subjected to serum neutralization test revealed low antibody titers ( $\leq 2$  to 8) reflecting poor immune status in the tested animals Table (3).

Table 3: BEF immune status of tested cattle

Items	Mean serum neutralizing BEF antibody titer*						Non-valid
	0	$\leq 2$	2	4	8	16	
Number of samples	61	1	6	12	15	0	8
Percentage	64.2	1.05	6.3	12.6	15.8	0	
Total %**	3.8	0.06	0.38	0.75	0.94	0	

\*Mean serum neutralizing BEF antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID<sub>50</sub> of BEF virus

\*\*Total percentage= percentage of the tested sample from the total number of farm cattle

c) *Virus isolation*

Inoculation of buffy coat and blood plasma samples in the brain of suckling mice induced specific signs of BEF infection in mice represented by paralysis of the limbs; cyanosis and death within three days post

inoculation. In addition to CPE of BEF virus (Figures 5 & 6) when inoculated in BHK21 cell culture characterized by cell rounding and cell aggregation followed by detachment of the cell sheet within 3-4 days post cell infection.

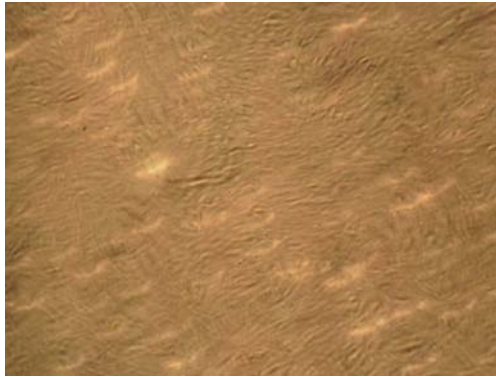


Fig. 5: Normal BHK21 cell culture (H&E 100Xs)

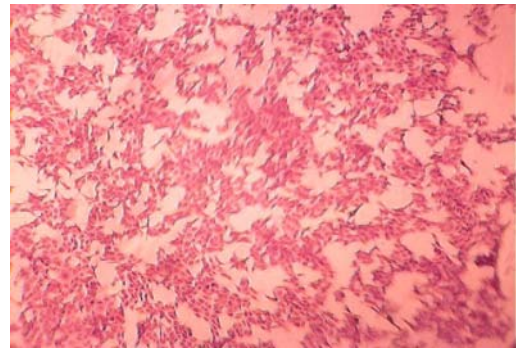


Fig. 6: BHK21 cell culture infected with the isolated BEF virus showing cell rounding; cell aggregation and cell detachment (H&E 100Xs)

d) *Virus identification*

Application of virus neutralization test and direct fluorescent antibody technique (Figure 7) using specific anti-BEFV serum and fluorescence conjugate antibodies confirmed that the obtained isolate is BEF virus.

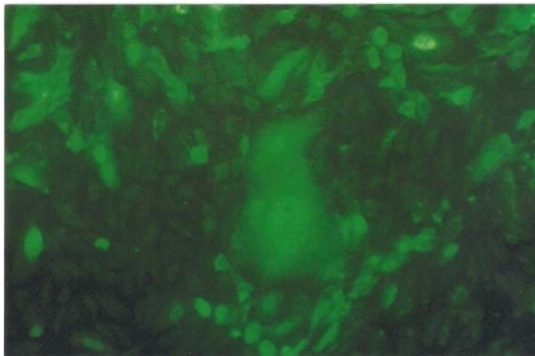


Fig. 7: Positive FAT carried out on BHK21 cell culture infected with the isolated BEF virus showing intra cytoplasmic apple green reaction (100Xs)

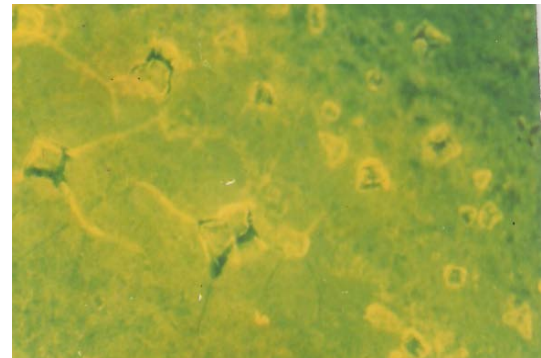


Fig. 8: Negative FAT carried out normal BHK21 cell culture (100Xs)



e) *Polymerase chain reaction*

BEFVs were identified with RT-PCR using specific primers. The cDNA was amplified producing a clear single band 523 base pairs (BP) in length on the

agarose gel stained with ethidium bromide (Figure -9). Positive samples included sample-1 (Buffy coat), sample-2 (plasma) and sample-3 (tissue culture propagated virus).

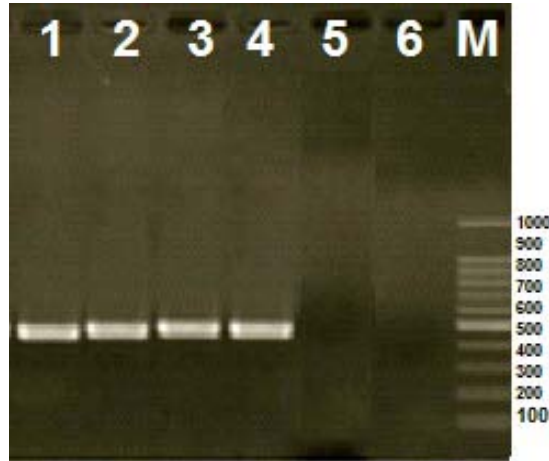


Fig. 9: RT-PCR for detection of bovine ephemeral fever

Lane (1): Buffy coat. Lane (2): plasma sample. Lane (3): tissue culture propagated virus. Lane (4) positive control lane: (5- 6): negative samples. Marker: 100 bp ladder.

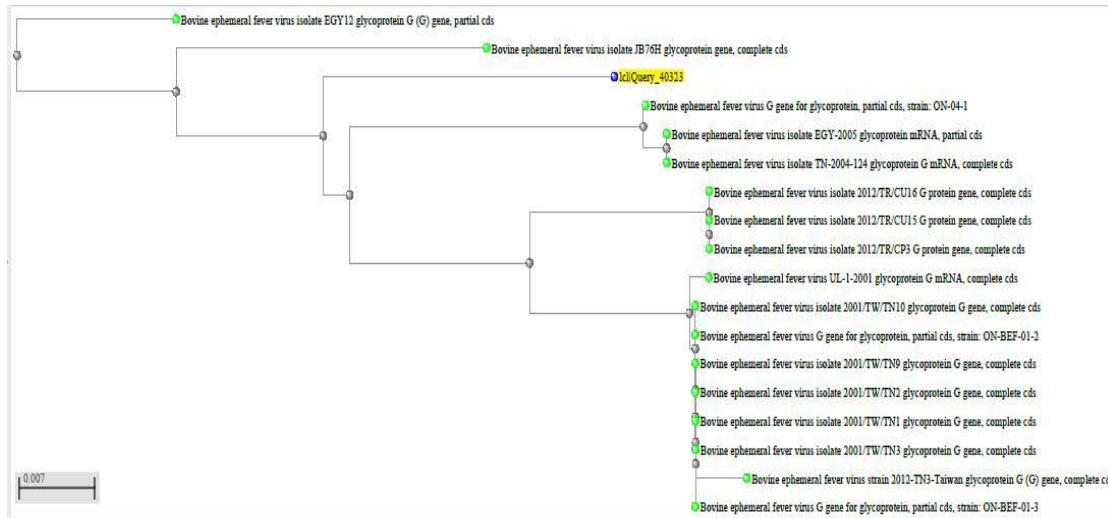


Fig. 10: Phylogenetic tree showing the relationship among BEFV depending on the virus partial code gene Sequence

Partial nucleotides sequences of BEFV was obtained and gave the easiness to select from BEF viruses partial and complete sequences found on gene bank to align (Figure 10) and to construct the phylogenetic tree.

Phylogenetic analysis of the sequences identity revealed that the obtained recent isolate of BEFV (Zagazig-2015) is closely related as 90% to BEFV/ isolate EGY- 2005 glycoprotein mRNA partial cds; BEFV/isolate TN-2004-124 glycoprotein mRNA, complete cds and BEFV/ isolate UL-1-2001 glycoprotein mRNA complete cds but as 88% with BEFV/ isolate EGY- 2012 glycoprotein G(G) gene partial cds.

IV. DISCUSSION

BEF is an important viral disease that causes severe economic losses in cattle herds. These losses are due to decrease milk production which usually doesn't return to the normal level at convalescence, mortality rates 1-10%, abortion in some cases, temporary loss of body weight, culling due to infertility in bulls,

The recorded clinical symptoms on affected cattle in this study including fever, hurried respiration; lameness and recumbency came in agreement with those reported by (Hassan et al. 1991, Nawal et al.

2001). Complication of the disease was recorded in some cases and manifested by severe respiratory signs, paralysis and subcutaneous emphysema. Similar signs were recorded by (Hassan 2000, Zaghawa et al. 2000). Cases of mastitis, abortion at the late stage of pregnancy and temporary infertility of bulls were reported by (Nandi S. and Negi, B.S. 1999).

Pathology and clinical signs related to BEFV infection are thought to be mainly attributed to increasing the vascular permeability and the cytokine storm resulting from the inflammatory response associated with the diseases (St George 1993). Pulmonary and subcutaneous emphysema may be attributed to nutritional selenium deficiency (Odiawo 1989).

In this study, the morbidity rate of the disease was 6.4% which is considered low compared with the morbidity recorded in Saudi Arabia during 1996 (59%) (Abu Elzein et al. 1997). The difference between two rats may be attributed to the previous vaccination of animals in the present study. Animals showed the clinical signs of the diseases which led to the suppression of their immunity. Also, the absence of the clinical signs of the diseases in calves in this farm may be due to colostrum antibody come from naturally infected or vaccinated dams (St. George et al. 1986).

The short incubation period, rapid onset and recovery of the BEF disease may be interpreted by the key role of released neutralizing antibodies in protection against the diseases (St. George, 1985).

Regarding the level of BEF antibodies in the examined vaccinated and affected cattle; serum neutralization test revealed that such animals had poor immune status with the antibody titer of  $\leq 2^{-8}$  (table-3) leading them to be susceptible for virus infection. In this respect (Ting et al. 2014 and Ting et al. 2016) concluded that when the titer of BEF neutralizing antibodies was equal to 5 or 6 animals became susceptible to virus infection showing typical symptoms of BEF and revaccination should be carried out. The protective level of BEF antibodies should not be less than 32 as demonstrated by (Daoud et al. 2001<sup>a</sup>, Amani 2006 and Aziz-Boaron et al. 2014).

Also, the infection in vaccinated cattle could be attributed to vaccination failure which may be due to the stress of pregnancy and high milk production on the general health of vaccinated cows. Also, the vaccination regime in the farm may play a significant role in the vaccination failure as the farm used the live BEFV vaccine in two doses with 28 days interval. This may consider as a load on the immune system of the vaccinated animals. (Inaba et al. 1974) recorded that vaccination with live attenuated vaccine followed by booster dose of inactivated vaccine lead to stronger immunity and more durable neutralizing antibodies response than using the live vaccine alone or two doses of inactivated vaccine.

On a contrast another side it was noticed that the used BEF vaccine might consider as a potent inducing 92.8% protection (103 infected animals and 12 dead cases from total 1600). Similar findings were obtained by (Daoud et al. 2001a and Elbehwar et al. 2010) who stated that the use of live BEF vaccine induced high levels of specific BEF neutralizing antibodies.

It was successful to isolate BEF virus from the buffy coat and blood plasma in suckling mice inducing limb paralysis; cyanosis and death within 3-4 days post infection in agreement with (Habbak 2005 and Abd El-Azeim, 2008).

It was found that inoculation of BHK21 cell culture with the obtained virus isolate induced specific CPE of BEF which characterized by cell rounding, cell aggregation followed by detachment of the cell sheet (photo-6) in agreement with what reported that BEF virus was isolated and propagated on different cell cultures as Vero cells (Soad et al. 2001); Vero, BHK and MDBK (Azab et al. 2002 and Zaghawa et al. 2006) and BHK (Zheng et al. 2011).

On the other side, the results of direct FAT came to confirm the presence of BEF virus showing the intracyto plastic fluorescent reaction (figure-7). The use of direct FAT for detection of BEF virus in cell culture was established by (Habbak2005 and Abd El-Azeim 2008) obtaining similar results.

Reverse transcriptase polymerase chain reaction (RT-PCR) has been developed with many advantages as it is possible to detect as little as a fragment of viral RNA from infected tissue by ethidium bromide staining after 35 cycles of PCR (Stram et al. 2005). The application of RT-PCR on isolated field virus yielded a clear single specific band on the agarose gel stained with ethidium bromide. The amplified DNA fragment corresponds to 523 BP. PCR confirms the diagnosis of BEF infection as a sensitive, specific and valuable rapid diagnosis of viral diseases.

Depending on the obtained results, it could be concluded that BEF diseases still cause a risk to the Cattle industry in spite of using a good vaccine (inducing 92.8% protection- rate under normal condition) so much attention should be paid to the risk factors of the disease.

## ACKNOWLEDGMENT

Great thanks for faculty of Veterinary Medicine, Zagazig Univ., and Veterinary Serum and Vaccine Research Institute, Abassia, Cairo for their fund.

## REFERENCES RÉFÉRENCES REFERENCIAS

1. *Abd El-Azeim, M.A. 2008. A contribution to bovine ephemeral fever virus isolation methods. M. V.Sc. (Virology), Fac. Vet. Med. Kafr El-shikh Univ.*

2. *Abu Elzein, E.M.E., Gameel, A.A., Al Afaleq, I.A.I. and Bukhari, A.* 1997. Bovine ephemeral fever in Saudi Arabia. *Veterinary Record*, 140: 630-631
3. *Amani, A.S.* 2006. Comparative studies on the immunogenicity of inactivated bovine ephemeral fever vaccine using oil and aluminum hydroxid gel as adjuvants. *Minufiya Vet. J.* 4: 389-394.
4. *Azab, A.M., Khodeir, M.H., Attyat, M.K. and El-Gallad, S.B.* 2002. Susceptibility of different cell cultures to bovine ephemeral fever virus. 6<sup>th</sup> Vet Med Zag Conf. 41-55.
5. *Aziz-Boaron, O., Gleser, D., Yadin, H., Gelman, B., Kedmi, M., Galon, N. and Klement, E.* 2014. The effectiveness of an inactivated bovine ephemeral fever virus vaccine. *Vet. Microbiol.* 17: 173 (1-2): 1-8.
6. *Aziz-Boaron, O., Klausner, Z., Hasoksuz, M., Shenkar, J., Gafni, O., Gelman, B., David, D. and Klement, E.,* 2012. Circulation of bovine ephemeral fever in the Middle East-strong evidence for transmission by winds and animal transport. *Veterinary Microbiology*, 158: 300–7.
7. *Bastawecy, I.M., Mahmoud, N.A., Eweis, M. and Salem, S.A.H.* 2009. Evaluation of different diagnostic techniques of bovine ephemeral fever in cattle Egypt. *J. Comp. Path. & Clinic. Path.* Vol. 23 (2): 7 – 17.
8. *Cybinski, D.H., Walker, P.J., Byrne, K.A. and Zakrzewski, H.* 1990. Mapping of antigenic sites on the bovine ephemeral fever virus glycoprotein using monoclonal antibodies. *J. Gen. Virol.* 71:2065–2072.
9. *Daoud, A.M., Saber, M.S., Taha, M.M., Azab, A. and Soad, M.S.* 2001a. Preparation of inactivated bovine ephemeral fever vaccine (BEF) in Egypt. *Beni-Suef Vet. Med. J.* XI (2): 619-626.
10. *Daoud, A.M., Soad, M.S., Azab, A. and Taha, M.M.* 2001b. Preparation of attenuated bovine ephemeral fever (BEF) vaccine. *Beni-Suef Vet. Med. J.* XI (2): 627-634.
11. *Daoud, A.M., Yonuis, E.E., El-Sawalhy, A.A., Sayed-Ahmed, M.Z. and Khodier, M.H.,* 2005. Some studies on bovine ephemeral fever in buffaloes. The fourth international scientific conference, Fac. Vet. Med. Mansoura Univ., Mansoura, Egypt 5-6 April: 687-702.
12. *Davies, F.G., Moussa, A. and Barsoum, G.* 1993. The 1990–1991 epidemic of ephemeral fever in Egypt and the potential for spread to the Mediterranean region. In: *Proceedings of the 1st International Symposium on Bovine Ephemeral Fever and Related Rhabdoviruses*, Beijing, August 1992. Australian center for international agricultural research proceeding 44, pp 54-56.
13. *Elbehwar, A.M., Nermein, G.S., Saad, M.A., Ibrahim, M.M., Magda, S.M., Anhar, A., Khodeir, M.H.* 2010. Comparative evaluation of the potency of traditionally inactivated bovine ephemeral fever vaccine and that one inactivated on the time of vaccination. 14th Sci Cong Fac Vet. Med. Assiut Univ. Egypt. 51-60..
14. *El-Habbak, A. S.* 2005. Comparative studies on rabies and Bovine ephemeral fever viruses. Master Thesis (Virology) Fac. Vet. Med Moshtohor Zagazig Univ.
15. *Hamoda, F.K., Khalaf-Allah, S.S. and Khodeir, M.H.* 2002. Some clinical, epidemiological and laboratory studies on bovine ephemeral fever (Three days sickness). *Vet. Med. J. Giza.* 50 (2):203-220.
16. *Hassan, H.B., El-Sanaf, N.A., Hafez, M.A., Ragab, A.M. and Fathia, M.M.* 1991. Clinico-diagnostic studies on bovine ephemeral fever with detection of its virus for the first time in Egypt. *First sci cong Egypt Soc. Cattle Dis.* 77-85.
17. *Hassan, H.Y.* 2000. An outbreak of bovine ephemeral fever in egypt during 2000. II-clinic-chemical and hematological alteration before and after symptomatic treatment trials. 9<sup>th</sup> Sc.Cong. 2000, Fac. of Vet .Med. Assiut Uni. Egypt.
18. *Inaba, Y., Kurogi, H., Takahash, A., Sato, K., Omori, T., Goto, Y., Hanaki, T., Yamamoto, M., Kishi, S., Kodama, K., Harada, K., Matumoto, M.* 1974. Vaccination of cattle against bovine ephemeral fever with live attenuated virus followed by killed virus. *Arch. Gesamte Virusforsch* 44:121–132.
19. *Nandi, S. and Negi, B.S.* 1999. Bovine ephemeral fever. A review. *Comparative Immunology, microbiology and infectious diseases.* 22:81-91.
20. *Nawal, M.A., Lamia, A.A., Shahein, M.A.* 2001. Isolation and identification of three- day sickness virus in Egypt. *Vet Med J Giza*, 2 49 (3): 425-432.
21. *Odiawo, G.O.* 1989. The relationship between selenium deficiency and development of pulmonary and subcutaneous emphysema in bovine ephemeral fever virus infected cattle. *On derstepoort j. vet. Res.* 56 (2):123-5.
22. *Radostitis, O.M., Gay, C.C., Hinchiff, K.W. and Constable, P.D.* 2007 *Veterinary medicine, a text book of the diseases of cattle, horse, sheep and goats*, 10<sup>th</sup> Edition, Philadelphia, WB Saunders Com. LTD, London.
23. *Ragbagliati, D.S.* 1924. Three day's fever or stiff sickness in cattle. *Vet. Rec.* 4:503–505.
24. *Rosenberger, G., Dirksen, G., Grunder, H., Grunert, E., Krause, D. and Stober, M.* 1979. *Clinical examination of cattle.* 2<sup>nd</sup> Edition. Verlag Paul Parey, Berlin and Hamburg
25. *Singh, K.V., Osman, O.A., Ivon, F.E. and Thanaa, I. B.* 1967. Colostral transfer of rinderpest neutralizing antibodies to offspring of vaccinated dams. *Can. J. Comp. Med. Vet. Sci.* 32: 295-298
26. *Soad, M.S., Taha, M.M., Samir, S.S. and Daoud, M.M.* 2001. Isolation and identification of bovine

- ephemeral fever virus in Egypt. Beni-Suef Vet. Med. J, XI (2): 609-617
27. *St. George, T.D.* 1985. Studies on the pathogenesis of bovine ephemeral fever in sentinel cattle. I. Virology and serology. *Vet Microbiol* 10:493-504.
  28. *St George, T.D.* 1993. The natural history of ephemeral fever of cattle. In: St George, T.D., Uren, M.F., Young, P.L., Hoffmann, D. (eds) Proceedings of the 1st International Symposium on Bovine Ephemeral Fever and Related Rhabdoviruses, Beijing, August 1992. Australian Centre for International Agricultural Research Proceedings 44: 13-19.
  29. *St. George, T.D., Uren, M.F. and Zakrzewski, H.* 1986. The pathogenesis and treatment of bovine ephemeral fever. In: St George TD, Kay BH, Blok J (eds) Proceedings of the 4th Symposium on Arbovirus Research in Australia, Brisbane. CSIRO-QIMR, pp 303-307
  30. *Stram, Y., Kuznetzova, L. and Levin, A.* 2005. A real-time RT-quantative (q) for the detection of bovine ephemeral fever virus. *J. Virology Methods.* 130: 1-6
  31. *Ting, L.J., Lee, M.S., Lee, S.H., Tsai, H.J. and Lee, F.* 2014. Relationship of bovine ephemeral fever epizootics to population immunity and virus variation. *Vet. Micro.* 173: 241-248
  32. *Ting, L.J., Lee, M.S., Lin, Y.L., Cheng, M.C. and Lee, F.* 2016. Short communication invasion of exotic bovine ephemeral fever virus into Taiwan in 2013-2014. *Vet. Micro.* 182: 15-17
  33. *Uren, M.F., George, M.F. and Murphy, G.M.* 1992. Studies on the pathogenesis of bovine ephemeral fever in experimental cattle. III. Virological biochemical data. *Vet. Microbiol.*, 30: 297-307
  34. *Walker, P.J.* 2005. Bovine ephemeral fever in Australia and the world. *Curr. Top. Microbiol. Immunol.* 292:57-80
  35. *Walker, P.J., Byrne, K.A., Cybinski, D.H., Doolan, D.L. and Wang, Y.*1991. Proteins of bovine ephemeral fever virus. *Journal of General Virology.* 72: 67-74
  36. *Walker, P.J., Klement, E.* 2015. Epidemiology and control of bovine ephemeral fever. *Vet Res.* 46:124
  37. *Yoneda, A., Tuchiya, K., Takashima, Y., Arakawa, T., Tsuji, N., Hayashi, Y. and Matsumoto, Y.* 2008. Protection of mice from rabies by intranasal immunization with inactivated rabies virus. *Exp. Anim.* 57 (1): 1-9
  38. *Zaghawa, A., Akela, M.A., Khadr, A.M. and Hassan, H.Y.* 2000. An outbreak of bovine ephemeral fever in egypt during 2000. I- clinical and epidemiological investigation. 9<sup>th</sup> Sc.Cong. Fac. of Vet .Med. Assiut Uni. Egypt.pp 346-353
  39. *Zaghawa, A., El-Fayoumy, M.M. and El- Balla, I.S.* 2006. Retrospective, clinical, laboratory and electron microscopic studies on bovine ephemeral fever in governmental farm in Menoufia. *Menoufia Vet J.* 4 (1): 91-100
  40. *Zaher, K.S., Ahmed, W.M.* 2011. Investigation on bovine ephemeral fever virus in Egyptian cows and buffaloes with emphasis on isolation and identification of afield strain. *Global Veterinaria,* 6 (5):447-452.
  41. *Zheng, F., Guo-Zhen, L., Jizhang, Z., Guanghua, W., Xiaolan, C., Xiaowei, G. and Changqing, Q.* 2011. A reverse-transcription, loop-mediated isothermal amplification assay for detection of bovine ephemeral fever virus in the blood of infected cattle. *Journal of Virological Methods,* 171: 306-309.



GLOBAL JOURNAL OF MEDICAL RESEARCH: G  
VETERINARY SCIENCE AND VETERINARY MEDICINE  
Volume 18 Issue 2 Version 1.0 Year 2018  
Type: Double Blind Peer Reviewed International Research Journal  
Publisher: Global Journals  
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# The Effects of Light on Egg Yield in Quail (*Coturnix coturnix*) under Laboratory Conditions

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**Abstract-** An egg has all the nutrients that people need. They have the richest proteins found in foods, contain proteins that cannot be secreted by the body and must be taken from the outside. The present study was conducted to show how aging and lighting can affect the yield and quality of egg. In this study, six female quails under 40 groups were used to investigate the egg yield and weight of the egg that starts with the first ovulation and for a period of 12 months. Egg counts and weight of quails that were kept in separate and individual cages. Two groups were investigated for 20W fluorescence light and daylight conditions separately. Yearly average egg yield of 282.42 calculated as population average was distributed along days 0-124, 125-248 and 249-372 as 39.85%, 34.66%, and 25.49% respectively. The ratios of the percentage of eggs obtained between days 125-248 and 249-372 to day's 0-124 were calculated as 92.59% and 72.53% respectively. It is concluded that egg yield with an average of 282.42 eggs per quail for 12 months following the age of first ovulation was stable until month 8.

**Keywords:** egg, light, wavelength, quality, yield.

**GJMR-G Classification:** NLMC Code: WA 360



*Strictly as per the compliance and regulations of:*





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Harun Cerit <sup>α</sup>, Hidir Demir <sup>ο</sup> & Engin Kozak <sup>ρ</sup>

**Abstract-** An egg has all the nutrients that people need. They have the richest proteins found in foods, contain proteins that cannot be secreted by the body and must be taken from the outside. The present study was conducted to show how aging and lighting can affect the yield and quality of egg. In this study, six female quails under 40 groups were used to investigate the egg yield and weight of the egg that starts with the first ovulation and for a period of 12 months. Egg counts and weight of quails that were kept in separate and individual cages. Two groups were investigated for 20W florescence light and daylight conditions separately. Yearly average egg yield of 282.42 calculated as population average was distributed along days 0-124, 125-248 and 249-372 as 39.85%, 34.66%, and 25.49% respectively. The ratios of the percentage of eggs obtained between days 125-248 and 249-372 to day's 0-124 were calculated as 92.59% and 72.53% respectively. It is concluded that egg yield with an average of 282.42 eggs per quail for 12 months following the age of first ovulation was stable until month 8.

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## I. INTRODUCTION

### a) Egg Yield and Egg Weight

Eggs are particularly important for humans as a source of nutrition and for birds as a way to maintain the survival and continuation of their species. As such, it would be desirable for farm animals to have high egg yields along with their ability to draw benefit from food as well as rapid development. While producers nowadays look for egg yields both high in quantity and quality while remaining economically viable, consumers are more interested in tangible features such as weight and size. One would not be wrong to think that bigger eggs would be more nutritious, albeit, it would also be necessary to assume that there would be a change in egg weight and the relative values of egg white <sup>[1, 2, 3]</sup>.

However, aging can have an impact on the quantity, chemical composition and physical structure of eggs in birds. In quails, aging leads to an increase in egg quantity and weight which in turn alter the inner and outer structure of the egg <sup>[2, 4]</sup>. It is noted that the increase in egg weight alters the egg's inner composition, decreases the percentage of egg yolk and at the same rate, increases the percentage of egg white.

*Author α ο ρ:* This article is an excerpt of the study supported by the Istanbul University Scientific Researches Project Unit with Issue Number of Bilims eiletkinlik/6787/2010.  
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Changes in egg's inner and outer composition effectively determine the final results obtained after incubation <sup>[5]</sup>.

The effects of aging are important in the determination of the duration during which a herd of quails kept either for breeding or egg production is economically viable. Wilson who raised quails by keeping them in separate and individual cages, feeding them with turkey starting feed, under 14-hour lighting conditions and investigated the egg yield for 52 weeks found that at the first month, egg yield was 70% whereas it reached and peaked at 80% by the second month<sup>[6]</sup>. In the same study, the average egg weight corresponded was found to be 7% of the animal's weight which was 9.1g. Sreenivasaiah and Joshi, in their study where they investigated the effects of age and season on the egg production and weight in quails, found that for quails that hatched during winter and summer the egg yields were 63.9% and 69.3% respectively, the average egg weights were 9.47 and 10.15, respectively and didn't find any significant effect of seasons and age on egg yield <sup>[7]</sup>. Kobayashi, in their study where they studied the effect of 14-hour long and continuous lighting and light intensity on quails, found the egg yield percentages for the intensities of 69cd and 104cd to be 89% and 90% respectively for 14-hour lighting, 82%-88% respectively for 24-hour lighting <sup>[8]</sup>.

In a study conducted by Okamoto on two experimental groups and one control group with a weight-based selection every six weeks for 14 weeks, found the average yield ratios to be around 82.4%, 77.0% and 93.1% <sup>[9]</sup>.

In another study done by Gebhardt-Henrich and Marks, they investigated egg weight under two different feeding conditions: ad-libitum and limited feeding. Under these two conditions, the average egg weight for the control group was found to be 9.25g and 8.62g respectively, whereas for the group on which a weight-based selection was done every four weeks, the values were 11.57g and 11.19g respectively <sup>[10]</sup>.

Uluocak who investigated the effects of raising egg-producing quails with or without a male on egg yield reported that, for the group weighing 180g, egg yield during the 10-week period following the first ovulation was 88.09% for the group with a male, and 89.18% for the group without a male, whereas egg weight was 11.05 and 10.92 respectively <sup>[11]</sup>.



Uluocak who also studied the effects of ageing on egg features in quails found that the average egg weight values for 2nd, 3rd, 4th and 5th months to be 10.3g, 11.6g, 11.9g and 12.3g respectively, noting a significant change in egg weight and quality with age for the duration of the investigation period<sup>[11]</sup>.

In a study conducted by Altinel on the relationship between egg quality and several characteristics of interest and the effects of the age of ovulation on the latter, a feeding program was applied with feeds containing 22% raw protein and 3100 kcal/kg metabolic energy for 5 week safter hatching and 16% raw protein and 2900 kcal/kg metabolic energy for the remainder of the study. Average egg weights for the 1st, 2nd and 3rd months after the first year of hatching were 11.108g, 11.643g, and 11.960g respectively while average egg weight for the 14-week period was 11.491. In the same study, it was reported that the values for total egg weight, yolk, white and shell weights increased with age, peaking at week 16<sup>[2]</sup>.

There aren't enough studies regarding the effects of lighting duration on quails. The aim of this study is to understand the effects of lighting on the egg yield and weight of ovulating quails.

## II. MATERIAL & METHOD

Starting with the first ovulation and for 12 months, egg count and weight of quails that were kept in separate and individual cages. Six female quails under 40 groups were used to investigate the egg yield and weight. Daily egg yield per quail was calculated as

the ratio of daily egg production over the number of caged quails. For each quail, the ratio of monthly egg yield over yearly egg yield was calculated individually. The month with the highest egg yield was designated as the peak yield and the ratios between egg yields from non-peak months and the peak yield were calculated. The lasting potential of egg yield was studied by dividing the yield periods in 3; days 0-124, 125-248 and 249-372 and was calculated by dividing the egg yields of days 125-248 and 249-372 with that of the first 124 days. 72,864 quail eggs were used for egg weight determination.

*Group 1:* The animals were kept in rooms lit without any interruption via 2x20W fluorescent lamps for 5 weeks.

*Group 2 (Control Group):* Rooms were lit via regular daylight with a normal duration for five weeks.

## III. RESULTS AND DISCUSSION

In this study, egg yields and weights of quails starting with their age of first ovulation were studied. After the determination of quails' age of first ovulation, they were kept in individual cages until the end of the ovulation period. Egg quantities and weights were recorded each day for the duration of the ovulation period. Table 1 shows the egg yields of 258 quails during the 12-month period as well as the evaluation of variation between the average values and Graph 2 shows the variations in egg yield and weight during the same period.

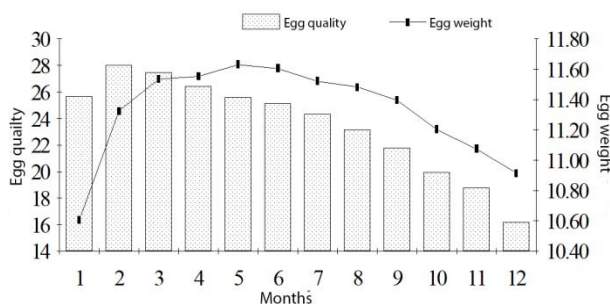
*Table1:* Egg yield and weight parameters of quails for a 1-year ovulation period

Ovulation period (months)	Egg Count (n)	Egg Yield (Quantity)		Yield Percentage (%)	Egg Weight (g)	
		x	s <sub>x</sub>		x	s <sub>x</sub>
1	6618	25.65 <sup>bc</sup>	0.313	84.10	10.60 <sup>h</sup>	0.017
2	7229	28.02 <sup>a</sup>	0.265	91.87	11.33 <sup>d</sup>	0.013
3	7083	27.45 <sup>ab</sup>	0.342	90.01	11.53 <sup>bc</sup>	0.015
4	6814	26.41 <sup>abc</sup>	0.363	86.59	11.55 <sup>abc</sup>	0.014
5	6600	25.58 <sup>bc</sup>	0.386	83.87	11.63 <sup>a</sup>	0.022
6	6486	25.14 <sup>cd</sup>	0.433	82.43	11.61 <sup>ab</sup>	0.021
7	6275	24.32 <sup>cd</sup>	0.435	79.74	11.53 <sup>bc</sup>	0.014
8	5979	23.17 <sup>de</sup>	0.492	75.98	11.49 <sup>c</sup>	0.022
9	5617	21.77 <sup>ef</sup>	0.599	71.38	11.39 <sup>d</sup>	0.023
10	5144	19.94 <sup>fg</sup>	0.621	65.37	11.20 <sup>e</sup>	0.026
11	4847	18.79 <sup>g</sup>	0.649	61.60	11.07 <sup>f</sup>	0.016
12	4172	16.17 <sup>h</sup>	0.678	53.02	10.91 <sup>g</sup>	0.018
Total	72864	282.42	3.659	76.87	11.34	0.005

*a-h:* Averages with differing superscripts are significantly different ( $P < 0.001$ ).

Monthly egg production of the quails for 12 months is 6618, 7229, 7083, 6814, 6600, 6486, 6275, 5979, 5617, 5144, 4847 and 4172 eggs respectively. Monthly yield averages per quail are 25.65, 28.12, 27.45,

26.41, 25.58, 25.14, 24.32, 23.17, 21.77, 19.94, 18.79 and 16.17 eggs respectively with an overall of 282.42 eggs for the whole 12-month period.



Graph 1: Egg yields and weights of quails during ovulation

As shown in Table 1 and Graph 1, egg yield reaches its highest at month 2 and especially after month 5, it shows a slow decline with aging until the end of the ovulation period. There is no statistically significant variation in the egg yield between months 2 and 5. Graph 2 also shows us that reaching a peak in egg yield in such a short time indicates the presence of a highly stable egg yield in quails for the first six months. Egg production continuity of 92.59% for the first eight months also supports this finding. The month with the highest yield observed in this study is in accord with the findings reported [6, 12, 13].

The egg yield ratio of 76.87% per quail for 12 months is found to be higher than the findings reported by Sreenivasaiah and Joshi [7], lower than those reported by Ludrowsky [15], and similar to the results given by Sundaram [17]. The egg yield of 86.59% at the fourth month after the age of first ovulation was found to be

higher than the egg yield values of groups subjected to selection that was reported [9,19,20,21], lower than the egg yield value of the control group reported and similar to the egg yield values reported [8,22].

The value reported by Kocak for six-month period is similar to the findings in this study. [23]

The egg yield values reported by Prabakaranin their study where they raised quails both in pairs and as a group were lower than the findings of this study for the same periods [24].

Table 2 shows the monthly egg yield distribution of the year-long ovulations of quails whereas Table 3 shows the egg yield continuity. The monthly egg yields appeared to be at their highest at month 2. As a result, the egg yield value of 258 quails for the 2nd month was taken as the peak value, and the ratios between monthly egg yield values and the peak value were calculated (Table 2).

Table 2: Monthly distribution of annual egg yield

Ovulation period (month)	Quail count	Egg Yield (quantity)		Monthly/1-Year Egg Yield Ratio	Monthly/ Highest Yield Ratio
		Daylight	Light		
1	258	25.65	26.8	9.08	0.92
2	258	28.02	29.7	9.92	1.00
3	258	27.45	29.5	9.72	0.98
4	258	26.41	28.1	9.35	0.94
5	258	25.58	27.3	9.06	0.91
6	258	25.14	26.4	8.90	0.90
7	258	24.32	26.1	8.61	0.87
8	258	23.17	25.4	8.21	0.83
9	258	21.77	22.6	7.71	0.78
10	258	19.94		7.06	0.71
11	258	18.79		6.65	0.67
12	258	16.17		5.73	0.58
Total	258	282.42		100.00	-

Yearly average egg yield of 282.42 calculated as population average was distributed along days 0-124, 125-248 and 249-372 as 39.85%, 34.66%, and 25.49% respectively. The ratios of the percentage of eggs obtained between days 125-248 and 249-372 to

day's 0-124 were calculated as 92.59% and 72.53% respectively (Table 3). According to these results, the egg yield continuity of quails appears to develop quite well especially between days 0-248.

Table 3: Egg yield continuity

Ovulation Period (days)	Egg yield (eggs)		Individual egg yield/ Total egg yield		Individual egg yield/ Egg yields between days0-124	
	x	s <sub>x</sub>	%	s <sub>x</sub>	%	s <sub>x</sub>
0-124	107.53	0.091	39.85	0.063		
125-248	98.22	0.141	34.66	0.036	92.59	0.187
249-372	76.67	0.255	25.49	0.075	72.53	0.279

Table 4: Degrees of heritability of egg yields of the quail population

Characteristic of Interest	Group #	Offspring #	x	s <sub>x</sub>	h <sup>2</sup>	s <sub>h</sub> <sup>2</sup>
Egg yield (eggs)	40	258	282.42	3.659	0.19	0.105

When compared to the average estimated degree of heritability of quails, the degree of heritability of egg yield estimated at 0.19 for this population demonstrates the unfeasibility of selection for the improvement of this property.

In this study, the monthly average of egg yields for the period of 12-months were 10.60g, 11.33g, 11.53g, 11.55g, 11.63g, 11.61g, 11.53g, 11.49g, 11.39g, 11.20g, 11.07g, and 10.91g respectively with an overall average of 11.34g (Table 1). Steady growth in egg weight can be observed from first ovulation until month 6 (Graph 2). A decrease in egg weight was observed in accordance with the decline in egg yield following month 6. Egg weight also reached its peak at month 5. The peaks have later date than the one reported by Yannakopoulos and Tserveni-Gousi<sup>[4]</sup> and in accord with the one reported by Ludrowsky<sup>[15]</sup>. However, the month after which egg weight started to decrease is later than those reported by the abovementioned authors. It can be seen in Graph 2 and Table 1 that there is no statistically significant variation in egg weight between months 3 and 7 despite an observed decline in egg yield. As a result of aging, a steady decrease in egg weight was observed following month 8.

#### IV. RESULTS

Among quails investigated in this study, the continuity of egg yield with an average of 282.42 eggs per quail for 12 months following the age of first ovulation was stable until month 8. This value steadily decreased after this period as a result of aging. Increase in egg weight appears to be in accord with egg yield. Based on these findings, it can be concluded that it would be economically viable to keep the population of quails for egg production between the age of first ovulation and the 8th month of ovulation.

When the estimated degrees of heritability of the egg yields of the quail (*Coturnix coturnix japonica*) population found at the University of Istanbul Faculty of Veterinary, Zootechny, Quail Research Unit are taken into account, it can be concluded that a selection made based on individual animal weight values at week 5 would be more effective in creating a faster genetic progression when compared to other weeks.

This study as a whole can be said to possess preliminary information regarding the determination of selection programs suitable for quail husbandry. Furthermore, knowing that it is necessary to study phenotypic and genetic correlations to create successful selection programs, this study demonstrates the need for further research with a wider scope. Finally, findings and results obtained within this study have the potential to shed light on the establishment of more viable conditions and principles regarding quail husbandry.

#### REFERENCES RÉFÉRENCES REFERENCIAS

1. Beylyavin, G.G. "Physical Characteristics of Intact and Cracked Eggs." Tandfonline, British Poultry Science, 3 Apr. 2008.
2. Altinel, A., et al. "Japon bıldırcınlarında (*Coturnix coturnix Japonica*) yumurta kalitesi ve özellikleri üzerinde araştırmalar." İstanbul Univ Vet Fak Derg 22.1 (1996): 203-213.
3. Alkan, Sezai, et al. "Effects of selection for body weight and egg production on egg quality traits in Japanese quails (*Coturnix coturnix japonica*) of different lines and relationships between these traits." Kafkas Universities Veterinary Faculties Dergisi 16.2 (2010): 239-244.
4. L.TSERVENI-GOUSHI, A. "Research Note: Effect of Breeder Quail Age and Egg Weight on Chick

- Weight." OUP Academic, Oxford University Press, 1 Sept. 1987.
5. Soares, M.C, and L Roque. "Effects of Eggshell Quality and Broiler Breeder Age on Hatchability." OUP Academic, Oxford University Press, 1 Dec. 1994.
  6. Wilson, et al. "Evaluation of Coturnix (Japanese Quail) as Pilot Animal for Poultry." OUP Academic, Oxford University Press, 1 May 1961.
  7. Sreenivasaiah, P. V., and H. B. Joshi. "Influence of hatching season on egg production characteristics in Japanese quails (Coturnix coturnix Japonica)." Kerala Journal of Veterinary Science (India) (1986).
  8. KOBAYASHI, Shin, et al. "Influence of light intensity on egg production in Japanese quail under 14L: 10D and continuous lighting." Japanese poultry science 26.4 (1989): 235-244.
  9. OKAMOTO, Satoru, Shin KOBAYASHI, and Teruo MATSUO. "Feed conversion to body weight gain and egg production in large and small Japanese quail lines selected for 6-week body weight." Japanese Poultry Science 26.4 (1989): 227-234.
  10. GEBHARDT-HENRICH, SABINE G., and H. L. Marks. "Effects of feed restriction on growth and reproduction in randombred and selected lines of Japanese quail." Poultry science 74.2 (1995): 402-406.
  11. Unlocks, A. N., et al. "bıldırcın yumurtalarında yaşla birlikte bazı kalite özelliklerindeki değişim." Hayvancılık 296 Ulusal Kongresi, Bildiriler 1 (1996): 438-445.
  12. Woodard, Allen Earl, Hans Abplanalp, and Wilbor Owens Wilson. "Japanese quail husbandry in the laboratory (Coturnix coturnix japonica)." Japanese quail husbandry in the laboratory (Coturnix coturnix japonica). (1965).
  13. Gildersleeve, R. P., et al. "Egg production in four generations of paired Japanese quail." Poultry science 66.2 (1987): 227-230.
  14. Sachdev, A. K., S. D. Ahuja, and Ram Gopal. "FEED consumption, egg-production and b egg-quality traits as influenced by cage-tier locations of japanese quail." indian journal of animal sciences 59.7 (1989): 860-865.
  15. Ludrovsky, F., Bodo, I., Janan, J., Rill, Z. and Takacs, E.:Comparison of egg production in various types of Japanese quails. Proceedings 19th World's Poultry Congress, Amsterdam, 19-24 September, 3, 384.
  16. Tikk, V., and H. Tikk. "The quail industry of Estonia." World's Poultry Science Journal 49.1 (1993): 65-68.
  17. Sundaram, T. S. T. (1989): Comparative egg production efficiency of chickens and ducks, quails. Poultry International, 28 (5), 60.
  18. Chrappa, V., V. Sabo, and K. Boda. "Feeding (recycling) of quail's excrements with different energy contents in feed mixtures for Japanese quails." Zivocisna Vyroba-UZPI (Czech Republic) (1993).
  19. Yücelen, Y. ve Alarslan, F. Ö. (1986): Enerji düzeyleri farklı rasyonların bıldırcınlarda yumurta verimi ve yumurtadan çıkış gücü üzerine etkileri. Ankara Üniv. Ziraat Fak. Yıllığı, 37 (2), 240-250.
  20. Sarıççek, B. Z., M. Sarıca, and G. Erener. "Değişik bitkisel protein kaynaklarının bıldırcınların verim özelliklerine etkileri (I. Gelişme özellikleri)." Ondokuzmayıs Üniv. Zir. Fak. Derg 9.3 (1994): 119-127.
  21. Darden, J. R., and H. L. Marks. "Divergent selection for growth in Japanese quail under split and completes nutritional environments. 1. Genetic and correlated responses to selection." Poultry Science 67.4 (1988): 519-529.
  22. Uluocak, A. N., Okan, F. ve Ayaşan, T. (1996a): Yumurtacı bıldırcınların erkekli veya erkeksiz barındırılmalarının yogurt verim özelliklerine etkileri. Hayvancılık '96 Ulusal Kongresi, 18-20 Eylül, Bildiriler.
  23. Koçak, Ç. Ö. Altan, and Y. Akbaş. "Japon bıldırcınlarının çeşitli verim özellikleri üzerinde araştırmalar." Tr. J. Vet. Anim. Sci 19 (1995): 65-71.
  24. Prabakaran, R., et al. "Effect of system of mating and season on the reproductive performance of Japanese quail." Indian Journal of Poultry Science 27.2 (1992): 100-102.





GLOBAL JOURNAL OF MEDICAL RESEARCH: G  
VETERINARY SCIENCE AND VETERINARY MEDICINE  
Volume 18 Issue 2 Version 1.0 Year 2018  
Type: Double Blind Peer Reviewed International Research Journal  
Publisher: Global Journals  
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Enhanced Immune Responses of Trivalent Foot and Mouth Disease Vaccine using Montanide Oil and Clinoptilolite Adjuvants in Cattle

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**Abstract-** Adjuvant play an important role in the efficacy of vaccines, the protective immune response produced by vaccines can vary according to the kinds of adjuvant. The comprehensive sero-immunological study was conducted to reveal the adjuvant's effect of Clinoptilolite and oil on the immune response of trivalent Foot and mouth disease (FMD) vaccine in cattle. This study was conducted in five cattle groups; The first group was vaccinated intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 µg/dose) vaccine, The second group was vaccinated with FMD (Oil + Clinoptilolite) vaccine and Third group was vaccinated with FMD oil vaccine while the fourth group were non vaccinated used as negative control and fifth group were used for safety test. Then conducted tests to compare the enhancement in cattle immunity. The humeral and cellular immune responses were monitored in different tested groups.

**Keywords:** FMD virus, vaccine, clinoptilolite, XTT, SNT, and ELISA.

**GJMR-G Classification:** NLMC Code: QW 70



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# Enhanced Immune Responses of Trivalent Foot and Mouth Disease Vaccine using Montanide Oil and Clinoptilolite Adjuvants in Cattle

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**Abstract-** Adjuvant play an important role in the efficacy of vaccines, the protective immune response produced by vaccines can vary according to the kinds of adjuvant. The comprehensive sero-immunological study was conducted to reveal the adjuvant's effect of Clinoptilolite and oil on the immune response of trivalent Foot and mouth disease (FMD) vaccine in cattle. This study was conducted in five cattle groups; The first group was vaccinated intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 µg/dose) vaccine, The second group was vaccinated with FMD (Oil + Clinoptilolite) vaccine and Third group was vaccinated with FMD oil vaccine while the fourth group were non vaccinated used as negative control and fifth group were used for safety test. Then conducted tests to compare the enhancement in cattle immunity. The humeral and cellular immune responses were monitored in different tested groups. The obtained results indicated that the incorporation of Clinoptilolite into inactivated FMD vaccine induces an increase of the specific protective immune response. Higher and longer period of immune responses were found in cattle vaccinated with both Montanide oil and Clinoptilolite adjuvanted vaccine up to 40 weeks, while those vaccinated with Clinoptilolite or oil vaccine showed protected immunity up to 32 weeks respectively. Finally, we recommended that using of Clinoptilolite with oil as a potential adjuvant in FMD vaccine.

**Keywords:** FMD virus, vaccine, clinoptilolite, XTT, SNT, and ELISA.

## I. INTRODUCTION

Foot-and-mouth disease (FMD) is an acute infectious disease that infects cloven-hoofed mammals, such as pigs, cattle, cattle and goats (Dar et al., 2013). The causative agent is a single-stranded positive-sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. The virus has seven serological types, identified as; O, A, C, SAT1, SAT2, SAT3 and Asia1 (Dar et al., 2013).

FMD is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout, and teats, with high morbidity and low mortality (Rodriguez and Grubman 2009).

In Egypt, the disease is enzootic, and outbreaks have been reported since 1950, Type O was the most prevalent since 1960 (Zahran 1960, Farag et al., 2005

And Satya 2009). FMDV serotype A was isolated during 2006 in Egypt through live animals importation where sever clinical signs were recorded among cattle and buffaloes Abed El-Rahman (2006). Also FMDV serotype SAT2 was recorded in Egypt (Shawky et al., 2013 and Nader et al., 2014).

Control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination is effective in limiting the spread of FMD (Depa et al., (2012).

The vaccine adjuvant is the very important factor which stimulates specific components of either cellular or humeral immune response Lombard (2007), Fakhry et al., (2012) and Sonia et al., (2015). Most foot-and-mouth disease vaccines are made of BEI (binary Ethyleneimine) inactivated virus that is adjuvanted with oil adjuvant.

The in-house produced vaccine by Veterinary Serum and vaccine Research Institute (VSVRI) is the Montanide ISA 206 trivalent inactivated vaccine which Consists of three FMDV serotypes (O/ Pan Asia1, A Iran/05 and SAT2/EGY?2012.

Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humeral or cell-mediated (Lombard et al., 2007). Continuous improvement of formulations to obtain the highly immunogenic vaccine, The improvement not only depend on the antigen payload, but also selecting the ideal or the most suitable adjuvant is one of the important tools in improving the efficacy of the FMD vaccine. Adjuvant is one which can stimulate the humeral immune response early (onset), and promote the production of high antibody titers that would long duration. It should also stimulate the cellular immune response (Park 2013).

The oil adjuvant has the capability for generating a rapid, high and long-lasting immune response. Generally, the Montanide

Series of oil adjuvant (SEPPIC, France) has a immunological effect for inactivated vaccine in different susceptible animals (Fakhry et al., 2012, Dar et al., 2013, and Ehab et al., 2015).

Clinoptilolite is a natural, non-toxic that has monoclinic crystal structure symmetry (Mansouri et al 2013). Also Clinoptilolite not classified as to their carcinogenicity to humans and animal (Dong et al.,

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2003). Clinoptilolite has been extensively tested for toxicity in a wide range of animals, including rats, mice, hamsters, beagles, and pigs appear to lack toxic effects unless ingested in very large quantities (European Parliament 1997), it does not have any side effect (Ray Sahelian 2016).

Clinoptilolite is a micro mineral particle that in earlier studies has shown adjuvant activity against different antigens. Clinoptilolite is safe and effective (Garces 1999 and Rhodes2010). Clinoptilolites play an important role in regulating the immune system. (Aikoh et al., 1998) have reported that silica, silicates, and aluminosilicates act as nonspecific immunostimulators similarly to super antigens. Super antigens are a class of immunostimulatory and disease-causing proteins of bacterial and viral origin with the ability to activate relatively large fractions (5-20%) of the T cell population, as well as humoral immune responses.

The purpose of this study was to evaluate the efficacy of Clinoptilolites in addition to ISA 206 as an adjuvant of inactivated trivalent FMDV, to stimulate the immune response.

## II. MATERIALS AND METHODS

### a) Animals

#### i. Cattle

21 cattle were clinically healthy and free from antibodies against FMDV.

#### ii. Unweaned baby mice

30 Swiss Albino suckling mice (three to five days old were) classified into six groups, used in safety test of inactivated virus and vaccines and supplied by the Lab. animal's farm of Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

### b) FMD virus Strains

Local FMDV strains (O /pan Asia2, A/ Iran 05 and SAT2/ Egypt 2012) were isolated and identified by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. and confirmed by Pirbright (FMD-WRL), United Kingdom. FMDV were propagated in BHK21 cell line in roller bottles (Huang et al., 2011), each virus had an infectivity titer of 10<sup>8</sup> TCID<sub>50</sub>/ml as described by (Reed and Muench 1938). These viruses were used as virus mitogens in the lymphocyte proliferation assay, vaccine preparation and SNT

### c) Inactivation of FMD virus

FMD virus strains were inactivated with mixture of 1 mM binary ethyl enamine (BEI) and 0.04% formaldehyde according to the method described by (Sarkar et al., 2017) sodium this sulfate 20% was added to the virus samples to the inactivated virus to neutralize the BEI in a final concentration of 2%. Sodium bisulfite 20% was added after inactivation process to neutralize the excess of formalin in final concentration of 2%.

### d) Adjuvants

#### i. Montanoid Oil

ISA 206 Montanide Oil was obtained from Seppic, Paris, France.

#### ii. Clinoptilolite

The fine powder of natural clinoptilolite was obtained by Micronisiertes Klinoptilolith –Hochwertigs Naturminera, Germany.

### e) Formulation of the prepared vaccines

#### i. Vaccine 1: Clinoptilolites adjuvant vaccine

Trivalent inactivated FMD with 1 µg/doses of Clinoptilolites according to (Mansouri et al., 2013, and (Hiam and Assem 2014).

#### ii. Vaccine 2: Oil and Clinoptilolites adjuvant vaccine

Trivalent inactivated FMD with Montanide ISA 206 +1µg/doses of Clinoptilolites according to (Alhawary et al., 2017)

#### iii. Vaccine 3

Trivalent inactivated FMD with Montanide ISA 206 according to (Barnett et al., 1999).

### f) Evaluation of the prepared vaccine formulations: Sterility and safety testing

The vaccines were cultured on Sabouraud's, nutrient agar; thioglycolate broth, phenol dextrose media and mycoplasma medium. The tested vaccines were free from any aerobic, anaerobic bacteria and fungal contaminants. The Safety of inactivated virus and vaccines were done according to (OIE 2013).

#### i. Evaluation of Cellular Immunity

Heparinized blood samples were obtained from vaccinated and control non- vaccinated animals at 0, 3, 7, 14, 21, 28, 35 and 42 days post vaccination.

Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated using cell proliferation kit (XTT kit) according to EL-Naggar (2012).

#### ii. Evaluation of humeral immune response of vaccinated animals

Serum samples were collected from the vaccinated and non-vaccinated cattle weekly post-vaccination for one month then every 2 weeks post vaccination up to 40 weeks for evaluation of antibody titers against FMDV strains (O /pan Asia2, A/Iran 05 and SAT2/Egypt 2012) in serum samples were measured using the neutralization assay as described previously (OIE 2012) and indirect ELISA according to (Voller et al., 1976).

### g) Experimental Design

21 cattle were classified into five groups, five animals for each first three groups. The first group was vaccinated with 3 ml intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 µg/dose) vaccine, the second group was vaccinated with 3 ml FMD

(oil + clinoptilolite) vaccine and Third group was vaccinated with 3ml FMD oil vaccine. While the fourth group (three animal) were none vaccinated used as negative control and fifth group (three animal) were used for safety test.

### III. RESULTS AND DISCUSSION

Foot and Mouth Disease (FMD) is an acute disease caused by Foot and Mouth Disease Virus (FMDV) which causes economy losses (Orsel et al., 2007). In endemic areas the vaccination of animals is effective in control and limiting the spread of FMD.

FMD vaccines can be defined as a specific formulation of chemically inactivated virus strains and mix with a suitable adjuvant.

Selecting the suitable vaccine formulation is dependent on several factors as the onset of protection and the duration of protection against FMD.

The effective formulation of inactivated FMD vaccines requires adjuvant Clinoptilolite, and Montanide ISA 206 mineral oil-based formulations have been widely employed in experimental studies to obtain a vaccine that stimulates a rapid and long-lasting protective immune response, the formulated vaccines are safe for animal use.

In this work, we studied the effect of natural Clinoptilolite particles to induce specific and protective immune response against foot and mouth disease.

The formulation Clinoptilolites-FMDV is non toxic with adjuvant activity (Batista et al., 2010). Vaccine formulations containing the adjuvant could promote the presentation of the virus so it could increase the immune response and the protection (Batista, et al., 2010 and Fakhry et al., 2012).

Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated using Lymphocyte blastogenesis using XTT assay) according to (Scudiero et al., 1988).

The obtained results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by  $\Delta OD$  (Delta Optical Density) were as follow: 3 ml intramuscularly (I/M) with trivalent FMD Clinoptilolite (1  $\mu\text{g}/\text{dose}$ ) vaccine. The second group was vaccinated with 3 ml FMD (oil + Clinoptilolite) vaccine and Third group was vaccinated with 3ml FMD oil vaccine.

In group 1 (trivalent FMD Clinoptilolite vaccine): Delta Optical Density was (0.517) by using FMD viruses at 3rd -day post vaccination(DPV) and still rise reached its highest level (1.557) at 3rd -week post vaccination(WPV) and continue high within examination time 35 DPV.

In group 2 (trivalent FMD oil + Clinoptilolite vaccine: Delta Optical Density was (0.515) by using FMD viruses at 3rd -DPV and still rise reached its highest level (1.665) at 2nd - WPV, and continue high within 35 DPV then declined.

In group 3 (trivalent FMD oil vaccine): Delta Optical Density was (0.473) by using FMD viruses at 3rd - DPV and still rise reached its highest level (1.136) at 3rd - WPV then declined gradually as shown in Table No. (1).

From Tables (1) showed the results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by  $\Delta OD$  (Delta Optical Density) appeared to be supported by (Sharma et al., 1984) they reported that cell mediated immune response was a constitute of immune response against FMD virus, and in agreement in some points with (Mercedes et al., 1996, El-Watany et al., 1999, Sonia et al., 2010 and El-Din, W et al., 2014) whose found that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV was greater than by mitogens (PHA) and appeared the highest increase in 1st and 2nd -WPV, while disagreed with (El-Watany et al., 1999). The obtained results were in agreement with (des 2010) who mentioned that Clinoptilolite (David 2013), our results also were supported by (Rholite enhance cell mediated immune response.

#### a) *Tracing the antibody titer against FMDV serotypes (O, A&SAT2)*

The SNT and ELISA data (Tables 2&3) show differences in the onset, intensity and duration of the FMD serotype O, A & SAT2 antibodies elicited by the different vaccine formulations. Concerning the onset of protection, it is clear that FMD Clinoptilolite vaccine (group1 and FMD Clinoptilolite + oil vaccine (group3) reach the protective level at 2nd WPV early than group (2) FMD oil vaccine which reach protective level at 3rd WPV. The results revealed that SNT titers for FMD vaccines, go in hand with the results obtained are consistent with the statement of (Wisniewski et al., 1972) they explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion. The peak of antibody titre in all groups at 10-12 WPV and continues with protective level till 32th WPV in FMD Clinoptilolite vaccine and FMD oil vaccine groups while in FMD Clinoptilolite+ oil vaccine group till 40th WPV. The results agreed with (Kreimir et al., 2000, and Rhodes 2010) who showed that adjuvant properties of Clinoptilolite as potent adjuvant induced higher antibody titers than the antigen alone or vaccine adjuvanted with Montanide oil and improved the potency of adjuvants. Results supported also by (Batista et al., 2010) they found that Clinoptilolite help the vaccine work more effectively, increasing antibody production. Who found that Clinoptilolite might help the vaccine work more effectively, increasing antibody production, also Clinoptilolite improved B-cells function, improved mucosal and humoral immunity and protective activity also helped vaccine for induction strong immunity when used as adjuvant. Our results also go in hand with the results obtained were consistent with the statement of



(Hamblin et al., 1986) who explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non-infectious virus.

Finally, it can conclude that: The usage of Clinoptilolite as an adjuvant alone or preferable with ISA 206 oil in inactivated FMD trivalent vaccine induces long lasting immunity than that induced with oil adjuvant alone and improve both cellular and humoral immunity and resulted in earlier and more long lasting immunity, also it gave an early immunity when it used alone.

So, it is recommended to use FMD inactivated vaccine adjuvanted with oil and Clinoptilolite in companying of vaccination to control FMD.

## REFERENCES RÉFÉRENCES REFERENCIAS

1. *Abdel- Rahman, A. O.; Farag, M. A.; Samira El- Kilany; Eman, M. A.; Manal Abo El- Yazed and Zeidan, S. (2006): Isolation and Identification of FMDV during an outbreak of 2006 in Egypt. Kafr El- Sheikh Vet. Med. J.; 4(1): 2006.*
2. *Aikoh T, Tomokuni a, Matsukii T, Hyodoh F, Ueki H, Otsuki T, Ueki A (1998): Activation-induced cell death in human peripheral blood lymphocytes after stimulation with silicate in vitro. Int J Oncol 12:1355-1359.*
3. *Batista A, Quattrocchi V, Olivera V, Langellotti C, Pappalardo JS, Di Giacomo S, Mongini C, Portuondo DI, Zamorano P (2010): Adjuvant effect of Cliptox on the protective immune response induced by an inactivated vaccine against foot and mouth disease virus in mice. Vaccine. 2010 Aug 31; 28(38):6361-6. doi: 0.1016/j.vaccine .2010. 06. 098. Epub 2010 Jul 14.*
4. *Depa, P.M., Dimri, U., Sharma, M.C. and Tiwari, R. (2012): Update on epidemiology and control of foot and mouth disease – A menace to international trade and global animal enterprise. Vet. World, 5(11): 694-70.*
5. *Dar P, Kalaivanan R, Sied N, Mamo B, Kishore S, Suryanarayana W (2013): Montanide ISA 201 adjuvanted FMD vaccine induces improved immune responses and protection in cattle. Vaccine 2013; 31:3327–32.*
6. *David, 2013. Evaluation of Calcium Phosphate Nanoparticles Mineralized with Proteins and Peptides for Use as Adjuvants in Protein and Nucleic Acid Vaccines. Doctor of Philosophy University of Washington 2013.*
7. *Dong Li, Chunxue Zhou, Daliang She, Pinghua Li, Pu Sun, Xingwen Bai, Yingli Chen, Baoxia Xie, Zaixin Liu (2013): The comparison of the efficacy of swine FMD vaccine emulsified with oil adjuvant of ISA 201 VG or ISA 206 VG, Journal of Biosciences and Medicines, 2013, 1, 22-25.*
8. *Ehab El-Sayed Ibrahim, Wael Mossad Gamal, Amr Ismail Hassan, Safy El-Din Mahdy, Akram Zakria Hegazy and Magdy Mahmoud Abdel-Atty (2015): Comparative study on the immunopotentiator effect of ISA 201, ISA 61, ISA 50, ISA 206 used in trivalent foot and mouth disease vaccine .Veterinary World, EISSN: 2231-0916 , P.(1189-1198).*
9. *El-Din, W.M., Ibrahim, E.E., Daoud, H. and Ali, S.M. (2014) Humeral and cellular immune response of Egyptian trivalent foot and mouth disease oil vaccine in cattle. Res. Opin. Anim. Vet. Sci., 4(4): 178-185.*
10. *EL-Watany, H.; Shawky, M.M.; Roshdy, O.M. and El-Kelany, S. 1999. Relationship between cellular and humoral immunity responses in animal vaccinated with FMD vaccine. Zagazig Vet. J., 27(1): 49-57.*
11. *EL-Naggar, H. 2012. Preparation of inactivated lyophilized NDV vaccine, M.V.Sc in Veterinary Science (Virology). Cairo University.*
12. *European Parliament (1997): Application for the Approval of Clinoptilolite Regulation (EC) No.258/97 of the European Parliament and of the Council of 27th January 1997.*
13. *Fakhry, H.M., Rizk, S.A., Abu-Elnaga, H.I., Deghaidy, W., Talaat, A.A. and Hegazi, A.Z. (2012): Field application of bivalent foot and mouth disease vaccine adjuvanted with Montanide ISA (25, 50, 206) and IMS (1113-3015) as an alternative to aluminum hydroxide gel. Egypt. J. Virol., 9(1): 123-136.*
14. *Farag, M.A., Aggour, M.A. and Daoud, A.M. (2005): ELISA as a rapid method for detecting the correlation between the field isolates of foot and mouth disease and the current used vaccine strain in Egypt. Vet. Med. J. Giza., 53(4): 949-955.*
15. *Garces JM (1999): Observations on Clinoptilolite applications. In: Treacz MMJ, Marcus BK, Misher ME, Higgins JB (eds) Proceedings of the 12th International Conference on Clinoptilolites. Materials Research Society, Warrendale, pp 551-566.*
16. *Hamblin C, Barnett I T R and Crowther J R (1986): Anew Enzyme-Linked Immuno Sorbent Assay (ELISA) for the detection of antibodies against FMD virus. II Application. Journal of immunological methods, 93: 123-129.*
17. *Hiam M. Fakhry and Assem A A (2014): study of zeolite as immune stimulant in foot and mouth disease trivalent oil adjuvant vaccine in cattle. 5th international conference of virology (December 9-12/2014), Egyptian J. virol. Vol. 11 (1) 60-69, (2014).*
18. *Hiam F. Alhawary, Assem M. Abobaker, Sonia A. Rizk, Akram Z. Hegazi, Abobaker A. Agoor (2017): Field application of trivalent foot and mouth disease vaccine adjuvant with Zeolite. Alhawary et al., J. of Virol. Sci., Vol. 2: 32-41, 2017.*

19. Huang, X., Li, Y., Fang, H. and Zheng, C. 2011: Establishment of persistent infection with foot and mouth disease virus in BHK-21 cells. *Virology Journal*, 8: 169.
20. Kre Imir Paveli , Mirko Had Ija , Ljiljana Bedrica , Jasminka Paveli , Ivan Điki Maakati , Marijeta Kralj , Maja Herak Bosnar , Sanja Kapitanovi, Marija Poljak-Blailmun Kri Anac , Ranko Stojkovi, Mislav Jurin, Boris Suboti And Miroslav Oli (2000): Natural Clinoptilolite clinoptilolite: new adjuvant in anticancer therapy. Received: 17 April 2000/ Accepted: 15 October 2000 / Published online 1-2.
21. Mercedes G. V., Timothy D., Trevor C., Martin R. and R. Michael E. P. (1996): Recognition of foot-and-mouth disease virus and its capsid protein VP1 by bovine peripheral T lymphocytes. *Journal of General Virology* (1996), 77, 727-735.
22. Lombard, M.; Pastoret, P.P. and Moulin, A.M. (2007): A brief history of vaccines and vaccination. *Rev. Sci. Tech.* 26 (1):29-48.
23. Mansouri Nabiollah , Navid Rikhtegar, Homayon Ahmad Panahi, Farideh Atabi and Behrouz Karimi Shahraki 2013: Porosity, characterization and structural properties of natural zeolite – clinoptilolite – as a sorbent. *Environment Protection Engineering*, Vol. 39 (139-152).
24. Nader M. Sobhy, Sunil K. Mor, Mohammed E.M. Mohammed, Iman M. Bastawecy, Hiam M. Fakhry, and Sagar M. Goyal 2014: Phylogenetic analysis of Egyptian foot and mouth disease virus endemic strains in 2013. 5th international conference of virology (December 9-12/2014), *Egyptian J. virol.* Vol. 11 (1) 49-59, (2014).
25. OIE 2012: Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France: 2012. Foot and mouth disease. Chapter 2.1.5.
26. OIE. (2013): OIE/FAO Foot-and-Mouth Disease Reference Laboratory Network, Annual Report, 2013
27. Orsel, K.; deJong, M.C.; Bouma, A.; Stegeman, J.A. and Dekker, A. (2007): Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* 2 21;25(34):6381-91
28. Park JH. (2013): Requirements for improved vaccines against foot-and-mouth disease epidemics. *Clin Exp Vaccine Res* 2013; 2:8–1.
29. Pluimers, F.H. (2004): Foot-and-Mouth disease control using vaccination: the Dutch experience in 2001.
30. Reed, L.J. and Muench, H. (1938): A simple method of estimating 50% end points. *Am. J. Hyg.*, 27: 493-497.
31. Ray Sahelian, M.D. (2016): Zeolite supplement benefit and side effects, a review, does it have benefits to the body. A review of Medline in 15 March 2016.
32. Rhodes CJ (2010): Properties and applications of Clinoptilolite. *Sci Prog*; 93(Pt3):223-84. Review PMID.
33. Rodriguez, L.L. and Grubman, M.J. (2009): Foot and mouth disease virus vaccines. *Vaccine*, 27 Suppl 4: D90-4.
34. Sarkar A, Tamil Selvan RP, Kishore S, Ganesh K and, Bhanuprakash V 2017 : Comparison of different inactivation methods on the stability of Indian vaccine strains of foot and mouth disease virus. *Biologicals*. 2017 Jul; 48:10-23.
35. Scudiero, D.A., Shoemaker, R.H., Paull, K.D., Monks, A., Tierney, S., Nofziger, T.H., Currens, M.J., Seniff, D. and Boyd, M.R. 1988. Evaluation of soluble tetrazolium/Formazan Assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Research Journal*, 48: 4827-4833.
36. Shawky M., Abd El-Aty M., Hiam. M. Fakhry, Hind M. Daoud, Ehab El-Sayed I., Wael Mossad G., Sonia A. Rizk, Abu-Elnaga H., Mohamed A. A., Abd El-kreem A. and Farouk E. M. (2013) : Isolation and Molecular Characterization of Foot and Mouth Disease SAT2 Virus during Outbreak 2012 in Egypt. *J Vet Adv*2013, 3(2): 60-68.
37. Sonia A Rizk, Hiam M Fakhry and Abu-Elnaga H I (2010): Comparative study of T cell proliferative response in cattle vaccinated with FMD vaccine using Cell titre-Aqueous one solution non radioactive assay (MTS). *Zag. Vet. J.* (ISSN. 1110-1458) Vol. 38, No. 4 pp. 188-195 (2010).
38. Sonia, A.Rizk., Abo Bakr Agoor, Ekbal Farok, Hind daoud and Hiam M Fakhry. (2015): Enhancing effects of Calcium phosphate nanoparticles adjuvant on the Immune response in cattle vaccinated with Foot and Mouth Disease trivalent vaccine. *Egyptian J. virology* vol.12 - 53- 66, (2015).
39. Sharma, M.C., Pathak, M.N. Hung, M.N., Nhi D.L. and Vuc, N.V. (1984). Report on the outbreak of foot and mouth disease in buffaloes in the southern part of Vietnam. *Veterinary viral diseases*: 302–305.
40. Satya, P. (2009): Vaccination against foot-and-mouth disease virus: Strategies and effectiveness. *Expert Rev. Vaccines*, 8(3): 347-365.
41. Tsai CP, Pan CH, and Liu MY, Lin YL, Chen CM, Huang TS, (2000): Molecular epidemiological studies on foot-and-mouth disease type O Taiwan viruses from the 1997 epidemic. *Vet Microbiol* 2000; 74:207–1.
42. Voller A, Bid Well D and Bartleha (1976): Micro plate enzyme immuno assay for the immuno diagnosis of virus infection. In *Manual of Clinical Immunology*, Chapter 69, edited by N. Rose and H. Friedman, pp. 506- 512. American Society for Microbiology.
43. Wisniewski J., Kobusiewicz T., Baronowski C., and Jankowski J.,(1972): Determination of the level of immunity in cattle on the basis of neutralizing

antibodies after the use of a Frenkel type FMD vaccine. Medycyna Wet 28 (10): 586-588.

44. Zahran, G.E.D. (1960): Foot and mouth disease in southern region of URA. Bull. Off. Int. Epiz., 13: 390-393.0.

Table 1: Comparative delta optical density of the cell-mediated immune response of cattle, vaccinated with trivalent FMD vaccines using lymphocyte Proliferation (XTT) Assay

Time post vaccination	ΔOD in buffy coat in Vaccinated cattle			Control non vaccinated animal
	Group1 clinoptilolite	Group 2 (oil and clinoptilolite)	Group 3 (Oil)	
Pre vaccination	0.0488	0.0466	0.044	0.064
3 <sup>rd</sup> day	0.5178	0.515	0.4736	0.065
1 week	0.8508	0.866	0.490	0.056
2 week	1.468	1.655	1.136	0.069
3 week	1.5572	1.660	0.856	0.067
4 week	1.257	1.459	0.777	0.065
5 week	1.257	0.934	0.676	0.064
6 week	0.827	0.848	0.627	0.065
7 week	0.599	0.819	0.463	0.066

Table 2: Mean of serum antibody titers against type (O), (A) & SAT 2 in cattle vaccinated with trivalent FMD vaccines using SNT expressed log<sub>10</sub>

Weeks post vaccination	Cattle groups vaccinated with trivalent FMD vaccines									Non vaccinated Group
	FMD Clinoptilolite vaccine			FMD Clinoptilolite+ oil vaccine			FMD oil vaccine			
	Mean antibody titer against FMD virus strains									
	FMD (O)	FMD (A)	FMD (SAT2)	FMD (O)	FMD (A)	FMD (SAT2)	FMD (O)	FMD (A)	FMD (SAT2)	
Pre vacc	0.15	0	0.3	0.15	0.27	0.27	0.15	0.3	0.3	0.3
1	1.1	1.05	1.2	0.9	0.9	0.9	1.2	1.05	1.2	0.3
2	1.65	1.8	1.8	1.14	1.29	1.38	1.65	1.8	1.8	0.3
3	2.1	2.1	1.95	1.71	1.8	1.77	1.8	2.1	2.15	0.3
4	2.4	2.4	2.4	1.95	2.1	1.8	2.4	2.4	2.55	0.3
6	2.7	2.7	2.7	2.34	2.25	2.1	2.7	2.7	2.85	0.3
8	2.85	2.85	2.85	2.58	2.7	2.37	3.0	3.0	3.0	0.3
10	2.85	3.15	3.0	2.82	2.82	2.7	3.3	3.15	3.15	0.3
12	2.55	2.85	3.0	3.0	3.0	3.0	3.3	3.15	3.3	0.3
14	2.55	2.7	2.85	2.8	2.8	2.70	3.0	3.0	3.0	0.3
16	2.4	2.4	2.55	2.6	2.6	2.49	2.85	2.85	2.9	0.3
20	2.1	2.1	2.4	2.5	2.4	2.37	2.6	2.7	2.85	0.3
24	1.8	1.8	2.1	2.37	2.13	2.25	2.4	2.55	2.7	0.3
28	1.65	1.65	1.8	2.13	2.04	2.16	2.25	2.4	2.4	0.3
32	1.5	1.5	1.65	1.83	1.77	1.7	2.1	2.1	2.1	0.3
36	1.05	1.05	1.2	1.35	1.17	1.20	1.65	1.8	1.8	0.3
40	0.75	0.6	0.75	0.9	0.75	0.6	1.5	1.65	1.5	0.3

**Table 3:** Antibody titers of cattle vaccinated with inactivated trivalent FMD vaccine using ELISA against FMDV. Serotype (O, A and SAT2)

Time post vaccination	ELISA titers of vaccinated animal groups									Control group
	FMD Clinoptilolite vaccine			FMD Clinoptilolite+ oil vaccine			FMD oil vaccine			
	O	A	SAT2	O	A	SAT2	O	A	SAT 2	
0	0.18*	0.21	0.21	0.11	0.21	0.21	0.24	0.27	0.27	0.3
1 week	1.93	1.95	1.93	1.70	1.70	1.69	1.50	1.50	1.50	0.0
2 week	2.12	2.12	2.11	1.97	1.99	1.96	1.90	1.92	1.90	0.0
3 week	2.42	2.42	2.41	2.61	2.62	2.61	2.19	2.19	2.16	0.3
4 week	2.47	2.47	2.46	2.43	2.49	2.48	2.43	2.43	2.43	0.6
6 week	2.73	2.73	2.73	2.73	2.79	2.79	2.44	2.44	2.44	0.7
8 week	2.92	2.92	2.92	2.92	2.95	2.95	2.80	2.80	2.78	0.6
10 week	3.12	3.15	3.13	3.32	3.34	3.33	2.90	2.92	2.92	0.6
12 week	3.15	3.15	3.15	3.15	3.19	3.19	3.10	3.10	3.10	0.6
14 week	2.85	2.85	2.85	2.97	2.99	2.99	2.49	2.49	2.49	0.0
16 week	2.67	2.67	2.67	2.75	2.78	2.76	2.52	2.52	2.52	0.6
18 week	2.66	2.66	2.65	2.69	2.71	2.71	2.43	2.43	2.43	0.0
20 week	2.34	2.34	2.34	2.60	2.62	2.62	2.19	2.19	2.19	0.6
22 week	2.31	2.32	2.32	2.44	2.46	2.46	2.10	2.11	2.11	0.7
24 week	2.34	2.34	2.34	2.43	2.46	2.46	2.09	2.10	2.10	0.3
26 week	2.11	2.19	2.19	2.43	2.45	2.43	1.99	1.99	1.99	0.7
28 week	2.11	2.15	2.15	2.43	2.44	2.44	1.93	1.93	1.93	0.3
30 week	2.10	2.12	2.12	2.34	2.36	2.36	1.93	1.93	1.93	0.3
32 week	1.95	1.98	1.97	2.27	2.29	2.29	1.94	1.94	1.92	0.9
34 week	1.93	1.95	1.95	2.10	2.11	2.10	1.72	1.72	1.69	0.3
36 week	1.91	1.92	1.92	1.97	1.99	1.99	1.46	1.46	1.46	0.6
38 week	1.71	1.72	1.71	1.97	1.98	1.96	1.45	1.45	1.42	0.6
40 week	1.59	1.61	1.61	1.95	1.95	1.92	1.41	1.41	1.39	0.9







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GLOBAL JOURNAL OF MEDICAL RESEARCH: G  
VETERINARY SCIENCE AND VETERINARY MEDICINE  
Volume 18 Issue 2 Version 1.0 Year 2018  
Type: Double Blind Peer Reviewed International Research Journal  
Publisher: Global Journals  
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

# Survey on Pathological Lesion and its Financial Losses in Ovine Slaughtered at Jimma Municipal Abattoir, Jimma, Ethiopia

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**Abstract-** A cross-sectional study was conducted from October 2016 to July 2017 on ovine slaughtered at at Jimma municipal abattoir with the aim of identifying main pathological lesions causing organs and carcass condemnation, and associated direct financial losses. In this, 384 sheep were recruited to the study using systematic random sampling and standard antemortem (AM), and postmortem inspection (PMI) procedures were employed. Nasal discharge, tick infestation, coughing, lameness, emaciation, depression and salivation are recorded as the major AM findings of the current study. Accordingly, 47 (12.2%) sheep showed signs of diseases and abnormalities; of which 23 (6%) were conditionally approved whereas 11 (2.9%) sheep were unfit and judged to be detained and rejected. In the present study age, body conditions and geographic origin of the animals were considered as study variables, and the results showed BCS and age groups had statistically higher ( $p \leq 0.05$ ) rejection probabilities.

**Keywords:** abattoir, financial loss, lesion, organ condemnation, PMI, sheep, jimma.

**GJMR-G Classification:** NLMC Code: WB 141



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# Survey on Pathological Lesion and its Financial Losses in Ovine Slaughtered at Jimma Municipal Abattoir, Jimma, Ethiopia

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**Abstract-** A cross-sectional study was conducted from October 2016 to July 2017 on ovine slaughtered at Jimma municipal abattoir with the aim of identifying main pathological lesions causing organs and carcass condemnation, and associated direct financial losses. In this, 384 sheep were recruited to the study using systematic random sampling and standard antemortem (AM), and postmortem inspection (PMI) procedures were employed. Nasal discharge, tick infestation, coughing, lameness, emaciation, depression and salivation are recorded as the major AM findings of the current study. Accordingly, 47 (12.2%) sheep showed signs of diseases and abnormalities; of which 23 (6%) were conditionally approved whereas 11 (2.9%) sheep were unfit and judged to be detained and rejected. In the present study age, body conditions and geographic origin of the animals were considered as study variables, and the results showed BCS and age groups had statistically higher ( $p \leq 0.05$ ) rejection probabilities. PM examination findings indicated a total of 192 lesions were encountered, of which 48.9%, 29.2%, 6.8%, and 5.7% lesions were recorded from livers, lungs, GIT, and hearts and kidneys, respectively. During the PM inspection, both total and partial condemnation judgments were passed on organs and carcass appeared with a sign of abnormality. C. tuniculosis 58 (30.2%), calcification 36(18.7%), abscess 31(16.2%), hydatid cyst 23 (12%), hepatitis 10 (5.2%), pericarditis 4(2%), bruise 3(1.6%) and nephritis 2(1%) were found to be the major pathological lesions recorded. In two sheep all organs and carcass were totally condemned as their entire bodies appeared yellowish. The direct loss due to the condemnation of organs and carcasses at Jimma municipal abattoir was also investigated in slaughtered sheep, and there were about 56,576 USD losses per year. In conclusion, this study has identified the pathological lesions affecting edible organs and meat, and then rendering them unfit for human consumption. The study also estimated pathological lesions associated direct financial losses at Jimma abattoir. Therefore, further studies focusing on the primary causes of the abnormalities were recommended in the study area.

**Keywords:** abattoir, financial loss, lesion, organ condemnation, PMI, sheep, jimma.

## I. INTRODUCTION

The livestock sector globally is highly dynamic, contributes 40% of the global value of agricultural output and support the livelihoods and food security of almost a billion people (Thornton, 2010).

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Within African society, small ruminant comprises a greater proportion of the total wealth of the rural families, because of the low input requirements such as low initial capital, fewer resources and maintenance cost. They are also able to produce milk and meat in readily usable quantities using marginal lands and poor pasture and crop residues. Furthermore, their production cycle makes them need only short periods to reconstitute flocks after a disaster and respond quickly to the demand (Genet by, 1991). Ethiopia is the leading African country in livestock population, having around 34-40 million TLU (Tropical livestock unit) out of which 17% and 12% cattle and small ruminants, respectively, are found in Ethiopia (Ministry of information (MOI), 2005). According to (Development, ppStatis, 2009), the population of sheep and goats in Ethiopia is estimated to be 26.1 and 21.7 million respectively. It was the third largest number of sheep and goat among African nations and rank eighth in the world (Alemu and Merkel, 2008).

They generate cash income from export of meat, edible organs, skins and live animals (Ibrahim, 1998). There is also a high domestic meat demand from these animals, particularly during religious festivals. Even though this sub-sector contributes much to the national economy, its development is hampered by various constraints. These include endemic animal diseases, insufficient nutrition, poor husbandry, and lack of sufficient infrastructure, trained labor and government policies (PACE, 2003). Each year a large loss results from the death of animals and weight loss during transportation; and condemnation of edible organs and carcasses at slaughter.

Abattoir meat inspection is essential to remove gross abnormalities from meat and its products, to prevent the distribution of contaminated meat and to assist detecting and eradication of certain livestock diseases. More specifically, ante mortem inspection attempts to avoid introduction of clinically diseased animals into slaughter house and also serves to obtain information that will be useful in making sound post mortem inspection. Likewise, postmortem inspection is the center around which meat hygiene revolves since it provides information essential for evaluation of clinical signs and pathological process that affect the wholesomeness of meat (Herenda, et al., 1994).

As the meat is the sources of protein to a human being, it should be clean and free from diseases of particular importance to the public such as tuberculosis and cysticercosis. Meat is also condemned at slaughterhouse to break the chain of some zoonoses which are not transmitted to man directly via meat like hydatidosis and other important diseases of animals such as fasciolosis (Arbabi and Hooshy, 2006; Fufa *et al.*, 2010).

Each year a significant economic loss results from mortality, poor weight gain, condemnation of edible organs and carcasses at slaughter. This production loss in the livestock industry is estimated at more than 900 million USD annually (Jacob, 1979; Abebe, 1995; Jobreet *et al.*, 1996). The major causes of pathological lesion during PMI of slaughtered ovine at abattoir are the disease caused by parasites, bacterial and other abnormalities. The final judgment as to action to be taken with an organ, the carcass or part of a carcass is based on the total evidence produced by the visual observation, palpation and incision (Teka, 1997).

Abattoir data is an important option for observing the diseases of both economic and public health importance (Arbabi and Hooshy, 2006; Fufa, *et al.*, 2010). Nowadays, several modern abattoirs like: HELMEX, ELFORA, Metehara, Modjo and Luna are established in Ethiopia. This increase in a number of slaughterhouse shows that increase in demand for meat supply, but the provisions have been challenging due to diseases, production problems and other factors. Given this, proper evaluation of financial losses due to organ condemnation resulting from various diseases at abattoirs is needed (Ezana, 2007). It is necessary to have enough information on a pathological lesion that causes organs and carcass condemnation at the abattoir. Hence, having information on where and how to reduce the losses that may be caused by the various abnormalities (lesions/pathology). Various studies (Jembere, 2002; Yimam, 2003; Aseffa, 2005; Get chew, 2008; Regessaet *et al.*, 2013) were carried out in the country in this regard to know the causes and losses associated. However, in Jimma there are no recorded studies conducted on major causes and financial losses associated with organs and carcass condemnation along with survey on pathological lesions. Therefore, the objectives of this study were to:

- Identify major pathological lesions causing organs and carcass condemnation in slaughtered sheep at Jimma municipal abattoir and,
- Estimate the direct financial losses attributed to condemned organs and carcass in sheep.

## II. MATERIALS AND METHODS

### a) Study Area

The study was conducted from November 2016 to July 2017 at Jimma municipal abattoir in Jimma zone.

Jimma two is found in Oromia region south-western part of Ethiopia at a distance of 346 km away from Addis Ababa and lies between 36°50'E longitude and 7°40'N latitude at an average elevation of 1750 meter above sea level. Jimma is the largest city in south-western Ethiopia. It is special zone of the Oromia Regional state and is surrounded by different Jimma woreda. The climate of the area is characterized by humid tropical with bimodal heavy rainfall which is uniform in amount and distribution, ranging from 1200 to 2800 mm per year, with short and main seasons occurring from mid-February to May and June to September, respectively. The rainy season extends from mid-February to early October. Temperatures at Jimma are in a comfortable range, with the daily mean staying between 20°C and 25°C year-round. The total human populations of Jimma town was about 174, 446(88, 766 males and 85, 680 females). The livestock population of the area was reported to be about 2, 016, 823cattle, 942, 908 sheep, 288, 411 goats, 74, 574 horses, 49, 489 donkey, 28, 371 mules, 1, 139, 735 poultry and 418, 831bee hives (GOR, 2006).

### b) Study Population

The study animals were sheep brought Jimma municipal abattoir and destined for slaughter. All animals were male and belonged to indigenous breeds kept under extensive management system. Sheep destined for slaughter had come from different parts of the weredas in the Jimma zone such as Dedo, Serbo, Saqa and Bilida inspected by standard AM, and PMI.

### c) Study Design

A cross-sectional study using systemic random sampling technique was conducted from December 2016 to April 2017 to determine the pathological lesion that causes organs and carcass condemnation and to estimate the magnitude of direct financial loss attributed in sheep slaughtered at Jimma abattoir.

### d) Sampling Method

#### i. Sample size determination

In this study, systematic random sampling method was applied to include study animals, and study animals were grouped into young (under 1 years and three months) and adult above this based on the eruption of one or more incisor teeth according to Vattaet *et al.* (2005). Since there was no published work on lesion survey from Jimma abattoir, 50% expected prevalence is considered to calculate the total sample size with 95% CI, 5% level of precision (Thrush field, 2007). The sample size was 384 and determined using the formula given by

$$N = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$$

Where N= required sample size, P<sub>exp</sub>= expected prevalence and d is desired absolute



precision. Accordingly, the total sheep included in the study were 384.

e) *Abattoir Survey*

In the cross-sectional study of active abattoir survey, both AM and PMI were carried out in accordance by the procedures of Ethiopian Meat Inspection Regulation (Belina and Melese, 2017).

i. *Ante-mortem Inspection (AMI)*

The AMI (pre-slaughter examinations) of ovine was conducted at lairage both in motion and at rest and information related to study variables such as the behavior of an animal, age, BCS and origin and were recorded. At the same time, various signs of diseases and abnormalities were inspected with physical animal examination and its judgment were approved, conditionally approved, detained and rejected. Study animals were grouped into young and adult age groups according to standard dentation method (Vattaet *al.*, 2005).

ii. *Post Mortem inspection (PMI)*

During PMI all internal organs (liver, lungs, heart, kidney, gastro-intestinal tract), and carcasses were thoroughly inspected by visualization, palpation and making systematic incisions for the presence of cysts, parasites and other abnormalities. Pathological lesions were differentiated and judged according to guidelines on meat inspection for developing countries

as totally fit for human consumption, and conditionally approved, and totally or partially condemned when unfit for human consumption (FAO, 1993).

f) *Assessment of direct Financial Loss*

In the current study, the total financial loss due to organs and carcass condemnation was computed by considering the condemnation rate or percentage of each edible organ and carcass, average number of animals slaughtered in the abattoir per year from retrospective data of the abattoir. The average weight of each organ and carcasses in kg, average current local market price of major organs and carcass, and each condemned organ was counted to estimate the financial loss. The average current local market price of each organ and mutton was collected by questionnaire from the butcherries in Jimma town for ease of computing the loss.

The retail average market prices obtained from butcher shops found in Jimma town in ETB were: Liver=30, lung=20, kidney=15, heart=18, GIT=90 ,whole carcass=4000 and mutton =150ETB/kg. In the case when there was whole carcass plus organs (whole body) rejection at PM, the average price of sheep came for slaughter was considered (4,000 ETB). The direct loss is calculated according to the procedures described by Ogurinate and Ogunrinade (1980), and the formula:

$$Los = MAK * PL \sum_{i=1}^{i=n} (PiCi), \text{ where}$$

LOS is direct annual financial loss due to organs and carcass loss, MAK is annual average number of sheep slaughtered at Jimma abattoir, PL is overall prevalence of lesion, Pi is prevalence of each organ and carcass condemned, Ci is average market price of each organ and 1kg mutton at butcher shops of the Jimma town.

The direct financial loss was expressed in US Dollar (\$) based on the current currency exchange rate of 1 USD = 22.5 Ethiopian Birr (ETB).

g) *Data Analysis*

The active abattoir data, and questionnaire survey were entered into Microsoft Excel- 2016 spread sheet and the process of coding, cleaning and validating was done on this sheet and analyzed using SPSS version 20. For the data from PMI, descriptive statistics were used to determine organ and carcass condemnation rates, defined as the proportion of organs and carcasses condemned to the total number of organs and carcasses examined. Each financial loss was also calculated. Possible variation between rejection rates of specific organs, age groups and origin, were taken into consideration.

### III. RESULTS

a) *Abattoir Survey*

i. *Ante mortem Inspection (AMI)*

Detail AMI was conducted on a total of 384 sheep destined for slaughter at Jimma municipal abattoir and 47 (12.2%) of ovine were found to have different abnormalities. Nasal discharge, coughing, tick infestation, depression, emaciation, and lameness were those frequently observed among signs of diseases encountered in both age groups. The result also showed 6% (23/384) animals were conditionally passed for slaughter because of abnormalities such as lameness, respiratory problem, and their collection with tick infestation. On the other hand 2% (8/384) sheep were unfit for human consumption and rejected during AMI. Since, they showed two and more signs of diseases such as emaciation with nasal discharge and depression 1 % ( 4/384), salivation and salivation with coughing 1 % ( 4/384) were the major cause of rejection (Table 1).

The AMI result also depicted of 28 sheep with poor body conditions 46.43% were found to have one or more sign/s of illness whereas in those with good BCS

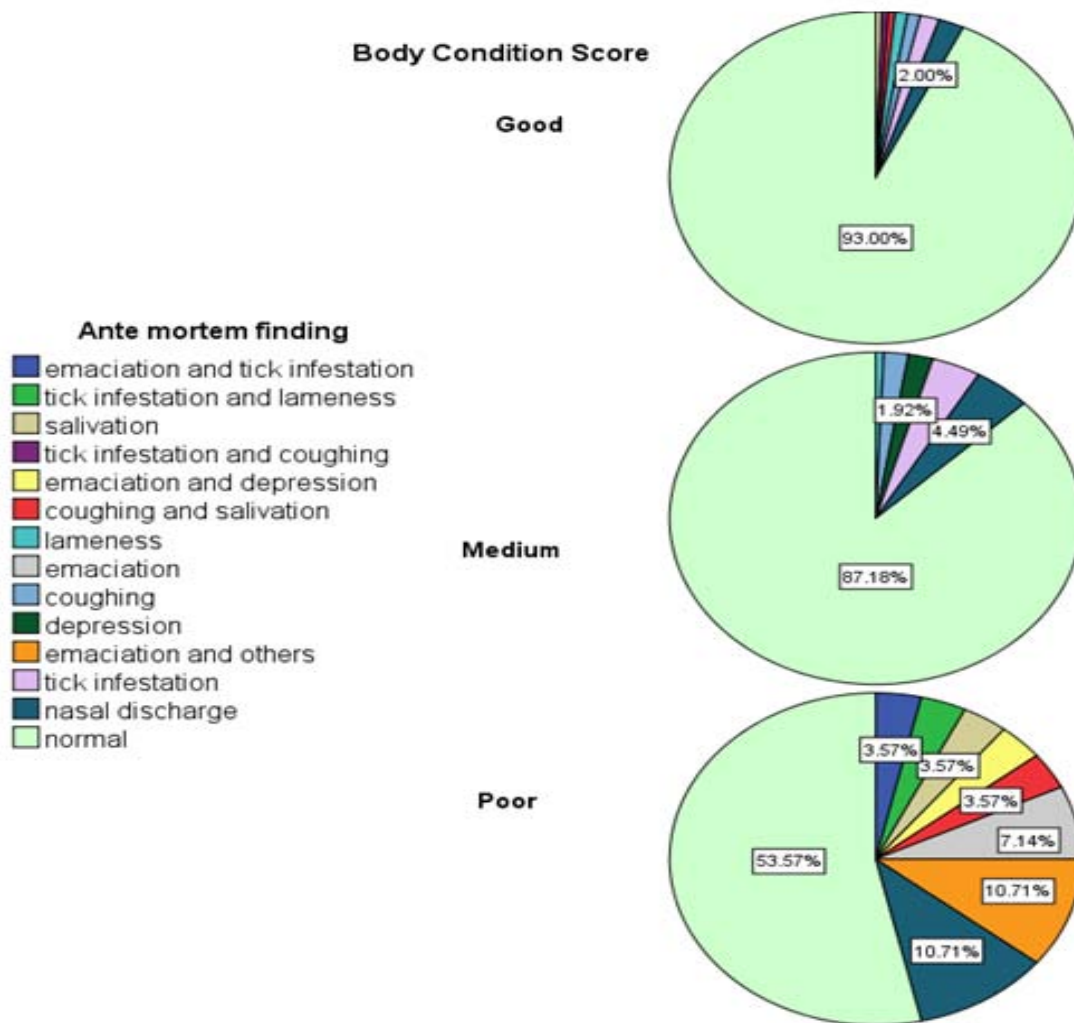
only 7% of the sheep were showed sign/s of diseases (Graph. 1)

In the current study, four different decisions were passed as AM judgments where apparently healthy sheep were passed for slaughter (91%), and others showed mild signoff illness and conditionally

approved (6%) whereas 2.9% of sheep were detained and rejected as unfit for slaughter. Rejection rate was significantly higher ( $p \leq 0.05$ ) in young animals with poor BCS than in adult animals with good and medium BCS (Table 2).

Table 1: Abnormalities encountered during AM inspection within Age groups and Origin of the animals

Ante-mortem Finding	Age (%)			Origin (%)				
	Adult	Young	Sub Total	Dedo	Bilida	Seka	Serbo	Sub Total
Apparently healthy	171(85.9)	166(89.7)	337(87.8)	127(88)	65(84)	99(89)	46(88.5)	337(87.8)
Depression	2(1)	1(0.5)	3(0.8)	2(1.4)	1(1.3)	0(0)	0(0)	3(0.78)
Salivation	1(0.5)	1(0.5)	2(0.5)	0(0)	1(1.3)	1(0.9)	0(0)	2(0.5)
Emaciation and nasal discharge	1(0.5)	1(0.5)	2(0.5)	1(0.7)	0(0)	0(0)	1(2)	2(0.5)
Tick infestation and coughing	2(1)	0(0)	2(0.5)	1(0.7)	0(0)	1(0.9)	0(0)	2(0.5)
Coughing	3(1.5)	2(1.1)	5(1.3)	0(0)	1(1.3)	4(3.6)	0(0)	5(1.3)
Lameness	3(1.5)	1(0.5)	4(1)	0(0)	2(2.6)	1(0.9)	0(0)	4(1)
Coughing and salivation	0(0)	2(1.1)	2(0.5)	0(0)	1(1.3)	1(0.9)	0(0)	2(0.5)
Tick infestation	5(2.5)	5(2.7)	10(2.6)	2(1.4)	3(4)	1(0.9)	3(5.8)	10(2.6)
Emaciation and depression	1(0.5)	1(0.5)	2(0.5)	2(1.4)	0(0)	0(0)	0(0)	2(0.5)
Nasal discharge	10(5)	4(2.2)	14(3.6)	8(5.6)	2(2.6)	2(1.8)	2(3.8)	14(3.6)
Tick infestation and lameness	0(0)	1(0.5)	1(0.3)	1(0.7)	0(0)	1(0.9)	0(0)	1(0.26)
Total	199	185	384	144	77	111	52	384



Graph 1: Proportion of ante-mortem finding by animals' boay condition score

Table 2: Proportion of animals rejected and passed the AMI judgments by age group and BCS at the abattoirs

AMI judgment	Age (%)		Body condition score (%)			Subtotal (%)
	Young	adult	Good	Medium	Poor	
Rejected	6(3.2)	2(1.0)	2(1.0)	0	6(21.4)	8(2)
Detained	0	4(2.0)	0	1(0.64)	3(10.7)	4(1)
Cond. Approved	8(4.3)	13(6.5)	10(5)	9(5.8)	4(14.3)	23(6)
Approved	171(92.4)	178(89.5)	188(94)	146(93.6)	15(53.6)	349(91)
Total	185	199	200	156	28	384
X(P value)	0.05				0.00	

ii. *Postmortem Inspection (PMI)*

Among animals that had been examined during AMI 373 were slaughtered and subjected to PMI following standard postmortem procedure and a total of 192 gross pathological lesions leading to partial and total condemnation of organs and carcasses were recorded. Among these abnormalities, lesions were frequently encountered from liver; and of which Cyst cercus teniculus (34%) and calcification accounted 25.5%. These followed by abscess (13.8%), hepatitis (10.6%), cirrhosis (7.4%) and hydatid cyst (7.4%), fasciolosis (5.3%) and stelezia hepatica (3.2%).

A total of 56 lungs were also condemned as they were affected by teniculus (35.7%), Hydatid cyst (21.4%), marbling lesion (17.8%), Emphysema and calcification (16%), pneumonia (12.5%), abscess (3.6%) and 5% with unidentified lesions. In this study abscessation was also inspected in other organs like heart, kidney and GIT, and carcass (Table 3).

Out of a total of 11 hearts condemned (Table 4), hydatid cyst and pericarditis recorded as major causes contributing 45.5% and 36.4% which followed by abscess (18.2%) and discoloration (0.9%). Renal problems were observed in 11 kidney examined and 54.5% found to be caused by abscess whereas 27.3% and 18.2% were due to calcification and nephritis and other unidentified causes, respectively. 13 GITs were also encountered as with abnormalities like cyst cercus teniculus and abscess and foreign bodies, and they were subjected to total and partial condemnation accordingly.

The major pathological conditions for carcass rejection from local market were bruising accounting for 42.9%. Out of 7 rejections judgments 2 were total and the rest 5 were partial (Table: 3; and 4).

Table 3: Relative percentages of pathological lesions resulted in condemnations of organs and or carcasses at the abattoir

Organ affected	Causes/ lesion	PMJ (%)			P value
		*AP	PC	TC	
Liver	Teniculus	0	14(23)	2(1.8)	0.00
	Calcification	1(16.7)	12(19.8)	3(2.7)	
	Discoloration	0	1(1.6)	3(2.7)	
	Hydatid cyst	0	0	2(1.8)	
	Fasciolosis	0	0	3(2.7)	
	Cirrhosis	0	0	7(6.3)	
	Abscess	0	1(1.6)	3(2.7)	
	Hepatitis	0	0	7(6.3)	
	Stelezia hepatica	0	0	3(2.7)	
	Teniculus and calcification	0	2(3.3)	0	
	Teniculus and abscess	0	1(1.6)	1(0.9)	
	Teniculus and hepatitis	0	0	1(0.9)	
	Calcification and fasciolosis	0	0	2(1.8)	
	Hepatitis and calcification	0	0	1(0.9)	
	Abscess and hepatitis	0	0	1(0.9)	
	Teniculus and hydatid	0	0	1(0.9)	
	More than three lesions	0	1(1.6)	2(1.8)	
	Lung	Calcification	1(16.7)	3(4.9)	
Teniculus		0	3(4.9)	1(0.9)	
Marbling		0	3(4.9)	6(5.4)	
Pneumonia		0	0	5(4.5)	
Hydatid cyst		0	0	5(4.5)	
Emphysema		0	1(1.6)	2(1.8)	
Abscess		0	0	1(0.9)	
Emphysema and teniculus		0	0	6(5.4)	
Marbling and teniculus		0	0	1(0.9)	
Teniculus and pneumonia		0	0	2(1.8)	
Hydatid and calcification		0	0	1(0.9)	
Unidentified Lesion		1(16.7)	0	2(1.8)	

Heart	Discoloration	0	0	1(0.9)	1(0.56)	0.00
	Pericarditis	0	0	4(3.6)	4(2.3)	
	hydatid cyst	0	0	3(2.7)	3(1.7)	
Kidney	Abscess	0	1(1.6)	3(2.7)	4(2.3)	
	Calcification	1(16.7)	1(1.6)	0	2(1.12)	
	Nephritis	0	0(0)	2(1.8)	2(1.12)	
	Discoloration	0	0	1(0.9)	1(0.56)	
	Unidentified	0	0	2(1.8)	2(1.12)	
GIT	Tenicolosis	0	2(3.3)	1(0.9)	3(1.7)	
	Foreign body	2(33.3)	2(3.3)	0	4(2.3)	
	Abscess	0	1(1.6)	2(1.8)	3(1.7)	
Carcass	Bruise	0	2(3.3)	0	2(1.12)	
	Abscess	0	2(3.3)	0	2(1.12)	
	Hydatid and Bruise	0	1(1.6)	0	1(0.56)	
	Discoloration	0	0	2(1.8)	2(1.12)	
liver and kidney	Calcification	0	1(1.6)	0	1(0.56)	
	Abscess	0	0	2(1.8)	2(1.12)	
heart and lung	Hydatid cyst and abscess	0	0	1(0.9)	1(0.56)	
lung and kidney	Hydatid cyst	0	0	1(0.9)	1(0.56)	
Liver and Lung	Calcification	0	1(1.6)	0	1(0.56)	
	Tenicolosis	0	3(4.9)	2(1.8)	5(2.8)	
	Hydatid cyst	0	0	1(0.9)	1(0.56)	
	Hydatid and calcification	0	0	1(0.9)	1(0.56)	
	Tenicolosis and hydatid	0	0	2(1.8)	2(1.12)	
Liver and GIT	Tenicolosis	0	2(3.3)	0	2(1.12)	
	Tenicolosis and abscess	0	0	1(0.9)	1(0.56)	
Liver and GIT	Abscess	0	0	1(0.9)	1(0.56)	
	Abscess	0	0	1(0.9)	1(0.56)	
Liver and heart	Abscess	0	0	1(0.9)	1(0.56)	
	Abscess	0	0	2(1.8)	2(1.12)	
<b>Total</b>		<b>6(3.4)</b>	<b>61(99.8)</b>	<b>111(99.9)</b>	<b>178(100)</b>	

\*AP=organs conditionally passed for human consumption

Table 4: Summary of direct financial losses and organs and carcass condemned at abattoir

Condemnation status	Organ condemned						Carcass
	Liver	GIT	Lung	Heart	Kidney		
PC	39	7	14	0	3	5(3kg)	68
TC	55	6	42	11	8	2*	124
Total	94	13	56	11	11	7	192
Price in US \$	99.33	38	43.556	8.8	6.33		370.8

There was a condition of examining, a single to multiple lesions per organ e.g., we examined teniculousis and Calcification from a single liver; and all are recorded as different lesions.

\*=Whole carcass plus organs totally rejected at PM; except in carcass, PC indicates 50% loss.

#### b) Assessment of Direct Financial Losses

The direct financial loss was computed based on average cost/ price of individual condemned organs and carcasses during the study period, applying the formula given by Ogurinate and Ogunrinade (1980). The study indicated that there had been a total loss of 12,729,960ETB which is 56,576 Undue to a partial and total condemnation of organs and carcass at slaughterhouse annually. The study result also indicated there was a total condemnation of 2 (1.3%) whole carcasses (carcass plus organs) (Table 4). For the calculation, alive price of one sheep is considered as 4000ETB for total carcass condemnation.

## IV. DISCUSSIONS

The AM and PM inspections were conducted in the abattoir for the purpose of identifying an abnormality and removing animals' products with pathological lesions which were unsafe for human consumption and having poor aesthetic values (Van Llogtestijn, 1993; Grace *et al.*, 1999).

In this research, out of 384 study animals 11 (2.9%) were rejected and detained as unfit for human consumption suspecting different zoonotic diseases such as rabies in the case of salivation with high fever and tuberculosis in animals with a sign of emaciation with high coughing, depression and nasal discharge. Similarly, suspecting rabies in the case of salivation and bovine TB in animals with sign of emaciation with high depression and coughing was reported by Belina and Melees (2017). Radostits *et al.*, (2000) stated that in domestic ruminants, cobalt deficiency results in appetite and loss of body weight, emaciation,



weakness, decreased growth, unthrifty appearance, diarrhea, and anemia. Again during the AMI, 23(6%) of sheep were found to be showing signs of abnormalities such as lameness, tick infestation along with coughing and nasal discharge frequently encountered and passed with judgments of conditional approval where due attention was given for whole body part and specific organ at postmortem examinations.

One of the causes of lameness was trauma caused by hitting with a thick stick during driving to abattoir on foot and inappropriate vehicles and loading and off-loading negligence during transportation to marketplaces and to the abattoir. During the AMI examinations, it was found that respiratory disorders were higher than other abnormalities encountered during the AMI 14(3.7%) nasal discharge and 5(1.3%) coughing. The respiratory signs such as the presence of nasal discharge and coughing were most probably related to stress due to lack of feed and water that may lead to immune suppression enhancing opportunistic pathogens. On the other hand, overcrowding during transportation is also a source of stress (Get chew, 2008). In agreement to the current study, coughing, depression and lameness are frequently observed abnormalities encountered during AMI (Man defroet *al.*, 2015) at Elfora Export Abattoir, Ethiopia.

The rejection rate was significantly higher ( $p < 0.05$ ) for those poor body conditions than good and medium body conditions (Table 2). Because of poor BC by itself may be due to unidentified abnormalities that increase rejection probability. Jibatet *al.* (2008) studied and determined the rate of organs and carcasses condemned and the associated annual financial loss at HELMEX abattoir in Ethiopia and they reported out of 2688 sheep and goats examined 188(7%) carcasses were condemned due to poor body condition cases.

On the other hand, there was a significant difference ( $p = 0.051$ ) within the age groups of animals in rejection at AM more young than the adult which were 3.2% (6/185) and 1% (2/199) respectively. It may due to difficulty in protection from stress, shortage of feed and water, not getting enough rest. Herenda *et al.* (2000) stated that leanness (Poorness) is often observed in case of poor quality pasture and young growing animals which have had protein-deficient diet.

In the present study, organ condemnation rate showed that, liver and lung were the most frequently affected organs with the highest condemnation rate followed by GIT, kidney and heart and carcass significantly ( $p = 0.00$ ), which is 94(48.9%), 56(29.2%), 13(6.8%), 11(5.7%), 11(5.7%) and 7(3.6%) respectively. This finding is in agreement with reports of Cadmus and Adesokan (2009) who recorded that lungs (45.7%) and the liver (32.9%) were the most affected organs with the kidney (0.02%) and the heart (0.01%) being the least. The current study introduced that parasites are the major causes of organ condemnations. Parasitic causes

like, Cyst cercus teniculousis, hydatidosis, fasciolosis and Stelezia hepatica were found to be the major parasitic conditions responsible for organ condemnation. There was no statistical difference in the rate of organ and carcass condemnation from parasitic infestation considering the age and origin of animals. This shows that parasitic diseases of sheep are widely spread in all age groups and everywhere in the country.

The presence of small ruminant hydatidosis at slaughterhouse has been documented in Ethiopia. (Bekeleet *al.*, 1988) reported a prevalence rate of 16.4% in sheep which is higher than the finding in this study (7.4%). Similarly (Jobreet *al.*, 1996) reported prevalence rate of 11% and 6% from South Omo and DebreZeit slaughterhouses, respectively in sheep and goats.

In present finding, hydatid cysts were more frequently observed in lungs than liver of sheep (6.3%) and (3.6%) respectively. Additionally, similar findings were also reported by different authors (Khanet *al.*, 2001, Dalimaiet *al.*, 2002, Daryaniet *al.*, 2007). However, the most common site for hydatid cyst was the liver followed by the lungs in the Middle East (Kamhawiet *al.*, 1996). Lungs are most commonly affected by hydatidosis because at old age the liver capillaries are dilated, and most cysts passed directly to the lung. Secondly, the cyst passes to the lung via the thoracic duct without involving the liver (Gracey, 1986). And also, many researchers reported that liver and lung are the most commonly affected organs by hydatid cyst (Abunna and Hordofa, 2013; Denbarga, 2011; Jobre, 1996). The reason being that lung, and liver contain highest capillary bed in the body and therefore, the majority of the oncospheres were filtered out and trapped in the fine blood capillaries and only small number of oncospheres reaches the remaining organs (Gracey, 1986). In present study also lungs and liver 12(6.3%) and 7(3.6%) respectively, were affected by hydatidosis.

Out of 94 condemned liver teniculousis is the most frequent cause of organ lesion (34%) followed by calcification (25.5%), abscess 13(13.8%), hepatitis 10(10.6%), cirrhosis 7(7.4%), discoloration 4(4.3%), more than 3 lesion on liver 3(3.2%) and parasites like teniculousis, Stelezia hepatica, Fasciola species and hydatid cyst were found to be the major causes that rendered liver rejection from the local market (Table 3). Fascioliasis constitutes both economic and public health constrains to ruminant production. It is caused by two trematode species, Fasciola hepatica and *F. gigantica*, which develop in different livestock species mainly sheep and cattle; but, also in many other domestic herbivores (Gracey, and Collins, 1992). The reported prevalence of Fasciolaspp. (5.3%) was lower than other studies in bovine, like Belina and Melese (2017) study result showed fasciolosis and hydatidosis alone contributed 690(35.1%) gross pathological lesions.

Previous studies have indicated a higher economic loss resulting from a condemnation of edible organs and carcasses due to parasitic causes (Negategizeet *et al.*, 1993; Jembere, 2002; Jibat, 2006). In the current study, these parasitic causes of liver lesion might be due to improper wasting of condemned organ and the stray dog feed it at abattoir and selling of infected offal for dog which is final host for teniculousis and hydatidosis and stay them. Sissayet *et al.*, (2008) studied the prevalence and seasonal incidence of cestodeparasite infections of sheep in Eastern Ethiopia for two years (2003- 2005). Duringthis period, viscera including liver, lungs, heart, kidneys and the gastro-intestinal tract were collected from 655 sheep slaughtered at four abattoirs. One of the most prevalent metacestodes was *C. teniculousis*. In sheep, the overall prevalence was 79% for *C. teniculousis*.

The causes for calcification abscess, hepatitis, cirrhosis and discoloration were difficult to identify grosslyand it may be due to systemic infectious diseases. Calcification is also another lesion that we encountered; it can be caused by injury, infection, and autoimmune disorders. Large-scale tissue damage is associated with extensive loss of cells, a situation referred to as tissue necrosis. The death of tissue in a specific area of the body leads to the release of signaling factors that attracts cells to clean up and heals the dead tissue. This process, known as an inflammatory response, attracts calcium into the damaged area as it heals (Carne, 2010).This study indicates 24(12.5%), 9(4.7%) and 3(1.6%) of Liver, lung, and kidney, respectively were affected by calcification.

Abscess was also a pathological condition; which is a collection of pus circumscribed by fibrous tissues. It occurs with great frequency throughout many organs and the carcasses of the meat animals and may be associated with a general condition or be found as isolated lesions (Libby, 1975). In present study 13(6.8%), 6(3.1%), 6(3.1%), 2(1%) and 2(1%) of liver, GIT, kidney, heart and carcass were affected by abscess. In agreement with (FSIS, 2009) stated that caseous lymphadenitis is a disease of sheep and goats caused by the *C.Pseudotuberculosis*. Postmortem findings may include, enlarged abscessed lymph nodes with greenish white-yellow caseous exudate, which tends to become dry and granular, cross-sections of lesions contain remnants of connective tissue capsules (resembles the concentric rings seen on the cut surface of an onion). Lesions found in many lymph nodes, especially the subiliac, superficial cervical, deep popliteal, tracheobronchial, and mediastinal lymph nodes, as well as lungs, heart, liver, spleen, and kidneys. (Asrat, 2004) stated that occasionally the worms penetrate the bile duct wall into the liver parenchyma causing liver abscesses.

The study conducted in Gondar abattoir (Meseleet *et al.*, 2012) and Nekemte (Moje *et al.*, 2014)

also revealed that livers and lungs are the most rejected organs by PM inspection and fasciolosis and hydatidosis are the major causes of rejections. However, in the current study different calcifications, cirrhosis, hepatitis, abscessations, emphysema, pneumonic lesions, marbling (contagious caprine pleuropneumonia (CCPP)) lesion, nephritis, foreign body, traumatic lesions and others nonparasitic abnormalities and unidentified lesion contributed to a condemnation of organs and carcasses were investigated.

Lungs were condemned because of *C. teniculousis*, hydatid cysts, marbling, emphysema, calcification-,pneumonia, other unknown caused lesion and abscess which were (35.7%), (21.4%), (17.8%),(16%), (16%), (12.5%), (5%) and (3.6%) respectively. *C. teniculousis* accounts for 35.7% as a principal cause of lung condemnation in sheep this might because of increased number of a stray dog in the area, the principal cause of lung condemnation was parasitic. However, the report observed during a retrospective study (Reassert *et al.*, 2013) reported pneumonia as a principal cause of lung condemnation in central Ethiopia accounting for 42.1% (Get chew, 2008).In current study, from the total lungs inspected higher 56 (29.2%) lungs were condemned. It may because of the animals unable to resist stress within a short period of time during transportation along way on foot, shortage of feed and water, stress due to hitting of animal by personnel who driving animal to market from the farmer and to abattoir and does not getting sufficient amount of rest at lair age may causes this respiratory problem.

FSIS (2009) reported that pneumonia is an inflammatory condition of the lungs that maybe caused by infectious agents, parasites, physical trauma, or foreign material in halation. In similar reports pneumonia might also be as a result of endemic diseases of sheep and goats such as pasteurellos are which is triggered by stress, contagious caprinepleuropneumonia (Radiostitis *et al.*, 2007). The other cause was marbled appearance (CCPP) lesions: CCPP is a disease peculiar to shoat and takes the form of a chronic inflammation of the lungs and pleura. It is not communicable to man and the carcass and the lungs found to be positive for CCPP can be passed for human consumption after a partial condemnation of the diseased part Gracey and Collins (1992).On the other hand, Emphysema is an abnormal and permanent enlargement of air spaces distal to terminal bronchioles with destruction of their alveolar walls, whereas oedema is a nonspecific lesion in which interstitium and alveoli are accumulated with fluid (Carne, 2010).

Also, different lesions of infectious and noninfectious causes like abscess, pericarditis, nephritis, and discoloration were found to be important causes for the condemnation of edible organs like liver,

heart, and kidney. Similarly the same causes were found at central Ethiopia (Get chew, 2008; Regassaet *al.*, 2013) and in goats slaughtered at Nigeria (Ojo, 1992).

In the present study, out of the 11 (5.7%) kidneys condemned abscess 6 (54.5%) account, whereas calcification accounts 3(27.2%), nephritis 2(18.2%) and other unidentified causes 2(18.2%) (Table 3). In this study abscess was a principal cause of kidney condemnation, however, the result in (Dejeneet *al.*, 2013) study revealed out of the 57 (6.71%) kidneys condemned Nephritis 20 (2.35%) accounting for 11 (2.59%) and 9 (2.12%) kidneys in Ovine and Caprine respectively, was the principal cause of condemnation. Radostitiset *al.*, (2000) stated that embolic nephritis occurs after septicemia or bacteremia when bacteria lodge in renal tissue.

The major causes of heart condemnation were found to be pericarditis, hydatid cyst and abscess. Out of the total of 11 (5.7%) hearts condemned due to gross abnormalities, pericarditis contributes about 4 (36.4%) and hydatid cyst also contribute 4(36.4%) and abscess 3(27.3%) out of condemned organs Table 3. The main cause of lesion in GIT condemnation primarily parasitic *C. teniculus* and abscess 6(46.2%) and foreign body 4(30.8%). As a septic lesion, whenever localized abscess is found, partial condemnation is recommended Gracey and Collins (1992).

The main management practices that rendered organs and carcasses unfit for human consumption were bruising of the carcass mainly brought about by not proper handling of animals during transportation to the slaughterhouses by hitting the animal with thick stick and mechanical damage to organs due to faulty evisceration especially liver. Apart from affecting carcass value, bruising has also animal welfare implications as excessive use of sticks while driving to the abattoir, mishandling of animals during loading and unloading, improper transport vehicle and at slaughter could be responsible causes (Mungubeet *al.*, 2006). It is stated that bruising of animals during transport is the major source of economic loss in Africa and Asia (Mitchell and slough, 1980). In the present study out of 7 carcass condemnations, 2 (28.6%) whole carcass was also totally condemned due to the yellowish discoloration, suspecting liver disease which may toxicity, systemic disease causing prehepatic and hepatic jaundice. Herendaet *al.*(2000)stated that icterus is the result of an abnormal accumulation of bile pigment, bilirubin, or of hemoglobin in the blood. Jaundice is divided into three main categories. Prehepatic jaundice occurs following an excessive destruction of red blood cells. Tick-borne diseases such as Babesiaovis and Anaplasmosis cause this type of icterus. Hepatic jaundice occurs due to direct damage to liver cells as seen in liver cirrhosis, systemic infections, and in chemical and plant poisoning. In sheep, jaundice may have been caused by phytogetic

chronic copper poisoning. Obstructive jaundice occurs when the drainage of the bile pigment bilirubin is blocked from entry into the intestine.

However, parasitic *C. teniculus* and Stelezia hepatic have no public health importance; they are considered as the important cause of economic loss in the meat industry since viscera harboring them are rejected for aesthetic reasons. The threat these parasites pose to small ruminants' meat industry in Ethiopia is evident due to the present situation of improper disposal of offal at abattoirs and backyard slaughter. The presence of freely roaming stray dogs on grazing land together with livestock and the deeply-rooted habit of feeding dogs with offal, including sheep heads, are important risk factors. This may lead to the perpetuation of the life cycle between intermediate hosts (sheep) and the final hosts (dogs) for *C. teniculus* and hydatidosis.

The financial loss in the abattoir was high, in this study analyzed those losses through condemnation of organs and carcass from local market. A total loss of(56,576 USD USD)was incurred in the abattoir. Carcass condemnation accounts highest part of the losses of the total direct losses whereas liver, lung, GIT, heart and kidney takes, respectively.

The indirect losses from body weight gain, mortality at the farms, public health implications (cause of treatment for a human when diseased upon eating of the affected edible organ which is zoonotic) were not included in the analysis in this study. Thus, the total financial loss attributable to diseases of ovine and, hence, abattoir wastage could be much higher. The economic analysis of livestock diseases in Ethiopia is scarce and inadequate because of lack of information on the prevalence and partly by the complexity of the analysis. Negategizeet *al.*(1993) have reported a financial loss associated with a liver condemnation due to ovine fasciolosis alone in the central highlands of Ethiopia amounting to be 2.3 million Ethiopian Birr (460, 000 USD). Similarly Jobreet *al.*(1996) have estimated a total annual loss of 1.3 million Ethiopian Birr (260 000 USD) resulting from offal condemnation and carcass weight loss

## V. CONCLUSION AND RECOMMENDATIONS

The current study revealed that, during the study period different signs of diseases, and abnormalities leading to conditional approval, rejection and detain of animals were encountered at AM inspection. In lesion survey, a total of 192 gross pathological lesions resulting in partial and total condemnations of liver, lung, kidney, heart, GIT and carcass were investigated. Different calcifications, parasitic teniculus, hydatidosis, fasciolosis, Stelezia hepatica, pneumonic lesions, abscess, cirrhosis, marbling (CCPP), emphysema, hepatitis, nephritis, pericarditis, bruising (mechanical damage),

discoloration and foreign body were the main abnormalities recorded as causes of (56,576 USD) losses. There is perpetuation of the life cycle between intermediate hosts (sheep) and the final hosts (dogs) for *C. teniculis* and hydatidosis by wasting the condemned organ near abattoir; and sometimes selling of affected organ. The results of the study showed that teniculis and calcification were the two most frequently examined conditions, contributing 58(30.2%) of gross pathological lesions.

Based on this conclusion, the following recommendations are recommended:

- Awareness should be created for the animal attendants, farmers, customers, abattoir workers and butchers regarding the public health significance of diseases of animal origin and the related losses
- The government must empower veterinarians and other meat inspector more in passing professional judgments and, avoid complains of investors working in meat industry sector on inspectors judgments
- Immediate, safe and controlled elimination of all condemned abattoir materials and the sale of contaminated.

### REFERENCES RÉFÉRENCES REFERENCIAS

1. Abebe, G. 1995. ILRI, Addis Ababa, pp: 133-138 *In*: Proceeding of an international conference of Current status of veterinary education and health research in Ethiopia *In* Veterinary Medicine impact on health and nutrition in Africa.
2. Abunna F., Hordofa D. 2013. Major Causes of Organ Condemnation for Cattle and its Financial Impact at Wolaita Soddo Municipality Abattoir, Southern Ethiopia. *Global Veterinaria* 11: 730-734.
3. Alemu Y., Merkel R.C. (2008). Sheep and Goat Production Handbook for Ethiopia: Ethiopia Sheep and Goat Productivity Improvement Program/ ESGPIP/, Ministry of Agriculture and Rural: 15-53.
4. Arbabi M., Hooshy H. 2006. Survey of Regions might have accounted for variation of the Echinococcosis and Hydatidosis in Kashan Region, Prevalence in different areas of a country Central Iran. *Iran J Public Health* 35: 75-81.
5. Aseffa, M. 2005. Parasitic Causes of Carcass/Organ Condemnation at Asella Municipality Abattoir. Unpublished DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.
6. Asrat, M. 2004. Infection prevalence of ovine fasciolo is in irrigation schemes along the Upper Awash River Basin and effects of strategic an thelminic treatment in selected upstream areas, Addis Ababa University School of Graduate Studies, Department of Biology. *M. Biomed. Sci.*
7. Bekele T., Mukassa M.E., Kasali O.B. 1988. The prevalence of cysticercosis and Hydatidosis in Ethiopian sheep. *Vet Parasitol.* 28: 267-270.
8. Belina, D. and Melese, M. 2017. Pathological lesion survey and financial losses associated with organs and carcass condemnation in cattle slaughtered at selected abattoirs in Ethiopia. *Indian J. Vet. Pathol*, 41(1): 1-11.
9. Cadmus, S.I.B., Adesokan, H.K. 2009. Causes and implications of bovine organs/offal condemnations in some abattoirs in Western Nigeria *Trop. Anim. Health Prod.*, [http://dx.doi.org/ 10.1007/s11250-009-9334-7](http://dx.doi.org/10.1007/s11250-009-9334-7).
10. Carne, H.R. 2010. Veterinary pathology hand book. Integrated Cased Based Applied Pathology. Vets 3011.
11. Dalimai A., Motamedi G.H., Hosseini M., Mohammad a B., Malaki H. 2002. Echinococcosis /hydatidosis in western Iran. *Vet Parasitol.* 105: 161-171.
12. Daryani A., Alaei R., Arab R., Sharif M., Dehghan M.H. 2007. The Prevalence, Intensity and Viability of Hdatidcysts in Slaughtered Animals in the Ardavil Province Northwest Iran's *Helminthol.* 81: 13-17.
13. Dejene S., Abebe B., Degefu H. 2013. Study on the Major Health Problems That Causes Carcass and Organs Condemnation at Hashim's Export Abattoir, Debrezeit, Ethiopia *Global Veterinaria* 11 (4): 362-371.
14. Denbarga Y., Demewez G., Sheferaw D. 2011. Major Causes of Organ Condemnation and Financial Significance of Cattle Slaughtered at Gondar Elfora Abattoir, Northern Ethiopia. *Global Veterinaria* 7: 487-490.
15. Development, pp Statistical Agency (CSA), (2009). Central Statistical Authority Federal Democratic Republic of Ethiopia Agricultural Samples Enumeration Statistical Abstract, Pp 446-539. Central the Federal Democratic Republic of Ethiopia.
16. Ethiopian Ministry of Agriculture Meat Inspection Regulations (EMAMIR), 1972. Legal Notice No. 428 Negarite Gazeta. Addis Ababa, Ethiopia.
17. Ezana, G., 2007. Major disease of export oriented livestock in export abattoirs in /around adalibenwereda, Debrezeit, Ethiopia.
18. FAO, 1993. Agro State Data. Statistical Division, [www.fao.org/economic/ess/publications/ ess-year book /en/](http://www.fao.org/economic/ess/publications/ess-year-book/en/) Rome, Italy.
19. FSIS, 2009. Public Veterinarian Training USDA FSIS Center for Learning Multi-speci Disposition Basics with a Public Health Focus 1/29/09.
20. Fufa A., Loma A., Bekele M., Alemayehu R. 2010. Bovine fasciolosis: coprological, abattoir survey and its economic impact due to liver condemnation at Sodo Municipal abattoir, Southern Ethiopia. *Trop Anim Health Prod.* 42: 289-292.



21. Get chew, E.W. 2008. Major Diseases of Export Oriented Livestock in Export Abattoirs in/Around Ada Liben Woreda, Debrezeit. Online DVM Thesis, Faculty of Veterinary Medicine, Haramaya University, Ethiopia. [http:// mahider. ilri.org/ handle / 10568/695](http://mahider.ilri.org/handle/10568/695).
22. Gatenby, R. M. 1991. Sheep. The tropical agriculturalist. MACMILLAN, ACCT. pp. 6-10. London and Basingstoke, education Ltd
23. GOR (Government of Oromia), 2006. Oromia regional government socio-economic profile. Government of Oromia Region. Addis Ababa, Ethiopia.
24. Gracey J.F., Collins D.S. 1992. Meat Hygiene. 9th ed. Pp: 339-421. In: Baillier Tindall. London.
25. Gracey, J.F. 1986. Meat Hygiene. 8th ed. pp. 119-350. In: Baillier Tindall. London.
26. Gracey J.F., Collins D.S. and Huey R.J. 1999. Meat hygiene. 10th Edition. pp: 758. In: London. W. B. Saunders Company Ltd.
27. Herenda D., Chambers P.G., Ettriqui A., Seneviratna P. and Dasilvat T.J.P. 1994. Manual on meat Inspection for developing countries, pp: 139-148.
28. Herenda D.; Chambers P.; Ettriqui A.; Seneviratna P. and da Silva T. 2000. Manual on meat inspection for developing countries. *FAO Anim. Prod. Health*, 119.
29. Ibrahim, H., 1998. Small Ruminant Production Techniques. pp: 207. ILRI Manual 3. ILRI (International Livestock Research Institute), Nairobi, Kenya.
30. Jacob, L., 1979. Seminar for animal health officials. Ministry of agriculture and settlement, Animals and Fisheries authority. Addis Ababa, Ethiopia
31. Jembere, S., 2002: A Survey of Causes of Organs/Carcass Condemnation in Slaughtered Cattle at Nazareth Abattoir. Unpublished DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, DebreZeit
32. Jibat, T., 2006. Causes of Organ and Carcass Condemnation in small ruminants slaughtered at HELMEX Abattoir. pp. 9-11. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.
33. Jibat, T., Ejeta, G., Asafw, Y. and Wudie, A. 2008. Causes of abattoir condemnation in apparently healthy slaughtered sheep and goats at HELMEX abattoir, DebreZeit, Ethiopia, *Rev. Méd. Vét.*, 159 (5): 305-31
34. Jobre Y., Lebago F., Tiruneh R., Abebe G., Dorchie Ph. 1996. Hydatidosis in their selected regions in Ethiopia: an assessment trail on its prevalence, economic and public health importance. *Rev Med Vet* 147: 797-804.
35. Kamhawi S., Hijjawi N., Abu-Ghazaleh A., Abbas M. 1996. Prevalence of Hydatid Cyst in Livestock from Five Regions in Jorda. *Ann Trop Med Hyg.* 147: 797-804
36. Khan A.H., El-Buni A.A., Ali M.Y. 2001. Fertility of the Cyst of Echinococcus Granulosus in Domestic Herbivore from Benghazi.
37. Libya, and the Reactivity of Antigens Produced from them. *Ann Trop Med Parasitol.* 95: 337-342. Libby, J.A., 1975. Meat Hygiene. 4th ed. Pp. 40-168. In: Lea and Febiger, Philadelphia
38. Mandefro A., Aragaw K., Hailu B., Alemayehu G., Chala G. 2015. Major Cause of Organ and Carcass Condemnation and Its Financial Loss at Bishoftu Elfora Export Abattoir. *International Journal of Nutrition and Food Sciences.* 4: 364-372. doi: 10.11648/j.ijnfs.20150403.24
39. Mesele G., Guadu T., Bogale B., Chanie M. 2012. Pathological Conditions Causing Organ and Carcass Condemnation and Their Financial Losses in Cattle slaughtered in Gondar, Northwest Ethiopia. *Afr J Basic Appl Sci*, 4: 200-2012
40. Ministry of information (MOI), 2005. Export products of Ethiopia. Press release of Ministry of information, department of press and Audio visual. Addis Ababa
41. Mitchell, J. R. and Slough, C.A.B. 1980. Helminthes in meat inspection. 56-62. In: Guide to meat inspection in tropics: Common Wealth Agricultural Burro.
42. Moje N., Abdeta D., Kebede S., Terfa T., Desissa F., Regassa A. 2014. Major Causes of Organs and Carcass Condemnation in Cattle Slaughtered at Nekemte Municipality Abattoir, East Wollega, and Ethiopia. *Global Veterinaria* 13: 278-284.
43. Mungube E.O., Bauni S. M., Tenhagen B. A, Wamae L. W., Nginyi J. M., Mugambi J. M. 2006. The prevalence and economic significance of Fasciolagigantica and Stilesia hepatica in slaughtered animals in the semi-arid coastal Kenya. *Trop Anim Hlth Prod.* 3388:475-483.
44. Negategize P.K., Tekleye B. and Get chew J. 1993. Financial losses caused by ovine fasciolosis in Ethiopian highlands. *Trop. Anim. Hlth. Prod.*, 23: 155-167.
45. Negategize, P.K.J., Bekele, T., Tilahun, G., 1993. Financial losses caused by ovine fasciolosis in the Ethiopian highlands. *Trop. Anim. Health Prod.* 25: 155-161.
46. Ojo, S., 1992. A Survey of Pathological Conditions in the Slaughter Goats. pp. 1-4. In: Zaria Slaughterhouse. Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.
47. PACE-Ethiopia, 2003. Experiences and the way forward on community Based animal health service delivery in Ethiopia. p. 6. In: Proceedings of workshop held in Addis Ababa, Ethiopia.
48. Radiostitis O.M., Gay C.C., Hincheliff K.W. and Constable, P.D. 2007. Veterinary Medicine. A text book of the disease of cattle, horse, sheep, pigs,



and goats. 10th ed. Pp 391-517. *In: Ballier Tindals*. London.

49. Radostits O., Gay C., Blood D. and Hinchcliff K. (2000). *Veterinary Medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses*. 9<sup>th</sup> Ed. 1877pp. London: Harcourt, Publishers Ltd.
50. Regassa A., Moje N., Megersaa B., Beyene D., Sheferaw D., Debela D., Abunnaa F., Skjerved E. 2013. Major causes of organs and carcass condemnation in small ruminants slaughtered at Luna Export Abattoir, Oromia Regional State, Ethiopia. *Preventive Veterinary Medicine*, 110: 139– 148.
51. Sissay M., Ugglä A. and Waller P. 2008. Prevalence and seasonal incidence of larval and adult cestode infections of sheep and goats in eastern Ethiopia. *Trop. Anim. Hlth. Prod.* 40(6):387-94.
52. Teka, G., 1997. Meat hygiene .pp: 99-113. *In: Food hygiene principles and methods of food born disease control with special reference to Ethiopia*.
53. Thornton, P., 2010. Livestock production: recent trends, future prospects. *Royal Soc.*, 365(1554): 2853-2867.
54. Hrushfield, M. 2007. *Veterinary Epidemiology*. Third Edition, Oxford. Pp 244-245, 249-251 and 258-259. *In: Blackwell Science Ltd*.
55. Van Iontestijn, J.G. 1993. Integrated quality. Meat safety: A new approach. *Meats Focus International*, 2:123-128.
56. Vatta A.F., Gumede S.A. Harrison L.J.S., Krecek R.C., Letty B.A., Mapeyi N. Pearson R.A. 2005. Goat keepers Veterinary Manual. Onderstepoort Veterinary Institute, Private Bag Onderstepoort, *South Africa. Veterinary Research*, 6: 42.
57. Yimam, M. 2003. Major Causes of Organ Condemnation in Ruminants Slaughtered at Gondar Abattoir, North West Ethiopia. Unpublished DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit.





GLOBAL JOURNAL OF MEDICAL RESEARCH: G  
VETERINARY SCIENCE AND VETERINARY MEDICINE  
Volume 18 Issue 2 Version 1.0 Year 2018  
Type: Double Blind Peer Reviewed International Research Journal  
Publisher: Global Journals  
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

## A Review on Small Ruminants Brucellosis

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**Abstract-** Brucellosis is an important zoonotic disease that causes huge economic losses to the livestock owners and is of great public health concern worldwide. It is a chronic infectious disease of livestock, rodents, marine animals and human beings. Brucellosis affects both public and animal health as well as production, and is widespread in many regions of the world. The disease is caused by non-motile, facultative intracellular Cocco-bacilli of genus *Brucella*. The two specific isolates of *Brucella*, *Brucella melitensis* and *Brucella ovis*, cause brucellosis in small ruminants. *Brucella ovis* causes the disease in sheep while *B. melitensis* is the etiologic agent of brucellosis in man, sheep and goats. Direct contact with infected animal secretions, inhalation of the organism, ingestion of contaminated food, and poor hygienic practices favor the transmission of brucellosis between animals and humans. Brucellosis affects the reproductive tract of animals which is manifested by late term abortions, retention of placenta in the case of female animals, epididymitis and orchitis in males. The disease is also characterized by infertility and reduced milk production. The diagnosis of brucellosis focuses on culture, serological tests and molecular investigations. Because of the high relapse rate associated with the disease, the use of a multidrug therapy is recommended. Brucellosis can be prevented by implementing appropriate animal-disease-control measures; avoiding the consumption of undercooked meat and unpasteurized dairy products; and using appropriate barrier precautions to exclude exposure to aerosols in humans.

**Keywords:** *brucellosis, brucella melitensis, brucella ovis, small ruminants, zoonosis, humans.*

**GJMR-G Classification:** *NLMC Code: WC 310*



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# A Review on Small Ruminants Brucellosis

Tewodros Alemneh <sup>α</sup> & Dawit Akeberegñ <sup>ο</sup>

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## I. INTRODUCTION

Small ruminants, which account for more than half of the domesticated ruminants in the world, are an important component of the farming systems in most developing countries (Gebremedhin et al., 2015). Recent studies in different regions of the world indicate that the global population of small ruminants increased from 1.35 billion to 1.94 billion (Tedeschi et al., 2011).

Small ruminants are an integral part of livestock keeping in developing countries, especially in Sub-Saharan Africa that are mainly kept for immediate cash sources, milk, meat, wool, manure, and saving or risk distribution. Small ruminants also have various social and cultural functions that vary among different cultures, socio-economies, agro-ecologies, and locations in tropical and subtropical Africa (Gobena, 2016). Sheep

and goats have many advantages over large ruminants for most smallholder farmers, including among others: fewer feed costs, quicker turnover, easy management and appropriate size at slaughter (Zahra et al., 2014). They also have greater tolerance to less favorable conditions, as they suffer far less in mortality during periods of drought than large ruminants. Also, breeders prefer sheep and goats as the risk of losing large ruminants is too high (Zahra et al., 2014).

Ethiopia is one of the African countries with the largest small ruminant population in the continent (Abebe 2013). A recent estimate indicates that there are about 27.35 million sheep and 28.16 million goats in the country (CSA, 2014). Almost all of the small ruminant populations comprise of local breeds. The CSA data further indicates that of those who own small ruminants, about 64% and 58% own less than five heads of sheep and goats, respectively (Gebremedhin et al., 2015).

Despite the importance of small ruminants in the livelihoods of producers, the current productivity of goats and sheep in developing countries remains low, mainly due to under-feeding, poor management system and diseases (Gizaw 2010). Brucellosis is one of the infectious diseases considered as most constraints for sheep and goats productivity (Tewodros and Dawit, 2015). Brucellosis is an economically important and widespread zoonosis in the world caused by bacteria of the genus *Brucella*, which tend to infect specific animal species (Awah-Ndukum et al., 2018).

Brucellosis occurs worldwide in domestic animals such as cattle, sheep, goats, camels and pigs and creates a high economic problem for both the intensive and extensive livestock production system in the tropics and a threat to public health. It shows that brucellosis causes high economic losses in the livestock industry. Economic losses stem from breeding efficiency, loss of offspring, reduced meat and milk production as well as an impediment to free animal movements and export of animals and their products (Tewodros and Dawit, 2015).

Brucellosis is a zoonotic infectious disease affecting a wide range of species of animals and humans with an estimated half a million human cases reported annually (Kelkay et al., 2017). Cattle, goats, pigs, sheep, horses, and dogs play a significant role in the transmission of this disease to man. It is also defined as a contagious systemic bacterial disease primarily of ruminants, characterized by inflammation of the genital organs and fetal membranes, abortion, sterility, and formation of localized lesions in the

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lymphatic system and joints (Awah-Ndukum et al., 2018). Additionally, the disease also poses a major constraint to international trading of animal and animal products (Seleem et al., 2010). As the problem often goes undetected, identification of infected herd and animals is of prime importance for the control of the disease. Having large livestock resource at hand coupled with an intermingling of livestock species may cause uninfected animals to easily get exposed to the disease from multiple sources such as abortion discharges and direct contact with infected animals. Mixed farming especially raising goats and sheep along with cattle was also reported by many researchers to be a risk factor for *Brucella* transmission between different animal species (Padilla et al., 2010, Godfroid et al., 2013).

Therefore, this review is undertaken with the objectives of compiling currently available information worldwide about brucellosis in small ruminants and humans, and creating awareness regarding the disease to animal producers, product users, researchers, and investigators.

## II. ORIGIN AND CLASSIFICATION

### a) *Brucella and Brucellosis*

Brucellosis is an infectious disease caused by the genus *Brucella*. It is a disease of worldwide importance and affects some animal species (Tegegn et al., 2016). *Brucellais smallcocci*, Cocco-bacilli or short rods, they are 0.5-0.7 by 0.6-1.5 pm in size, non-sporulating and non-encapsulated and nonacid-fast bacteria which stain gram-negative. Brucellosis is an important zoonotic disease of worldwide importance and affects some animal species. The causal organism is first isolated by Bruce in 1870 from the liver of a patient died of undulant fever (Malta fever) (Hirsh et al., 2004, Khan and Zahoor, 2018). The old classification of the genus included six species, namely, *Brucella (B) abortus* (cattle), *B. melitensis* (sheep and goats), *B. suis* (pigs), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (El Abdin et al., 2014). Latter a marine species has been noted as *B. maris* (Sohn et al., 2003). There are two other species that affect marine mammals, and they are *B. ceti* and *B. pinnipedialis* (Foster et al., 2007).

Complete genome sequences of *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, and *B. ovis* are available while many other strains are being sequenced. They are considered to be very similar in size and genetic makeup (Sriranganathan et al., 2009). *B. melitensis* is predominantly found in goats and is the most widely distributed of all the *Brucella* species. It is also the most pathogenic and virulent species for humans and affects almost all domestic animals and many wild animals (Benkirane, 2006). *B. abortus* is mainly found in cattle and buffaloes, *B. suis* in pigs, *B. ovis* in sheep and *B. canis* in dogs (Seleem et al., 2010).

The different species cannot be distinguished from each other morphologically. For microscopic demonstration in or outside of tissues, selective staining methods are applied which can show the tiny bacteria (Stamper Hansen's staining). For culturing *Brucella* require complex media. They grow best if special peptones, like tryptose and trypticase-soya-peptone, are added to the medium at a neutral PH and 3-10% CO<sub>2</sub> atmosphere with an incubation temperature of 37°C is required. Delicate translucent colonies of 2-3 mm in diameter grow on blood or glucose-agar. *Brucella ovis* (*B. ovis*) always grows in the M-(mucoid) form, *Brucella abortus* (*B. abortus*) and *B. melitensis* grow at the beginning in the S-(smooth) form later dissociate into the R-(rough) and the M-form (Radostits et al., 2007).

## III. BRUCELLOSIS IN SMALL RUMINANTS

*Brucella melitensis* is the most important cause of brucellosis which primarily affects sheep and goats and also very pathogenic for human beings. The disease is also caused by *B. ovis* which severely affects sheep. Although the disease has preferred hosts, the bacteria can cross-infect other domestic animals. Hence, sporadic infections in small ruminants can also be caused by *B. abortus* or *B. suis*, but such cases are rare (OIE, 2012a; Kelkay et al., 2017).

The species *B. melitensis* is the causal organism of brucellosis in small ruminants and undulating or "Malta fever" in humans. *Brucella melitensis* primarily affect the reproductive tract of sheep and goats, and it is characterized by abortion, retained fetal membrane and to a lesser extent, impaired fertility. Although *B. melitensis* infects mainly sheep and goats and its zoonotic importance, plays a significant role in the national economy and the public health of many developing countries. Before *B. melitensis* was recognized as the same symptoms in regions hindering the Mediterranean was known as tibiris andulans (Radostits et al., 2007).

The disease caused by the infection of sheep with *B. ovis* is characterized by infertility in rams due to epididymitis. Abortion and neonatal mortality are also caused by the infection (Radostits et al., 2007).

## IV. EPIDEMIOLOGY

### a) *Occurrence and Geographical Distribution*

Brucellosis is of major economic importance in most countries of the world, and it affects approximately 50% of the livestock population worldwide and continues to increase in distribution (OIE, 2012a). Small ruminant brucellosis has been shown to occur worldwide. It is mostly present in Mediterranean countries, the Middle East, Asia, India, China, Mexico and parts of Latin America (OIE, 2009) (Table 1-3). *Brucella ovis* has been reported in parts of Eastern Europe, Africa (Table 4), Western State of the

United States of America (USA), New Zealand and Australia, it does not occur in the United Kingdom (UK) (Foster et al., 2017) (Table 1-3).

*Brucella melitensis* and *B. ovis* create an economic problem for the intensive and extensive animal production systems of the tropics. While the disease has been eradicated in most industrial countries, especially in Europe, through intensive schemes of control and eradication, its occurrence is increasing in developing countries in an even

aggravating epizootological situation. This depends on the policy of many developing countries of importing exotic high production breeds without having the required veterinary infrastructure and the appropriate level of development of the socioeconomic situation of the animal holder. Furthermore, the increasing international animal trade with increasing movements of animals and the trend towards intensification of animal production favor the spread and transmission of the infection (Radostits et al., 2007).

Table 1: The Distribution of *Brucella Melitensis* and *Brucella Ovis* in Asian Countries

Continent / Country / Region	Distribution		Reference
	<i>Brucella Melitensis</i>	<i>Brucella Ovis</i>	
<b>Asia</b>			
Armenia	Present	Not Reported	Oie, 2009
Azerbaijan	„	„	Oie, 2009
China	Restricted Distribution	„	Oie, 2009
Georgia (Republic Of)	Last Reported In 1991	„	Oie Handistatus, 2005
Iran	Present	„	Oie, 2009
Iraq	„	„	Oie, 2009
Israel	„	„	Oie, 2009
Jordan	„	„	„
Kuwait	„	„	„
Kyrgyzstan	Restricted Distribution	Restricted Distribution	„
Lebanon	Present	Not Reported	„
Malaysia	„	„	„
Mongolia	„	„	„
Oman	„	„	„
Pakistan	Restricted Distribution	„	„
Qatar	Present	„	„
Saudi Arabia	„	Present	„
Syria	„	Not Reported	„
Tajikistan	„	„	„
Thailand	„	„	„
Turkey	„	„	„
Uzbekistan	„	Not Reported	Oie Handistatus, 2005
Yemen	„	„	„

Table 2: Brucellosis Infected Countries in North, Central and South America Continents

Continent / Country / Region	Distribution		Reference
	<i>Brucella Melitensis</i>	<i>Brucella Ovis</i>	
<b>North and Central America</b>			
Canada	Not Reported	Absent	Oie, 2009
Mexico	Present	Not Reported	Oie, 2009
Usa	Not Reported	Restricted Distribution	„
United States Virgin Islands	Present	Not Reported	Ahlet Al., 1993
<b>South America</b>			
Argentina	Present	Restricted Distribution	Oie, 2009
Chile	Not Reported	Restricted Distribution	„
Sao Paulo	Present	Not Reported	Gouvêaet Al., 1989
Peru	Restricted Distribution	„	Oie, 2009
Uruguay	Not Reported	Present	„



Table 3: Distribution of *Brucella Melitensis* and *Brucella Ovis* in Europe and Oceania

Continent / Country / Region	Distribution		Reference
	<i>Brucella Melitensis</i>	<i>Brucella Ovis</i>	
<b>Europe</b>			
Albania	Present		Oie, 2009
Andorra	„	Not Reported	Oie Handistatus, 2005
Bulgaria	„	Present	Oie, 2009
Croatia	„	„	„
Cyprus	„	Not Reported	„
Gibraltar	„	„	Yantzis, 1984
Greece	„	„	Oie, 2009
Hungary	Not Reported	Present	„
Italy	Restricted Distribution	Not Reported	„
Macedonia	„	„	Oie, 2009
Malta	„	„	Abela, 1999, Oie, 2009
Moldova	„	„	Oie Handistatus, 2005
Portugal	„	„	Oie, 2009
Romania	Not Reported	Present	„
Russian	„	„	„
Serbia	„	Not Reported	„
Slovenia	Not Reported	Present	„
Spain	Present	Restricted Distribution	„
Yugoslavia	„	Not Reported	Oie Handistatus, 2005
<b>Oceania</b>			
Australia	Not Reported	Present	Oie, 2009
New Caledonia	„	„	„
New Zealand	„	„	„

Adopted from: CABI (2017b) for *B. Melitensis* and CABI (2017a) for *B. Ovis*.

Table 4: Distribution of *Brucella Melitensis* and *Brucella Ovis* in Africa

Country	<i>Brucella Melitensis</i>	<i>Brucella Ovis</i>
Egypt	+	ND
Ethiopia	+	ND
Kenya	+	ND
Sudan	+	ND
Somalia	+	ND
Eritrea	+	+
Libya	+	ND
Lesotho	ND	+
Algeria	+	+
Tunisia	++	ND
Namibia	+	+
Niger	+	+
Nigeria	+	ND
Cotd' Ivor	ND	+
Zimbabwe	+	+
Botswana	ND	+
South Africa	+	+

Source: (FAO, 2010; OIE, 2012b)

(++: High prevalence, +: Sporadic low prevalence, ND: No Data)

b) Occurrence in Ethiopia

The states of brucellosis of the small ruminant in Ethiopia are not well known or are not more than mere report. This may be due to the lack of attention given to small ruminant production sector. The absence of research activity in animal diseases, poor veterinary

development, lack of awareness of the economic and zoonotic impact of the disease have contributed to the less amount of information observed. Though limited, sero-surveillances carried out so far indicate that brucellosis may be one of the important diseases in goat rising communities. A sero-surveillance study carried out

in small ruminants in different regions clearly demonstrated that the disease exists in Ethiopia. According to the current sero-surveillance findings of the disease in the country, low infection rate was recorded at Bahir Dar Town of Amhara Regional State (Ferede et al., 2011) and the highest was reported at Tellalok District of

Afar Regional State (Tadeg et al., 2015) (Table 5). The existence of the disease was also confirmed and reported in Southern Nations Nationalities and Peoples Regional state (SNNPRS) of Ethiopia, according to the annual report of Soda Regional Veterinary Laboratory in the year 2005 (Table 5).

Table 5: The Epidemiology of Small Ruminant Brucellosis in Ethiopia

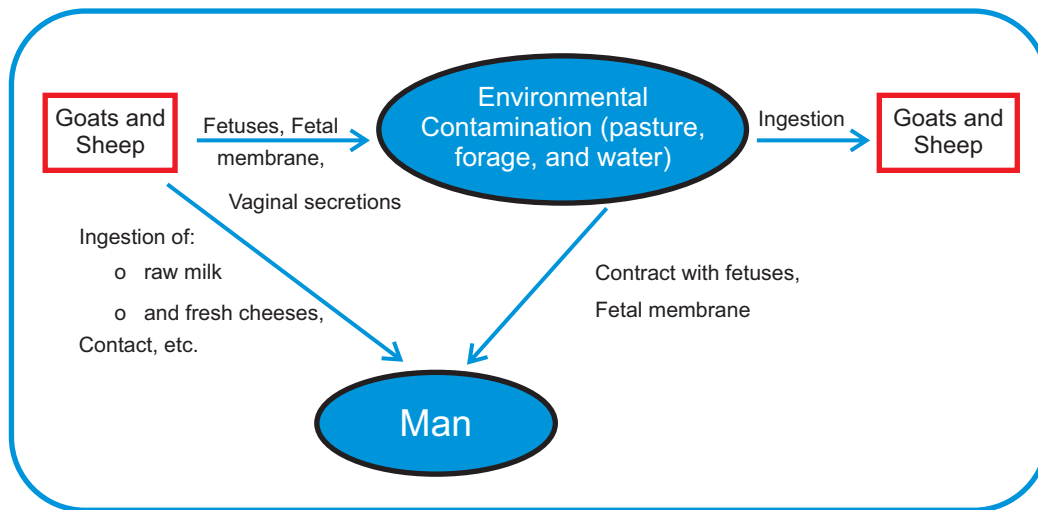
Sr. No.	Study Area	Region	Prevalence	Source
1	Tellalok District	Afar	13.7%	Tadeg et al., 2015
2	Chifra and Ewa Districts	Afar	12.35%	Tegegn et al., 2016
3	Yabello districts of Borena Zone,	Oromia	8.1%	Wubishet et al., 2018
4	Liban District of Guji Zone	Oromia	6.2%	Wubishet et al., 2017
5	Southern Zone of Tigray	Tigray	3.5%	Teklue et al., 2013
6	Selected Export Abattoirs	Addis Ababa	2.7%	Nigatu et al., 2014
7	Werer Agricultural Research Center	Afar	2.25%	Bezabih and Bulto, 2015
8	Selected Pastoral and Agro-pastoral Lowlands of Ethiopia	Somali and Oromia	1.9%	Sintayehu et al., 2015
9	Southern and Central Ethiopia	SNNP and Oromia	1.9 %	Asmare et al., 2012
10	Tselemti Districts	Tigray	1.79%	Kelkay et al., 2017
	South Wollo	Amhara	1.5%	Yesuf et al., 2011
11	Three Selected districts of Jijiga Zone	Somali	1.37%	Mohammed et al., 2017
12	In and Around Kombolcha	Amhara	0.7%	Tewodros and Dawit, 2015
13	In and Around Bahir Dar	Amhara	0.4 %	Ferede et al., 2011

c) Modes of Transmission

The primary route of infection is through ingestion of contaminated feed and water, inhalation during overcrowding, contact through intact skin and conjunctiva, lambs may be infected while in the uterus or by suckling infected milk of their mother. Venereal transmissions by the infected ram to susceptible ewes appear to be rare. Transmission may occur by artificial insemination (Radostits et al., 2007). Transmission between animals occurs readily after massive exposure to aborted materials, contaminated placenta and postpartum discharge in an infected female. In sheep, the degree of infection of milk and in uterine exudates is much lesser than goats. Studies indicate that 70-90%

cause of Brucella infection occurs via the skin and mucus membrane by direct contact (Franc et al., 2018) (See the mode of transmissions in figure 1).

Transmission to man is as a result of contact with infected animal carcasses, aborted fetus, placenta, consumption of unpasteurized milk and cheese. It is common to observe human cases that are in contact with goats in an area where active brucellosis outbreak occurs. Raw vegetable and water contaminated with the extra of infected animals can also serve as a source of infection. Brucella organisms can remain viable in milk, water, and damp soil for up to four months (Radostits et al., 2007).



Sources: WHO (2006)

Fig. 1: Small Ruminant Brucellosis (B. Melitensis) Mode of Transmission

#### d) Communicability of the Disease between Humans

Brucellosis is not usually transmit from person to person. Rarely, bacteria have been transmitting by bone marrow transplantation, blood transfusion or sexual intercourse (Wikipedia, 2018). Rare congenital infections have also been documented. In some cases, the infant appeared to be infected through the placenta and in others by the ingestion of breast milk. Brucellosis was reported in an obstetrician infected infants respiratory tract at birth (Saxena et al., 2018).

### V. ZOO NOTIC ASPECTS OF BRUCELLOSIS

It is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE) as one of the widest spread zoonosis in the world. Reported incidence in endemic disease areas varies widely, from < 0.01 to > 200 per 100,000 populations (Franco et al., 2007).

The bacterium *B. melitensis* is the most invasive and pathogenic for humans among the three classical species (*B. abortus*, *B. melitensis* and *B. suis*) of the genus *Brucella*. Brucellosis remains a most serious zoonosis in areas of the world where *B. melitensis* is enzootic in goats and sheep, and the resulting disease in human is severe and long lasting (Radostits et al., 2007). Human brucellosis is widely distributed all over the world, with regions of high endemicity such as Mediterranean, Middle East, Latin America and parts of Asia (Khan et al., 2018).

Brucellosis due to *B. melitensis* is a zoonotic disease causing a debilitating illness in human. Symptoms of acute brucellosis caused by *B. melitensis* are flu-like and highly nonspecific. Chronic brucellosis is an insidious/dangerous disease with vague/unclear symptoms that might be confused with other disease affecting various organ systems (Kelkay et al., 2017).

The risk for infection is high in cultures that cohabit with their animals or when weak, infected newborn animals are brought in to the house for warmth and intensive care. Flaw milk and cheese products from infected goats and sheep provide a risk for human and were the mechanism for the occurrence of Malta fever that initiated the definition of the disease (Radostits et al., 2007).

### VI. RISK FACTORS

The factors influencing the epidemiology of brucellosis infection in any geographical location can be classified into factors associated with the transmission of the disease between herds and factors influencing the maintenance and spread of infection within the herd (WHO, 2006). Factors associated with brucellosis include host factors (age, sex, and breed), agent and extrinsic factors (environmental factors) including management and ecology (Güven et al., 2013, Hotez et al., 2012).

It is widely accepted that susceptibility increases with sexual development and pregnancy (Güven et al., 2013). Kids and lambs may become infected before or soon after birth, and tend to become free from infection before reaching breeding age, occasionally infection persist much longer (WHO, 2006). *Brucella melitensis* infection causes disease only in adult (sexually mature) females and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response (Radostits et al., 2007).

In *B. melitensis* infection males of sheep and goat are less susceptible than females. *Brucella ovis* has a great affinity for the reproductive tract of the male than the female. Breeding ewes with infected rams seldom cause the disease in ewe and incidence of abortion is low (WHO, 2006).

Animals of an exotic breed and their hybrid are found to be at higher risk. This may associate with better producers and intensively managed (Rossetti et al., 2017). Most breeds of goats are fully susceptible to *B. melitensis*. There is great variation in the susceptibility of different breeds of sheep, where Malta sheep are very resistant whereas fat-tailed sheep are very susceptible (WHO, 2006).

*Brucella* is intracellular bacteria, hence has protection from the innate host defense and from therapeutics, moreover in quiescent state does not cause formation of humeral antibodies (Güven et al., 2013).

Humidity and PH of the environment influence the survival of *B. melitensis*. The organism is sensitive to direct sunlight, disinfectant, and pasteurization (WHO, 2006).

*Brucella* survives for up to 4 months in milk, urine, water and damp soil under proper environmental condition (WHO, 2006). Disinfectants like caustic soda, formalin 2%, and Lysol 1% destroy *Brucella* (Radostits et al., 2007).

The husbandry systems as well as environmental conditions greatly influence the spread of infection. Thus lambing in the dark, crowded enclosures is more favorable to spread than lambing in the open air in a dry environment. The spread of infection between flocks generally follows the movement or gathering of infected animals. The main risk for introducing the disease into a previously non-infected area is by the purchase of infected animals. In several countries, there is a strong correlation between the prevalence of brucellosis in small ruminants and the practice of transhumance (Khan et al., 2018).

### VII. PATHOGENESIS

The initiation of *Brucella* infection depends on exposure dose, virulence of *Brucella* species and the natural resistance of the animal to the organism (Radostits et al., 2007, Sharifi et al., 2015). Resistance to

infection is on the basis of host's ability to prevent the establishment of infection by the distraction of the invading organism. Invading Brucellais usually localized in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticuloendothelial tissue and the infiltration of inflammatory cells. Survival of the first line of defense by the bacteria results in local infection and the escape of Brucella from the lymph nodes into the blood (Tadeg et al., 2015). During the bacteremic case, which may last 2-8 weeks, bones, joints, eyes, and brain can be infected, but the bacteria are most frequently isolated from super mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus (Radostits et al., 2007).

There is preferential localization to the reproductive tract of the pregnant animals. Unknown factors in the gravid uterus collectively referred to as allantoic fluid factors, stimulate the growth of Brucella. Erythritol, a four-carbon alcohol, is considered to be one of these factors. Abortion is associated with the extensive replication of the brucellae within the chorioallantoic trophoblasts that form a vital component of the placenta. This massive intracellular replication ruptures the infected trophoblasts and allows the bacteria direct access to the fetus. The resulting loss of placental integrity and fetal infection lead to termination of the pregnancy or the premature birth of a weak and infected calf (Hotez et al., 2012). Localization in the placenta leads to the development of placentitis with subsequent abortion. After an abortion, the uterine infection persists for up to 5 months, and mammary gland may remain infected first years (Radostits et al., 2007, Saxena et al., 2018).

There is initial bacteremia, often with a mild systemic reaction, and the organism can be isolated from the internal organs of animals slaughtered after experimental infection. However, systemic disease is not a feature of the natural disease, and clinical disease results from localization in this area results in sperm stasis and extravasations with a subsequent immunological reaction which is usually in the tail and unilateral, causing a spermatocele and therefore reduced fertility. Not all infected rams have palpable lesions in the epididymis and infection can also establish in the seminal vesicles. In either case, it is shed in the ejaculate. Testicular and epididymal lesions can be palpated at about nine weeks after infection but may occur earlier in some rams. A significant proportion of infected rams have no palpable lesions but still excrete the organism (Radostits et al., 2007).

This disease is well described by its original name undulant fever. The disease does not have precise symptoms besides general malaise, making it difficult to diagnose clinically. Brucellosis is characterized by acute fever, sweats, headaches, and flu-like symptoms in the humans (Franco et al., 2007). It is believed that brucellosis causes fewer spontaneous

abortions than it does in animals because of the absence of erythritol in the human placenta and fetus. An additional reason for the lesser role of Brucella infection in human is the presence of anti-Brucella activity in human amniotic fluid (Hotez et al., 2012).

## VIII. CLINICAL FINDINGS

The primary clinical manifestations of brucellosis are related to the reproductive tract. The biggest problem of Brucella infection is the uncertain incubation period, which may vary between 15 days to month and years depending on the invasion site, infective dose, and others (FAO, 2010). The only symptom noted under natural infection is abortion. Infected goats show abortion and sometimes mastitis, with reduced milk production. Abortion usually occurs at 3-4 month of pregnancy. Goats that have aborted once are not likely to occur the second time, but sheep may abort a second time. Retention of the fetal membrane may or may not occur (FAO, 2010). Goats shed Brucella in milk for years, but sheep may shed during one or more lactation period. Excretion in the vaginal fluid and urine may last for the 4-6 month (FAO, 2010). The first reactions in males are a marked deterioration in the quality of the semen together with the presence of leukocytes and Brucella. Acute edema and inflammation of the scrotum may follow, a systemic reaction, including fever, depression, and increased respiratory rate, accompanies the local reaction. Regression of the acute syndrome is followed later a long latent period, by the development of palpable lesion in the epididymis and tunica of one or both testicles. The epididymis is enlarged and hard, more commonly at the tail, the scrotal tunics are thickened and hardened, and the testicles are usually atrophic. The groove between the testicle and epididymis may be obliterated. There is usually no clinical sign in the ewe, but in some flocks, infection causes abortion and the birth of weak and stillborn lambs and kids, associated with microscopic placentitis (Radostits et al., 2007).

## IX. NECROPSY FINDINGS

The abortus is characterized by thickening and edema, sometimes restricted to only a part of the placenta, firm, elevated yellow-white plaques in the intercotyledonary areas. The average degree of abnormality of the cotyledons, which is in the acute stages are much-enlarged, firm and yellow-white in color. When abortion occurs, the organism can be isolated from the placenta and the stomach and lungs of the lamb. Although placentitis is uncommon, it is occasionally seen in infected ewes (Radostits et al., 2007).

Some aborted fetuses appear normal others are autolysis or have variable amounts of subcutaneous edema and bloodstained fluid in the body cavities. In ruminant fetuses, the spleen and liver may enlarge, and

the lungs may exhibit/show sign of pneumonia and fibrous pleuritis. Abortion caused by *Brucella* species are typically accompanied by placentitis. The cotyledons may be red, yellow, normal or necrotic. In small ruminants, the intercotyledonary region is typically leathery, with a wet appearance and focal thickening. There may be exudates on the surface. In adults, granulomatous to purulent lesions may found in the male and female reproductive tract, mammary gland, supra mammary lymph node, other lymphoid tissues, bones, joints and other tissues and organs. Mild to severe endometritis may be seen after an abortion, and males can have unilateral or bilateral epididymitis and/or orchitis (Saxena et al., 2018). In rams infected with *B. ovis*, lesions are usually limited to epididymis and orchitis. Epididymal enlargement can be unilateral or bilateral, and the tail is affected more than the head or body. Fibrous atrophy can occur in the testis. The tunica vaginalis is often thickened and fibrous and can have extensive adhesions. In the acute stage, there is inflammatory edema in the loose scrotal fascia, exudates in the tunica vaginalis and early granulation tissue formation. In the chronic stage, the tunics of the testes become thickened and fibrous, and adhesions develop between them (Radostits et al., 2000, Saxena et al., 2018).

Brucellosis is responsible for massive economic losses around the world especially in developing countries where accurate data are not available to truly assess the loss. Losses are usually due to culling of animals, abortion, infertility, reduced milk production, treatments costs of animals, vaccines, market losses, losses due to missed reproductive cycles, hospitalizations for human cases and administrative costs by governments in an attempt to control or eradicate the infection. In Latin America, annual economic losses were \$600 million for ruminant brucellosis, and in the United States, the eradication program spent \$3.5 billion between 1934 and 1997 and loss due to reduced milk production in 1952 amounting to about \$400 million (Bamaiyet al., 2014). In assessing the economic impact of brucellosis in case of a bioterrorist attack, it will have an economic impact of \$477.7 million per 100,000 persons exposed. Many other losses due to loss of foetus, decreased milk yield, infertility, interference with farrowing management and sequential seasonal calving, joint infections, weakling calves, disease in man and others could not be accounted for financially but are likely to run into millions of dollars annually (Bamaiyet al., 2014) (figure 2).

X. ECONOMIC IMPACT OF BRUCELLOSIS

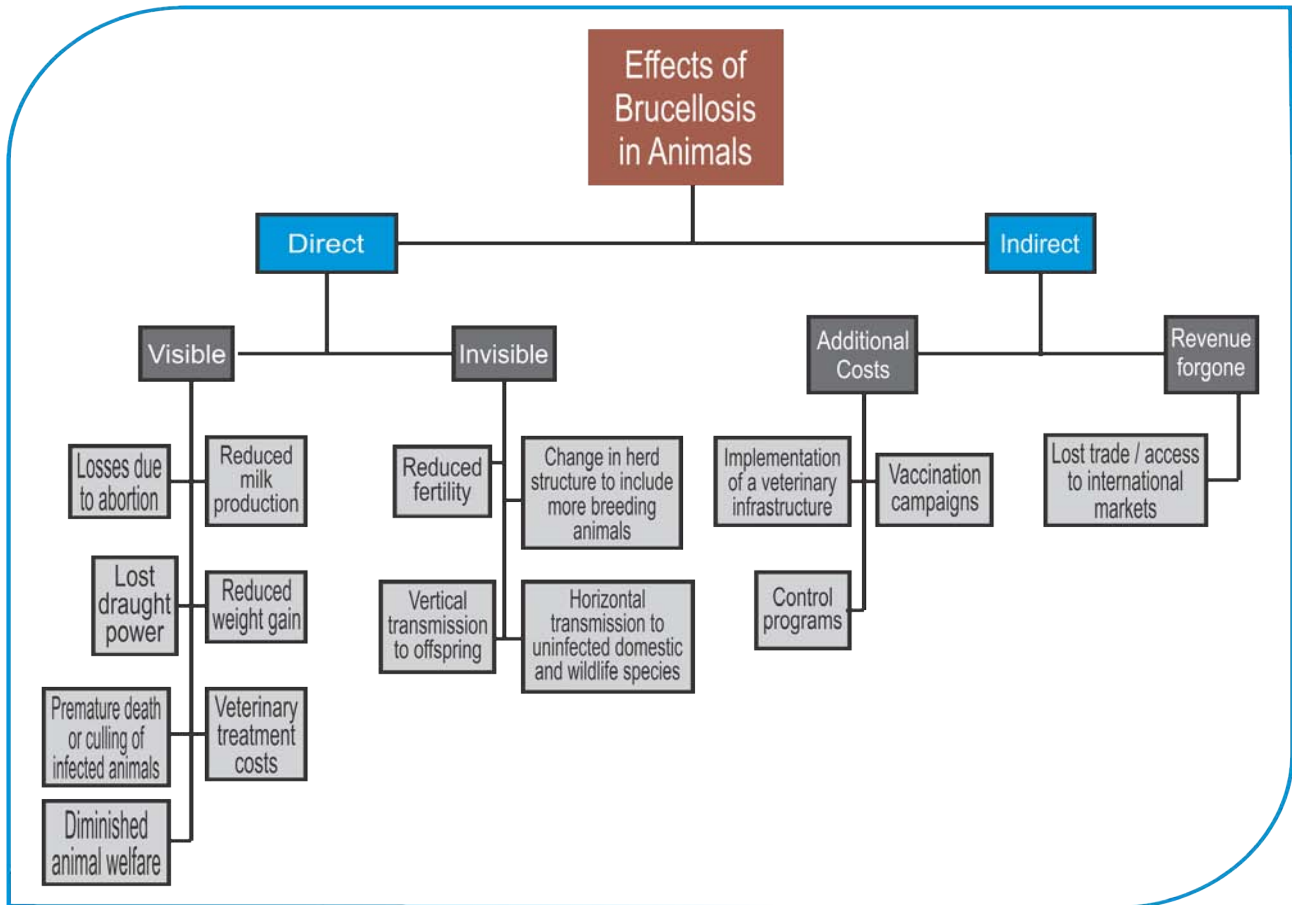


Fig. 2: Framework to Assess the Effect of Livestock Brucellosis in Regions where the Disease is Endemic (Franc et al., 2018)



## XI. DIAGNOSIS

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk (Tewodros and Dawit, 2015).

Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens (WHO, 2006). The isolation of *Brucella* is definitive proof that the animal is infected, but not all infected animals give a positive culture, and the methods and facilities that must be employed are not always readily available (Khan et al., 2018). The detection of an antibody or a hypersensitivity reaction provides only a provisional diagnosis, but in practice is the most feasible and economical means of diagnosis. False positive reactions to serological tests can occur through some factors, including vaccination, and this must be borne in mind when interpreting results. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily an active infection, and may also result from vaccination (WHO, 2006).

Laboratory diagnosis based on direct examination of clinical specimens using modified acid-fast stains, bacterial culture and serology (Arifet al., 2018). However, achievement of a reliable diagnosis of brucellosis is a tedious process since isolation is affected by some factors, such as high fastidious nature of *Brucella*, the presence of a lesser number of viable organisms in the sample and delay in the sample submission (leading to putrefaction). Also, a prolonged incubation period may lead to a failure in its isolation (Hanci et al., 2017).

Direct demonstration of the causal organism can be done microscopically with staining examination, fluorescence serologically, cultural on special nutrients, in animal experiments with guinea pigs. Primary bacteriological diagnosis can be made on smears from vaginal swabs, milk, placentas or aborted fetuses stained with stamps method. Confirmation on appropriate culture and selective media is recommended. Spleen and lymph nodes are most reliable from necropsy material. Polymerase chain reaction (PCR) is potentially a useful method on samples containing a low number of *Brucella* (Musallam et al., 2016).

Some of the most used diagnostic tests by indirect demonstration of the pathogen are Card test (C1) is the most suitable for detecting infected flocks and for a survey. It is simple and rapid and does not

require laboratory facilities (Khan et al., 2018). Milk ring test (MR7) this procedure is valuable in screening dairy cows and has limitations in the diagnosis of caprine and ovine brucellosis. A serious disadvantage of the test is that its use is limited to milking animals. Allergic skin test (A81) is characteristic of brucellosis in man and some animals and appears through the delayed type of hypersensitivity to *Brucella* allergens in generalized infection, and the sensitivity may persist for several years (Musallam et al., 2016).

Individual Serologic Tests are Serum Agglutination Tube Test (SAT), Complement Fixation Test (CFT) this serologic test has a relatively high sensitivity and Specificity and is superior to the SATT. The CFT indicates active *Brucella* infection better than any other serologic test. It detects mostly IgG antibodies which are present in both acute and chronic stages of brucellosis (Musallam et al., 2016).

The use of the Rose Bengal Plate Test (RBPT) is easy to perform and considered as valuable screening test though less effective than CFT at detecting brucellosis in individual sheep and goats. The CFT is considered to be the most effective test for diagnosing brucellosis in small ruminants (WHO, 2006).

Most studies agree that the ELISA is as specific as the CFT, but it is more sensitive. Yet, for a reliable diagnosis of infected animals studies suggest using the ELISA in combination with other tests (Mohseni et al., 2017). Other studies consider the ELISA suitable for screening flocks of sheep and goats for brucellosis (Curro et al., 2012).

## XII. TREATMENT

As a general rule, treatment of infected livestock is not attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs (Yousefi-Nooraie et al., 2012).

Even though the complex nature of brucellosis makes it difficult to treat, long-term treatment with an antibiotic is thought to be beneficial. In most cases, antibiotics in combination are found to be more effective against the infection, however, the state of the disease still does not lose its importance (Falagas and Bliziotis, 2006, Moon, 2014). Several conventional antibiotics including tetracycline, trimethoprim - sulfamethoxazole, amino-glycosides, rifampicin, quinolones, chloramphenicol, doxycycline, and streptomycin are commonly used in clinics (Saltoglu et al., 2002, Geyik et al., 2002). In several cases, the application of antibiotics in a specific order has given best results. Likewise, a case reported that treatment with doxycycline for six months, followed by streptomycin for three weeks was found very effective against brucellosis in human (Yousefi-Nooraie et al., 2012). Another study reported that the alkaloid columbamine in combination with

jatrorrhizine was more effective against brucellosis caused by *B. abortus* compared to a combination of streptomycin and rifampicin (Azimi et al., 2018). The World Health Organization recommends that acute brucellosis cases be treated with oral doxycycline and rifampicin (600 mg for six weeks) (Ersoy et al., 2005). However, rifampicin monotherapy is in common practice for treating brucellosis in pregnant women, and combined therapy of sulphamethoxazole and trimethoprim is recommended for children (Karabayet al., 2004). In underdeveloped countries, treatment of cattle is not a common practice, however, the infected animals are isolated, culled or slaughtered to prevent the spreading of infection to other herd and at substantial veterinary costs. In China, a case of subdural empyema complicated by intracerebral abscess due to *Brucella* infection was effectively treated with antibiotic therapy (ceftriaxone, doxycycline, rifapentine) (Zhang et al., 2017). In line with this, several reports suggested the combination therapy of doxycycline and rifampicin for six weeks is enough to eradicate *Brucella* infection, as well as associated complications (Meng et al., 2018, Kaya et al., 2018). This combination of doxycycline and rifampicin has also been proven experimentally (Yang et al., 2018). As a result of continued efforts by the scientific community to develop an effective therapeutics, *Caryopterismongolica* Bunge (Lamiaceae) has been tested in combination with doxycycline (Tsevelmaa et al., 2018, Saxena et al., 2018). Even though several therapeutics are in practice which makes the disease manageable, an effective therapeutic is required for the complete treatment of brucellosis (Khan et al., 2018).

Humans are treated with antibiotics (doxycycline with rifampicine). Relapses are, however, possible (Solis and Solera, 2012). In experimentally infected rams the combined administration of chlortetracycline (800mg intravenously) and streptomycin (1gram subcutaneously) injected daily for 21 days, eliminated infection. Streptomycin alone and streptomycin plus sulfadimidine were not satisfactory. Treatment is economically practicable only in valuable rams and must be instituted before irreparable damage to the epididymis has occurred. A dose of 1000 mg of long-acting tetracycline give every three days for the period of 6 weeks achieved a cure rate of 75% (Radostiis et al., 2007).

### XIII. CONTROL AND PREVENTION

As the ultimate source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must be based on elimination of such contact. The obvious way to do this-elimination of the disease from animals is often beyond the financial and human resources of many developing countries. The technical and social difficulties involved in eradicating *B. melitensis* from small ruminants have

even taxed the resources of some developed countries. In many situations, there is little alternative but to attempt to minimize the impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene. The lack of safe, effective, widely available vaccines approved for human use means that prophylaxis currently plays little part in the prevention of human disease (WHO, 2006).

Prevention and control of brucellosis can be adopted realistically through an understanding of local and regional variations in animal husbandry practices, social customs, infrastructures and epidemiological patterns of the disease (Dorneles et al., 2015). The common approaches used to control brucellosis includes quarantine of imported stock and decide for or against immunization of the negative animals (Radostiis et al., 2007).

Eradication by test and slaughter principles are based on the magnitude of disease prevalence and economic status at countries and handling hygienic disposal of aborted fetuses, fetal membrane and discharges with subsequent disinfection of contaminated area (WHO, 2006).

Control measures must include hygiene at lambing and the disposal of infected or reactor animals. Separate pens for lambing ewes, which can be cleaned and disinfected, early weaning of lambs from their dams, and their environment and vaccination, are recommended. In endemic areas, all placentas and dead fetuses should be buried as a routine practice. The need to test and cull, introduced and resident animals likely to be carriers is recommended, but difficult to be effective because of the inaccuracy of the tests. Because of the possibility that lambs may be infected at birth and carry the disease for life, it may be more economical to dispose off the entire flock (Radostiis et al., 2007).

The experience from all over the world, that vaccination is in most situations the only practical method of control of brucellosis in sheep and goats. Immunization with effective vaccines helps to get the infection under control, limit its spread, prevent human infections and reduce economic losses (Musallam et al., 2016). Most workers agree that the smooth live organisms of *B. abortus* strain 19 and *B. melitensis* Rev 1 have many advantages over inactivated vaccines. Their limitations, including interference with diagnostic tests, are well known. However, they provide good protection on a herd per flock basis by reducing clinical symptoms (exposure potential) and elevating to induce sexually mature animals. The reduced doses also reduce the physiologic and serologic effects. It is illogical to restrict the use of vaccines among mature animals where there are no controls on infected populations (Donev, 2010).

#### XIV. CONCLUSIONS

Brucellosis is thought to be widespread zoonotic infectious disease that highly affects the health and economy of animals and humans in the world. The two species of *Brucella*, *B. ovis* and *B. melitensis*, are the main causative agents of infection to sheep and goats, respectively. The latter, is also the one in which, greatly contributes the infection to humans.

The disease is serious, therefore, proper veterinary legislation must be implemented and policies regarding animal health need to be encouraged. Current and modern awareness on brucellosis should be delivered to farmers, veterinary professionals, and health educators, especially for rural populations, which will help to prevail over the dispersal of *Brucella* infection worldwide. Effective and relatively safe vaccines should be available to provide long-term protection against brucellosis in both animals and humans.

In general, to combat the disease, there should be proper management practices such as rearing of brucellosis free animals, isolating and restricting movement of infected and/or suspected animals, following the guidelines of incineration or burial for proper disposal of animal discharges and wastes, formulating a schedule for cleansing and disinfection of animal houses, feeding and watering troughs, and understanding proper hygienic practices in all stages.

#### ACKNOWLEDGEMENTS

The Authors greatly acknowledge those who participated either directly or indirectly in supplying materials and logistics that highly contributed for the success of this work.

#### Conflicting of Interest:

Authors declare that no conflict of interest in the publication of this work.

#### REFERENCES RÉFÉRENCES REFERENCIAS

1. Abebe, Y., Melaku, S. and Tegegne, A (2013). Assessment of sheep marketing system in Burie district, North Western Ethiopia. *Wudpecker Journal of Agricultural Research* 2 (3): 97-102.
2. Abela B. (1999). Epidemiology and control of brucellosis in ruminants from 1986 to 1996 in Malta. *Revue Scientifique et Technique - Office International des épizooties*, 18 (3): 648-659, 20 ref.
3. Ahl A. S, Bartlett P. C, Frerichs W. M (1993). Serological evidence for the presence of *Brucella* antibodies in sheep and goats on Saint Croix, U.S. Virgin Islands. *Revue d'élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46 (½): 61-63, [Actes de la 2e Réunion Biennale de la Society for Tropical Veterinary Medicine, 2-6 février 1993, Saint François, Guadeloupe.], 14 Ref.
4. Arif, S., Heller, J., Hernandez-Jover, M., McGill, M.D., Thomson, C.P. (2018) Evaluation of three serological tests for diagnosis of bovine brucellosis in smallholder farms in Pakistan by estimating sensitivity and specificity using Bayesian latent class analysis. *Prev. Vet. Med.* 149, 21-28. [CrossRef] [PubMed].
5. Asmare K, Megersa B, Denbarga Y, Abebe G, Taye A, Bekele J, Bekele T, Gelaye E, Zewdu E, Agonafir A, Ayelet G, Skjerve E (2012) A study on seroprevalence of caprine brucellosis under three livestock production systems in southern and central Ethiopia. *Trop Anim Health Prod.* 45: 555-560, doi 10.1007/s11250-012-0258-2.
6. Awah-Ndukum J, Mouiche M. M. M, Bayang H. N, NguNgwa N, Assana E, Feussom K. J. M, Manchang T. K, Zoli P.A (2018). Seroprevalence and Associated Risk Factors of Brucellosis among Indigenous Cattle in the Adamawa and North Regions of Cameroon. *Vet Med Int.* 3468596.
7. Azimi, G., Hakakian, A., Ghanadian, M., Joumaa, A., Alamian, S. Bioassay - directed isolation of quaternary benzylisoquinolines from *Berberisintegerrima* with bactericidal activity against *Brucella abortus*. *Res. Pharm. Sci.* 2018, 13, 149-158. [PubMed].
8. Bamaiyi P. H., Hassan L., Khairani-bejo S., Zainal A.M. (2014). Updates on Brucellosis in Malaysia and South Asia. *Malaysian Journal of Veterinary Research.* (5): 71-82.
9. Bezabih M. K, Bulto W. C. (2015). Seroprevalence of small ruminant brucellosis in Werer Agricultural Research Center, Afar Region, North East Ethiopia. *Acad. J. Microbiol. Res.* 3 (2): 031-035.
10. CABI. (2017a, 11 09). Brucellosis (*Brucella ovis*). Retrieved 06 14, 2018, from <https://www.cabi.org/isc/datasheet/90731>.
11. CABI. (2017b, 11 09). Brucellosis (*Brucella melitensis*). Retrieved 06 14, 2018, from <https://www.cabi.org/isc/datasheet/90734>.
12. Central Statistical Agency (CSA) (2014). Federal Democratic Republic of Ethiopia, Central Statistical Agency, Agricultural sample survey 2013/14 (2006 E.C). Vol II. Report on livestock and livestock characteristics (Private Peasant Holdings). *Statistical Bull.* PP. 573.
13. Currò, V., Marineo, S., Vicari, D., Galuppo, L., Galluzzo, P., Nifosì, D., Pugliese, M., Migliazzo, A., Torina, A., Caracappa, S. The isolation of *Brucella* spp. from sheep and goat farms in Sicily. *Small Rumin. Res.* 2012, 106, S2-S5.
14. Donev, D. (2010) Brucellosis control and eradication in the south eastern European countries: Current status and perspective strategies. *Maced. J. Med. Sci.* 3, 220. [CrossRef].

15. Dorneles, E. M., Sriranganathan, N., Lage, A. P. (2015). Recent advances in *Brucella abortus* vaccines. *Vet. Res.* 46, 76. [Cross Ref] [Pub Med].
16. El abdin S. Z., Angara T. E., Elfadil A. A., El Sanousi E. M., Ibrahim A. M. (). Prevalence and Risk Factors of Ruminants Brucellosis in Jabel Aolia Locality, Sudan. *Sudan Journal of Science and Technology* 15 (2): 60-72.
17. Ersoy, Y., Sonmez, E., Tevfik, R. M., But, D. A. Comparison of three different combination therapies in the treatment of human brucellosis. *Trop. Doct.* 2005, 35, 210-212. [Cross Ref] [Pub Med].
18. Falagas, M. E., Bliziotis, I. A. Quinolones for treatment of human brucellosis: Critical review of the evidence from microbiological and clinical studies. *Antimicrob. Agents Chemother.* 2006, 50, 22-33. [Cross Ref] [Pub Med].
19. Ferede Y, Mengesha D, Mekonen G, Hilemeleket M. (2011). Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar, North West Ethiopia. *Ethiop. Vet. J.*, 15 (2), 35-44.
20. Food and Agriculture Organization of the United Nations. (2010). *Brucella melitensis* in Eurasia and the Middle East. FAO Animal Production and Health Proceedings. No. 10 Rome.
21. Foster, J. T., Walker, M. F., Rannals, D. B., Hussain, H. M., Drees, P. K., Tiller, V. R., Hoffmaster, R. A., Al-Rawahi, A., Keim, P., Saqib, M (2017). African lineage *Brucella melitensis* isolates from Omani livestock. *Front. Microbiol.* 8, 2702. [Cross Ref] [Pub Med].
22. Franc, K. A, Krecek, R. C, Häsler, B. N, Arenas-Gamboa, A. M (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health* 18: 125.
23. Franco, M. P., Mulder, M., Gilman, H. R., Smits, L. H (2007). Human brucellosis. *Lancet Infect. Dis.* 7, 775-786. [Cross Ref].
24. Franco, M. P., Mulder, M., Gilman, H. R., Smits, L. H. (2007) Human brucellosis. *Lancet Infect. Dis.* 7, 775-786. [Cross Ref].
25. Gebremedhin, B., Hoekstra, D., Tegegne, A., Shiferaw, K. and Bogale, A (2015). Factors determining household market participation in small ruminant production in the highlands of Ethiopia. LIVES Working Paper 2. Nairobi, Kenya: International Livestock Research Institute.
26. Geyik, M. F., Gur, A., Nas, K., Cevik, R., Sarac, J., Dikici, B., Ayaz, C. Musculoskeletal involvement of brucellosis in different age groups: A study of 195 cases. *Swiss Med. Wkly.* 2002, 132, 98-105. [Pub Med].
27. Gizaw S (2010) Sheep and goat production and marketing systems in Ethiopia: Characteristics and strategies for improvement (No. 23). ILRI (aka ILCA and ILRAD).
28. Gobena, M. M (2016). Review on Small Ruminant Production, Marketing and Constraints in Ethiopia. *Advances in Life Science and Technology*, V (48): 28-34.
29. Godfroid J, Al Dahouk S, Pappas G, Roth F, Matope G, Muma J, Marcotty T, Pfeiffer D, Skjerve E (2013). A "One Health" surveillance and control of brucellosis in developing countries: Moving away from improvisation. *Comp. Immunol. Microbiol. Infect. Dis.* 36 (3): 241-248.
30. Gouvêa G, Roxo E, Lima MABS, Homerich D, 1989. Occurrence of brucellosis in goats from Sao Paulo State. *Arquivos do Instituto Biológico (Sao Paulo)*, 56 (Supl.): 60.
31. Guven, T., Ugurlu, K., Ergonul, O., Celikbas, K. A., Gok, E. S., Comoglu, S., Baykam, N., Dokuzoguz, B. Neurobrucellosis: Clinical and diagnostic features. *Clin. Infect. Dis.* 2013, 56, 1407-1412. [Cross Ref] [Pub Med].
32. Hanci, H., Igan, H., Uyanik, M. H. Evaluation of a new and rapid serologic test for detecting brucellosis: *Brucella* Coombs gel test. *Pak. J. Biol. Sci.* 2017, 20, 108-112. [Cross Ref] [Pub Med].
33. Hirsh D. C, Maclachlan N. J, Walker R. L (2004) *Veterinary Microbiology*. Black well science USA 105-112.
34. Hotez, P. J., Savioli, L., Fenwick, A. Neglected tropical diseases of the Middle East and North Africa: Review of their prevalence, distribution, and opportunities for control. *PLoS Negl. Trop. Dis.* 2012, 6, e1475. [Cross Ref] [Pub Med].
35. Karabay, O., Sencan, I., Kayas, D., Sahin, I. Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: A randomized clinical trial [ISRCTN11871179]. *BMC Infect. Dis.* 2004, 4, 18. [Cross Ref] [Pub Med].
36. Kaya, S., Elaldi, N., Deveci, O., Eskazan, E.A., Bekcibasi, M., Hosoglu, S. Cytopenia in adult brucellosis patients. *Indian J. Med. Res.* 2018, 147, 73-80. [Cross Ref] [PubMed].
37. Kelkay, M. Z., Gugsu, G., Hagos, Y., & Taddelle, H. (2017). Sero-prevalence and associated risk factors for *Brucella* sero-positivity among small ruminants in Tselemti districts, Northern Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 9 (11), 320-326.
38. Khan M. Z, Zahoor M (2018). An Overview of Brucellosis in Cattle and Humans, and its Serological and Molecular Diagnosis in Control Strategies. *Trop. Med. Infect. Dis.* 3, 65.
39. Meng, F., Pan, X., Tong, W. Rifampicin versus streptomycin for brucellosis treatment in humans: A meta-analysis of randomized controlled trials. *PLoS One* 2018, 13, e0191993. [Cross Ref] [Pub Med].
40. Mohseni, K., Mirnejad, R., Piranfar, V., Mirkalantari, S. A comparative evaluation of ELISA, PCR, and



- serum agglutination tests for diagnosis of *Brucella* using human serum. *Iran J. Pathol.* 2017, 12, 371-376. [Pub Med].
41. Moon, M. S. Tuberculosis of spine: Current views in diagnosis and management. *Asian Spine J.* 2014, 8, 97-111. [Cross Ref] [Pub Med].
  42. Musallam, I. I., Abo-Shehada, N. M., Hegazy, M. Y., Holt, R. H., Guitian, J. F. Systematic review of brucellosis in the Middle East: Disease frequency in ruminants and humans and risk factors for human infection. *Epidemiol. Infect.* 2016, 144, 671-685. [Cross Ref] [Pub Med].
  43. Neharika Saxena, Balbir Bagicha Singh and Hari Mohan Saxena. (2018). Brucellosis in Sheep and Goats and Its Serodiagnosis and Epidemiology. *Int. J. Curr. Microbiol. App. Sci.* 7(01): 1848-1877. doi: <https://doi.org/10.20546/ijcmas.2018.701.225>.
  44. Nigatu S, Deneke M, Kassa T (2014). Sero-Prevalence of Brucellosis in Sheep and Goat Destined for Slaughter in Selected Export Abattoirs, Ethiopia. *African Journal of Basic & Applied Sciences* 6 (3): 82-86.
  45. Office International des Epizooties Terrestrial Manual (OIE) (2012a). Bovine brucellosis (Chapter 2.4.3). In: OIE Manual of diagnostic tests and vaccines for terrestrial animals. Paris: Off. Int. Epizoot. 616-650.
  46. OIE (2009a). World Animal Health Information Database - Version: 1.4. World Animal Health Information Database. Paris, France: World Organization for Animal Health. <http://www.oie.int>.
  47. OIE (2012b). World Animal Health Information Database. Version 2. World Animal Health Information Database. Paris, France: World Organisation for Animal Health. [http://www.oie.int/wahis\\_2/public/wahid.php/Wahidhome/Home](http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home).
  48. OIE Handistatus, (2005). World Animal Health Publication and Handistatus II (data set for 2004). Paris, France: Office International des Epizooties.
  49. Padilla Poiester F, Nielsen K, Ernesto Samartino L, Ling Yu W (2010). Diagnosis of Brucellosis. *Open Vet. Sci. J.* 4: 46.
  50. Radostits O. M, Gay C, Blood D. C, Hinchcliff K. W (2007). *Veterinary Medicine: a text book of the Disease of cattle, sheep, pigs, goats and horse.* Harcourt publishers limited, London. PP. 882-885.
  51. Rossetti, C. A., Arenas-Gamboa, A. M., Maurizio, E. Caprine brucellosis: A historically neglected disease with significant impact on public health. *PLoS Negl. Trop. Dis.* 2017, 11, e0005692. [Cross Ref] [Pub Med].
  52. Saltoglu, N., Tasova, Y., Inal, S. A., Seki, T., Aksu, S. H. Efficacy of rifampicin plus doxycycline versus rifampicin plus quinolone in the treatment of brucellosis. *Saudi Med. J.* 2002, 23, 921-924. [Pub Med].
  53. Saxena, H. M., Raj, S. A novel immunotherapy of Brucellosis in cows monitored non-invasively through a specific biomarker. *PLoS Negl. Trop. Dis.* 2018, 12, e0006393. [Cross Ref] [Pub Med].
  54. Seleem M. N, Boyle S. M, Sriranganathan N (2010). Brucellosis: A re-emerging zoonosis. *Vet. Microbiol.* 140: 392-398.
  55. Sharifi H, Mashayekhi K, Tavakoli MM (2015). Risk facts of small ruminant brucellosis: a cross-sectional study in Southeast Iran 2012. *Human & Veterinary Medicine. International Journal of the Bioflux Society* 7: 42-45.
  56. Sintayehu G, Melesse B, Abayneh A. D, Sintayehu A, Melaku S (2015). Epidemiological survey of brucellosis in sheep and goats in selected pastoral and agro-pastoral lowlands of Ethiopia. *Rev. Sci. Tech. Off. Int. Epiz.*, 2015, 34 (3).
  57. Sohn, A. H., Probert, W. S., Glaser, C. A. (2003). Human neurobrucellosis with intra cerebral granuloma caused by a marine mammal *Brucella* spp. *Emerging Infectious Diseases Journal*, 9: 485-488.
  58. Solis Garcia delPozo, J., Solera, J. Systematic review and meta-analysis of randomized clinical trials in the treatment of human brucellosis. *PLoS ONE* 2012, 7, e32090. [Cross Ref] [Pub Med].
  59. Tadeg W. M, Gudeta F. R, Mekonen T. Y, Asfaw Y. T, Birru A. L, Reda A. A (2015). Seroprevalence of small ruminant brucellosis and its effect on reproduction at Tallalak district of Afar region, Ethiopia. *J. Vet. Med. Anim. Health* 7(4): 111-116.
  60. Tedeschi, L. O., Nicholson, C. F. and Rich E. 2011. Using system dynamics modelling approach to develop management tools for animal production with emphasis on small ruminants. *Small Ruminant Research* 98: 102-110.
  61. Tegegn A. H, Feleke A, Adugna W, Melaku S. K. (2016). Small Ruminant Brucellosis and Public Health Awareness in Two Districts of Afar Region, Ethiopia. *J. Vet. Sci. Technol.* 7 (335): 2.
  62. Teklue T, Tolosa T, Tuli G, Beyene B, Hailu B (2013). Sero-prevalence and risk factors study of brucellosis in small ruminants in Southern Zone of Tigray Region, Northern Ethiopia. *Trop. Anim. Health Prod.* 45: 1809-1815.
  63. Tewodros A. E, Dawit A. A (2015). Sero-Prevalence of Small Ruminant Brucellosis in and around Kombolcha, Amhara Regional State, North-Eastern Ethiopia. *J. Vet. Sci. Med. Diagn.* 4 (5).
  64. Tsevelmaa, N., Narangerel, B., Odgerel, O., Dariimaa, D., Batkhuu, J. Anti-*Brucella* activity of *Caryopterismongolica* Bunge root extract against *Brucella melitensis* infection in mice. *BMC Complement. Altern. Med.* 2018, 18, 144.
  65. Wikipedia. (2018, 05 24). *Brucella*. Retrieved 06 10, 2018, from <https://en.m.wikipedia.org/wiki/Brucella>.



66. World Health Organization (WHO) (2006). Brucellosis in humans and animals. WHO Library Cataloguing-in-Publication Data.
67. Wubishet Z, Golo D, Chala F, Shubisa A, Huqa L, Godana H, Ararsa D. Sero-Prevalence Of Brucellosis In Goats And Community Awareness In Liban District Of Guji Zone, Oromia Regional State, Southern Ethiopia. *JOJ Pub Health*. 2017, 2 (3): 555587. DOI: 10.19080/JOJPH.2017.02.555587.
68. Wubishet Z, Sadik K, Abdala B, Mokonin B, Getachew T, Getachew K. Small Ruminant Brucellosis and Awareness of Pastoralists Community about Zoonotic Importance of the Disease in Yabello districts of Borena Zone Oromia Regional State, Southern Ethiopia. *Curr Trends Biomedical Eng & Biosci*. 2018, 12 (1): 555827. DOI: 10.19080/CTBEB.2018.12.555827.
69. Yang, H. X., Feng, J. J., Zhang, X. Q., Hao, E. R., Yao, X. S., Zhao, R., Piao, R. D., Cui, Y. B., Jiang, H. A case report of spontaneous abortion caused by *Brucella melitensis* biovar 3. *Infect. Dis. Poverty* 2018, 7, 31. [Cross Ref] [Pub Med].
70. Yantzis D, 1985. *Brucella melitensis* in Greece: current situation report. *Brucella melitensis*. CEC seminar, Brussels, November 1984, 43-46, [Current Topics in Veterinary Medicine and Animal Science volume 32], 12 Ref.
71. Yesuf M, Alemu S, Temesgen W, Mazengiac H, Negussie H (2010). Seroprevalence of Ovine Brucellosis in South Wollo, North Eastern Ethiopia. *American-Eurasian J. Agric. & Environ. Sci.*, 9 (3): 288-291.
72. Yousefi-Nooraie, R., Mortaz-Hejri, S., Mehrani, M., Sadeghipour, P. Antibiotics for treating human brucellosis. *Cochrane Database Syst. Rev*. 2012, 10, Cd007179. [Cross Ref] [Pub Med].
73. Zahra, A., Mulema, A., Colverson, K., Odongo, D., and Rischkowsky, B. (2014). A review of Ethiopia small ruminant value chains from a gender perspective. Nairobi: ILRI and ICARDA. PP. 1-38.
74. Zhang, J., Chen, Z., Xie, L., Zhao, C., Zhao, H., Fu, C., Chen, G., Hao, Z., Wang, L., Li, W. Treatment of a subdural empyema complicated by intracerebral abscess due to *Brucella* infection. *Braz. J. Med. Biol. Res*. 2017, 50, e5712. [Cross Ref] [Pub Med].

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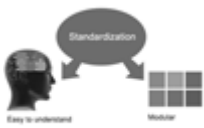
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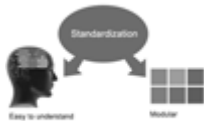
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Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct*, along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
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7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

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- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

### Acknowledgments

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## PREPARING YOUR MANUSCRIPT

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



### ***Manuscript Style Instruction (Optional)***

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

### ***Structure and Format of Manuscript***

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- 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.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



## FORMAT STRUCTURE

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

All manuscripts submitted to Global Journals should include:

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The title page must carry an informative 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) where the work was carried out.

### **Author details**

The full postal address of any related author(s) must be specified.

### **Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

### **Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning 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 a research paper?" Then consider synonyms for the important words.

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

### **Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

### **Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

### **Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

### **Tables, Figures, and Figure Legends**

Tables: Tables should be 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. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



## Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

### PREPARATION OF ELETRONIC FIGURES FOR PUBLICATION

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). 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.

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### TIPS FOR WRITING A GOOD QUALITY MEDICAL RESEARCH PAPER

**1. Choosing the topic:** In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

**2. Think like evaluators:** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. 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.

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

**4. Use of computer is recommended:** As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

**5. Use the internet for help:** An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.





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

**7. Revise what you wrote:** When you write anything, always read it, summarize it, and then finalize it.

**8. Make every effort:** Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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

**11. Pick a good study spot:** Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

**12. Know what you know:** Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

**13. Use good grammar:** Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

**14. 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 for your topic. You may also maintain your arguments with records.

**15. Never start at the last minute:** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

**17. Never copy others' work:** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

**18. Go to seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

**19. Refresh your mind after intervals:** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



**20. Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

**21. Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

**22. Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

**23. Upon 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 through which your research is going to be in print for 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 of 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 criteria peer reviewers will use for grading the final paper.

### **Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

*The introduction:* This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

### **The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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 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 avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

### **Title page:**

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

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite 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 approaches 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. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a 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 limit the initial two items to no more than one line each.

*Reason for writing the article—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 for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

### **Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity 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 of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets 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 for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

#### **Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show 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:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

#### **Methods:**

- Report the method and not the 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—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

#### **Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and 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 as 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. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

**Content:**

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

**What to stay away from:**

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

**Approach:**

As always, use past tense when you submit 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 section.

**Figures and tables:**

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully 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 the prospect, and let it drop at that. Make a decision as to whether each premise is supported or 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 an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of 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 other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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ISSN 9755896



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