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Navicular Bone of Horses

Controlling Gastro-Intestinal

Highlights

Major Transboundary Disease

Percoll Density Centrifugation

Discovering Thoughts, Inventing Future

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Relationship between the Distal Phalanx Angle and Radiographic Changes in the Navicular Bone of Horses: A Radiological Study

By Cristobal Dörner, Pablo Fueyo & Rodrigo Olave

Riding School of the Chilean Army

Abstract- The aim of this study was to determine the relationship between the distal phalanx angle and the radiological condition of the navicular bone and establish a database of reference values for hoof radiographic A retrospective study was measurements in Chilean horses. performed considering radiographic examinations on 146 feet from 92 horses. Linear and angle measurements of the hoof capsule and distal phalanx were obtained and compared statistically. Radiographic condition of the navicular bone was determined and statistically compared with the radiographic hoof values. Additionally, horses were categorized by breed to elucidate differences between breeds.There was a significant negative correlation between the palmar angle and the navicular score. Also, there was a significant negative correlation between the hoof angle and the navicular score. There were significant statistical differences between the distal phalanx angle, weight-bearing surface of the toe and second phalanx length when compared by breed.The information gathered in this study can help to prevent the presentation or the advance of the radiological changes in the navicular bone. A radiographic-guided shoeing should always be considered. Additionally, the present study provides a database of normal values of the hoof capsule in Chilean horses that can be used by veterinarians and farriers as a guideline for routine and orthopedic shoeing.

Keywords: *horse, foot, palmar angle, navicular disease, radiographs.*

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Relationship between the Distal Phalanx Angle and Radiographic Changes in the Navicular Bone of Horses: A Radiological Study

Cristobal Dörner [α], Pablo Fueyo [σ] & Rodrigo Olave [ρ]

Abstract- The aim of this study was to determine the relationship between the distal phalanx angle and the radiological condition of the navicular bone and establish a database of reference values for hoof radiographic measurements in Chilean horses. A retrospective study was performed considering radiographic examinations on 146 feet from 92 horses. Linear and angle measurements of the hoof capsule and distal phalanx were obtained and compared statistically. Radiographic condition of the navicular bone was determined and statistically compared with the radiographic hoof values. Additionally, horses were categorized by breed to elucidate differences between breeds.There was a significant negative correlation between the palmar angle and the navicular score. Also, there was a significant negative correlation between the hoof angle and the navicular score. There were significant statistical differences between the distal phalanx angle, weight-bearing surface of the toe and second phalanx length when compared by breed.The information gathered in this study can help to prevent the presentation or the advance of the radiological changes in the navicular bone. A radiographic-guided shoeing should always be considered. Additionally, the present study provides a database of normal values of the hoof capsule in Chilean horses that can be used by veterinarians and farriers as a guideline for routine and orthopedic shoeing.

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I. Introduction

Foot pain is described as the most common cause of forelimb lameness in sport horses(Dyson2011[a]) and has been associated with poor hoof balance and conformation (Turner 1986). Some authors have been suggested that changes in foot conformation increase the load on the palmar aspect of the foot and so the navicular bone is overstressed predisposing to foot pain and lameness. Unfortunately, there is limited information about the relation between the distal phalanx (P3) orientation within the hoof and the radiological changes in the navicular bone.

A condition that causes foot pain is the one called "Negative palmar angle syndrome (NPAS)". This is a term in which the solar or palmar and/or plantar margin of P3 has a negative angle in relation to the ground surface, and sole depth under the dorsal-distal margin of P3 is greater than that under the palmar processes when viewed on a lateral radiographs (Floyd 2010). The term "long toe, low heel" has been used to describe this condition and has been accepted as being abnormal among veterinarians and farriers. Conversely, a recent study in horses with foot pain indicated the variations in shape of the distal phalanx were not accurately predicted by external characteristics of the hoof capsule (Dyson *et al* 2011). On the other hand, a marked correlation between hoof conformation and forces applied to the equine foot has been also described (Eliashar *et al* 2004). According to the aforementioned, in many cases it is very difficult to predict an abnormal condition in the structures inside the hooves when only the external hoof capsule is seen.

In normal conditions, the distal phalanx solar angle or palmar angle with the surface range between 2^{o} – 10^{o} (Parks 2003). As the border of the distal phalanx is the insertion point of the deep digital flexor tendon (DDFT), a change in its orientation, increases the DDFT tensile force and subsequently the force it exerts on the navicular bone during the different phases of a stride (Wilson *et al* 2001; Weaver *et al* 2009; de Zani *et al* 2016). Furthermore, biomechanical overloadexerted over the navicular bone has shown to be harmful and may result from aged related accumulation of workload (Dik *et al* 2001). Accordingly, in theory the more tensile forces exerted to the navicular bone (biomechanical overload) the moreradiographic changes should be found (Dik *et al* 2001; Wilson *et al* 2001; Weaver *et al* 2009). The changes seen in the navicular bone in horses presenting navicular disease are well documented (Verschooten *et al* 1989; Wright 1993; Buttler *et al* 2000; Dyson 2011[b]; Komosa *et al* 2013).

In this study, we hypothesized that the compressive force exerted by the DDFT over the navicular bone due to an abnormal distal phalanx angle,as described by different authors (Wilson *et al* 2001, Eliashar *et al* 2004; Weaver *et al* 2009; Holroyd *et al* 2013), has a correlation with the radiographic changes observed in the navicular bone. Additionally, we provided a database of normal values of the hoof capsule in Chilean horses that can be used by veterinarians and farriers as a guideline for routine and orthopedic shoeing.

Author α: Equine Hospital, Riding School of the Chilean Army, Quillota, Chile. e-mail: cdorner@gmail.com

II. Materials and Methods

a) Horse Selection

One hundred and forty-six feet (146) from horses were used for the study (54.79% from Chilean Criollo horses and 45.21% from Warmbloods horses) (385 – 590 kg bwt) in routine work and shoeing status examined consecutively between July 2015 and December 2016. All horses were assessed for lameness. Sound horses were immediately enrolled for the study. Lame horses selected for this study had lameness abolished after a palmar digital nerve block was performed, using 1.5 mL mepivacaine 2% (Vetacaine™)[a]injected just proximal to the lateral cartilages of the distal phalanx. Horses presenting with laminitis or lameness located anywhere else were excluded from the study. Horses selected should have been trimmed within 5 weeks. Additionally, age, gender and breed were recorded.

To establish the normal reference values for the hoof radiographic measurements of the Chilean Criollo horses and to investigate the differences of hoof radiographic measurements between Chilean Horses and Warmblood horses, horses were categorized by breed.

b) Radiographic Image Adcquisition

Eighty forelimbs from Chilean horses (left front n=40; right front n=40) and sixty-six forelimbs from Warmblood horses (left front n=33; right front n=33) were radiographed obtaining a total sample of one hundred and forty-six feet (n=146). All radiographic examinations were performed after standard foot preparation as describe by Buttler *et al* (2000). Radiographic views selected to evaluate horses` feet were based on previous studies performed in different breeds (Dyson *et al* 2011; Dyson 2011[b]; Thieme *et al* 2015; Wright 1993). Lateromedial, 60° dorso proximal oblique navicular (upright pedal) and palmaro proximal – palmaro distal (Navicular Skyline) radiographic views were obtained. For lateromedial view, the foot to be examined was placed on a block 6 cm high and the x-ray beam was centered approximately 1 cm distal to the coronary band, midway between the dorsal and palmar aspects of the hoof. The x-ray generator was set at 76 Kvp and 1.2 mAs. For the 60° dorsoproximal navicular view, the hoof was placed over the x-ray tunnel in a square stance and the x-ray beam was centered in the coronary band and the x-ray generator was set at 78 Kvp and 1.6 mAs. The last radiographic view was obtained with the limb over the tunnel and placed backwards and the x-ray beam was centered between the heel bulbs following the pastern angle and the x-ray generator was set at 80 Kvp and 2.0 mAs.Radiographs were obtained using a digital x-ray machine (Envision G2 DR panel)[b] and a Poskom PXP-20HF x-ray generator.

c) Image Analysis

Radiographs were analyzed using an image analysis software (Metron-DVM 7.05 for windows)[c]. Following the instructions of the program, 10 parameters on the LM view were measured. The following measurements were obtained: Palmar angle, descent of the distal phalanx, distance of the distal phalanx to ground, hoof angle, proximal HL zone, distal HL zone, percentage of the weight-bearing surface of the toe, coffin joint angle, pastern joint angle, length of the middle phalanx (Figure 1 and Figure 2).To determine the radiographic condition of the navicular bone ("navicular score") a standardized classification was used as described by Dyson (2011[b]) (Table 1).

d) Data Analysis

Statistical analyses were run on a specialized statistical software (SPSS Inc, version 19 for windows)[d]. A Kolmogorov-smirnov test was performed to assess whether the data were normally distributed. A t-student test for independent variables was used to compare the data between breeds. All measurements were compared to determine whether they were significantly different between groups. A Spearman correlation test was run to determine the association between the radiographic hoof values and the radiographic score of the navicular bone. The significance level was set at $p<0.05$.

III. Results

One hundred and forty-six feet (146) from horses were used for the study (47.83% geldings; 42.39% mares; 9.78% stallions). Eighty feet were from Chilean Criollo horses and sixty-six feet were from Warmblood horses (29.53% Holsteiner; 10.16% Selle Frances; 5.52% Warmblood cross). The mean ±standard deviations (s.d.)and t-student test of the data obtained for radiological hoof values for Chilean and Warmblood horses are summarized in table 2.

There was significant difference between groups for palmar angle, toe/support %, third phalanx distance to the ground, and length of the middle phalanx determined radiologically (table 2). Warmblood horses have a smaller palmar angle (3.39± 3.37) than Chilean Criollo horses (6.46± 3.88)(p= 0.000) as well as the toe/support % (65.12± 5.48 and 67.35± 5.78 respectively, p= 0.033). Additionally, there was a significant difference in the length of the middle phalanx in which the Chilean Criollo horses have a shorter middle phalanx bone (3.99± 0.53, p value 0.000). The other measurements determined radiologically showed no difference between breeds (table 2).

Table 3 summarizes means ±s.d. and t-student test results when horses were assessed by limb, showing no statistical differences when right and left legs were compared between each other. This situation was seen in both breeds.

Additionally, the mean ±s.d. for the navicular score for each breed was analyzed. Chilean Criollo horses (0.95 ± 0.80) showed a lesser value when compared with Warmblood horses (1.23 ± 0.83). These results were statistically different ($p = 0.038$).

Each measurement determined radiologically was correlated with its respective navicular score. The palmar angle and hoof angle (rho= -0.190, $p = 0.024$) showed a weak negative correlation with the navicular score (rho= -0.173, $p = 0.041$) (table 4). The other parameters measured did not show significant association with the navicular score.

IV. Discussion

This study was performed in order to establish the relationship between the distal phalanx angle within the hoof capsule and the radiological condition score of the navicular bone.Additionally,a database of reference values of the radiographic hoof values from the Chilean Criollo horses were obtained and compared with the values obtained from Warmblood horses. Hoof trimming has shown a remarkable influence on hoof conformation and in some measurements that describe the position of the third phalanx within the hoof capsule (Kummer*et al*2006) so in our study,horses were excluded when the feet had not been trimmed within 5 weeks.

The selection and use of Metron software for this study was based on the previous results obtained where it was determined that Metron software can be used to objectively measure most of the parameters predefined by the software (Vargas Rocha*et al* 2004).

Chilean Criollo horses showed a larger palmar angle when compared with Warmblood horses, finding somehow expecteddue to the described lower palmar angle of Warmbloods compared to other breeds (Kummer*et al* 2006). Toe/support % was larger in Chilean horses and thus they should have a better capacity to dissipate the ground reaction forces within the hoof capsule compared with Warmbloods. Nonetheless, one study showed no differences when the presentation of catastrophic pathologies and toe/support % were compared (Kane *et al* 1998). According to the *FédérationEquestreInernationale* (2017), a Pony is a small horsewhose height at the withersdoes not exceed 148 cms. Chilean horsesare considered as Ponies due to their height (<145 cms),so a shorter middle phalanx compared to Warmblood horses was expected. Chilean horses tend to have narrow, upright, and small feet relative to their body size (Reckmann 1999; Vergara 2012), hence the larger palmar angle in Chilean horses is mostly due to the hoof conformation and its relation with the inner structures of the hoof capsule (Dyson *et al* 2011). When the radiographic navicular score was obtained, Chilean horses showed a lower mean score than Warmblood horses used in this study. The above situation was suspected according to the hoof and palmar angle obtained, where larger palmar angle has been associated with a smaller probability to present navicular bone or DDFT lesions (Holroyd *et al*2013). This situation is most likely related to the forces exerted by the DDFT to the navicular bone (Wilson *et al* 2001, Eliashar*et al* 2004, Weaver *et al*2009).

According to the results of our study, there was a significant negative correlation between the navicular score and the palmar angle. There was also a significant negative correlation between the navicular score and the hoof angle. The aforementioned results, were in accordance to our expectations and these may be the reflection of the increased force exerted by the DDFT due to a highest moment arm force (Wilson*et al*2001) to the navicular bone when the hoof presents a low palmar angle (neutral to negative) (Floyd 2010).This situation has also being documented by Weaver *et al* (2009)were they topographically map pressure distribution across the palmar surface of the navicular bone in response to forces applied by the deep digital flexor tendon (DDFT). This study showed and evaluated the effect of raising the heels in vitro showing the relationship between the DDFT and navicular disease.Moreover, Eliashar*et al* (2004) concluded that an increase in the palmar angle by 1° would decrease the force of the DDFT on the navicular bone by 4%, supporting the biomechanical overload suffered by the navicular bone when an abnormally low palmar angle is present. Additionally, no significant correlation has been found between heel collapse and the palmar angle (Floyd 2010) thus the radiographic evaluation to determine the hoof inner structures measurements is mandatory.Considering biomechanical and risk factors for development of navicular disease, the palmar angle of the distal phalanx should play an important role in the presentation of the disease. According to Dik and van den Broek (1995) and Dik*et al* (2001), horses presenting with different palmar angles should present different shapes of the navicular bone based on a shape-dependent distribution of the forces exerted on the navicular bone. For example, navicular bone shape 1 and 2 are associated with overloading of the distal interphalangeal joint, and navicular bone shape 3 is related with strain of the collateral ligament of the navicular bone (Dik and van den Broek1995).

As this study did not evaluated the correlation between the palmar anglewith the presentation of clinical navicular disease,further investigation is required in this matter. Nonetheless, recent studies has shown very interesting data regarding correlations between radiographic measurements of the foot and abnormalities of specific structures found with magnetic resonance imaging (MRI) (de Zani*et al*2016). Moreover, it has been documented that the larger the palmar angle, the smaller the likelihood of a DDFT or navicular bone lesion (Holroyd *et al*2013).

In conclusion, this study contributes to the informationalready available in the literature helping to have a better understanding of changes suffered by inner structures of the hoof capsule. We have documented the reference hoof values from the Chilean Criollo horses and at the same time we have shown a few difference between this breed and Warmblood horses. Additionally, we have demonstrated that there is a significant statistical correlation between the radiographic navicular score and the palmar angle. Given these results, a radiological evaluation of horse's feet before and after shoeing is always recommended. To fully understand the implication of the changes suffered on the palmar angle in horses presenting navicular disease, further investigation is needed.

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References

1. Buttler J, Colles C, Dyson S, Kold S, Poulos P. 2000.Foot, Pastern andFetlock.In:Buttler J, Colles C, Dyson S, Kold S, Poulos P (eds). *Clinical Radiology of the Horse*, 2nd ed, Wiley-Blackwell, Oxford. Pp 53-187.
2. De Zani D, Polidori C, Di Giancamillo M, Zani D. 2016. Correlation of radiographic measurements of structures of the equine foot with lesions detected on magnetic resonance imaging.*Equine Vet. J.* 48, 165-171.
3. Dik K and van den Broek J. 1995. Role of navicular bone shape in the pathogenesis of navicular disease: a radiological study. *Equine Vet. J.*27, 390-393.
4. Dik K, van den Belt A, van den Broek J. 2001. Relationships of age and shape of the navicular bone to the development of navicular disease: a radiological study. *Equine Vet. J.*33, 172-175.
5. Dyson S. 2011[a].Navicular disease. In: M. Ross and S. Dyson (eds). *Diagnosis and Management of Lameness in the Horse*, 2nd ed, W.B. Saunders, St Louis. pp 324-342.
6. Dyson S. 2011[b].Radiological interpretation of the navicular bone.*Equine Vet. Educ.* 23, 73-87.
7. Dyson S, Tranquille C, Collins S, Parkin D, Murray R. 2011.An investigation of the relationships between angle and shapes of the hoof capsule and the distal phalanx.*Equine Vet. J.* 43, 295-301.
8. Eliashar E, McGuigan M, Wilson A. 2004. Relationship of foot conformation and force applied to the navicular bone of sound horses at the trot. *Equine Vet. J.* 36, 431-435.
9. FEI, FédérationEquestreInternationale, Switzerland. 2017. Veterinary Regulations, 13th Edition, Chapter IV, Article 1042.
10. Floyd A. 2010. Use of a grading system to facilitate treatment and prognosis in horses with negative palmar angle (heel collapse): 107 cases. *J. Equine Vet. Sci.* 30, 666-675.
11. Holroyd K, Dixon J, Mair T, Bolas N, Bolt D, David F, Weller R. 2013.Variation in footconformation in lame horseswithdifferentfootlesions. *Vet. J.* 195, 361-365.
12. Kane A, Strover S, Gardner I, Bock K, Case J,*et al.* 1998. Hoof size, shape, and balance as possible risk factors for catastrophic musculoesqueletal injury in Thoroughbred racehorses. *Am. J. Vet. Res.*59,1545-1552.
13. Komosa M, Purzyc H, Frąckowiak H. 2013. Changes in Navicular Bone (ossesamoideumdistale) Shape in Horses as a Result of Pathological Alterations. *Folia Biologica* 61, 1-10.
14. Kummer M, Geyer H, Imboden I, Aüer J, Lischer C. 2006. The effect of hoof trimming on radiographic measurements of the front feet of normal Warmblood horses. *The Vet. J.* 172, 58-66.
15. Parks A. 2003.The foot and shoeing. In: *Diagnosis and Management of Lameness in the Horse*, 2nd edn.,Eds: M. Ross and S. Dyson, W.B. Saunders, St Louis. pp 250-271.
16. Reckmann O. 1999. Evaluación de Aplomos, Cascos y Herraje en caballosfina sangre criollo Chileno. Memoria deTítulo, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile.
17. Thieme K, Ehrle A, Lischer C. 2015.Radiographic measurements of the hooves of normal ponies.*The Vet. J.* 206, 332-337.
18. Turner TA. 1986. Navicular disease management: Shoeing principles, *Proceedings of the 32nd Annual Convention of the American Association of Equine Practitioners,* Nashville, Tennessee, USA, pp. 625–633.
19. Vargas Rocha J, Lischer C, Kummer M, Hässig M, Aüer J. 2004. Evaluating the Measuring software package Metron-PX for morphometric description of equine hoof radiographs. *J. Equine Vet. Sci.* 24, 347-354.
20. Vergara I. 2012. Descripción de cascos, herrajes y aplomos en caballoschilenos en la Región de La Araucanía, Temuco-Chile. Memoria deTítulo, Facultad Silvoagropecuaria, Universidad Mayor, Temuco, Chile.
21. Verschooten F, Roels J, Lampo P, Desmet P, De Moor A, Picavet T. 1989. Radiographic measurements from the lateromedial projection of the equine foot with navicular disease.*Res. Vet. Sci.* 46, 15-21.
22. Weaver M, Shaw D, Munaiwa G, Fitzpatrick D, Bellenger C. 2009.Pressure distribution between the deep digital flexor tendon and the navicular bone, and the effect of raising the heels in vitro.*Vet. Comp. Orthop .Traumatol.* 22, 278-282.

23. Wilson A, McGuigan M, Fouracre L, MacMahon L. 2001. The force and contact stress on the navicular bone during trot locomotion in sound horses and horses with navicular disease. *Equine Vet. J.* 33, 159-165.

24. Wright I. 1993. A study of 118 cases of navicular disease: radiological features. *Equine Vet. J.* 25, 493-500.

Table 1: Radiographic findings and classification of the navicular bone (Dyson 2011)

Grade	Condition	Radiographic Findings
0	Excellent	Good corticomedullary demarcation; fine trabecular pattern. Flexor cortex of uniform thickness and opacity. No lucent zones along the distal border of the bone, or <6 narrow conical lucent zones along the horizontal distal border.
1	Good	As above, but lucent zones on the distal border of the navicular bone more variable in shape.
2	Fair	Slightly poor definition between the palmar cortex and the medulla due to subcortical increased opacity. Several (<8) lucent zones of variable shape along the distal horizontal border. Mild entheseophyte formation on the proximal border of the navicular bone. Proximal or distal extension of the flexor border of the navicular bone.
3	Poor	Poor corticomedullary definition due to increased opacity of the medulla. Thickening of the dorsal and flexor cortices. Poorly defined lucent areas in the flexor cortex of the bone. Many (>7) radiolucent zones along the distal horizontal or sloping border. Lucent zones along the proximal border of the bone. Large entheseophyte formation on the proximal border of the bone. Radiopaque fragment on the distal border of the navicular bone.
4	Bad	Large cyst-like lesion within the medulla. Lucent region in the flexor cortex. New bone on the flexor cortex of the navicular bone.

Table 2: Radiological values of the hoof of both groups expressed as the mean ± s.d. and the results of t-Student test

	Chilean Horses	Warmblood Horses	
Parameters	Mean ± s.d.	Mean ± s.d.	*P* value
Palmar angle	6.46± 3.88 [a]	3.39± 3.37 [a]	0.000
P3 descent	0.38 ± 0.49	0.53 ± 0.60	0.096
P3 dist. to ground	1.86 ± 0.54 [a]	2.02 ± 0.56 [a]	0.048
Hoof angle	51.38 ± 5.96	51.21 ± 4.34	0.885
Prox. HL zone	1.51 ± 0.33	1.58 ± 0.26	0.204
Dist. HL zone	1.47 ± 0.42	1.43 ± 0.24	0.405
Toe/Support %	67.35± 5.78 [a]	65.12± 5.48 [a]	0.033
Coffin joint angle	9.89 ± 6.71	9.24 ± 7.13	0.791
Pastern joint angle	2.47 ± 4.66	3.36 ± 5.21	0.354
Length of P2	3.99± 0.53 [a]	4.66 ± 0.33 [a]	0.000

P3, distal phalanx; HL, hoof-lamella; P2, middle phalanx.
[a]Significant difference $P < 0.05$

Table 3: The mean ± s.d. values for radiological hoof parameters for Chilean and Warmblood horses when assessed by limb and results of t-Student test

	Chilean Horses			Warmblood Horses		
	LF	RF		LF	RF	
Parameters	Mean ± s.d.	Mean ± s.d.	*P* value	Mean ± s.d.	Mean ± s.d.	*P* value
Palmar angle	6.69 ± 4.02	6.19 ± 3.94	0.474	3.59 ± 3.28	3.05 ± 3.48	0.768
P3 descent	0.45 ± 0.48	0.32 ± 0.51	0.321	0.53 ± 0.65	0.56 ± 0.56	0.588
P3 dist. to ground	1.83 ± 0.56	1.87 ± 0.52	0.754	2.08 ± 0.56	1.97 ± 0.58	0.500
Hoof angle	51.99 ± 2.37	50.64 ± 8.41	0.360	51.17 ± 4.08	51.24 ± 4.75	0.713
Prox. HL zone	1.52 ± 0.33	1.53 ± 0.34	0.991	1.57 ± 0.23	1.61 ± 0.27	0.823
Dist. HL zone	1.47 ± 0.32	1.50 ± 0.51	0.865	1.42 ± 0.25	1.45 ± 0.23	0.710
Toe/Support %	67.00 ± 6.34	67.49 ± 5.53	0.736	65.41 ± 5.27	64.84 ± 5.85	0.949
Coffin joint angle	9.20 ± 6.44	9.91 ± 6.92	0.846	8.98 ± 7.19	9.51 ± 7.28	0.751
Pastern joint angle	3.28 ± 4.46	1.96 ± 4.39	0.320	3.66 ± 4.81	1.98 ± 5.41	0.147
Length of P2	4.00 ± 0.53	3.98 ± 0.55	0.988	4.63 ± 0.33	4.71 ± 0.33	0.892

P3, distal phalanx; HL, hoof-lamella; P2, middle phalanx; LF, left front limb; RF, right front limb.

Table 4: Results from the Spearman correlation test assessing correlations between the hoof values obtained from all horses and the radiological score of the navicular bone

Parameters	Rho Spearman	*P* value
Palmar angle & Navicular score	-0.173	0.041 [a]
P3 descent & Navicular score	-0.136	0.108
P3 dist. to ground & Navicular score	0.096	0.259
Hoof angle & Navicular score	-0.190	0.024 [a]
Prox. HL zone & Navicular score	-0.045	0.595
Dist. HL zone & Navicular score	-0.058	0.498
Toe/Support % & Navicular score	0.118	0.165
Coffin joint angle & Navicular score	0.074	0.386
Pastern joint angle & Navicular score	0.021	0.801
Length of P2 & Navicular score	0.113	0.182

P3, distal phalanx; HL, hoof-lamella; P2, middle phalanx.
[a] Significant difference $P < 0.05$

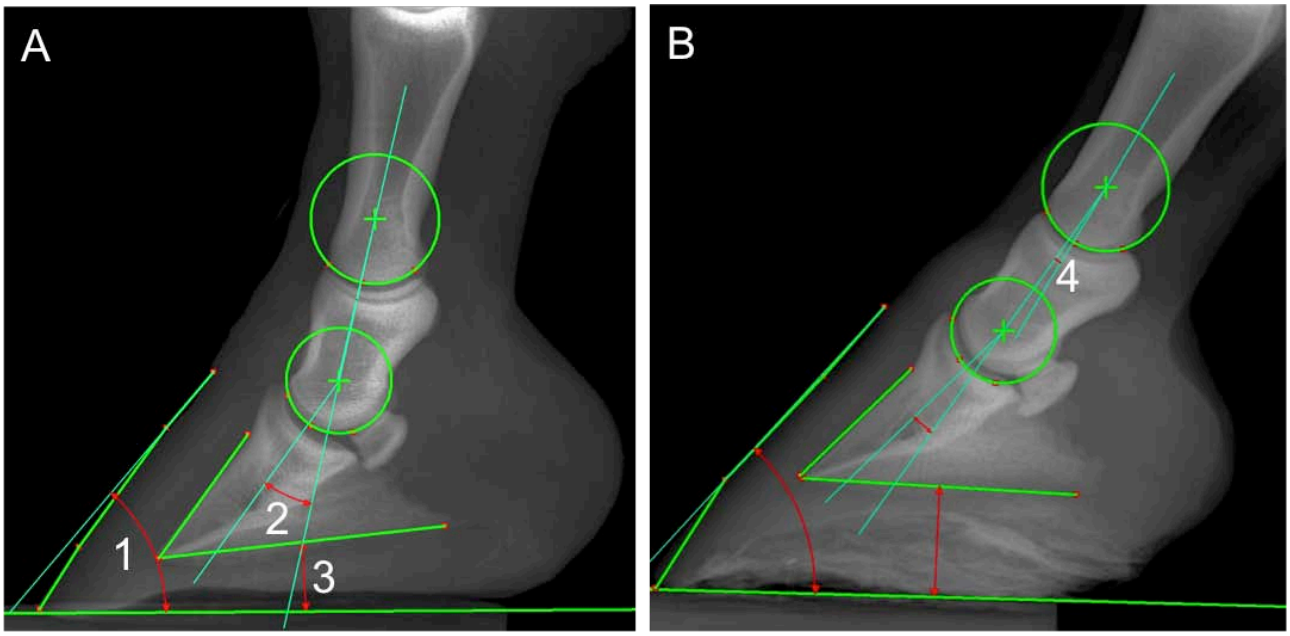

Figure 1: Angle measurements using Metron software. A. Normal palmar angle. Podophalangeal axis is broken backwards. B. Notice the negative palmar angle. There is an overgrown hoof capsule, classical upright conformation seen in Chilean Criollo horses. 1, hoof angle.2, coffin joint angle.3, palmar angle. 4, pastern joint angle.

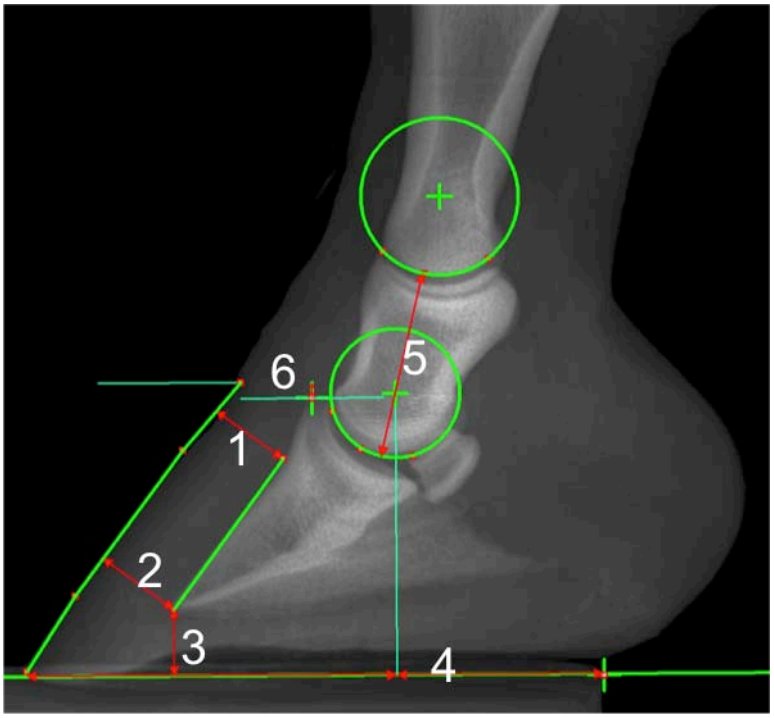

Figure 2: Linear measurements using Metron software.1, proximal HL zone.2, distal HL zone.3, distance of the distal phalanx to ground.4, percentage of the weight-bearing surface of the toe.5, length of the middle phalanx. 6. descent of the distal phalanx.

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Combined Use of Herb Extract as Anthelmintic for Controlling Gastro-Intestinal Parasites and Hemoto-Biochemical Effect on Sheep

By Shankar Biswas, Jotan Kar, Md Bayzid & Sabuj Kanti Nath

Chittagong Veterinary and Animal Sciences University

Abstract- This study was conducted on sheep for the evaluation of anthelmintic efficacy of some selected indigenous medicinal plants comparison with synthetic anthelmintic of ivermectin (0.1%). Three (3) medicinal plants Neem (Azadirachta indica), Bitter gourd (Momordica charantia) and Clove (Eugeniu caryophyllus) were selected. Crude aqueous extracts (CAE) and Crude methanol extract (CME) were prepared separately for in vivo screening against gastro instestinal parasites in sheep during July to December, 2015. Sixteen (16) naturally gastrointestinal parasites infested sheep (>840 EPG) were selected age between 6 to 24 months. The sheep population was divided into four (4) groups (A, B, C and D) which consisted of four (4) sheep in each group. Group A was control and group B, C and D were treated groups. Stock solution was prepared by following the herbs extraction standard procedure. Combined herbal anthelmintic dose was prepared at the ratio of 1: 2 : 2 (neem: bitter gourd: clove) to get better result instead of single use. In vivo screening, the extracts efficacy was observed @ 1ml/kg body weight at concentration (100 mg/ml) on day 0 and 7. The egg per gram (EPG) load was counted by using the McMaster egg counting technique on day 0, 7, 14, 21 and 28. Continuous reduction of EPG load observed at post-treatment period of group B, C and D (89%, 86 % and 90.7 %), respectively on day 28, in compared to day 0. Significant differences ($p \leq 0.05$) were observed among the treated groups. Conversely, in control group A, the EPG load sharply increased, ranging from 947.5 at day 0 to 1572.5 at day 28 but the differences were not significantly differed ($p \geq 0.05$). The Hb (%), PCV, TLC and TEC increased and ESR (mm/1st hr) decreased that was significant ($p \leq 0.05$) among the treated groups. In leukocytes count, the eosinophils (6.2%) and basophils (0.2%) decreased at the 28th day.

Keywords: gastrointestinal parasites, medicinal plants, pharmacokinetics, extracts, ivermectin, GIT, EPG.

GJMR-G Classification: NLMC Code: QW 70

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Combined Use of Herb Extract as Anthelmintic for Controlling Gastro-Intestinal Parasites and Hemoto-Biochemical Effect on Sheep

Shankar Biswas [α], Jotan Kar [σ], Md Bayzid [ρ] & Sabuj Kanti Nath [ω]

Abstract- This study was conducted on sheep for the evaluation of anthelmintic efficacy of some selected indigenous medicinal plants comparison with synthetic anthelmintic of ivermectin (0.1%). Three (3) medicinal plants Neem (*Azadirachta indica*), Bitter gourd (*Momordica charantia*) and Clove (*Eugeniu caryophyllus)* were selected. Crude aqueous extracts (CAE) and Crude methanol extract (CME) were prepared separately for *in vivo* screening against gastro instestinal parasites in sheep during July to December, 2015. Sixteen (16) naturally gastrointestinal parasites infested sheep (>840 EPG) were selected age between 6 to 24 months. The sheep population was divided into four (4) groups (A, B, C and D) which consisted of four (4) sheep in each group. Group A was control and group B, C and D were treated groups. Stock solution was prepared by following the herbs extraction standard procedure. Combined herbal anthelmintic dose was prepared at the ratio of 1: 2 : 2 (neem: bitter gourd: clove) to get better result instead of single use. In vivo screening, the extracts efficacy was observed @ 1ml/kg body weight at concentration (100 mg/ml) on day 0 and 7. The egg per gram (EPG) load was counted by using the McMaster egg counting technique on day 0, 7, 14, 21 and 28. Continuous reduction of EPG load observed at post-treatment period of group B, C and D (89%, 86 % and 90.7 %), respectively on day 28, in compared to day 0. Significant differences ($p \leq 0.05$) were observed among the treated groups. Conversely, in control group A, the EPG load sharply increased, ranging from 947.5 at day 0 to 1572.5 at day 28 but the differences were not significantly differed ($p \geq 0.05$). The Hb (%), PCV, TLC and TEC increased and ESR (mm/1st hr) decreased that was significant ($p \leq 0.05$) among the treated groups. In leukocytes count, the eosinophils (6.2%) and basophils (0.2%) decreased at the 28th day. The levels of AST, ALT and creatinine also varied significantly ($p \leq 0.05$) among the treated groups on days 7, 14, 21 and 28 and no toxicogenic effects was found. These findings revealed that adult gastrointestinal parasites are more vulnerable to selected indigenous herbs, no harmful effects on animal body and further may use as herbal anthelmintic at 1ml/kg (100 mg/ml).

Keywords: gastrointestinal parasites, medicinal plants, pharmacokinetics, extracts, ivermectin, GIT, EPG.

Author α: Department of Physiology, Biochemistry & Pharmacology. e-mail: sabujkantinath@gmail.com
Author σ: Department of Microbiology and Veterinary Public Health.
Author ρ: Faculty of Veterinary Medicine.
Author ω: Department of Animal Science and Animal Nutrition, Chittagong Veterinary and Animal Sciences University, Chittagong, Khulshi-4225, Bangladesh.

I. Introduction

Helminthosis is a parasitic disease of animal that are major problems of livestock production throughout the world, particularly in tropical and subtropical areas (Hussain *et al.*, 2010). Bangladesh is an agro-based developing country of South Asia which has huge livestock population. Livestock is an important sector which plays important contribution to solve unemployment, poverty alleviation, promote human health by supplying animal protein sources with high calorie value in the forms of meat and milk and help to achieve the sustainable development goals (SDGs). But parasites hinder the growth of livestock production and it has been identified as one of the important limiting factors in small ruminant specially in sheep farming (Hussain *et al.*, 2010). It is estimated over 90% of the endoparasitism cases in small ruminants are due to such as *Haemonchus contortus and Trichostrongylus axei* whose are found in the abomasums of small ruminants (Sani *et al.*, 1990). Other most common gastrointestinal parasites are *Paramphistomus* spp, *Gastrothillus* spp, *Cooperia* spp in sheep (Eysker and Ploege, 2000). Clinically it is manifested by reduced weight, roughness hair, anaemic condition and lowered meat and milk production (Githigia *et al.*, 2005). For controlling of helminthes a lot of chemicals have been used in most of the part of the world. Frequently use to livestock development which grow resistance against chemical anthelmintics (Papadopoulos *et al.*, 2012). This view has renewed the interested to study of medicinal plants for the development of novel anthelmintics. Plants have been used for human benefit from time immemorial (Koehn and Carter, 2005). According to the World Health Organization (WHO, 2008), almost 80% of Asia's population has incorporated into their primary modality of health care by using traditional medicine, which has compounds derived from medicinal plants (Hossain *et al.*, 2003). The use of plants as medicine is slowly increasing day by day in the world because they have minor or no side effects (Jordan et al., 2010). Bangladesh is endowed with vast resources of medicinal plants. About 5000 plant species have been estimated to be present in this country and most of them are reported be used in

traditional medicines for the health care of the millions of people of this country (Rahman *et al*., 2010). Neem (*Azadirachta indica*) is a tropical evergreen tree native to Indian sub-continent (Girish and Bhat, 2008). The various parts of neem such as fruits, seeds, leaves, bark and roots are used as antiseptic, anthelmintic, antibacterial, antiviral, antiulcer and antifungal, insecticides, pesticides and agrochemicals (Brahmachari, 2004). It has been recommended for using against gastro-intestinal nematodes and related problems in many parts of the world (Biswas *et al*., 2002; Subapriya and Nagini, 2005). Bitter gourd *(Momordica charantia*) is a traditional medicine of India sub-continent are used to relieve diabetes, as a stomachic, laxative, emetic, anthelmintic agent, for the treatment of cough, respiratory diseases, hyperglycemia, increasing milk flow, intestinal parasites, jaundice, kidney stones (Sampath and Bhowmik, 2010). Clove (*Eugeniu caryophyllus*) used as carminative and to increase hydrochloric acid in the stomach that improve peristalsis (Chaieb *et al*., 2007). Clove has been used a natural anthelmintic digestive stimulant (Patil *et al*., 2014). A large number of chemical anthelmintics are now available but most of them are expensive, anthelmintic resistance, high price value and adverse effects (Hannan *et al*., 2003). The multiple drug resistance not only increases morbidity and mortality but also increase expenditure and prevention and control of parasitic diseases are becoming very difficult day by day. In Bangladesh very limited research works have been conducted on the use of medicinal plants as anthelmintic. This present study was considered with the following objectives i) To evaluate the *in vivo* anthelmintic efficacy from *Azadirachta indica*, *Momordica charantia* and *Eugeniu caryophyllus* against GIT parasites in sheep. ii) To find out the combine *in vivo* efficacy at different concentration from methanol and aqueous treated extract. iii) To evaluate the effects of herb extracts on animal body by analysis the heamatological (Hb, PVC, ESR, TEC TLC and DLC) and biochemical (AST, ALT and creatinine) parameters.

II. Materials and Methods

a) Study area, study period and study design

The study area was included the sheep farm, a small gabble type farm housing during July to December 2015. An intervention study was conducted on in-vivo screening of herbs extract by using the three indigenous medicinal plants (Neem, Bitter gourd and clove) against gastrointestinal parasites in sheep.

b) Collection and processing of plant materials

Fresh leaves of neem *(Azadirchta indica*) Bitter gourd (*Momordica charantia*) fruits and dry clove (*Eugeniu caryophyllus)* were collected from the local area. Neem and bitter gourd washed thoroughly into running tap water to ensure removing of extraneous dusts materials (Sujan *et al*., 2008). Then cut into small pieces and taken a plastic jar. Then perform air-dried and finally sun dried for 3 days on the roof by covering a piece of cloth as prevention oxidation such as antioxidants and others chemical components (Amin *et al*., 2009). Clove was cleaned and be prepared for use. Dust was prepared from the dried leaves by using blender, mortar and pestle. Dried bitter gourd and clove dust was prepared with the help of a blender (Sujan *et al*., 2008). A 25-mesh diameter seize was prepared to obtain fine dust and were preserved them into air-tight plastic container until being used (Amin *et al*., 2009).

c) Preparation of Crude methanol extract (CME)

Crude methanol extract (CME) was prepared from the selected three medicinal herbs according to the standard herb extraction methods (Gilani *et al*., 2004). Ten (10) gm of each category of dusts were taken into a 500ml beaker and separately mixed with 100ml 70% aqueous methanol. Then the mixtures were stirred for 30 minute by a magnetic stirrer (6000 rpm) and left as such for next 24 hrs (Amin *et al*., 2009). The extracts were filtered through a fine cloth and final filtration was done through filter paper (Whatman No. 1) (Hussain *et al*., 2010). Evaporation of water from filtrate by using a vacuum rotary evaporator at 50^0C till it reached the final volume of 10 ml (Amin *et al*., 2009). Stored in a refrigerator in air tightly corked-labeled bottle at 4^0C temperature until use (Hussain *et al*., 2010).

d) Preparation of Crude aqueous extract (CAE)

Crude aqueous extract (CAE) was prepared by using the selected herbs according to the standard herb extraction methods (Gilani *et al*., 2004). Half kilogram (kg) of each two category (neem and bitter gourd) plants parts and 250 gm of clove were taken separately and washed thoroughly in the running tape water. Each sample was dried in room temperature at 30 minutes and then bitter gourd was cut into small pieces. Then 50 gm of neem leaves was taken in blender's plastic pocket and mixed with 300 ml distilled water and prepared juice (Anonymous, 1996). Then the juice was filtered through a fine piece of porous cloth and final filtration was done by using the filter paper (Whatman No. 1) (Amin *et al*., 2009). The juice performed evaporation by using evaporator at 50^0C till it reached the final volume of 10 ml as condense form. Stored in air tightly corked-labeled bottle at 4^0C temperature in a refrigerator until use (Hussain *et al*., 2010).

e) Preparation of stock solution

Each category of condensed crude aqueous and crude methanol extracts was mixed separately at the ratio of 1: 2: 2 (neem: bitter gourd: clove) as formation of final stock solution. Then preserved in air tight corked-labeled bottle and stored at 4^0C temperature in a refrigerator (Hussain *et al*., 2010). Stock solution was used by diluting with required amount distilled water.

f) Herbal anthelmintic dose

Herbal anthelmintic dose was prepared for *in vivo* screening by adding required amount of distilled water after weighting stock solution (Amin *et al*., 2009). For in *vivo* screening combine herbal anthelmintic dose was given 1 ml/kg (100mg/ml) body weight for this study.

g) Sampling Strategy

A total number of 33 sheep of both sexes (male and female) and different age (6-24 month) were selected by taking interview with the help of prepared questionnaire. Highly infected (>840 EPG) sixteen (16) sheep were used for this present study. The sheep were divided into four (4) groups; each group was consisted of four (4) populations with the mean EPG are 947.5, 918.7, 923.7 and 911.5 for group A, B, C and D, respectively. Group A was represented as infected control group and B, C and D were treated groups.

h) Treatment intervention, Dose and Dosing

This study was investigated the herbs extracts dose was 1 ml/kg body weight at the concentration of 100 mg/ml (Amin *et al*., 2009). Ivermectin (1%) was used at 0.2 mg/kg body weight at sub cuteneous route in group B. 1 ml/ kg (100mg/ml) body weight was used as herbal anthelmintic doses in group C and D on day 0 and 7.

i) In vivo screening of plant extracts for anthelmintic efficacy

Oral administration of crude aqueous extract (CAE) and crude methanol extract (CME) at 1 mg/kg were performed and compared with ivermectin (Acimec®- ACI Pharmaceuticals Ltd.) on day 0, 7, 14, 21 and 28 by McMaster egg counting technique. The efficacy of different treatment was determined by faecal egg count reduction test. The effect of herbs extracts on animal body specially circulatory and visceral organs effects were determined by analysising the haemato-biochemical parameters.

j) Collection, preservation and transportation of samples

Faecal and blood samples were collected from each sheep at day 0, 7, 14, 21 and 28 of the pre and post treatment period. Fresh eight gm fecal samples were collected from rectum in the morning before they are fed and then put the samples immediately into a sterile container containing six ml formalin. Blood samples were collected from jugular vein of each sheep and four ml blood placed into vacutainer tube, containing ethylene diamine tetra-acetic acid (EDTA) and four (4) ml placed in another vacutainer tube without containing EDTA. Samples were then being immediately transferred by transport media to laboratory through ice eskie and stored temporarily in refrigerator before laboratory evaluation.

k) Examination of fecal samples for parasitic egg count

In each case, three gm of fresh faeces was accurately weighed and mixed in 42ml of saturated salt solution (Sodium chloride-400gm, water-10000ml; specific gravity-1.2) while the number of eggs per gram of faeces was obtained by multiplying the total number of eggs counted in the two squares of the counting chambers of the McMaster slide by the dilution factor of 50. Externeous particles were removed and residue was left pass through. Homogenus distribution was performed by well stirring. McMaster slide was filled by using a Pasteur pipette and remove the bubbles. Then second counting chamber was filled in the same way. Then egg floated up and sticks to the cover glass. Characteristics of eggs were identified using standard parasitological criteria described by Soulsby (1986). Then egg was counted by using microscope at low magnification.

$$\text{Number in one gram} = \frac{\text{Number in two chambers}}{0.3} \times \text{Dilution factor}$$

$$\text{*Dilution factor} = \frac{\text{Total volume of suspension in ml}}{\text{Total volume of faeces}}$$

l) Determination of the drug efficacy

During the pre and post-treatment period EPG and clinical performance were monitored. Faeces were examined on day 0, 7, 14, 21 and 28 of post-treatment period. Efficacy of the drug was calculated as per described formula by Moskey and Harwood (1941).

$$\text{Percent efficacy} = \frac{\text{EPG of faeces before treatment} - \text{EPG of faeces after treatment}}{\text{EPG before treatment}} \times 100$$

m) Evaluation of haematological parameters

EDTA containing blood samples were used to determine the haematological parameters such as Hb, TEC, TLC and DLC with the help of microscope at day 0, 7, 14 and 28 during the treatment period.

n) Evaluation of biochemical parameters

The activities of biochemical parameters like as AST, ALT and creatinine concentration were determined at day 0 and 7, 14 and 28 of post treatment. Serum was separated by centrifugation at 3000 rpm for 15 minurtes. The separated serum was used for the estimation of biochemical parameters. AST and ALT activity was determined according to the method described by Reitman and Frankel (1957). Creatinine was determined by the method described by Husdan and Rapoport (1968).

o) Statistical Analysis

The experimental Data were entered into a spread sheet of the MS Excel-2007 Program. Data were sorted and cleaned using the Excel program before exporting to STATA-11 (STATA Corp, USA) for analysis. Descriptive statistics were performed to express the each category as percentage, mean and standard error (SE). *p* values of≤0.01 and ≤0.05 were considered statistically significant.

III. Results

a) In-vivo screening of ivermectin, crude aqueous extracts (CAE) and crude methanol extract (CME) with their efficacy

The efficacy was observed and compared with the control group A (non-treated) and group B with C and D groups. The efficacy of group C and D was determined at the concentration of 100 mg/ml. Efficacy of ivermectin and herbs extract was considered based on declination of EPG count. The average EPG loads per gm faeces sample were 947.5, 918.7, 923.7 and 911.5 in the group A, B, C and D, respectively on day 0 of the pre-treatment. The EPG load were reduced in different post-treatment period and reached 109 (89 % reduction), 130.7 (86 % reduction) and 84 (90.7 % reduction) for group B, C and D, respectively on day 28, compared to the results obtained at day 0. Highly significant differences ($p \leq 0.01$) were observed among the treated groups. The highest reduction of EPG was observed on day 28 irrespective of treatment groups (Table-1). Conversely, in the control group, the EPG load sharply increased, ranging from 947.5 at day 0 to 1572.5 at day 28 but the differences were not significantly differed ($p \geq 0.05$).

Table 1: Efficacy of ivermectin, crude aqueous extracts (CAE) and crude methanol extract (CME) of neem, Bitter gourd and clove based on reduction of EPG

Group	Treatment	Pre-treatment	Post-treatment			
		Day 0 (Mean±SE)	Day 7 (Mean±SE)	Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day 28 (Mean±SE)
A	Control	947.5±26.2	990.5±19.8	1162.5±33	1442±26.8	1572.5±22.1
B	Ivermectin	918.7±27	586.5±20.5 (36 %)	294.2±26.1* (66.8 %)	157±15.7** (82.8 %)	109±11.6** (89 %)
C	Crude aqueous extracts (CAE)	923.7±18.8	505±26.4 (45.2 %)	306.5±17.2 (66.8 %)	236.7±34** (74.4 %)	130.7±17.1** (86 %)
D	Crude methanol extract (CME)	911.5±29.3	454.5±24.3 (50.1 %)	279.5±27.5** (69.3 %)	157±19.1** (82.7%)	84±6.9** (90.7 %)

Each group consists of four sheep.
SE= Standard error; * = significant differences ($p \leq 0.05$); **= highly significant differences ($p \leq 0.01$)

The maximum reduction rate was observed in Crude methanol extract (90.7 % reduction where the ivermectin treated group (89 % reduction) and crude aqueous extracts (86 % reduction).

b) Effects on haematological parameters

The Hb (gm/dl) in untreated control group it decreased from 8.4 at day 0 to 6.1 at day 28 post-treatment. The Hb contents were increased from 7.8 at day 0 to 8.6 at day 28, 7.9 at day 0 to 8.4 at day 28 and 8.2 at day 0 to 9.2 at day 28 in ivermectin, CAE and CME treated groups, respectively. The PCV contents were increased from 28.2 at day 0 to 34.2 at day 28, 29.2 at day 0 to 36.6 at day 28 and 28.6 at day 0 to 35.2 at day 28 in ivermectin, CAE and CME treated groups, respectively. The PCV of the untreated control group reduced significantly ($p \leq 0.01$) in different interval of the post-treatment, compared to 32.8 at the day 0, 23.2 at the day 28 (Table-3). The mean values of ESR (mm/1st hr) were 0.4, 0.7, 0.5 and 0.5 for group A, B, C and D,

respectively at day 0. TEC levels increased among the anthelmintic treated groups and reached from 6.8 at day 0 to 11.4 at day 28, 6.2 at day 0 to 9.2 at day 28 and 7.2 at day 0 to 10.8 at day 28, across the study period in ivermectin, CAE and CME treated groups (Table-2), correspondingly but the variation was not significant ($p \geq 0.05$). The mean value of TLC content decreased from 7.3 at day 0 to 5.4 at day 28. The TEC levels increased among the treated groups and reached from 6.2 at day 0 to 10.1 at day 28, 7.4 at day 0 to 9.6 at day 28 and 6.2 in day 0 to 8.5 at day 28 in ivermectin, CAE and CME treated groups, respectively.

Table 2: Effects of ivermectin, crude aqueous extracts (CAE) and crude methanol extract (CME) on Hb, PVC, ESR, TEC and TLC

Treatment	Parameters	Pre-treatment	Post - treatment			
		Day 0 (Mean±SE)	Day 7 (Mean±SE)	Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day 28 (Mean±SE)
Control	Hb	8.4±0.7	8.14±0.5**	8.06±0.4**	7.84±0.3*	6.11±0.2*
	PCV	32.4±01.2	29.8±0.67**	27.4±0.5 **	25.2±0.6**	23.2±0.7**
	ESR	0.7±0.2	0.1± 0.1*	0.1±0.2*	0.1±0.1*	0±0*
	TEC	7.90±0.3	7.45±0.3**	6.84±0.1**	6.67±0.3**	6.24±0.3**
	TLC	7.29±0.5	6.95±0.5**	6.44±0.5**	5.72±0.5**	5.34±0.5**
Ivermectin	Hb	7.8±0.7	7.78±0.6	7.8±0.5	8.2±0.3	8.6±0.3
	PCV	28.2±01.4	29±1.6	30.8±1.2	33.2±1.4	34.2±1.5
	ESR	0.7±0.2	0.1± 0.1*	0.1±0.1*	0.1±0.2*	0±0*
	TEC	6.82±0.6	7.87±0.7	8.97±0.7	9.84±0.6	11.41±0.7
	TLC	6.22±0.6	7.16±0.6	8.28±0.7	9.02±0.6	10.07±0.8
Crude aqueous extracts (CAE)	Hb	7.9±0.2	7.48±0.3	7.34±0.2	7.9±0.2	8.4±0.2
	PCV	29.2±01.2	29.8±1.1	31.2±1.2	33.4±0.9	36.6±0.7
	ESR	0.5±0.2	0.3±0.2	0.2±0.1*	0.1± 0.1*	0±0*
	TEC	6.17±0.3	6.75±0.3	7.57±0.4	8.31±0.5	9.20±0.5
	TLC	7.4±0.3	7.94±0.3	8.57±0.3	9.11±0.3	9.64±0.3
Crude methanol extract (CME)	Hb	8.4±0.7	8.5±0.7	8.6±0.6	8.9±0.4	9.2±0.4
	PCV	28.6±1.5	30.2±1.2	32.6±1.3	34.4±01.1	35.4±1.1
	ESR	0.5±0.2	0.2±0.1*	0.2±0.1*	0.1± 0.1*	0±0*
	TEC	7.16±0.5	7.88±0.4	8.92±0.4	9.97±0.2	10.84±0.1
	TLC	6.23±0.7	6.8±0.7	7.53±0.7	8.01±0.6	8.53±0.6

Each group consists of four sheep.
SE= Standard error; * = significant differences ($p \leq 0.05$); **= highly significant differences ($p \leq 0.01$)

c) Effects on differential lymphocyte count

The mean values of lymphocyte (%) were reduced in different post-treatment period and reached from 66.2, 65.2 and 63.2 at the day 0 to 51.7, 52.7 and 52.5 for group B, C and D, respectively on day 28 of post-treatment (Table 3). The average values of neutrophil (%) of sheep were 36.5, 36.7 and 36 at the day 0 and reached 26.75, 28.5 and 29.2 on day 28 of post-treatment of group B, C and D (Table 3). Highly significant differences ($p \leq 0.05$) were observed among treated groups. The average values of monocyte (%) of sheep were 1.5, 1.2 and 1.5 at the day 0 and reached 2.5, 2.2 and 2.5 on day 28 of post-treatment of group B, C and D (Table 3). Highly significant differences ($p \leq 0.05$) were observed among treated groups across the study period, compared to day 0. Conversely, in control group, the values of monocyte increased, ranging from 2.2 at day 0 to 0.2 at day 28. The eosinophil contents were decreased from 7 at day 0 to 5.7 at day 28, 6.7 at day 0 to 6 at day 28 and 7.2 at day 0 to 6.25 at day 28 in ivermectin, CAE and CME treated groups, respectively (Table 3). The eosinophil percentage of untreated control group increased significantly ($p \leq 0.05$) 8.2 at day 28, compared to 6.2day 0. The basophil contents were decreased from 0.5 at day 0 to 0.2 at day 28, 0.5 at day 0 to 0.2 at day 28 and 0.5 at day 0 to 0.2 at day 28 in the ivermectin, CAE and CME treated groups. The basophil of the untreated control group declined from 0.7 to 0 on day 28.

Table 3: Effects on differential lymphocytes count in sheep affected with gastro-intestinal parasitic infestation

Treatment	Parameters	Pre-treatment	Post -treatment			
		Day 0 (Mean±SE)	Day 7 (Mean±SE)	Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day 28 (Mean±SE)
Control	Lymphocyte	63±1.4	63.7±0.9	65.7±0.9	68.7±0.9	70.5±1.2
	Neutrophil	34.5±1.9	36±1.4	38.7±1.5	41.5±2.2	44.2±1.7
	Monocyte	2.25±0.5	1.5±0.5*	0.7±0.5**	0.5±0.5**	0.2±0.5**
	Eosinophil	6.2±0.9	6.5±0.5	6.5±0.5	7±0.8**	8.2±0.5**
	Basophil	0.7±0.6	0.5±0.5	0.2±0.5	0±0	0±0
Ivermectin	Lymphocyte	66.2±2.0	63.2±0.9*	61.2±0.9**	56.2±1.7**	51.7±1.7**
	Neutrophil	36.5±1.2	34.7±0.9	33.75±0.5**	30±1.6**	26.7±0.9**
	Monocyte	1.5±0.5	0.7±0.5	1±0.8	1.5±0.5	2.5±0.5
	Eosinophil	7±1.1	6.7±0.9	6.5±0.5	6±0.8	5.7±0.9
	Basophil	0.5±0.5	0.5±0.5	0.2±0.5	0.5±0.5	0.2±0.5
Crude aqueous extracts (CAE)	Lymphocyte	65.2±2.7	63.7±1.7	60.7±1.8*	55±2.5*	52.7±.95**
	Neutrophil	36.7±1.7	35.2±.55*	33.5±.57**	31.7±1.5**	28.5±2.6**
	Monocyte	1.25±0.5	0.8±0.5	1±0.8	1.7±0.5	2.2±0.5
	Eosinophil	6.7±0.9	6.5±0.5	6±1.1	6.25±1.2	6±0.8
	Basophil	0.5±0.5	0.5±0.5	0.2±0.5	0±0	0.2±0.5
Crude methanol extract (CME)	Lymphocyte	63.2±.9	62.5±1.7	60.1±1.9	56±1.6	52.5±1.2
	Neutrophil	36±1.6	34.5±1.2	33±1.1	31±1.5*	29.2±0.9*
	Monocyte	1.5±0.5	0.7±0.9**	1±0.8**	1.5±1.1**	2.5±0.5**
	Eosinophil	7.2±0.9	6.7±0.1	6.5±1.7	6.25±1.2	6.25±0.9
	Basophil	0.5±0.5	0.5±0.5	0.2±0.5	0±0	0.2±0.5

Each group consists of four sheep.
SE= Standard error; * = significant differences ($p \leq 0.05$); **= highly significant differences ($p \leq 0.01$)

d) Effects on biochemical parameters

The AST (U/L), ALT (U/L) and creatinine (mg/dl) values were differentiated among the treated and control groups. The levels of AST, ALT and creatinine varied significantly ($p \leq 0.01$) at different observational periods within the ivermectin, CAE and CAME treated groups. The result recommended that the AST, ALT and creatinine level decreased significantly in ivermectin, CAE and CAME treated groups on days 7, 14, 21 and 28 compared to day 0 (Table 4). The levels of AST, ALT and creatinine also varied significantly ($p \leq 0.01$) among the groups on days 7, 14, 21 and 28. The values of AST, ALT and creatinine were significantly lower in the treatment groups than in the untreated group across the study period.

Table 4: Effects on biochemical parameters in sheep affected with parasitic infestation

Treatment	Parameters	Pre-treatment	Post treatment			
		Day 0 (Mean±SE)	Day 7 (Mean±SE)	Day 14 (Mean±SE)	Day 21 (Mean±SE)	Day 28 (Mean±SE)
Control	AST	92.9±5.2	95.7±5.4	99.5±6.1	102.2±6.7	105.4±7.1
	ALT	21.7±1.5	22.9±1.4	24.4±1.3	25.2±1.7	26.5±1
	Creatinine	1.6±0.1	1.7±0.1	1.9±0.1	2.0±0.1	2.1±0.1
Ivermectin	AST	99.4±6.7	96.6±6.4**	93.2±6.0**	88.7±5.9**	85.4±5.8**
	ALT	24.5±2.1	22.1±2.0**	19.8±1.7**	17.6±1.6**	16.0±1.2**
	Creatinine	1.7±0.1	1.3±0.1**	1±0.1**	0.8±0.1**	0.7±0.1**
Crude aqueous extracts (CAE)	AST	90.9±3.1	87.4±2.7*	84.4±3.0*	80.6±2.7**	75.4±3**
	ALT	24.5±1.4	23.3±1.3**	21.1±1.0**	19.8±0.8**	18.4±0.5**
	Creatinine	1.8±0.1	1.5±0.2**	1.2±0.1**	1.0±0.2**	0.8±0.2**
Crude methanol extract (CME)	AST	95.7±9.4	92.7±9.5**	89.5±8.8**	86.5±8.4**	82.9±7.0**
	ALT	23.7±2.9	21.7±2.5**	19.5±2.2**	18.3±2.2**	17.1±2.0**
	Creatinine	1.8±0.0	1.6±0.1*	1.4±0.1**	1.1±0.1**	0.8±0.1**

Each group consists of four sheep.
SE= Standard error; * = significant differences ($p \leq 0.05$); **= highly significant differences ($p \leq 0.01$)

IV. Discussion

Efficacy was founded on the basis of reduction of EPG count in comparison with the control and ivermectin treated group with other group on the day o to 28 day. The efficacy of neem, Bitter gourd and clove at the form of crude aqueous and methanol treated Extract against parasitic infestation in sheep was satisfactory level which was determinated by *iv vitro* and *in vivo* anthelmintic activity. The present study showed higher efficacy at the concentration of 100 mg/ml than the concentration of 25 mg/ml and 50 mg/ml. The anthelmintic efficacy was compared with corresponded studies Bhalke *et al*., (2011); Sujon *et al*., (2008); Rabiu and Subhasish, (2011); Surendra *et al*., (2013) and Kumar *et al*., (2014) who found that neem leaves were 85.4% and 89.7% effective at the dose rate of 100 mg/kg body weight, respectively and ivermectin was effective 96.62% against gastrointestinal helminths in sheep. The current result is in agreement with Qamar *et al*., (2011), after oral administration were observed and compared with the ivermectin. Efficacies of drug and herbs extract were considered based on declination of EPG count. In the study the maximum reduction level of ivermectin treated group (89 %) efficacy was observed which was close to the following author activites. The result is also consistent with Sujon *et al.,* (2008) and Jaiswal *et al*., (2013) who found efficacy of Ivermectin and neem 94% and 81%, respectively. The maximum EPG reduction rate was observed in aqueous treated extracts 86 % reduction at the concentration of 100 mg/ml by oral administration of 1 ml per kg body weight. The control group A where the EPG count increased from 947.5 at the day 0 to 1572.5 at the day 28. On the other hands the maximum EPG reduction rate was observed in methanol treated extracts 88.6 % reduction at the concentration 100 mg/ml by oral administration of 1 ml per kg body weight. Costa *et al*., (2008) and Marta *et al.,* (2008) reported neem, bitter gourd and clove extract were very effective in eliminating parasitic infection in sheep. The other authors of the same kinds of work against gastrointestinal parasites were investigated Das *et al.,* (2000); Brelin, (2002); Mishra *et al*., (2005); Saha *et al*., (2006); Amin *et al*., (2008) found almost similar results. Frequently reduction of EPG indicates the effectiveness of both aqueous and methanol treated extracts against gastrointestinal parasites in sheep. So the present work proved the anthelemintic property of neem, bitter gourd and clove in response to the existence of popular reports of such activity in animals.

a) Effects on haematological parameters

The hematological parameters were analyzed on the comparison with control and treated groups (Table 2). The following investigated blood parameters such as PCV, Hb, TEC and TLC were improved significantly in parasites affected sheep with the anthelmintic (ivermectin) and selected herbs extracts treatment. Due to reduction of blood-sucking parasites (*Haemonchus* spp) and other gastrointestinal parasites infections in sheep the blood parameters such as Hb, PCV, TEC and TLC increasing day by day. The ESR percentages were decreased in control group due to blood cell destruction by comparison on the day 0 to day 28 (Table 2). The effectiveness of herbs extracts both aqueous and methanol treated indicated the stimulatory effect on the hemopoietic system. The rise in mean PCV after treatment might be associated with the increase of Hb%, as these parameters are closely interrelated with each other. The improvement of blood PCV, Hb, TEC and TLC levels in the treated sheep might be due to the elimination of external and internal parasites, which was expected. Similar kinds of improvement of these blood parameters after anthelmintic treatment have been previously reported in sheep Amin *et al*., (2008); Aruwayo *et al*., (2011) and Rahman (2002) observed extract of neem leaves increased TEC, Hb content on day 21 of post-treatment in goat. Similarly, (Rob *et al.,* 2004) stated that Hb content, PCV, TEC and TLC increased in sheep on day 28.(Kumar et al., 2003) reported that fall of Hb, PCV, TEC and TLC might be due to disturbance caused by worms rather than a direct blood lost. Reduction of ESR may be due to recovery from inflammation, which was produced by parasitic infestation. The result of the current study is consistent with Rahman *et al.,*(2009) who found the ESR values was increased up to 5.19 mm on day 28 in untreated groups. Similar findings have been reported by (Kumar *et al*., 2003) and Deka and Borah (2008). In the study ESR and PCV values significantly ($p \leq 0.05$) increased in treated group which is similar to the finding of Nayaka *et al.,* (2013). The effects of herbs extracts as anthelmintic in animal body were indicated that the Eosinophil, basophil were decresed on the day 0 to on the day 28. On the other hand the monocytes count was increased day by day. In this study has showed the eosinophil and basophil were decresed and monocyte levels were increased which indiacates that the herbs extracts have effectiveness against gastrointestinal parasites in sheep. The percentages of eosinophil, basophil were decresed and monocytes were increased after post-treatment in parasitic infections reported by (Aruwayo *et al.,* 2011). Similarly, Biu *et al.,* (2009) reported that the mean values for monocytes, basophils and eosinophils increased significantly with increasing dose of anthelmintics while mean values for lymphocytes and neutrophil decreased significantly.

b) Effects on biochemical parameters

Effect of herbs extracts on animal body in the levels of AST, ALT and creatinine in anthelmintic treated groups decreased, which indicates the removal of

parasites from the affected sheep. Furthermore, it indicates that treatment with ivermectin, aqueous and methanol treated extracts are not toxic to the liver and kidney. By external palpation of liver and kidney in sheep are normal in size and shape. No debilitating lesion was founded on the liver. These results are in near similar with earlier reports (Sokumbi and Egbunike, 2000; Gupta *et al.*,2005).

V. Conclusion

Efficacy of herb extracts and drugs were measured *in vitro* and *in vivo* after the preparation and use of various concentration viz. 25 mg/ml, 50 mg/ml and 100 mg/ml of crude aqueous extract (CAE) and crude methanol extract (CME). In vitro screening the anthelmintic efficacy (96.6%) of methanol extract at the concentration of 100 mg/ml was higher than the aqueous extracts (86.6%). Highly parasitic infested sheep (16) age between 6 to 24 months were selected based on EPG count (>840 EPG) indicating anemic condition. In vivo screening of aqueous extracts and methanol extract at the concentration of 100mg/ml were reasonably effective 86 % and 88.6 % reduction of EPG. By hemato-biochemical parameters analysis the percentages of eosinophil and basophil were decrease which indicates reduction of endoparasites and correction of anemia. Therefore, these herbs can be used as alternatives to conventional anthelmintic and this could reduce the unnecessary use of conventional drugs which make parasites more resistant to the drugs.

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References References Referencias

1. Akbar MA, Ahmed TU, Mondal MH. 2006. Improving animal productivity and reproductive efficiency: Development and testing medicated urea molasses until nutrient blocks in rural farms of Bangladesh. Improving animal productivity by supplementary feeding of multi nutrient blocks, controlling internal parasites and enhancing utilization of alternate feed resources (pp.14-27). Austria: International Atomic Energy Agency.
2. Al-Shaibani, I.R.M.S. 2009. Epidemiological study and evaluation of anthelmintics activity of indigenous plants on gastrointestinal nematodes of sheep in Hyderabad district.Sindh Agriculture University, Tandojam, Pakistan.
3. Amin MR, Mostofa M, Awal MA, Hossain A. 2008. Effects of neem (Azadirachta indica) leaves against gastrointestinal nematodes in cattle. Journal of Bangladesh Agricultural University, 6(1): 87-99.
4. Amin MR, Mostofa M, Hoque ME and Sayed MA. 2009. In vitro anthelmintic efficacy of some indigenous medicinal plants against gastrointestinal nematodes of cattle. Journal of Bangladesh Agricultural University, 7(1): 57–61.
5. Aruwayo A, Maigandi SA, Malami BS, Daneji AI. 2011. Haematological and Biochemical Parameters of Uda Lambs Fed Graded Levels of Alkali -Treated Neem Kernel Cake. Nigerian Journal of Basic and Applied Science, 19(2): 277-284.
6. Bernal J, Mendiola JA, Ibanez E, Cifuentes A. 2011. Advanced analysis of nutraceuticals. Journal of Pharmaceutical and Biomedical Analysis, 55: 758-774.
7. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. 2002. Biological activities and medicinal properties of Neem (*Azadirachta indica*) Clinical Science, 82: 1336-1345.
8. Biu A, Yusufu SD, Rabo JS. 2009. Studies on the effects of aqueous leaf extracts of neem (*Azadirachta indica* A. Juss) on haematological parameters in chicken. African Scientist, 10(4): 189-192.
9. Brahmachari G. 2004. Neem - An omnipotent plant: A retrospection. Chembiochem, 5: 408-421.
10. Brelin D. 2002. Evaluation of the neem tree (*Azadirachta indica*) as an alternative anthelmintic for helminth control in small ruminants in Malaysia. Minor Field Studies International Office, Swedish University of Agricultural Sciences. 196: 31.
11. Cava R, Nowak E, Taboada A, Marin-Iniesta F. 2007. Antimicrobial activity of clove and cinnamon essential oils against *Listeria monocytogenes* in pasteurized milk. International Journal Food Protection .70(12): 2757-2763.
12. Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K. and Bakhrouf, A. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum L.*). Phytother. Res. 2007, 21, 501-506.
13. Coffin DL. 1955. Manual of Veterinary Clinical Pathology. Third Ed. Coinstock Publishing Associates. *Inc. Ithaca* New York, 116-157.
14. Costa CTC, Bevilaqua CML, Camurca-Vasconcelos ALF, Maciel MV, Morais SM, Castro CMS, Braga RR, Oliveira LMB. 2008. In vitro ovicidal and larvicidal activity of *Azadirachta indica* extracts on *Haemonchus contortus*. Small Ruminant Research, 74: 284–287.
15. Eisa E, Mukhtar I. 2007. Antimicrobial activity of Neem (Azadirachta indica) callus and leaf extracts.

African Journal of Microbiology Research, 11(2): 53-61.
16. Eysker M, Ploeger HW. 2000. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitology, 120 (Suppl): S109–S119.
17. Gilani AH, Ghayur MN, Saify ZS, Ahmad SP, Choudary MI, Khalid A. 2004. Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. Life Science 75: 2377–2389.
18. Girish K, Bhat SS. 2008. Neem -A Green Treasure. Electronic Journal of Biology, 4(3):102-111.
19. Githigia, S.M, Thamsberg, S.M, Maing N. and Munyua, W.K.(2005). The epidemiology of gastrointestinal nematodes in goats in the low potential areas of Thinka district, Kenya. Animal Health Production Africa. 53,5-12.
20. Gupta RK, Kersari AN, Murthy PS, Chandra R, Tandon V, Watal G. 2005. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa L.* in experimental animals. *Journal of Ethnopharmacology* , 99(1): 75-51.
21. Hannan ASMA, Mostofa M, Hoque MA, Alim MA, Saifuddin AKM. 2003. Efficacy of ivermectin against naturally occurring gastrointestinal nematodes and ectoparasites of sheep. Bangladesh Journal of Animal Science, 32(2): 39-45.
22. Hossain Z. 2003. People's awareness about medicinal values of plants and prospect in Bangladesh. BRAC, Research and Evaluation division, Dhaka, Bangladesh.
23. Husdan H, Rapoport A.1968. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. *Clinical Chemistry,* 14: 222-238.
24. Hussain A, Khan MN, Sajid MS, Iqbal Z, Khan MK. 2010. In vitro screening of the leaves of *Musa paradisiacal* for anthelmintic activity. The Journal of Animal & Plant Sciences, 20(1): 5-8
25. IUCN. 2003. The World Conservation Union. Bio-ecological Zones of Bangladesh., Bangladesh.
26. Jaiswal AK, Sudan V, Shanker D, Jaiswal AK, Kumar P. 2013. Emergence of Ivermectin resistance in gastrointestinal nematodes of Emergence of Ivermectin resistance in gastrointestinal nematodes of goats in a semi-organized farm of Mathura district. Indian Veterinarski Arhiv, 83(3): 275-280.
27. Jordan SA, Cunningham DG, Marles RJ. 2010. Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. Toxicology and Applied Pharmacology, 243:198–216.
28. Joshi KK. 2001. Genetic Heritage of Medicinal and Aromatic Plants of Nepal Himalayas. Kathmandu: Buddha Academic Publishers and Distributors Pvt. Ltd.
29. Kala CP, Sajwan. 2007. Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building. Current Science, 93 (6): 797-806.
30. Koehn FF, Carter GT. 2005. The evolving role of natural products in drug discovery. Nature Reviews Drug Discovery, 4(3): 206-220.
31. Kumar DS, Sharathnath KV, Yogeswaran P, Harani A, Sudhakar K, Sudha P, et al. 2010. A medicinal potency of *Momordica charantia*. International Journal of Pharmacological Science3(1): 95-100
32. Kumar RV, Gupta VK. 2002. Thrust on Neem is need of today. In: Employment news, 20-26. New Delhi, India.
33. Marta MC, Souza, Claudi ML, Bevilaqa, Selene M, Morais, Cicero TC. 2008. Anthelmintic acetogenin from *Annona squamosa L. Annuals of the Brazilian academy of sciences*, 80(2): 271-277.
34. Mishra V, Parveen N, Singhal KC. 2005 Antifilarial activity of Azadirachta indica on cattle filarial parasite Setaria cervi. Fitoterapia, 76: 54-61.
35. Moskey HE, Harwood PD. 1941. Methods of evaluating the efficacy of anthelmintics. American Journal of Veterinary Research, 2: 55-59.
36. Papadopoulos E, Gallidis E, Ptochos S. 2012. Anthelmintic resistance in sheep in Europe: A selected review. Veterinary Parasitology, 189: 85– 88.
37. Patil, Kadam JS, Chavhan JA and Salunkhe AJ. 2014. Anthelmintic Activity of Ethanolic Bud Extract Of *Syzygium Aromaticum* Against *Pheritima Posthuma*. Journal of Medicinal Plant Research, 3 (4): 294-310.
38. Qamar MF, Maqbool A, Ahmad N. 2011. Comparative efficacy of allopathic, herbal, homeopathic and effective micro-organisms for the contol of haemonchosis in sheep & goats. Science International, (Lahore). 23(1):43-48.
39. Rabiu H, Subhasish M. 2011. Investigation of in Vitro Anthelmintic activity of *Azadirachta indica* leaves. International Journal of Drug Development & Research, 3 (4): 94-100.
40. Rahman M. 2002. In vitro and in vivo anthelmintic effects of some plants against gastrointestinal nematodes of goats. M.S. Thesis, submitted to the Department of Parasitology, Bangladesh Agricultural University, Mymensingh.
41. Saha BN, Islam W, Khan AR. 2006. Effect of Azadirachtin on the growth and development of the pulse beetle, *Callosobruchus chinensis* L. Journal of the Asiatic Society of Bangladesh, Science, 32 (1): 69-65.
42. Sani RA, Rajamanickam C. 1990. Gastrointestinal parasitism in small ruminants. In Proc.Workshop on Integrated tree cropping and small ruminant production system. Medan, Indonesia.

43. Schalm OW, Jain WC, Carrol EJ. 1975. Veterinary haematology, 3rd Edition Lea and Febieger, Philadelphia. p.807.
44. Simmons A, Leverton P, Elbert G. 1974. Normal laboratory values for differential white cell counts established by manual and automated cytochemical methods (Hemalog D). American *Journal* of *Clinical Pathology,* 27: 55.
45. Sokumbi OA, Egbunike GN. 2000. Physiological responses of growing rabbits to neem (*Azadirachta indica*) leaf meal based diets. Haematology and serum biochemistry. Tropical Journal of Animal Production Investment, 3: 81-87.
46. Subapriya R, Nagini S. 2005. Medicinal properties of neem leaves: a review. Current Medicinal Chemistry - Anti-Cancer Agents, 5: 149-156.
47. Sujon MA, Mostofa M, Jahan MS, Das AR, Rob S. 2008. Studies on medicinal plants against gastroinstestinal nematodes of goats. Bangladesh Journal of Veterinary Medicine, 6(2): 179-183.
48. World Health Organization (WHO). 2008. Traditional medicine. Available from: http://www.who.int/meadiacentre /factsheets/fs134/en/

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Equine Lung Worm: A Systematic Review

By Nuraddis Ibrahim
Jimma University

Abstract- Lungworms are parasitic nematode worms of the order Strongylidae that infest the lungs of vertebrates. Dictyocaulus arnfieldi is the true lungworm affecting donkeys, horses, mules and zebras and is found throughout the world. Dictyocaulus arnfeildi can cause severe coughing in horses and because patency is unusual in horse (but not in donkeys) differential diagnosis in disease due to other respiratory disease can be difficult. Adult Dictyocaulus worms are slender, medium sized roundworms, up to 8 centimeter long. Females are about one third longer than males. They have a whitish to grayish color. Dictyocaulus worms have a direct lifecycle, i.e. there are no intermediate hosts involved. The pathogenic effects of lungworm depends on their location within the respiratory tract, the number of infective larvae ingested, the animal immune status, on the nutritional status and age of the host. Despite the prevalence of patent D. arnifieldi infection in donkeys, overt clinical signs are rarely seen; however, on close examination slight hyperpnoea and harsh lung sounds may be detected. Diagnosis is based on clinical signs, epidemiology, presence of first-stage larvae in feces, and necropsy of animals in the same herd or flock. Bronchoscopy and radiography may be helpful. Larvae are not found in the faeces of animals in the prepatent or postpatent phases and usually not in the reinfection phenomenon. ELISA tests are available in some laboratories. Bronchial lavage can reveal Dictyocaulus arnifieldi infections in horses. The concern of lungworm in Ethiopia is increasing and is now to be a major problem of equines. Routine deworming of horses and donkeys may help prevent cross infection when kept together. Reducing pasture contamination with infective larvae is a key preventative measure that can be achieved to a large extent with adequate management measures. Rotational grazing with a change interval of 4 days and keeping the paddocks empty for at least 40 days significantly reduces pasture contamination.

Keywords: *coughing, dictyocaulus arnfeildi, lifecycle, lung, pathogenic effect.*

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

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Strictly as per the compliance and regulations of:

Equine Lung Worm: A Systematic Review

Nuraddis Ibrahim

Summary- Lungworms are parasitic nematode worms of the order *Strongylidae* that infest the lungs of vertebrates. *Dictyocaulus arnfieldi* is the true lungworm affecting donkeys, horses, mules and zebras and is found throughout the world. *Dictyocaulus arnfeildi* can cause severe coughing in horses and because patency is unusual in horse (but not in donkeys) differential diagnosis in disease due to other respiratory disease can be difficult. Adult *Dictyocaulus* worms are slender, medium sized roundworms, up to 8 centimeter long. Females are about one third longer than males. They have a whitish to grayish color. *Dictyocaulus* worms have a direct lifecycle, i.e. there are no intermediate hosts involved. The pathogenic effects of lungworm depends on their location within the respiratory tract, the number of infective larvae ingested, the animal immune status, on the nutritional status and age of the host. Despite the prevalence of patent *D. arnifieldi* infection in donkeys, overt clinical signs are rarely seen; however, on close examination slight hyperpnoea and harsh lung sounds may be detected. Diagnosis is based on clinical signs, epidemiology, presence of first-stage larvae in feces, and necropsy of animals in the same herd or flock. Bronchoscopy and radiography may be helpful. Larvae are not found in the faeces of animals in the prepatent or postpatent phases and usually not in the reinfection phenomenon. ELISA tests are available in some laboratories. Bronchial lavage can reveal *Dictyocaulus arnifieldi* infections in horses. The concern of lungworm in Ethiopia is increasing and is now to be a major problem of equines. Routine deworming of horses and donkeys may help prevent cross infection when kept together. Reducing pasture contamination with infective larvae is a key preventative measure that can be achieved to a large extent with adequate management measures. Rotational grazing with a change interval of 4 days and keeping the paddocks empty for at least 40 days significantly reduces pasture contamination.

Keywords: coughing, dictyocaulus arnfeildi, lifecycle, lung, pathogenic effect.

I. Introduction

Equines are one of the most important and mostly intimately associated with man. They have enormous contribution through their involvement in different social and economic sectors. Equines play an important role as working animals in many parts of the world, for packing, riding, carting and ploughing. Equine power is very crucial in both rural and urban transport system. This is because of its cheapness and availability and so provides the best alternative transport means in places where the road network is insufficiently developed and the landscape is rugged and mountainous and in the cities where narrow streets prevent easy delivery of merchandise (Feseha *et al.*, 1991).

In Ethiopia equines have been as animals of burden for long period of time and still render valuable services mostly as pack animals throughout the country particularly in areas where modern means of transportation are absent, unaffordable or inaccessible (Abayneh *et al.*, 2002).

In some areas of North West Kenya and Southern Ethiopia, donkey meat is a delicacy and the milk believed to treat whooping cough (Fred and Pascal, 2006).

Even though mules and donkeys have often been described as sturdy animals; they succumb to a variety of diseases and a number of other unhealthy circumstances. Among these, parasitic infection is a major cause of illness (Sapakota, 2009). Lungworms are widely distributed throughout the world providing nearly perfect conditions for their survival and development but are particularly common in countries with temperate climates, and in the highlands of tropical and subtropical countries. *Dictyocaulidae* are known to exist in East Africa and South Africa (Hansen and Perry, 1996).

Dictyocaulus arnfieldi is the true lungworm affecting donkeys, horses, ponies and zebras and is found throughout the world (Smith, 2009). Donkeys and their crosses (Mules) are the natural hosts for lungworm and the condition in horses is usually found in those that have been in the company of donkeys and mules (Rose and Hodgson, 2000). This review article supports researchers to more understand the equine lung worm disease and factors influencing the disease occurrence under Ethiopian condition. It also helps policy makers to draw sound decisions in order to improve the control policy. The review paper gives information to farmers and cattle rearing people regarding equine lung worm disease.

And therefore, the objectives of this paper are to give background information on the disease and recommend modern control measures.

II. Definition and Etiology of Lungworm

Lungworms are parasitic nematode worms of the order *Strongylidae* that infest the lungs of vertebrates. The taxonomy of this parasite is belonging to kingdom *Animalia*, phylum *Nematode*, class *Secernentea*, family *Dictyocaulidae*, genus *Dictyocaulus*

Author: Jimma University, School of Veterinary Medicine, Jimma, Ethiopia. e-mail: inuraddis@yahoo.com

and species of *Dictyocaulus arnfieldi* (Johnson *et al.,* 2003).

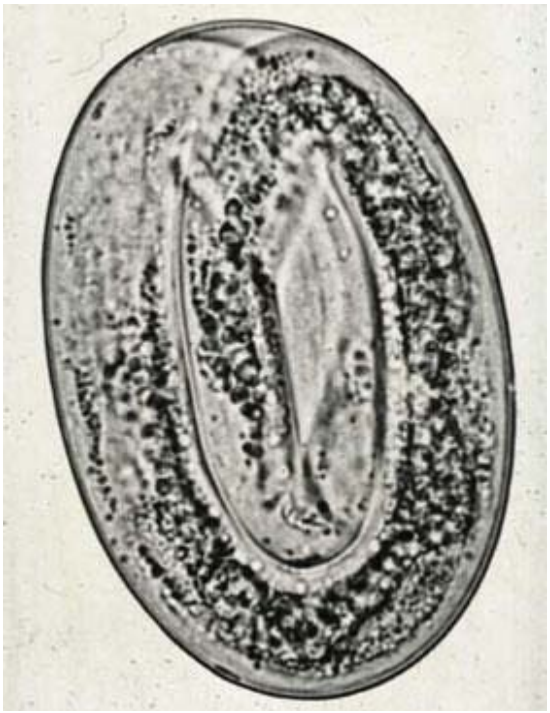
An infection of lower respiratory tract, usually resulting in bronchitis or pneumonia can be caused by several parasitic nematodes, including *D. viviparous* in cattle and deer; *D. arnfeildi* in horses and donkeys; *D. filaria*, *Protostrongylus rufescens,* and *Mullarius capillaries* in sheep and goats; *Metastrongylus apri* in pigs; *Filaroides (Oslerus) osleri* in dogs; and *Aelurostrongylus absrtusus* and *Capillaria aerophila* in cats, other lungworm infection occur but less common (Fraser, 2000).

Dictyocaulus arnfieldi is the true lungworm affecting donkeys, horses, mules and zebras and is found throughout the world (Smith, 2009). It is a relatively well adopted parasite of donkeys but tend to be quite pathogenic in horses, where this parasite is endemic (Bowman, 2003).

The first three lungworm listed above belong to superfamily *Trichostrongylidea* and have direct life cycle; others belong to *Metastrongylidea* and, except F.*osleri* and *C.aerophila* have indirect life cycle. Diseases caused by the three *Dictyocaulus* species are of most economic importance. The cattle lungworm *Dictyocaulus viviparous* is common in Northwest Europe and is the cause of severe outbreaks of "husk" or "hoose" in young grazing cattle. The lungworm of sheep and goat, *Dictyocaulus filarial* is less pathogenic but does cause losses especially in Mediterranean countries, although it also recognized as a pathogen in Australia, Europe and North America. *Dictyocaulus arnfeildi* can cause severe coughing in horses and because patency is unusual in horse (but not in donkeys) differential diagnosis in disease due to other respiratory disease can be difficult. *Mullarius capillaries* present worldwide and, while usually nonpathogenic in sheep, can cause severe signs in goats. Other lungworm infections generally cause occasional sporadic infection on many species in many countries (Fraser, 2000)

III. Morphology of *Dictyocaulus Arnfieldi*

Adult *Dictyocaulus* worms are slender, medium sized roundworms, up to 8 centimeter long. Females are about one third longer than males. They have a whitish to grayish color. As in other roundworms, the body of these worms is covered with a cuticle, which is flexible but rather tough. The worms have a tubular digestive system with two openings, the mouth and the anus. They also have a nervous system but no excretory organs and no circulatory system, i.e. neither a heart nor blood vessels. The female ovaries are large and the uteri end in an opening called the vulva. Males have a copulatory bursa with two short and thick spicules for attaching to the female during copulation. The eggs of *Dictyocaulus arnfieldi* are approximately 60x90 micrometers. They have an ovoid shape and contain a fully developed L1 larva (Junquera, 2014).



Source: (http://www.studyblue.com)

Figure 1: Egg of *Dictyocaulus arnfieldi*

Lungworm larvea are slender and 25 to 70 millimeters long. The *D. arnfeildi* larvae resemble those of *D.viviparous* but the tail ends in a small spine (Fraser, 2000).

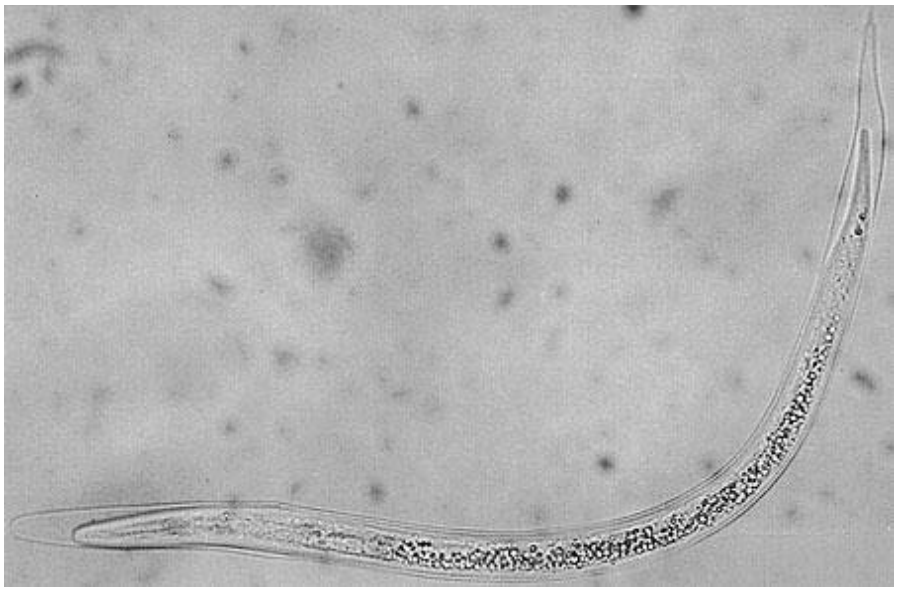


Figure 2: Larva of *Dictyocaulus arnfeildi*

Source: (http://www.studyblue.com)

a) Epidemiology

The epidemiology of lungworm disease is largely concerned with factors determining the number of intensive larvae on the pasture and the rate at which they accumulate. The third stage larvae are long living in damp and cool surroundings. Warm and wet summers give rise to heavier burdens in the follow autumn and spring. Horses are not the favorite host of this parasite and do not usually transmit the disease to other horses. In most instances, horses acquire this disease when pastured with donkeys (Blood *et al.*, 1999).

Under optimal condition the larvae may survive in the pasture for a year. They are quite resistant to cold although it generally delays their maturations. They can withstand temperature of 4-5 degree Celsius; Larvae can over winter in cold climates (Blood *et al.,* 2000). Most outbreak of verminous pneumonia occur during cool season specially autumn and early winter because the larvae stages of the causative worms tolerate and prefer low temperatures (Hansen and Perry, 1996).

The natural host of the parasite is donkey, and comparably, large numbers of parasites can accumulate in the lungs of this host without clinical signs. Donkeys and mules can act as a reservoir for horses (Beelitz *et al.,* 1996). *Pilobolus* fungi may play a role in the dissemination of *D. arnifieldi* larvae from faeces, as *D. viviparus. D. arnfieldi* is found worldwide, particularly in areas with heavy rainfall (Urquhart *et al.*, 1999).

b) Life Cycle

The detailed life cycle is not fully known, but is considered to be similar to that of bovine lungworm, *Dictyocaulus viviparus* except in the following respect. The adult worms are most often found in the small bronchi and their eggs, containing the first stage larvae, hatch soon after being passed in the faeces (Urquhart *et al.*, 1999).

Dictyocaulus worms have a direct lifecycle, i.e. there are no intermediate hosts involved. Adult females lay eggs in the airways of infected hosts. These eggs are transported to the pharynx within respiratory secretions. From the pharynx these eggs are coughed out, into the mouth to be swallowed or directly to the outside. Those that are swallowed release the L1 larvae in the gut, which are shed in the faeces. Once in the environment, L1-larvae develop to infective L3 larvae in about 1 week. These larvae show a low motility and remain close to the droppings. Animals become infected mainly while grazing, but infection can also happen indoors through contaminated hay or bedding. Once ingested and in the host's gut infective larvae penetrate into the gut's wall and reach the lymphatic nodules where the molt to L4 larvae. Through the thoracic duct and the jugular vein they reach the heart and are pumped to the lungs. Once in the lungs they are stopped in the lung capillaries, cross their wall and reach the bronchioles, bronchi or the trachea where they complete development to adult worms. The prepatent period (time between infection and first larvae shed with the faeces) lasts about 4 weeks. However, larvae in the lungs may become arrested (dormant, hypobiotic, inhibited) for up to 5 months. These larvae resume development at early spring and re-infect the pastures during the next season (Junquera, 2014).

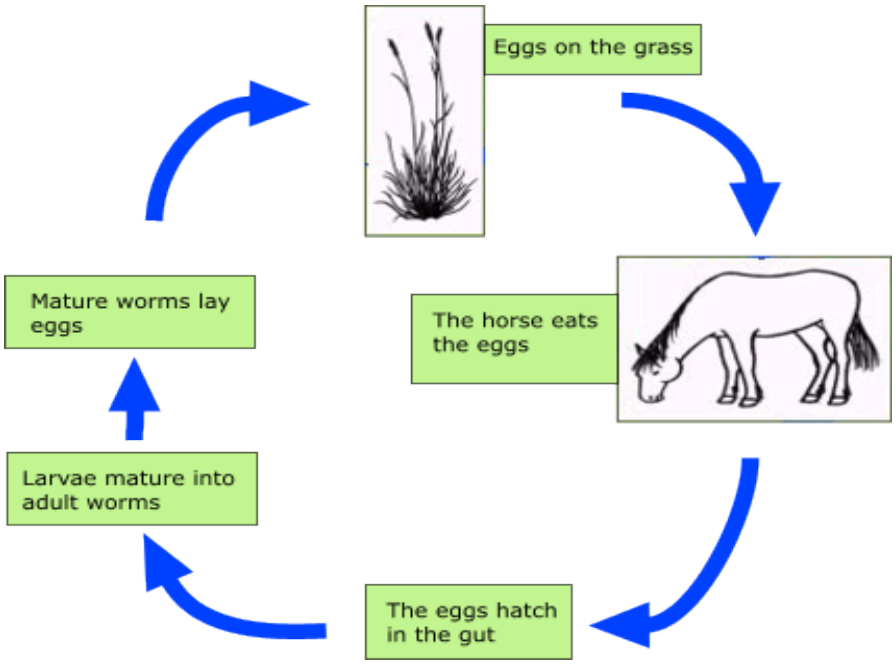


Figure 3: The lifecycle of *Dictyocaulus arnfieldi*

Source: http://www.merial.ph/SiteCollectionDocuments/equine

c) Pathogenesis

The pathogenic effects of lungworm depends on their location within the respiratory tract, the number of infective larvae ingested, the animal immune status, on the nutritional status and age of the host (Blood *et al.,* 1989; Fraser, 2000). Larvae migrating through the alveoli and bronchioles produce an inflammatory response, which may block small bronchi and bronchioles with inflammatory exudates. The bronchi contain fluid and immature, latter adult worms and the exudates they produce also block the bronchi. Secondary bacterial pneumonia and concurrent viral infections are of the complication of Dictyocaulosis (Howard, 1993). The major pathologic changes which results from primary infection may be divided into three stages. These are the prepatent stages, where blockage of small bronchi and bronchioles by eosinophilic exudates produced in response to the developing and migrating larvae. The patent stage, when adult worms cause bronchitis and primary pneumonia development occurs. The post patent phase is when adult worms are expelled and majority of animals gradually recover. The pathological changes seen in the lungs during necropsy are atelectasis, emphysema, petechial hemorrhage and lung consolidation (Aiello and Mays, 1998).

d) Clinical Signs

Despite the prevalence of patent *D. arnifieldi* infection in donkeys, overt clinical signs are rarely seen; however, on close examination slight hyperpnoea and harsh lung sounds may be detected. This absence of significant clinical abnormality may be partly a reflection of the fact that donkeys are rarely required to perform sustained exercise. Infection is much less prevalent in horses. However, patent infections may develop in foals and these are not usually associated with clinical signs. In older horses infections rarely become patent but are often associated with persistent coughing and an increased respiratory rate (Urquhart *et al*., 1999). Donkeys usually show no disease signs and can be silent carriers and shedders of this parasite, which causes clinical signs in horses (Johnson *et al.,* 2003).

e) Diagnosis

Diagnosis is based on clinical signs, epidemiology, presence of first-stage larvae in feces, and necropsy of animals in the same herd or flock. Bronchoscopy and radiography may be helpful. Larvae are not found in the faeces of animals in the prepatent or postpatent phases and usually not in the reinfection phenomenon. ELISA tests are available in some laboratories. Bronchial lavage can reveal *Dictyocaulus arnifieldi* infections in horses (Stuart, 2012).

Verminous pneumonia is easily confused clinically with bacterial bronchopneumonia, with acute and chronic interstitial pneumonia, and with viral pneumonia. The disease usually occurs in outbreak form in summer and autumn (Blood *et al.,* 1999). The diagnostic methods of lungworms are described as the following ways in details.

f) Clinical Diagnosis

Typical signs and symptoms are heavy coughing (often paroxysmal), accelerated and/or difficult breathing and nasal discharge. Affected animals lose

appetite and weight. Severe infections can also cause pneumonia (lung inflammation), emphysema (over inflation of the alveoli), and pulmonary edema (liquid accumulation in the airways). Adult livestock usually develops resistance and if re-infected may not show clinical signs but continue shedding larvae that contaminate their environment (Junquera, 2014).

g) Faecal Examination

A convenient method for recovering larvae is a modification of the Baermann technique in which large faecal samples (5-10 grams) are wrapped in tissue paper or cheese cloth and suspended or placed in water contained in a beaker. The water at the bottom of the beaker is examined for larvae after 4 hours; in heavy infections, larvae may be present within 30 minutes. Bronchial lavage can reveal *Dictyocaulus arnfieldi* infections in horses (Stuart, 2012).

h) Serological Diagnosis

Enzyme Linked Immuno Sorbent Assay (ELISA) test can demonstrate antibodies from five weeks after the animals have been exposed and it may be useful in identifying infected animals when heavy burdens of worms do not generate and larvae in the feces. This time need to perform an ELISA depends on the availability of antigen-coated microstate-plates. If such plates can be provided; the result can be obtained within four hours after the serum has been prepared. If not, plates have to be coated with antigen for up to 16 hours (Boon *et al.,* 1984).

IV. Necropsy Findings

The morphological change in the lungs include wide spread areas of collapsed tissue of dark pink color, hemorrhagic bronchitis with much fluid filling all the air passed and enlargement of the regional lymph nodes. Histologically, the characteristic lesions are edema, eosinophilic infiltration, debris and larvae in the bronchioles and alveoli. The most obvious lesions at necropsy are discrete patches of over inflation. The bronchial epithelium is hyperplasic and heavily infiltrated by inflammatory cells, particularly eosinophils (Reinecke, 1989).

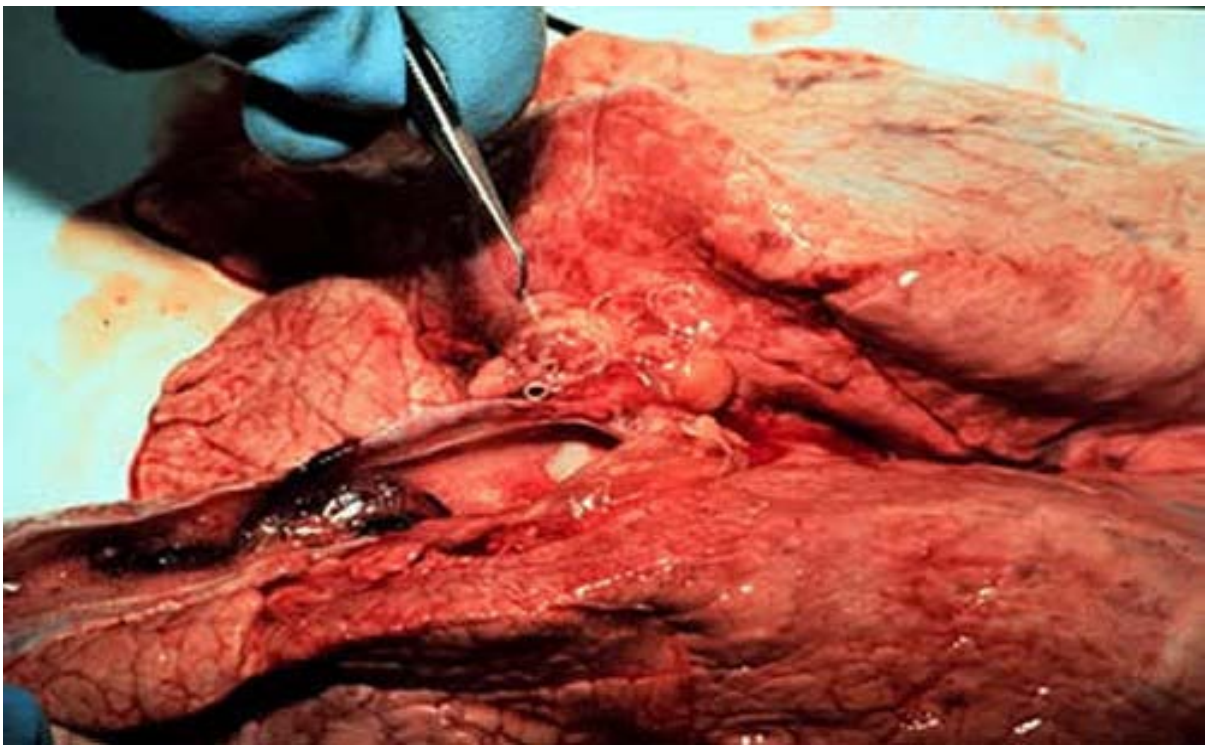


Figure 4: Necropsy finding of *Dictyocaulus arnifieldi* in lung of horse

Source: Staurt (2012)

a) Differential Diagnosis

On a clinical basis, bacterial pneumonia is considered as the first tentative diagnosis. Other probable tentative diagnoses are considered such as chronic hypersensitivity pneumonitis, chronic obstructive pulmonary disease, fungal pneumonia, immune mediated interstitial or vascular disease and unusual drug reactions as well as foreign body in the trachea (Burks, 1998).

b) Control and Preventions

Routine deworming of horses and donkeys may help prevent cross infection when kept together. Pastures that housed donkeys may be infected with lungworm larvae. As a result, horses and donkeys should not be grazed together (Johnson *et al.,* 2003).

Reducing pasture contamination with infective larvae is a key preventative measure that can be

achieved to a large extent with adequate management measures. Rotational grazing with a change interval of 4 days and keeping the paddocks empty for at least 40 days significantly reduces pasture contamination. This is due to the fact that larvae are susceptible to dryness and won't survive more than 4 or 5 weeks on pasture if they do not find an adequate host. Obviously, by very moist weather or where pastures are almost permanently moist survival may be longer. Alternate grazing with sheep and/or horses may be considered, since *Dictyocaulus* species are quite host-specific (for cattle, sheep & goats, horses). The longer the absence of the specific host, the higher will be the reduction of its specific lungworm. However, this may not be advisable in places infected with gastrointestinal roundworms that are simultaneously parasitic of cattle and sheep or horses. For their first grazing season it is highly advisable that young stock does not share the pastures with older stock that has been exposed earlier to infected grounds and can therefore shed larvae. It must also be avoided that young stock uses pastures already used by older stock during the same season. It must also be considered that heavy rains and flooding can disseminate infective larvae inside a property or from one property to neighboring ones. Keeping the pastures as dry as possible and keeping livestock away from places excessively humid are additional key measures to reduce the exposure of livestock to infective larvae. In endemic regions preventative strategic treatment of young stock is often recommended just prior to their first grazing season, followed by additional treatments depending on the infestation level of the pastures and the residual effect of the administered anthelmintic (Junquera, 2014).

Table 1: Different modern broad-spectrum anthelminthic drugs currently used against Lungworm

Drug groups	Anthelminthic drugs	Dose (mg/kg)	Routs of administration
Macrolides	Ivermectin	0.05	PO and SC
Benzimidazole	Oxfendazole	2.5	PO
	Fenbendazole	5.0	PO
	Albendazole	7.5	PO
	Febantele	10	PO
Imidathiazole	Levamisole	8.0	PO

Source: Blood et al. (2000)

c) Economic Impact of the Disease

The vitality and wellbeing of horses of all age are thread by a variety of internal parasites and the use of control ensures and the best performance (Power, 1992). Internal parasites are one of the greatest limiting factors to successful horse rising throughout the world. All horses at pasture become infected and suffer a wide range of harmful effects ranging from impaired development and performance to death despite the availability of large array of modern anthelmintic, parasite controls often fail to safeguard horse health. The main reason for these break downs are errors the choice of anthelmintic and in the time of treatment (Herd, 1987).

d) Prevalence of lungworm infection in different parts of Ethiopia

The concern of lungworm in Ethiopia is increasing and is now to be a major problem of equine in the central highlands of Ethiopia. However there were little preliminary findings of lungworm infection which were done by few researchers of the country (Table 2).

Table 2: Prevalence of lungworm infection in different parts of Ethiopia

S. No.	Site of study	Prevalence in %	Researcher name
2	North Wollo	17.5	Belay, 2005
4	Jimma	13.8	Tihitna et al., 2012
5	South eastern Ethiopia	42.7	Kamil et al. (2017)

V. Conclusion

Dictyocaulus arnfieldi is the true lungworm affecting donkeys, horses, ponies and zebras and is found throughout the world. The natural host of the parasite is donkey, and comparably, large numbers of parasites can accumulate in the lungs of this host without clinical signs. Donkeys and mules can act as a reservoir for horses. The epidemiology of lungworm disease is largely concerned with factors determining the number of intensive larvae on the pasture and the rate at which they accumulate. The third stage larvae are long living in damp and cool surroundings. Warm and wet summers give rise to heavier burdens in the follow autumn and spring. Pastures that housed donkeys may be infected with lungworm larvae. As a result, horses and donkeys should not be grazed together. In endemic regions preventative strategic treatment of young stock is often recommended just prior to their first grazing season, followed by additional treatments depending on the infestation level of the pastures and the residual effect of the administered anthelmintic.

References Références Referencias

1. Abayneh, T., Gebreab, F., Zekarias, B. and Tadesse, G., 2002. The potential role of Donkeys inland tillage in central Ethiopia. *Bulletin of Animal Health and production in Africa*. 50: 172-178.
2. Aiello, S. and Mays, E., 1998. The Merck Veterinary manual, 8th Ed., (Merck and Co Inc., White house station, NJ), Pp: 131-140.
3. Beelitz, P., Gobel, E. and Gothe, R., 1996. Endoparasites of donkeys and horses kept together in Upper Bavaria. Range of species and intensity of infestation. *Tier arztliche Praxis* 24: 471-475.
4. Belay, M., 2005. Preliminary study on helminithosis of equines in South and North Wollo zones. *Ethiopian Veterinary Journal* 9(2): 39-50.
5. Blood, D. C., Rodastitis, O. M. and Gay, C. C., 1989. Veterinary Medicine a text book of the disease of cattle, sheep, pigs, goats and horses 6th Ed., Balilliere Tindal, Pp: 1039-1044.
6. Blood, D.C. and Radostits, O.M., 1999. Veterinary Medicine, A text book of the disease of Cattle, Sheep, Pigs, Goats and Horses, 7th Ed., Bailliere Tindal, London, UK, Pp: 1064-1066.
7. Blood, D.C., Radiostitis, O.M., Gray and Hincheeliff, K.W., 2000. Veterinary Medicine a text of book of the disease of Cattle, Sheep, Pigs and Horses. 8th Ed., Harcourt Publisher Ltd, London, Pp: 1246-1253.
8. Boon, J., Kloosterman, N. and Vanderlander, T., 1984. The incidence of *D. viviparous* infection of cattle, Netherland, Surveys of sera collected in the field. Veterinary quarterly, 6th Ed., Pp: 13-17.
9. Bowman, D. D., 2003. Georgis, Parasitology for Veterinarians, 8th Ed., USA, New York Saunders, Pp: 166-168.
10. Burks, B.S., 1998. Parasitic pneumonitis in horses. *Compendium on Continuing Education for the Practicing Veterinarians* 20: 378-383.
11. Feseha, G., A. Mohammed and J.M. Yilma, 1991. Vermicular endoparasitism in donkeys of Debre Zeit and Menagesha, Ethiopia. Donkeys, mules and horses in tropical agricultural developement. Proc. Colloq. On donkeys, mules and horses. Eds. D. Fielding and R.A. Pearson, University of Edinburgh, Center for tropical veterinary medicine: UK., pp: 156-16.
12. Fraser, C. M., 2000. The Merck Veterinary Manual. A hand book of Diagnosis Therapy and Disease prevention and control for the Veterinarians 8th Ed., Merck and Co; Inc, Rahaway, NIT, USA, Pp: 851-852.
13. Fred, O. and Pascal, K., 2006. Extension approaches to improving the welfare of working equines. Kenya Network for Dissemination of Agricultural Technologies (KENDAT), Nairobi, Kenya, Pp: 1-28.
14. Hansen, J. and Perry, B., 1996. The Epidemiology, Diagnosis and control of Helminthes parasites of ruminants, ILRAD, Nairobi, Kenya, Pp: 83.
15. Herd, R. P., 1987. Epidemiology and control of equine strongylosis at new market. *Equine Veterinary Journal* 18: 447-452.
16. Howard, J., 1993. Current Veterinary therapy of food animal practice, 3rd Ed., W.B. Sunders Company Harcourt Brace Ivovanov, Pp: 673-675.
17. http://www.merial.ph/SiteCollectionDocuments/equine (Assessed on May 11, 2014).
18. http://www.studyblue.com/note/n/parasite-test…/1 (Assessed on May 11, 2014).
19. Johnson, M., MacKintosh, G., Labes, E., Taylor, J. and Wharton, A., 2003. "*Dictyocaulus* species. Cross infection between cattle and red deer". *New Zealand Veterinary Journal* 51(2): 93-98.
20. Junquera, P., 2014. *Dictyocaulus* species, parasitic lungworms of Cattle, Sheep, Goats and other Livestock. Biology, prevention and control. *Dictyocaulus filaria, Dictyocaulus viviparus, Dictyocaulus arnfieldi*. In: Merck Veterinary Manual, Merck and Co, Inc, Whitehouse Station, N.J., USA, Last Updated on Friday, January 03, 14:28.
21. Kamil A., Ibrahim N. and Yosef D., 2017. Prevalence of equine lungworm and associated risk factors in Sudie district, Oromia region, south eastern Ethiopia, African journal of agricultural research, Vol. 12(18), pp. 1526-1531, 2017, DOI: 10.5897/AJAR2016.11683, Article Number: FF0A79264107, ISSN 1991-637X, http://www.academicjournals.org/AJAR.
22. Power, D.G., 1992. The health of horse, Great Britain, Pp: 67-82.
23. Reinecke, R.K., 1989. *D.viviparous*, Veterinary Helminthology, professional publisher (Pty) Ltd, Pretoria, Pp: 178-180.
24. Rose, F.R. and Hodgson, R.D.S., 2000. Manual of Equine practice. 2nd Ed., USA, Saunders, Pp: 224.
25. Sapakota, C.R., 2009. A Report on Prevalence of Helminthes Parasites in Mules of Brick Kiln, Pp: 160.
26. Smith, P. B., 2009. Large animal internal medicine, 4th Ed., USA, Mosby Elsevier of Lalitpur District, Pp: 232-321.
27. Stuart, M.T, 2012. An overview of Lungworm Infection. In: Merck veterinary manual, Merck and Co; Inc, Whitehouse Station, N.J., USA, Last full review/revision on March.
28. Tihitna S., Basaznew B., Mersha C., Achenef, M., 2012. Ocuurrence of Lungworm Infection in Equines and their Associated Risk Factors. Glob. Vet. 8(1):35-38.
29. Urquhart, G.M., Armur, J.I., Dunn, A.M. and Jenninngs, and F.W., 1999. Veterinary parasitology 2nd Ed., Pp: 39-40.

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Major Transboundary Disease of Ruminants and their Economic Effect in Ethiopia

By Befikadu Seyoum & Endale Teshome
Haramaya University

Abstract- Trans-boundary animal diseases pose a serious risk to animal production and jeopardize international trade. The objectives of this paper were to give general overview about major trans boundary disease of ruminants and their economic effect in Ethiopia. Ethiopia has been facing devastating economic losses from major outbreaks of trans-boundary animal diseases (TADs) such as foot and mouth disease, contagious bovine pleuropneumonia, lumpy skin disease in cattle and pest des petites ruminants, contagious Caprine pleuropneumonia, sheep and goat pox, and brucellosis in small ruminants. These diseases impose major economic costs and risks to the country, the neighbors, and trading partners. Even though both the direct and indirect impact of these diseases causes devastating economic losses to the country, the indirect effect is more serious. The trade implication of TADs can cause a greater economic impact than the direct production losses themselves. The trade ban due to the existence of these major trans-boundary disease and other negative domestic impacts on agriculture and other sectors, can be raised as an example. Among other factors affecting the economic benefit of the country from livestock sector, increased outbreaks of highly contagious trans-boundary animal diseases (TADs) is considered as major cause of economy loses.

Keywords: ethiopia, economic lose, livestock, trans-boundary disease.

GJMR-G Classification: NLMC Code: WA 360

Strictly as per the compliance and regulations of:

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Befikadu Seyoum [α] & Endale Teshome [σ]

Abstract- Trans-boundary animal diseases pose a serious risk to animal production and jeopardize international trade. The objectives of this paper were to give general overview about major trans boundary disease of ruminants and their economic effect in Ethiopia. Ethiopia has been facing devastating economic losses from major outbreaks of trans-boundary animal diseases (TADs) such as foot and mouth disease, contagious bovine pleuropneumonia, lumpy skin disease in cattle and pest des petites ruminants, contagious Caprine pleuropneumonia, sheep and goat pox, and brucellosis in small ruminants. These diseases impose major economic costs and risks to the country, the neighbors, and trading partners. Even though both the direct and indirect impact of these diseases causes devastating economic losses to the country, the indirect effect is more serious. The trade implication of TADs can cause a greater economic impact than the direct production losses themselves. The trade ban due to the existence of these major trans-boundary disease and other negative domestic impacts on agriculture and other sectors, can be raised as an example. Among other factors affecting the economic benefit of the country from livestock sector, increased outbreaks of highly contagious trans-boundary animal diseases (TADs) is considered as major cause of economy loses. To obtain, expected value from animal sub-sector government policy and roles of Veterinary Services in function of TADs control, rapid detection and early response need improvement.

Keywords: ethiopia, economic lose, livestock, trans-boundary disease.

I. Introduction

Trans-boundary animal diseases (TADs) have been described as those diseases that are of significant economic, trade and food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries [1]. These diseases are highly contagious and have the potential for rapid spread, irrespective of national borders, causing serious socio- economic consequences [2]. With increasing globalization, the persistence of trans-boundary animal diseases(TADs) in the world poses a serious risk to the world animal agriculture and food security and jeopardizes international trade [3].

Author α: College of Veterinary Medicine, Haramaya University.
e-mail: befikaduseyoum92@gmail.com
Author σ: School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University.
e-mail: endaleteshome23@gmail.com

In recent decades, the world has been facing devastating economic losses to livestock farmers from major outbreaks of TADs, such as foot and mouth disease (FMD), in Europe, classical swine fever in the Caribbean and Europe(1996–2002), render pest (RP) in Africa in the 1980s, pest des petites ruminants in India and Bangladesh, contagious bovine pleura pneumonia in Eastern and Southern Africa (late 1990s), as well as Rift Valley fever in the Arabian Peninsula (2000)[4].

In Ethiopia, the aggregate annual economic losses from such animal diseases through direct mortality and reduce productive and reproductive performance were estimated at US$ 150 million, equivalent to three billion Ethiopian birr per year [5]. The overwhelming majority of morbidity and mortality is caused by a finite set of common and predictably occurring disease problems that are conditioned by local geography, climate, and animal management system [6].

In the past two to three decades, public health authorities in industrialized countries have been faced with an increasing number of food safety problems. The situation is equally serious in developing countries. In addition to known food borne diseases, public health communities are being challenged by the emergence of new or newly recognized types of food-borne illnesses, often with serious health and economic consequences. For example, result of the BSE crises, the world suffered economic losses of more than 10 billion U.S. dollars [7].

Ethiopia has estimated livestock population of 57.4 million cattle, 58.6 million sheep and goat [8].However, Livestock production system, particularly in pastoral areas, is mainly constrained by rampant animal disease and seasonal feed and water shortages, which can be up to a level of losing the entire livelihood of the pastoral households. Besides the direct losses incurred by the disease, the trans-boundary nature of most diseases, with potential risk of introduction of notifiable diseases, which are not yet reported from Ethiopia, and its high rate of transmissibility among different herds and/or between domestic animals and wildlife increases the risk. In particular concern to South Omo Zone area apart from other areas of the region, is that it shares boundaries with other countries, Kenya and South Sudan, and there is no real avoidance of movement of animals among the different pastoral agro-pastoral communities in these different countries, which makes the situation most favorable for the introduction and/or

transmission of trans-boundary diseases; some are known to be found in neighboring countries ,but, not in Ethiopia, like East Coast fever, Rift Valley fever, and Nairobi sheep disease [9].

In Ethiopia limited works has been done on this disease so far and few works have been reported on risk factors assessments, epidemiological aspects, seroprevalence and financial impacts in selected areas of the country. Therefore, the objective of this paper is to review major trans-boundary disease of ruminants and their economic effect in Ethiopia.

II. Literature Review

a) *Epidemiological Feature of Major Trans-boundary Diseases in Cattle*

Ethiopia is a resourceful country bestowed with the largest livestock resource in the Africa continent [10] with the potential to export substantial numbers of live animals and their products. Livestock is central to the Ethiopian economy, contributing for 20% of the GDP, supporting the livelihoods of 70% of the population and generating about 11% of annual export earnings [11]. However, the livestock sub-sector's contribution to the economy and foreign currency earnings in particular, is very low as per the country expectation and potential of the sectors. Some of the major factors contributing to the poor performance of the livestock sub-sector include the prevalence of highly contagious trans-boundary animal diseases (TADs) such as foot-and-mouth disease (FMD), lumpy skin disease (LSD) and contagious bovine pleura pneumonia (CBPP). These diseases continue to hinder international trade in live cattle and their products seriously in an era of globalization. Public concern is growing regarding the rapid trans-boundary spread of animal diseases through animals and animal products have forced importing countries to apply strict measures so that animals and their products exported should meet international sanitary phytosanitary (SPS) requirements [12].

i. *Foot and mouth disease*

Foot and Mouth Disease (FMD) also known as Aphtous fever, is a major global animal health problem [13]. It ranks first among the notifiable, list of infection animals disease. It is the most contagious trans-boundary animal disease (TAD) affecting cloven hoofed animals of domesticated and wildlife. Among species of the domesticated animals; cattle, sheep, goats, pigs and buffalo are susceptible. It is caused by RNA virus of genus Aphthous virus known as foot and mouth disease virus. There are seven recognized serotypes of FMD (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3), which differ in distribution across the world [14].

In Ethiopia, although its level of prevalence may have significant variations across the different farming systems and agro ecological zones of the country, FMD is endemic and known for its wider distribution. The records of the Ministry of Agriculture and Rural Development (MOARD) from 1997 to 2006 showed that FMD outbreak occurred everywhere throughout the country with highest incidence in the central part [15]. The sero-prevalence of FMD among Borana pastoral cattle in 2008 was reported to be 24.6% (14). Another study that covered broader areas of the country showed sero-positivity of 44.2% with 1.6% and 8.9% mortality and case fatality rates [16].

Endemic distributions of five of seven serotypes of FMDV are maintained in Ethiopia: serotype O, serotype A, serotype C, serotype SAT 2, and serotype SAT 1. Infection or vaccination against one serotype does not provide protection against the other serotypes [17; 18; 15].

The disease was first recorded in Ethiopia in 1957 when serotypes O and C were found [19; 20]. FMD is transmitted by a variety of methods between herds, countries and continents. In endemic areas, the most important method of spread is probably by direct contact between animals moving across state and national boundaries as trade or nomadic cattle. The routes of spread include inhalation of aerosolized virus, ingestion of contaminated feed, and entry of the virus through skin abrasions or mucous membranes [21].

In Ethiopia, it is believed that infected animal's movement is common method of spreads of FMD. The movement of animal health workers and artificial inseminators from one farm to the other without taking into consideration the disease situation suggest that these could have been suspected in a spread of virus. On top of these, poor hygienic conditions on the farms notably the absence of foot bath, management practices like failure to isolate infected animals from the healthy ones and the absence of quarantine for newly introduced animals are also open doors for introduction of the virus to a farm [22;23]. In the most favorable circumstances, it is now estimated that sufficient virus to initiate an infection can be wind borne as far as 250 km (156 miles) [24].

The morbidity rate in outbreaks of FMD in susceptible animal's involvement and complications such as secondary infection, exposure or malnutrition can rapid approach 100% but some strains are limited in their infectivity to particular species [25]. However, the case fatality is generally very low, about 2% in adults and 20% in young stock [26].

ii. *Lumpy skin disease*

Lumpy skin disease is one of the most economically significant trans-boundary, emerging viral diseases. It is a disease with a high morbidity and low mortality rate and affects cattle of all ages and breeds [27]. The disease is caused by Neethling virus prototype strain classified in the genus Capri poxvirus of family Poxviridae. It is acute to sub-acute infectious disease and cattle strain of *Capri poxvirus* does not infect and

transmit between sheep and goats [28; 29]. Lumpy skin disease occurs in different ecological and climatic zones and extends its boundaries to different areas [30]. It is currently endemic in most African countries and expanded to Middle East region [31].It has been endemic in Africa for more than 70 years occurring in a wide range of ecotypes. In Ethiopia the disease was first observed in the western part of the country (southwest of Lake Tana) in 1983. Recently, Lumpy skin disease is found almost inall the regions and agro ecological zones of the country [32 and33].

Lumpy skin disease is mechanically transmitted by different types of biting and blood feeding arthropods [34]. Direct contact could be a minor source of infection. LSDV occurs in cutaneous lesions, saliva, respiratory secretions, milk and semen. The virus is very resistant to inactivation, surviving in desiccated crusts for up to 35 days, and can remain viable for long periods in the environment and this favors its transmition for prolong period [35].

Outbreaks of LSD are highly associated with seasonal peak of mechanical vectors in wet and warm weather conditions in Ethiopia. Therefore, morbidity and mortality rates for LSD vary greatly in different endemic areas depending on the severity of strain, prevalence of insect vectors and susceptibility of the host.During its occurrence it causes significant economic problems as a result of reduced milk production, beef and draft animals' loss, abortion, infertility, loss of condition and damage to the hide [36 and 28].

iii. *Contagious bovine pleuropneumonia*

Contagious Bovine Pleuropneumonia (CBPP) is a highly infectious cattle disease, which is caused by *Mycoplasma mycoides subsp. mycoides SC* (small colony, bovine biotype), is one of the major constraints to cattle-raising and trade in Africa. Contagious bovine pleuropneumonia is widespread in pastoral areas of African countries [37]. According to Tambi *et al*, [38], Ethiopia is one of countries in which CBPP is endemically maintained all over the country with 25% morbidity and more than 10% mortality rate. The economic effects of CBPP in a cattle population are enormous often resulting into heavy losses. In Zambia CBPP devastated livestock production and reduced the cattle from 650,000 herds in 1997 to about 400,000 herds in 2006 [39].

Cattle movements are responsible for the transmission of the CBPP from one herd, region or country to others. Close, repeated contact is generally thought to be necessary for transmission. In addition to contact *M. mycoides SC* can also spread through aerosol route if the climatic conditions are favorable [37,40].

b) Epidemiological Feature of Major Trans-boundary Diseases in Small Ruminant

Small ruminants form an integral and important component of pattern of animal production. Because of factors such as their low cost, little feed requirement, manageable quantities of products and high reproductive rate, keeping sheep and goats is preferable than large ruminants[41].

Development of small ruminant production in Ethiopia is constrained by widely distributed disease, lack of feed and improper management. Among diseases contributing to the poor production of small ruminants, highly contagious trans-boundary animal diseases (TADs) such as Pest des petites ruminants (PPR), Sheep and Goat pox, Brucellosis and Contagious Caprine Pleuropneumonia (CCPP) are found to be common in the country [42]. These diseases continue to hinder international trade of live goat and sheep and their products seriously in an era of globalization [43].

i. *Peste des petites ruminants*

Peste des Petits Ruminants (PPR) is an acute, highly contagious, infectious and notifiable trans-boundary viral disease of domestic and wild small ruminants [44]. *Pest des petitis Ruminants virus* (PPRV), the causative agent, belongs to the genus Morbillivirus of the family Paramyxoviridae [45]. Currently, PPR occurs in most African countries situated in the wide belt between the Sahara and the Equator (including the Sudan, Ethiopia, Kenya and Uganda), the Middle East, and the Indian subcontinent [46]. It is a disease that threatens the national food security of affected countries and also results in economic losses due to sanitary related trade embargoes. The disease has high morbidity and mortality rates and significant economic impacts in developing countries [47].

There are often a number of risk factors that contribute to the overall risk of disease transmission in a particular community, production system or value chain [48]. These risk factors are often quite simple attributes of the sub-population such as the amount of movement, exchange of animals between households and flocks as a result of social practices and changes in economic conditions that exhibit seasonal patterns, distance from services, lack of large scale vaccination campaigns, altitude, season, and inter-species contact or interaction with wildlife [26]. In Ethiopia the morbidity and mortality rates from PPR can be up to 100% in severe outbreaks. In milder outbreaks, morbidity is still high but the mortality rate may be closer to 50% [49].

ii. *Contagious caprine pleuropneumonia*

Contagious Caprine pleuropneumonia (CCPP) is a highly fatal Caprine disease firstly reported in Algeria in 1873 [50]. It is a devastating disease of goats [51] included in the list of notifiable diseases of the Office International des Epizooties (OIE) and caused by *Mycoplasma capricolum subsp. Capripneumoniae*

(Mccp) [52]. It is a major threat to the goat farming industry in developing countries [53] and is pandemic in Africa, the Middle East and Asia [54].

This disease is the major trans-boundary disease in Ethiopia and characterized by fibrinous pleuro-pneumonia with increased straw colored pleural fluid in the infected lung [55]. The disease has been reported to affect only goat species and does not infect sheep [56]. In Ethiopia CCPP has been suspected to occur for a long period, especially in areas found at the vicinity of endemic areas of Kenya and Sudan. It has been confirmed to be present in Ethiopia since 1980s. The disease has been reported from almost all regional states of Ethiopia [57]. It is more prevalent in the arid and semi-arid low land of rift valley, Borena rangelands, South Omo, Afar and other pastoral areas of Ethiopia where about 70% the national goat population are existed. Sero prevalence rate from different authors varies from 6% to 77% in different parts of the countries [58].

CCPP is transmitted directly by an aero genic route through contaminated droplets. The outbreak of the disease follows the introduction of an infected animal into a group of susceptible goats [59]. Disease outbreak may occur after heavy rain, animal transportation over a long distance, poor climatic conditions and primary infections. This is because recovered carrier begins shedding the infectious agent during stress [56]. CCPP is a major cause of economic losses in the goat industry globally as these intracellular bacteria can infect domestic as well as wild breeds of goat [58,60,61), with 100% morbidity and 60–80% mortality rates [55].

iii. *Sheep and goat pox*

Sheep and goat pox (SGP) is viral diseases of sheep and goats characterized by fever, generalized papules or nodules, vesicles (rarely), internal lesions (particularly in the lungs) and death [62]. The virus that causes SGP is a *Capri poxvirus*, one of the largest viruses (170-260 nm by 300-450 nm) [63]. There is only one serotype of SGP virus (SGPV). Various strains of SGPV cause disease only in sheep, others only in goats, and some in both sheep and goats [64].

Sheep and Goat Pox (SGP) is one of the most important diseases of sheep and goats in Ethiopia following Pest des Petites Ruminants (PPR) and Contagious Caprine Pleuropneumonia (CCPP). This disease is among the commonest of the diseases that affect small ruminants entailing a huge economic loss and Office International des Epizooties (OIE) listed as trans- boundary disease of animal affecting the economy of the country through limiting international trade of animals and their products [65]. Morbidity rates in indigenous breeds can be 70-90% with mortality ranging from 5-10%. Mortality and morbidity rates in newly imported animals can reach 100% [66].

The most likely manner for SGP to enter a new area is by introduction of infected animals. Restrictions on the movement of animals and animal products (meat, hair, wool, and hides) are important to prevent SGP [67].

iv. *Brucellosis*

Brucellosis is an infectious bacterial disease that's caused by different species of Brucella. Each Brucella spp. has a preferred natural host that serves as a reservoir. Brucellosis in small ruminants is caused mainly by *B.melitensis*[68]. Brucella infection follows a very strict, host-related hierarchy of pathogenicity [69]. Thus, goats are the natural hosts of *B.melitensis* and sheep are preferred hosts of the pathogen [70]. Prevalence rates vary throughout and even within the same geographical zones operating different husbandry techniques [71].

This disease is common trans-boundary disease in Ethiopia that cause huge economic loses and trade restriction [72]. The herd level important risk factors for small ruminants brucellosis identified are large flock size, addition of new animals from unscreened sources, intensive system of management, history of abortion, grazing communal pasture, keeping sheep and goat together [73].

In Ethiopia, studies conducted on brucellosis in small ruminants indicated that; prevalence proportions of 1.5% in sheep and 1.3% in goats in the central highlands [74], prevalence proportions of 15% in sheep and 16.5% in goats in the Afar region[75] and 1.6% in sheep and 1.7% in goats in the Somali region [76]. The presence of this disease has also been reported in the Southern Nations, Nationalities and Peoples' Regional State and pastoral areas of Borana[77]. The disease is known by its high mortality rate in lambs and kids [78].

c) Factors Spread Trans-boundary Diseases

Traditionally, trade and travel have been instruments for disease spread. Now, changing climate across the globe is adding to the misery. Climate change is creating new ecological platform for the entry and establishment of diseases from one geographical region to another. Several new trans-boundary diseases emerge, and old diseases reemerge, exhibiting increased chances for unexpected spread to new regions, often over great distances[79].

Other common ways of spreading of trans-boundary diseases to a new geographical location are through entry of live diseased animals and contaminated animal products, importation of contaminated biological products such as vaccines or germplasm or via entry of infected people (in case of zoonotic diseases). Even migration of animals and birds, or natural spreading by insect vectors or wind currents, could also spread diseases across geographical border [80, 2].

d) Economic Effect of Trans-boundary Diseases

TADs impose major economic costs and risks to infected countries, their neighbors, and trading partners. The varying impact of TADs among stakeholders and the threat to existing and potential trade in wealthier countries complicates the question of appropriate control. For all livestock producers, the threat of TADs increases the risk of lost production and impacts on livelihood, increasing vulnerability to poverty particularly for small-scale producers. The impact of TADs and of their control varies depending on the virulence of the disease, number of animals at risk, dependency on livestock for livelihood, and method of control [81].

Direct effects of TADs on livestock productivity include reduced feed intake, changes in digestion and metabolism, increased morbidity and mortality and decreased rates of reproduction, weight gain, reduced draught power and manure and milk production. These have aggregate effects that limit economically important herd-management decisions regarding animal selection and optimal longevity. Many TADs have mortality rate 50-90% in susceptible animals [2]. For instance, in the wake of the render pest pandemic of 1887 was estimated to have killed about 90% of Ethiopian cattle and more than 10 million cattle on the continent as whole [82]. On other hand, the socio-economic significance of PPR is a result of heavy losses at production level and market effects along the value chain. It is estimated that 10% of the total impact of the disease is on trade and public expenditure and 90% on herd productivity [83]. In Ethiopia, FAO estimated that losses associated with PPR reached an average of US$ 375 per flock, with an average of 143 small ruminants per flock (an average loss of more than US$ 2 per animal) [84].

Indirect losses are often less visible than the obvious effects of clinical disease but may be equally or more important in their overall economic impact. Disease control has costs including vaccine purchase, vaccine delivery, disease surveillance, laboratory diagnosis and testing, quarantine and movement management, expensive antibiotic treatment [85]. Movement restrictions and local quarantines mean the closure of livestock markets and reduced or no opportunities for sale of live animals and possibly meat and other products. In addition to the measurable economic impact on a national economy the inability to sell one steer or some sheep or goats can bring severe hardship to a pastoral family with no other income of sources of support [86).

The trade implication of TADs can cause a greater economic impact than the direct production losses themselves [87]. The trade ban From February 1998 to April 1999, by Saudi Arabia and Other Gulf states of live animals from the Horn due to Rift Valley fever outbreak in Kenya is estimated to have cost US $32 million in lost exports and other negative domestic impacts on agriculture and other sectors such as transport and services [72]. In addition to this FMD is one of the major diseases in Ethiopia that hampering export of livestock and livestock products to the Middle East and other African countries, in which the country lost more than US$14 million [88]. These bans have disrupted trade patterns and dealt severe economic blows to the region. Following the 1998 ban, for instance, exports from the port of Berbera in Somaliland ,a major export point for Ethiopian livestock from Somali Region, dropped from nearly three million head in 1997 to just over one million in 1998, representing an export loss of approximately $100 million. As a result livestock prices in Ethiopia and Somalia fell by approximately 30 percent [89]. Traders have found ways of circumventing trade bans, for instance by exporting livestock to Yemen for re-export to Saudi Arabia, but, such measures do not address the root problem of SPS concerns from Gulf States. Indeed, the length of the bans suggests that Saudi Arabia and other Gulf States lack confidence in the Horn's disease surveillance and regulatory systems. The most recent ban was finally lifted in October 2009 [90].

Trans-boundary animal diseases have significant and measurable effects on human welfare in developing countries. Particularly in pastoral societies, livestock contribute directly or indirectly to food security and nutrition a source of protein, micronutrients, animal power and tradable asset [91].

e) Prevention and Control of Trans-boundary Diseases

Techniques and tools for the control of major TADs are already existed. They have been used successfully in many countries that most have been eradicated from or prevented from infecting North America, much of Europe, much of Southern Africa, Australia and New Zealand. In these countries there is now nothing other than sporadic and localized outbreaks which are usually quickly dealt with [92,93].

The following techniques are used for prevention and control of Trans-boundary disease these are: Preventing incidence of trans-boundary diseases and disease transmitting vectors and minimizing the movement of animals across the borders is essential. Also, prompt practice of quarantine protocol would reduce many trans-boundary diseases [94], Reducing man-made disasters that have adverse implications on climate [79], Interrupting the human-livestock wildlife transmission of infections, Breaking the cycle of disease transmission [95], Establishing regional biosecurity arrangement with capacity for early disease warning system for surveillance, monitoring and diagnosis of emerging disease threats [96], Undertaking animal breeding strategies to create disease resistant gene pools[97], Strengthening government policies to enhance agricultural/animal research and training, and

technology development [98], Ensuring appropriate preparedness and response capacity to any emerging disease and Intensification of international cooperation in preventing spread of TADs [96,99].

In addition to this the International Organization for Animal Health recognizes the improvement of national standards in animal health, should be parallel to the improvement of veterinary services in terms of increasing the capacity of early epidemiological detection, diagnosis and control of TAD [59]. Roles of Veterinary Services in function of TADs control, rapid detection and early response are crucial for the control of TADs. This function is highly linked with the transparent and timely notification of disease for effective control of such diseases at source. To achieve those, national Veterinary Services (VSs), as a public good, play quite important roles and need to be strengthened in various areas of their important mission, including human and financial resources, legislation for animal health and diagnostic and surveillance capability, and disease control measures. The World Organization for Animal Health, has supported Member Countries/ Territories to evaluate Performance of Veterinary Services, by applying the OIE Public Veterinary Service tool, which is designed to assist VSs to identify gaps and weaknesses regarding their ability to comply with OIE International Standards on animal health, to form a shared vision with stakeholders and to establish priorities and carry out strategic in control of TADs [100].

III. Conclusion and Recommendation

Trans-boundary diseases is becoming ever more important since it can spread throughout an entire region, impact trading partners and commerce, tourism, consumer confidence, and occur in distant countries, with devastating economic and livelihood consequences. With the globalization of trade and the increasing movements of people, these major crises will continue to menace the global animal and human populations. In Ethiopia the Livestock sub-sector's contribution to the economy and foreign currency earnings in particular, is very low as per the country expectation and potential of the sectors. Some of the major factors contributing to the poor performance of the livestock sub-sector include the prevalence of highly contagious trans-boundary animal diseases (TADs) such as foot-and-mouth disease (FMD), lumpy skin disease (LSD) and contagious bovine pleura pneumonia (CBPP) in cattle and pest des petites ruminants, contagious Caprine pleuropneumonia, sheep and goat pox, and brucellosis in small ruminants. These diseases continue to hinder international trade in live animal and their products seriously in an era of globalization.

Based on above conclusive remarks the following recommendations are forwarded:

- ✓ Strategies to improve veterinary service delivery by field staff and laboratories should be designed.
- ✓ In the medium to long term, health facilities and laboratories need to be better equipped and the number of veterinary staff in the public and private sectors should be increased.
- ✓ Rapid detection and early response are crucial for the control of TADs at source and national level.
- ✓ Government policies to enhance agricultural/animal research and training, and technology development should be strengthened.
- ✓ Animal movement from region to region should be controlled and quarantine should also be established.

References Références Referencias

1. Otte, M.J., Nugent, R., McLeod, A. (2004). Trans-boundary animal diseases: Assessment of socio-economic impacts and institutional responses. Livestock policy discussion paper No.9.FAO, Rome, Italy.
2. Seine, Z.C. (1998). Perspectives of Veterinary Services in Latin America in the face of globalization. Second FAO Electronic Conference on Veterinary Services, FAO.
3. FAO (1996). Prevention and control of transboundary animal diseases. Report of the FAO Expert Consultation on the Emergency Prevention System (EMPRES) for Trans boundary Animal and Plant Pests and Diseases (Livestock Diseases Programme) including the blueprint for global rinderpest eradication. 22–29 July 1996, Rome, Italy. Pp: 1-19.
4. Balkhy, H.H., Memish, Z.A. (2003). Rift Valley fever: an uninvited zoonosis in the Arabian Peninsula. *Int J Anti microb Agents.* 21: 153–157.
5. Berhanu, A. (2002).Welcome address: animal health and poverty reduction strategies. In: Proceedings of the 16th Annual Conference of the Ethiopian Veterinary Association (EVA), held 5–6 June, 2002, Ghion Hotel, Addis Ababa, Ethiopia, pp: 117-137.
6. EARO (1996). Animal health research program strategy (draft document). Addis Ababa, Ethiopia. pp: 3-23.
7. Verebeke, W. (2001).Consumer reactions and economic consequences of the BSE crisis. Verh.K. Acad.Geneeskd.Belg. 63: 483–492.
8. CSA (2016). Agricultural sample survey 2010/2011, Report on livestock and livestock characteristics. Central Statistical Agency of Ethiopia. Statistical Bulletin 505.
9. Pepin, M., Bouloy, M., Bird, B., Kemp, A., Paweska, J. (2010). Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet. Res. 41: 13-0593.

10. FAOSTAT (2007). Database (http:/faostat.fao.org). Accessed July 2011, Rome, Italy.
11. SPS-LMM (2010). Focus on Ethiopia's Meat and Live Animal Export. Trade Bulletin 1.
12. GebreMariam, S., Amare, S., Baker, D., Solomon, A. (2010). Diagnostic study of live cattle and beef production and marketing. Constraints and opportunities for enhancing the system. Addis Ababa, Ethiopia. pp: 4-21 20.
13. Hirsh, C.D., MacLauchlan, N.J., Walner, R.L. (2004). Veterinary Microbiology. 2nd ed. Black Well Science, pp: 341.
14. Mekonen, H., Beyene, D., Rufael, T., Feyisa, A., Abunna, F. (20011). Study on the prevalence of Foot and Mouth Disease in Borana and Guji Zones, Southern Ethiopia. Res. 4: 293-296.
15. Ayelet, G., Mahapatra, M., Gelaye, E., Gebreegziabher, B., Rufael, T., Sahle, M., Ferris N.P, Wadsworth, J., Hutchings, G.H., Knowles, N.J. (2009). Genetic Characterization of Foot-and-Mouth Disease Viruses, Ethiopia, 1981–2007. *Trans. Emerg. Infect.* Dis. 15: 1409-1417.
16. Negussie, H. H., Mazengia, M., Taye, S., Tassew, A. (2011). Incidence of foot and mouth disease and its effect on milk yield in dairy cattle at Andassa dairy farm, Northwest Ethiopia. Agri. Bio. J. N. America, 1: 543-565.
17. FAO (2007). Foot-and-Mouth Disease. Situation worldwide and major epidemiological events in 2005-2006.Intelligence information intervention1. Rome, Italy.
18. Rufael, T., Catley, A., Bogale, A., Sahle, M., Shiferaw, Y. (2008). Foot and Mouth Disease in Borana Pastoral system, Southern Ethiopia. Trop. Anim. Hlth. Prod. 40: 29–38.
19. Martel, J.L. (1974). Foot and mouth disease in Ethiopia. Distribution of viral serotypes. Rev. d'Elevageet de Med. Vet. des Pays Trop., 27: 169-175.
20. Martel, J.L., Gallon, C. (1975). Comparative serological study of the principal Foot-and mouth disease virus strains isolated in Ethiopia, 1969-1974. Rev. sci. tech. Off. int. Epiz 28: 287-29.
21. Aftosa, F. (2007). Foot and Mouth Disease, The center for food security and public health, Iowa State University. College of Veterinary Medicine. http://www.cfsph.iastate.edu. Accessed April 2012.
22. Quinn, P.J., Markey, B.K. (2003). Textbook of Concise review of veterinary microbiology.6th Ed USA, Blackwell Publisher, pp: 126. 23
23. Dejene, A. (2004). Foot and Mouth outbreak investigation in small holder and Commercial Dairy Farms in and around Addis Ababa. DVM Thesis, Faculty of Veterinary Medicine, Debre Zeit, pp: 30-39. 19
24. Radostits, O. M., Gay, C.C., Hinchcliff, K. W., ConsTable, P. D. (2006). Veterinary Medicine: A textbook of diseases of cattle, horses, sheep, pigs and goat. 10th Ed. WB Saunders Co., Philadelphia, USA.
25. MacLauchlan, N.J., Dubovi, E. (2011). FENER'S Veterinary Virology. 4th ed.USA.Acadamic Press, pp: 32.
26. Radostits, O.M., Blood, D.C., Gay, C.C. (2007).Veterinary Medicine, A Text Book of the Disease of Cattle, Sheep, Goats, Pigs and Horses. 8th ed. London: BalliereTindall, pp: 1223-1227.
27. Tuppurinen, E.S.M., Oura, C.A.L. (2011).Review: Lumpy Skin Disease: An Emerging Threat to Europe, the Middle East and Asia, Institute for Animal Health, Pirbright, Surrey, UK. pp: 13-24.
28. CFSPH, (2008) .The Center for Food Security and Public Health, Iowa State University, College of Veterinary Medicine and Institution of International cooperation in Animal Biologics, an *OIE colla. cent.* 26: 675-684.
29. OIE (2010). OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Lumpy skin disease.Chapter2.4.14.pp:768-778.
30. Davies, F.G. (1991). Lumpy skin disease of cattle: A growing problem in Africa and Near East. *Wor.Anim. Rev.* 68: 37-42.
31. Tuppurinen, E.S.M., Oura, C. A. L. (2012). Review: Lumpy Skin Disease: An Emerging Threat to Europe, the Middle East and Asia, Trans. Emerg. Dis. 59: 40–48.
32. Gari, G., Waret-Szkuta, A., Grosbois V., Jacquiet, P., Roger F. (2010). Risk factors associated with observed clinical lumpy skin disease in Ethiopia. Epidemiol. Infect. 138: 1657–1666.
33. Getachew, G., Grosbois, V., Waret-Szkuta, A., Babiuk, S., Jacquiet, P., Roger, F. (2012). Lumpy skin disease in Ethiopia: Seroprevalence study across different agro-climate zones.p:1
34. Chihota, C.M., Rennie, L.F., Kitching, R.P., Mellor, P.S. (2001). Mechanical transmission of lumpy skin disease virus by Aedesaegypti (Diptera. Culicidae). *Epidemiol. Infect.* 126: 317–321.
35. Rovid, S.A. (2008a). Lumpy Skin Disease. The center for food security and public health, Iowa State University. College of Veterinary Medicine. http://www.cfsph.iastate.edu. Accessed at April 2012.
36. Getachew, G., Waret-Szkuta, A., Grosbois, V., Jacquite, P. (2010). Risk Factors Associated with observed clinical lumpy skin disease in Ethiopia. PhD thesis. PP: 68-84
37. Masiga, W.N., Domenech, J., Windsor, R.S. (1996). Manifestation and epidemiology of contagious bovine pleuropneumonia in Africa. In Animal mycoplasmoses and control. Rev. sci. tech. Off. int. Epiz., 15 : 1283-1308.
38. Tambi, E.N., Maina, O.W., Mukhebi, A.W. and Randolph, T.F. (1999). Economic impact

assessment of Rinderpest control in Africa. Sc. Tech. Rev. Off. Int. Epiz. 18: 458-477. 24.
39. Hudeckova, R., SovJAK, R. (2006). Strategy of the contagious bovine pleuropneumonia eradication in the Western province of Zambia. Agric. Trop. EtSubtrop. 39: 233-23
40. Takele, G. (1998). Epidemiological Survey of CBPP in Awi and Western Gojam zone of Amhara Region and Comparison of CFT and C-ELISA for the Diagnosis of CBPP. MSc thesis Addis Ababa University and Free University of Berlin.
41. CSA (2013). Agricultural Sample Survey, 2012/13 (2005 E.C.), Volume II: Report on Livestock and livestock characteristics (Private peasant holdings). Statistical Bulletin 570. Addis Ababa: Central Statistical Agency (CSA), Federal Democratic Republic of Ethiopia.
42. Alemayehu, T. (2015). *Assessment on the Challenges of Meat Export in Ethiopia* (Doctoral dissertation, St. Mary's University).
43. Hailemariam, T., Legese, G., Alemu, D., Negassa, A. (2009). Market Structure and Function for Live Animal and Meat Exports in Some Selected Areas of Ethiopia. Research Report 79.
44. Abubakar, M., Ali, Q., Khan, HA. (2008). Prevalence and mortality rate of peste des petits ruminant (PPR): possible association with abortion in goat. *Trop Anim Health Prod.* 40: 317-321
45. Murphy, FA. Gibbs, EPJ., Horzinck, MC., Studdert, MJ. (1999). Paramyxoviridae. In Veterinary Virology. 3rd edition. USA, Academic Press, pp: 411-42
46. Osama, IM. (2010). Studies on Peste Des Petits Ruminants, Disease in the White Nile State, the Sudan. MVSc Thesis, Council of Animal Resources Research, Sudan Academy of Sciences, Cartum, Sudan.
47. Wambura, P. N. (2000). Serological evidence of the absence of peste des petits ruminants in Tanzania. Vet. Rec. 146: 473-4.
48. Elsawalhy, A., Jeffrey, C., Mariner, J., Dickens, C., Henry, W., Samuel, W., William, O., Phillip, T. (2010). Pan African Strategy for the Progressive Control of PPR. Bull. Anim. Hlth. Prod. Afr. 265: 185-193.
49. Anna, R.S, Roth, J. A. (2006). Emerging and Exotic Diseases of Animals, 3rd ed. Iowa State University, College of Veterinary Medicine, Ames, Iowa USA.
50. McMartin, DA., MacOwan, KJ.and Swift, LL. (1980). A century of classical contagious pleuropneumonia: fromo original description to aetiology. British Vet. J. 136: 507–515.
51. Bascunana, CR., Mattsson, JG., Bolske, G., Johansson, KE. (1994). Characterization of the 16S rRNA genes from Mycoplasma sp. strain F38 and development of an identification system based on PCR. *Jour Bacterio.*176: 2577–2586.
52. Manso-Silvan, L., Dupuy, V., Chu, Y., Thiaucourt, F. (2011). Multi-locus sequence analysis of Mycoplasma capricolum subsp. capripneumoniae for the molecular epidemiology of contagious caprine pleuropneumonia. Vet. Res., 42: 1–10. 21
53. Lorenzon, S., Wesonga H., Ygesu L., Tekleghiorgis T., Maikano Y., Angaya M., Hendrikx P. , Thiaucourt, F., (2002). Genetic evolution of Mycoplasma capricolum subsp. capripneumoniae strains and molecular epidemiology of contagious caprine pleuropneumonia by sequencing of locus H2. Vet. Micro. 85: 111–123.
54. Nicholas, R., Churchward, C. (2012). Contagious caprine pleuropneumonia: a new aspencts of an old disease. Trans. Emerg. Dis. 59: 189–196.
55. Rurangirwa, FR., McGuire, TC. (2012). contagious caprine pleuropneumonia: Diagnosis and control. Online retrieved from http://www.fao.org/wairdocs/ilri/x5473b/ x5473b11.htm#Top of Page.
56. Thiaucourt, F., Bolske, G. (1996). Contagious caprine pleuropneumonia and other pulmonary of int .Epiz. 15: 1397–1414.
57. Yigezu, L. M., Tarik, S., Aylet, G., Roger, F. (2004): Respiratory Mycoplasmoses in small Ruminant. Ethio.vet. 8:67-74
58. Nicholas, R. A. J. (2002): Contagious caprine pleuropneumoniae. In: Recent Advances in Goat Diseases. International Veterinary Information Service. Ithaca, UK. 22
59. OIE (2009).Contagious caprine pleuropneumonia. Online retrieved from: http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Contag ous_caprine_pleuro_FINAL.pdf.
60. Arif, A., Schulz, J., Thiaucourt, F., Taha, A., Hammer, S. (2007). Contagious caprine pleuropneumonia outbreak in captive wild ungulates at al wabra wildlife preservation, state of Qatar.*J. Z. Wild. Medi.* 38: 93–96.
61. Ostrowski, S., Thiaucourt, F., Amirbekov, M., Mahmadshoev, A., Manso-Silvan L., Dupuy V., Vahobov, D., Ziyoev, O., Michel, S. (2011). Fatal outbreak of Mycoplasma capricolum pneumonia in endangered markhors.Emerg.Infec. Dis. 17: 2338–2341.
62. Chaudhary, SS., Pandey, KD., Singh, RP., Verma, PC., Gupta, PK. (2009). A Vero cell derived combined vaccine against sheep pox and Peste des petits ruminants for sheep. *Vaccine* 27: 2548-2553.
63. Matthews, R.E.F. (1982). Classification and nomenclature of viruses.Intervirol.17: 1 -99.
64. Davies, F.G. (1996). Characteristics of a virus causing a pox disease of sheep and goats in Kenya, with observations on the epidemiology and control.*J. Hyg.* 76: 163-171.
65. OIE (2008b). Sheep and Goat Pox. Terrestrial Manual. Pp: 1058- 1068.

66. Spickler, A. R., Roth, J.A. (2006).Texbook of emerging and Exotic Diseases of Animals, 3rd ed. Iowa State University, Ames, IA, USA.
67. http://www.vet.uga.edu/vpp/gray_book/Handheld/sgp.htm (6 of 9)3/5/2004 4:17:01 AM. Accessed July 2008, Rome Italy.
68. Quinn, PJ., Carter, ME., Markey, B., Carter, GR. (1994). Texbook of Clinical Veterinary Microbiology 2nd Ed. Pp: 261-267, Wolfe Publishing, London, England.
69. Adams, L.G. (2002).The pathology of brucellosis reflects the outcome of the battle between the host genome and the *Brucella* genome. *Vet. Microbiol.* 90: 553-561.
70. Ocholi, R. A., Kwaga, J. K. P., Ajogi, I., Bale, J.O.O. (2005). Abortion due to Brucella abortus in sheep in Nigeria.Rev. Sc. tech. Off.inter. Epiz. 24: 973-979.
71. Bale, O.O.J., Nuru, S., Addo, P.B., Adeyinka, I.A. (2003) Bacteriological investigation of sheep and goat's milk for brucellosis in government farms in northern Nigeria. *Nigerian JourAnim Pro.*30:109-116.
72. Rossiter, P. B., Hammadi, N. L. (2009). Living with trans boundary animal diseases (TADs). Trop AnimHlth Prod, 41: 999–1004.
73. Minas, A. (2006). Control and eradication of brucellosis in small ruminants.Small Rum. Res. 62:101–107
74. Tekelye B., Kasali, O.B. (1990). Brucellosis in sheep and goats in Central Ethiopia. Bull. anim. Hlth. Prod. Afr. 38: 23-25. 25.
75. Yibeltal, M. (2005). A seroprevalence study of small ruminant brucellosis in selected sites of the Afar and Somali regions, Ethiopia.DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
76. Benkirane A. (2006). Ovine and caprine brucellosis: world distribution and control/eradication strategies in West Asia/North Africa region. *Small Rum. Res.* 62: 19-25.
77. Teshale, S., Aschalew, Z., Gelagay, A., Basu, A.K. (2006). – Preliminary study on prevalence of Brucella antibodies in sheep and goats in Borana, Southern Ethiopia. J. nat. Hist.2: 7-10
78. Quinn P.J., Carter, M.E., Markey, B., Carter, G.R. (1999). –Textbook of Clinical veterinary microbiology, 1st Ed. Mosby, Edinburgh, pp: 261-267.
79. FAO (2008). Expert meeting on climate related trans-boundary pests and diseases including relevant aquatic species. FAO headquarters, 25-27 February 2008. Rome, Italy.
80. FAO and OIE (2004). Joint FAO/OIE initiative. The global framework for the progressive control of trans-boundary animal diseases (GF-TADs).
81. Domenech, R.T., Thiaucourt, F., Dedieu, L., Maillard, J.C. (2003). Contagious bovine pleuropneumonia vaccines, historic highlights, present situation and hopes. Dev. Biol.114: 147–160.
82. Negussie, H., Kyule, M.N., Yami, M., Ayelet, G., Jenberie, S. T. (2010). Outbreak investigations and genetic characterization of foot-and-mouth disease virus in Ethiopia in 2008/2009. Trop. Anim. Hlth. Prod. 43: 235–243
83. DFID (2002). Annual Report, Department for International Development: pp: 1-73.
84. Wakhusama, S., Sinkala, Y., Chisemebele, C.C. (2011). Rapid assessment on peste des petits ruminants (ppr) situation in Zambia, VACNADA, AU-IBAR: pp: 1-16.
85. Tambi., N.E., Maina, W.O., Ndi, C. (2006). An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. Rev. sci. tech. Off. int. Epiz. 25: 999-012.
86. Holleman, C.F. (2002). The socio-economic implications of the livestock ban in Somalia. Famine Early Warning System Network: Nairobi, Kenya. pp: 62-78.
87. Thompson, D., Muiel, P., Russel, D., Osborne, P., Bromley, A., Rowlands, M., Creigh-Tyte, S., Brown, C. (2002). Economic costs of the foot-and-mouth disease in the United Kingdom in 2001. Rev. sci. tech. Off. int. Epiz . 21: 675–687
88. Leforban, Y. (2005). Report of a mission on foot and mouth disease in Ethiopia. Proposals for a strategic plan for a control program oriented to the export, 10–22 April 2005, Pp: 12–42.
89. Belachew, H. (2010). *GULFOOD Fair 2010: Promotional Forum for Ethiopian Agricultural Exports, March 2010.* Ethiopia Sanitary and Phytosanitary Systems Livestock and Meat Marketing System (SPS-LMM), Texas Agricultural Experiment Station (TAES)/Texas A&M University System. pp: 15
90. Berhanu, G., Adana, H., Kahsay, B. (2009). *Feed Marketing in Ethiopia: Results of Rapid Market Appraisal.* Improving Productivity and Market Success (IPMS) of Ethiopian farmers project, Working Paper 15. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp: 64
91. FAO (2002). Improved animal health for poverty reduction and sustainable livelihoods.FAO Animal Production and Health Paper, 153. Rome, Italy
92. Bruckner, GK., Vosloo, W., Du Plessis, BJA., Kloeck ,PELG., Connoway, L., Ekron, MD., Weaver, D.B., Dickason, C.J., Schreuder, F.J., Marais, T., Mogajane, M.E. (2002). Foot and Mouth Disease: the experience of South Africa. *Rev. Sci. tech. Off.int. Epiz.* 21: 751–764.
93. Pluimers, F.H., Akkerman, A.M., Van der Wal, P., Dekker, A., Bianchi, A. (2002). Lessons from the foot and mouth disease outbreak in the Netherlands in 2001. Rev. Sc.Tech.Off. Inter. des Epiz. 21: 751–764.

94. Martin, V., De Simone, L., Lubroth, J. (2007). Geographic information systems applied to the international surveillance and control of trans-boundary animal diseases, a focus on highly pathogenic avian influenza.Vet Ital. 43: 437-450
95. Siembieda, J. L., Kock, R. A., McCracken, T. A., Newman, S. H. (2011). The role of wildlife in trans-boundary animal diseases. Anim. Hlth. Res. Rev. 12: 95-111
96. Domenech, J., Lubroth, J., Eddi, C., Martin, V., Roger, F. (2006). Regional and international approaches on prevention and control of animal trans-boundary and emerging diseases. Ann N Y Acad. Sci.1081: 90-107.
97. Gibson, J.P., Bishop, S.C. (2005). Use of molecular markers to enhance resistance of livestock to disease: A global approach.OIE Sci. Tech. Rev. 24: 343-353
98. Rweyemamu, M.M., Musiime, J., Thomson, G., Pfeiffer, D., Peeler, E. (2006). Future control strategies for infectious animal diseases- Case study of the UK and sub-Saharan Africa. In: UK Government's foresight project, infectious diseases: preparing for the future, pp: 1-24...
99. Hitchcock, P., Chamberlain, A., Van Wagoner, M., Inglesby, T.V., Toole, T. (2007). Challenges to global surveillance and response to infectious disease outbreaks of international importance.Bios.Biot. 5: 206-227
100. Woodford, J. (2004). Synergies between Veterinarians and Para-professionals in the Public and Private Sectors: Rev. sci. tech. off. int. epiz. 23: 115-135.

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Use of Different Immunoresponse Assays for Evaluation of Live Attenuated Sheep Pox Vaccine in Comparison with Challenge Test

By Nermeen G. Shafik, Ibrahim M.M, Sonia A. Rizk & Ali A.M

Abstract- Sheep pox (SP) is one of the priorities, high-impact animal diseases in many developing countries, where live attenuated vaccines are routinely used against sheep pox virus (SPV). Sheep pox virus is a member of the family Poxviridae, genus Capri poxvirus. In this study, live attenuated Sheep pox vaccines were evaluated for humoral and cellular immunity using virus neutralization index (NI), ELISA and lymphocyte proliferation assay (XTT) beside routinely titration of life attenuated virus content of vaccine in Vero cell line which gives mean satisfactory TCID50/dose (3.34) for used vaccine batches, in addition to clinical examination of vaccinated sheep and also application of challenge test. Sixty susceptible lambs were divided into (10) groups and vaccinated with field and safety doses of (10) different batches of live attenuated vaccine intradermal (I/D) in tail fold while three lambs kept as control. The results showed that lymphocyte proliferation began to increase till reach to its peak (1.312) at 10th day post vaccination then decrease after that with re-increasing after challenge , serological assays results revealed that protective serum antibody titer started at 10th day post vaccination with mean titer (1.6 and 1.99), mean absorbance (1.56 and 2.02) and at three weeks the mean titer (2.35 and 2.61) , mean absorbance (2.43 and 2.51) for NI and ELISA respectively, also all vaccinated lambs showed satisfactory levels of protection against the virulent SPV through challenge test as SID50 more than (2.5) for all batches of vaccine.

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Nermeen G. Shafik [α], Ibrahim M.M [σ], Sonia A. Rizk [ρ] & Ali A.M [ω]

Abstract- Sheep pox (SP) is one of the priorities, high-impact animal diseases in many developing countries, where live attenuated vaccines are routinely used against sheep pox virus (SPV). Sheep pox virus is a member of the family Poxviridae, genus Capri poxvirus. In this study, live attenuated Sheep pox vaccines were evaluated for humoral and cellular immunity using virus neutralization index (NI), ELISA and lymphocyte proliferation assay (XTT) beside routinely titration of life attenuated virus content of vaccine in Vero cell line which gives mean satisfactory $TCID_{50}$/dose (3.34) for used vaccine batches, in addition to clinical examination of vaccinated sheep and also application of challenge test.

Sixty susceptible lambs were divided into (10) groups and vaccinated with field and safety doses of (10) different batches of live attenuated vaccine intradermal (I/D) in tail fold while three lambs kept as control. The results showed that lymphocyte proliferation began to increase till reach to its peak (1.312) at 10^{th} day post vaccination then decrease after that with re-increasing after challenge , serological assays results revealed that protective serum antibody titer started at 10^{th} day post vaccination with mean titer (1.6 and 1.99), mean absorbance (1.56 and 2.02) and at three weeks the mean titer (2.35 and 2.61) , mean absorbance (2.43 and 2.51) for NI and ELISA respectively, also all vaccinated lambs showed satisfactory levels of protection against the virulent SPV through challenge test as SID_{50} more than (2.5) for all batches of vaccine.

The results demonstrated that vaccine titration in Vero cell line and evaluation of humoral, cellular immuneresponses using different assays for vaccinated lambs were possible to be an accurate parameter for evaluation of life attenuated sheep pox vaccine equivalent the protective results obtained against a virulent SPV in challenge test.

I. Introduction

Sheep pox virus is a member of genus Capri poxvirus in the family Poxviridae (1). Sheep pox is a disease of sheep and goats characterized by pyrexia, generalized skin and internal pox lesions, and lymphadenopathy (2). Sheep pox and goat pox are ancient diseases that are currently endemic in the Middle East, the Indian subcontinent, and Central and Northern Africa. Kids and lambs are generally more susceptible than adults (3).

Vaccination has been considered to be the cheapest and sustainable means of disease control in the enzootic situation like India, Egypt and Middle East (4). Prophylaxis using attenuated vaccines is the choice of control measure as the immunity is long lasting (5). Vaccines are considered among the most valuable and cost-effective tools for the control of infectious diseases. The development of safe and effective vaccines for the prevention and control of emerging and neglected infectious diseases is an international priority (6) and (7).

In endemic countries a variety of attenuated live vaccines have been used against SPV. Live attenuated vaccine protection is mediated by both cellular and humoral immunity (8) and (9). The virus neutralization test is the most specific serological test for evaluation of immunity against SPV, also the enzyme linked Immunosorbent assay (ELISA) had already been proved to have great potentiality as a quantitative serological tool in the detection of antibodies against several viral infections including the pox viruses. It had been proved that the sensitivity and specificity of ELISA are superior to those of other serological tests (10) and (11).

A significant number of veterinary vaccine potency tests for serial release are conducted using in vitro methods. For live viral vaccines, these include culture techniques to quantify microbial content as an indicator of antigenic content of the vaccine (12) and (13).

Potency testing for inactivated veterinary vaccines has traditionally used challenge testing of vaccinated animals with live microbes to determine the quantity of vaccine necessary to provide adequate protection. Inadequately protected and control animals that become infected usually develop significant clinical signs of the disease and/or die. However, in recent years, antibody quantification procedures have been developed and validated and subsequently replaced the challenge test for several vaccines (14), (15) and (16).

The global veterinary vaccine industry continues to actively pursue in vitro assays and the reduction in the use of animals for in-process antigen measurement and finished product potency testing (17), (18) and (19)

The present work aims to use different immuneresponse assays for evaluation of live attenuated sheep pox vaccine as alternatives to challenge test.

Author α: e-mail: sonia_rizk@yahoo.com

II. Material and Methods

a) Virus

Virulent sheep pox virus, Egyptian strain of sheep pox virus was obtained from the Pox Department, VSVRI Abbassia, Cairo. The virus had been previously isolated from a local outbreak **(20)** and was used for challenge test.

b) Cell Culture

African Green Monkey Kidney cell line (VERO) was supplied by VSVRI, Abbasia Cairo and used for virus titration and serum neutralization test.

Attenuated sheep pox vaccine

10 batches of live attenuated sheep pox vaccine from Romanian strain of sheep pox virus years (2014, 2015 and 2016) stored at -20°C.

c) Animals

Sixty three susceptible native breed sheep 6 months old were screened using serum neutralization test and found to be free from antibodies against SPV.

d) Experimental Design

The experimental sheep were divided into ten groups (contain 6 animals/each) and each group divided into two subgroups as described in Table (1).Beside control group (Gp Co.) contains three animals, were kept unvaccinated as negative control.

Table (1): Experimental Design

Groups	Batches of SHEEP POX Vaccine	Sub Groups		Number of Sheep/Gp
		(a) Field dose	(b) 20X (Safety dose)	
Gp1	(2016)	3 Sheep	3 Sheep	6 Sheep
Gp2	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp3	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp4	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp5	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp6	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp7	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp8	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp9	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp10	(2015)	3 Sheep	3 Sheep	6 Sheep
Gp CO.	CONTROL	3 Sheep		Total # 63 Sheep

e) Evaluation of life attenuated sheep pox vaccine

1. Titration of live attenuated sheep pox in Vero cell Line by using tenfold serially dilutions of vaccine and calculation of tissue culture infective dose fifty / dose for each vaccinal batch *($TCID_{50}$/dose)*
2. *Potency field tests:* Ten groups of sheep were vaccinated by inoculated subcutaneously in the ventral aspect of the tail fold with the field and (20X) safety dose of different (10) batches of vaccine, beside one control group, kept unvaccinated as negative control. The animals were clinically observed daily to detect post-vaccinal reaction, and different blood samples were collected for cellular and humeral immune responses were evaluated.
3. *Challenge test:* was applied according to *(10);* 3 weeks post vaccination, all sheep groups and control group, inoculated with 0.5 ml of the virulent SPV through the intradermal route as five inoculums for each dilution of six tenfold serial diluted virus in both body sides of sheep. The challenged animals were kept in separate isolator under observation for (7) days, then exanimate for count of button shaped lesion and calculated sheep infected dose fifty *(SID_{50}).*

f) Samples

- Heparinized blood samples were collected from vaccinated and control animals before and after vaccination at different intervals (0, 3, 5, 7, 10, 14, 21 and 28 days) for application of the cellular immuneresponse assay.
- Whole blood samples for separation of serum were collected also for application of the humoral immuneresponse assay at different intervals (0, 3, 5, 7, 10, 14, 21 and 28 days).

g) Evaluation of cellular immune response of the vaccine Batches

The cellular immunity was evaluated by application of Lymphocyte blastogenesis assay. It was carried out according to **(21)** and **(22)** using XTT cell viability assay kit (AppiChem).

h) Evaluation of humoral immune response of the vaccine Batches

- *Serum neutralization test (SNT):* It was carried out using the microtitre technique according to (23) where SP antibody titer was expressed as neutralizing index (NI) according to (24).

- *Indirect ELISA:* It was performed to evaluate the humoral immune response according to the method described by **(25)** and the results were expressed by Mean of Absorbance (Ab).

III. Results

Table (2): Showed the titer values of life sheep pox virus of different Batches of vaccine using Vero cell line (T.C) which calculated as $TCID_{50}$.

Table (2): Titration of different Batches of SHEEP POX Vaccine in Vero Cell Line

Batches of SHEEPPOX Vaccine	Virus Titer of Vaccine ($TCID_{50}$/dose)	Virus Titer of Vaccine ($TCID_{50}$/1ml)	Lot Dose	CONCLUSION
1-(2016)	2.5	3.5	10 dose	Satisfactory
2-(2015)	2.7	3.7	10 dose	Satisfactory
3-(2015)	4.5	6.5	100 dose	Satisfactory
4-(2015)	4.3	5.3	10 dose	Satisfactory
5-(2015)	2.5	3.5	10 dose	Satisfactory
6-(2015)	4.1	5.1	10 dose	Satisfactory
7-(2015)	4.3	6.3	100 dose	Satisfactory
8-(2015)	3.3	5.3	100 dose	Satisfactory
9-(2015)	2.7	3.7	10 dose	Satisfactory
10-(2015)	2.5	3.5	10 dose	Satisfactory
Virus Control	2.1/0.1 ml	4.1/1ml	---	Control

Table (3-1) Showed the post vaccinal body temperature changes (thermal response) of all vaccinated animals and control ones through different follow up intervals of experiment and till application of challenge test. the thermal reaction elevated only in sheep groups of batches (2, 6 and 10) at 5th days post vaccination, while at 7th and 10th days the thermal reaction recorded in all sheep groups, and there was mild thermal reaction for all vaccinated groups while control unvaccinated group showed severe thermal reaction post challenge.

Table (3): Field follow up for Different Batches of SHEEP POX Vaccine post Vaccination

Days / Batches of SHEEP POX Vaccine	3-1- Thermal Response (Reaction)															
	Post Vaccination														Challenge Test in Sheep	Post Challenge
	Day 0		Day 3		Day 5		Day 7		Day 10		Day 14		Day 21			Day 28
	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x		F	20x
1-(2016)	-	-	-	-	-	-	+ +	++	+	+	-	-	-	-		-	-
2-(2015)	-	-	-	-	+	++	+ +	++	+	+	-	-	-	-		-	-
3-(2015)	-	-	-	-	-	-	+	++	+	+	-	-	-	-		-	-
4-(2015)	-	-	-	-	-	-	+ +	++	+	+	-	-	-	-		-	-
5-(2015)	-	-	-	-	-	-	+ +	++	+	+	-	-	-	-		-	-
6-(2015)	-	-	-	-	+	++	+ +	++	+	+	-	-	-	-		-	-
7-(2015)	-	-	-	-	-	-	+	++	+	+	-	-	-	-		-	-
8-(2015)	-	-	-	-	-	-	+ +	++	+	+	-	-	-	-		-	-
9-(2015)	-	-	-	-	-	-	+ +	++	+	+	-	-	-	-		-	-
10-(2015)	-	-	-	-	+	++	+ +	++	+	+	-	-	-	-		-	-
Controls	-		-		-		-		-		-		-			++	

* No Thermal reaction (-) (37.6 – 38.5) = Normal Temp. or very mild not over 0.5°c
*Mild Thermal reaction (+) (38.6 – 39.5)
*Severe Thermal reaction (+ +) (39.6 – 40.5)

Also clinical examination of all sheep groups explained in *Table (3-2)* Showed that small post vaccinal lesions or reactions in tail fold of sheep vaccinated with some batches and after 7 days of challenge test few numbers of intradermal buttons shaped lesions were observed in body sides of vaccinated sheep groups control unvaccinated sheep group.

Days / Batches of SHEEP POX Vaccine	3-2- Clinical Examination (Appearances of I/D Lesion)															
	Post Vaccination														Challenge Test in Sheep	Post Challenge
	Day 0		Day 3		Day 5		Day 7		Day 10		Day 14		Day 21			Day 28
	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x		F	20x
1-(2016)	-	-	-	-	-	-	±	+	-	-	-	-	-	-		V	V
2-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
3-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
4-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
5-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
6-(2015)	-	-	-	-	-	-	±	+	-	-	-	-	-	-		V	V
7-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
8-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
9-(2015)	-	-	-	-	-	-	±	+	-	-	-	-	-	-		V	V
10-(2015)	-	-	-	-	-	-	-	-	-	-	-	-	-	-		V	V
Controls	-		-		-		-		-		-		-			+++	

* No Lesion (-)
*Small or Mild reaction (±)
*Detected Skin reaction (+)
* Variable numbers (V)

Table (4): Showed the titers of Vaccine Batches using Challenge Test in Sheep after being challenged with virulent field strain of sheep pox virus, and calculated of SID_{50}.

Table (4): Titration of different Batches of SHEEP POX Vaccine using Challenge Test in Sheep

Batches of SHEEPPOX Vaccine	Post Challenge (I/D) Button Shaped Lesion	Titer of Vaccine Post Challenge Test in sheep [Average of sheep infective $dose_{50}$] (SID50)	Deference Between SID_{50} of Control & vaccinated Groups	CONCLUSION
1-(2016)	±	2.3	3	Satisfactory
2-(2015)	±	2.1	3.2	Satisfactory
3-(2015)	±	0.5	4.8	Satisfactory
4-(2015)	±	0.7	4.6	Satisfactory
5-(2015)	±	2.4	2.9	Satisfactory
6-(2015)	±	0.9	4.4	Satisfactory
7-(2015)	±	0.6	4.7	Satisfactory
8-(2015)	±	1.7	3.6	Satisfactory
9-(2015)	±	2.2	3.1	Satisfactory
10-(2015)	±	2.6	2.7	Satisfactory
Mean for all Batches	±	1.6	3.7	------
Virus Control	+++	5.3		control

NB. The Batch of SHEEP POX Vaccine considered satisfactory if the deference between SID_{50} of Control and Vaccinated Sheep is more than (2.5) after challenge.

Laboratory follow up of Different Batches of SHEEP POX Vaccine

Table (5): Showed the results of cell mediated immune response *(XTT)* expressed as the mean of absorbance and clarified that the lymphocyte proliferation.

Table (5): Cell mediated immune response (XTT)

Days	Cell mediated immune response(XTT) (Mean of Absorbance)															
	Post Vaccination														Challenge Test in Sheep	Post Challenge
Vaccine Batches	Day 0		Day 3		Day 5		Day 7		Day 10		Day 14		Day 21			Day 28
	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x		F	20x
1-(2016)	0.075	0.076	0.370	0.421	0.518	0.600	1.035	1.052	1.320	1.420	1.041	1.061	0.501	0.635		0.832	0.864
2-(2015)	0.071	0.073	0.365	0.410	0.517	0.590	1.032	1.048	1.290	1.400	1.036	1.058	0.490	0.580		0.820	0.859
3-(2015)	0.074	0.073	0.366	0.413	0.516	0.611	1.033	1.050	1.300	1.415	1.039	1.058	0.498	0.621		0.822	0.861
4-(2015)	0.072	0.074	0.369	0.419	0.515	0.632	1.031	1.052	1.315	1.421	1.040	1.060	0.500	0.610		0.825	0.860
5-(2015)	0.075	0.076	0.370	0.410	0.516	0.607	1.030	1.049	1.310	1.412	1.039	1.059	0.490	0.609		0.830	0.856
6-(2015)	0.073	0.073	0.370	0.411	0.517	0.590	1.036	1.050	1.321	1.420	1.042	1.061	0.501	0.612		0.823	0.858
7-(2015)	0.072	0.075	0.368	0.412	0.514	0.570	1.030	1.051	1.291	1.391	1.038	1.060	0.940	0.609		0.824	0.862
8-(2015)	0.074	0.072	0.372	0.420	0.518	0.622	1.037	1.053	1.325	1.429	1.042	1.067	0.507	0.617		0.830	0.856
9-(2015)	0.075	0.077	0.371	0.418	0.516	0.608	1.035	1.051	1.320	1.421	1.041	1.063	0.503	0.614		0.829	0.863
10-(2015)	0.070	0.071	0.370	0.423	0.517	0.633	1.032	1.054	1.326	1.416	1.040	1.064	0.508	0.610		0.833	0.857
Mean of means	0.073	0.074	0.369	0.416	0.516	0.606	1.0331	1.051	1.312	1.415	1.040	1.061	0.544	0.612		0.827	0.860
Controls	0.078		0.078		0.078		0.078		0.078		0.078		0.078			1.043	

* Cell mediated immune response of control animals (absorbance) not exceeded, just between 0.07 – 0.08 all over the time of study up to Challenge Test.

Humoral Immuneresponses

Table (6 and 7) showed the results of SNT and ELISA assays, expressed as mean NI and absorbance (Ab).

Table (6): Neutralizing antibody titers

Days	Humoral immunresponse (mean results of Serological Examination)(NI)															
	Post Vaccination														Challenge Test in Sheep	Post Challenge
Vacc Batches	Day 0		Day 3		Day 5		Day 7		Day 10		Day 14		Day 21			Day 28
	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x		F	20x
1-(2016)	0.3	0.2	0.5	0.7	0.8	1.1	1.2	1.6	1.5	1.9	1.9	2.3	2.3	2.6		2.0	2.2
2-(2015)	0.4	0.4	0.6	0.9	0.9	1.0	1.4	1.4	1.8	2.0	2.0	2.4	2.4	2.7		2.1	2.3
3-(2015)	0.2	0.3	0.4	0.8	0.7	1.2	1.4	1.4	1.7	1.9	1.9	2.3	2.3	2.6		2.0	2.2
4-(2015)	0.3	0.3	0.5	0.8	0.8	1.0	1.3	1.4	1.6	1.9	1.9	2.3	2.3	2.6		2.0	2.2
5-(2015)	0.4	0.4	0.6	0.9	0.9	1.2	1.4	1.4	1.6	1.9	1.9	2.3	2.3	2.6		2.0	2.2
6-(2015)	0.2	0.3	0.4	0.8	0.7	1.1	1.3	1.4	1.5	2.1	2.1	2.5	2.4	2.7		2.1	2.3
7-(2015)	0.3	0.3	0.5	0.8	0.8	1.2	1.4	1.4	1.6	2.1	2.1	2.4	2.4	2.6		2.1	2.2
8-(2015)	0.4	0.4	0.6	0.9	0.9	1.3	1.5	1.4	1.6	2.1	2.1	2.4	2.4	2.6		2.1	2.2
9-(2015)	0.3	0.4	0.5	0.9	0.8	1.2	1.4	1.4	1.5	2.1	2.1	2.3	2.4	2.5		2.1	2.1
10-(2015)	0.4	0.3	0.6	0.8	0.9	1.0	1.3	1.4	1.6	1.9	1.9	2.4	2.3	2.6		2.0	2.2
Mean of means	0.32	0.33	0.52	0.83	0.82	1.13	1.36	1.42	1.6	1.99	1.99	2.36	2.35	2.61		2.05	2.21
Controls	0.2		0.2		0.3		0.4		0.4		0.3		0.4			1.00	

* Neutralization Index (NI) $\geq$ 1.5 considered protective mean against Capri pox viruses (Cottral, 1978)
* Positive (NI) in Vaccinated and Safety groups starting from the 10th day post vaccination (about 2 weeks).

Table (7): ELISA Titer

Days / Batches Of SHEEP POX Vaccine	Humoral immune response (mean results of Serological Examination) (ELISA) (Mean of Absorbance)															
	Post Vaccination														Challenge Test in Sheep	Post Challenge
	Day 0		Day 3		Day 5		Day 7		Day 10		Day 14		Day 21			Day 28
	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x		F	20x
1-(2016)	0.36	0.44	0.43	0.73	0.54	1.02	0.68	1.60	1.50	2.20	2.30	2.50	2.35	2.55		2.25	2.45
2-(2015)	0.28	0.29	0.37	0.68	0.56	0.92	0.70	1.40	1.40	1.89	2.30	2.40	2.35	2.45		2.25	2.35
3-(2015)	0.46	0.28	0.41	0.65	0.60	0.90	0.83	1.09	1.60	1.90	2.35	2.40	2.40	2.45		2.30	2.35
4-(2015)	0.37	0.45	0.45	0.75	0.64	0.98	0.90	1.20	1.60	2.22	2.45	2.50	2.50	2.55		2.40	2.45
5-(2015)	0.28	0.35	0.36	0.70	0.62	0.92	0.98	1.30	1.70	1.98	2.40	2.45	2.45	2.50		2.35	2.40
6-(2015)	0.36	0.28	0.44	0.67	0.61	0.96	0.88	1.20	1.30	1.90	2.35	2.40	2.40	2.45		2.30	2.35
7-(2015)	0.25	0.45	0.35	0.75	0.59	0.97	0.90	1.50	1.60	2.20	2.40	2.50	2.45	2.55		2.30	2.45
8-(2015)	0.43	0.30	0.42	0.65	0.66	0.90	0.94	1.30	1.70	1.92	2.45	2.50	2.50	2.55		2.40	2.45
9-(2015)	0.26	0.38	0.36	0.70	0.67	0.94	0.97	1.50	1.80	1.94	2.45	2.50	2.50	2.55		2.40	2.45
10-(2015)	0.23	0.29	0.31	0.66	0.61	0.98	0.84	1.10	1.40	2.00	2.35	2.50	2.40	2.50		2.30	2.40
Mean of means	0.33	0.35	0.39	0.64	0.61	0.95	0.86	1.32	1.56	2.02	2.38	2.47	2.43	2.51		2.33	3.41
Controls	0.35		0.33		0.35		0.34		0.44		0.45		0.40			1.9	

* Mean of Absorbance $\geq$ one considered positive (protective samples)

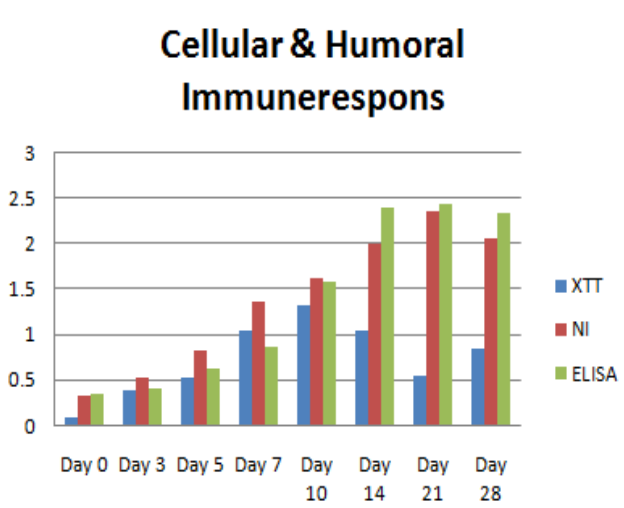
Table (8): Collective Immuneresponse Evaluation of Different Batches of SHEEP POX Vaccine

Days	Mean of means																			
Exam Assay	Post Vaccination																Post Challenge			
	Day 0		Day 3		Day 5		Day 7		Day 10		Day 14		Day 21		Day 28		Challenge Test in Sheep			
	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x	F	20x				
Cellular immune. (XTT)	0.073	0.074	0.369	0.416	0.516	0.606	1.033	1.051	1.312	1.415	1.040	1.061	0.544	0.612	0.827	0.860				
Controls	0.078		0.078		0.078		0.078		0.078		0.078		0.078		1.043					
Humoral immune. (NI)	0.32	0.33	0.52	0.83	0.82	1.13	1.36	1.42	1.6	1.99	1.99	2.36	2.35	2.61	2.05	2.21				
Controls	0.2		0.2		0.3				0.4		0.4		0.3		1.00					
Humoral immune. (ELISA)	0.33	0.35	0.39	0.64	0.61	0.95	0.86	1.32	1.56	2.02	2.38	2.47	2.43	2.51	2.33	3.41				
Controls	0.35		0.33		0.35		0.34		0.44		0.45		0.40		1.9					

Table (9): Collective virus Titeration for all vaccine Batches

Mean of	Virus Titer of Vaccine	Batches	CONCLUSION
Titer for all Batches in cells ($TCID_{50}$/dose)	3.34	10 Batches	Satisfactory
Virus Control	2.1	---	control
Titer for all Batches in Sheep (Challenge Test)	1.6	10 Batches	Satisfactory (as deference: 5.3 – 1.6 = 3.7 > 2.5)
Virus Control	5.3	---	control

1- Immuneresponse Assays



2- Titration in cell Line (Cell T) & Sheep (Sheep T)

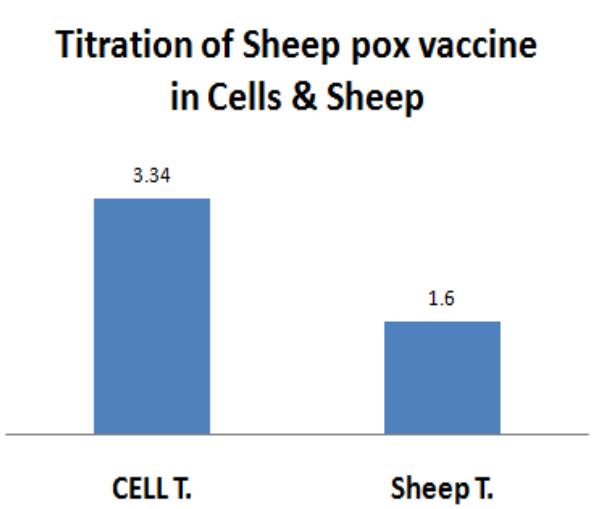


Fig. (1): Collective Evaluation Methods of Live Attenuated Sheep Pox Vaccine

IV. Discussion

Immunity to sheep pox involves both humoral and cellular responses (26). Antigens on the envelope and on the tubular elements of the virion surface stimulate protective antibodies. Even though it is the cell mediated immune response which eliminates the infection, antibodies limit the spread of the infection within the body. Neutralizing antibodies do play a significant role in the immunity as they have been shown to be an essential component of the protective immune response against sheep pox as the same was found to be absent in unvaccinated and pre-vaccinal serum samples (27) .Current evaluation of animal vaccines still focuses on the potency of final products in a batch-wise manner. All recent researches go in way to shafting from in-vivo to in- vitro for replacement the animal models, to ensure relevant quality attributes of vaccine batches by in-vitro evaluation of vaccines rather than by in-vivo potency tests on the final product (28).

For evaluating veterinary vaccines challenge studies were widely used under controlled conditions and sero-conversion studies, but the potency test in animals requires a large number of animals and involves unrelieved pain and suffering. A relevant in-vitro assay should provide a more accurate, reproducible, rapid, safe, vaccine potency test (29).

So, this study was performed for evaluation of live attenuated sheep pox vaccine by using different immuneresponse assays as alternatives to challenge test.

Table (2): Shows the titer values of life sheep pox virus of the ten different Batches of vaccine using Vero cell line (T.C) which calculated as ($TCID_{50}$) . The titer values were (≥ 2.5 $TCID_{50}$/ dose) for all batches in comparing with used control sheep pox virus (2.1 $TCID_{50}$/ 0.1ml) so all vaccine batches were considered Satisfactory on the level of tissue culture and these results agree with protocol of life attenuated sheep pox vaccine evaluation (30) and (31).

Table (3-1) Shows the post vaccinal body temperature changes (thermal response) of all vaccinated animals and control ones through different follow up intervals of experiment and till application of challenge test. The body temperature elevated only in sheep groups of batches (2, 6 and 10) at 5^{th} days post vaccination, while at 7^{th} and 10^{th} days the thermal reaction recorded in all sheep groups as the result of using the live attenuated vaccine. Also there was a mild thermal reaction for all vaccinated groups while control unvaccinated group showed severe thermal reaction post challenge due to the development of protective humoral and cellular immuneresponse of vaccinated sheep as shown in Tables (5,6 and 7) these results agree with (32).

Clinical examination of all sheep groups explained in Table (3-2) Showed that small post vaccinal lesions or reactions in inner side of tail fold for sheep vaccinated with batches (1, 6 and 9) only that disappeared within 3 days (salve regeration) , and there were no lesions or reactions in other groups. While after 7 days of challenge test few numbers of intradermal buttons shaped lesions were observed in both body sides of vaccinated sheep groups in comparing to huge number of buttons shaped lesions showed in control unvaccinated sheep group. .The mild post challenge

reactions appeared on the previously vaccinated animals, are due to the circulating antibodies derived through vaccination, which limits spread virus in animals (33) and (34),the results also were agreement with (35).

Table (4): Shows the titration of Vaccine Batches using Challenge Test in Sheep after being challenged with different dilutions of virulent field strain of sheep pox virus, then calculated as SID_{50} and the difference between the values SID_{50} of used control animal group and vaccinated groups were more than (2.5) for the vaccines and all batches considered satisfactory, these method of calculation and results were agree with (30) and (31).

It is known that sheep pox immunity depends mainly on the cell-mediated immune response in comparison to the humoral immune response (12) and (33).

The results of cell mediated immune response (XTT) expressed as the mean of absorbance in Table (5) showed the gradual increasing in lymphocyte proliferation as reached its peak on the 10th day (1.312 and 1.415) then decrease to lowest level at 21th day post vaccination (0.544 and 0.612) and re-increased to (0.827 and 0.860) post application of challenge test. These results agree with those of (36) and (37). Our results were in agreement with, (38) and (39) who reported the increase of lymphocyte activity by the 3rd day post vaccination and reached its peak on the 10th day then decreased.

Table (6 & 7) showed the results of SNT and ELISA assays. The humeral immune response increased gradually to be detected by the 10th day post vaccination as the mean NI was (1.6 and 1.99) more than protective level (>1.5) and mean absorbance of ELISA was (1.56 and 2.02) also more than protective level (> 1) then reached to the highest level mean of NI (2.35 and 2.61) and mean absorbance of ELISA (2.43 and 2.51) at the 21st day. These results also documented by (10) that reported neutralizing Index (NI) ≥1.5 considered protective mean against Capri pox viruses and were found by (27) and (40) , mentioned that serum neutralizing antibodies develop on the 2nd day and a significant rise of antibody titer was detected from the 21st to 42nd day post inoculation. Neutralization is very specific for almost all viruses (39). Results also harmonize with (41) and (42) who concluded that the serum neutralizing antibodies do play a significant role in the immunity against sheep pox and agree with (43) pox vaccines is the most effective immunogenic available and provide strong humoral immune response.

Table (8 & 9) and fig . (1) Showed the collective results obtained from all methods used for evaluation of live attenuated sheep pox vaccine either in-vivo or in-vetro. The pattern of these results indicated the presence of co-relation between different vaccine evaluation assays with the same value and accuracy to overcome and solve the safety problems and precautions of Challenge Test. (14), (15) and (16).

So the positive concordance found between the antibody levels and protection in tested lambs indicates that using immuneresponse assays as method for evaluation of live attenuated sheep pox vaccine appears to be as accurate as challenge test and presents several advantages in terms of costs and speed of issue of results.

We conclude that NI and ELISA as immuneresponse assays can be reliable measure of the efficacy of vaccine batches, provided that a good correlation has been demonstrated between protective immunity and resistance to challenge in vivo. So NI and ELISA can be used as alternatives to challenge test.

References References Referencias

1. Buller, RM, Arif, BM, Black, DN (2005): Poxviridae. In: Virus Taxonomy: Eight Report of the International Committee on the Taxonomy of Viruses. Elsevier Academic Press, Oxford; pp 117.
2. Kitching, R.P. (2004): Sheep pox and goat pox. In: Coetzer JAW, Tustin RC (Eds). Infectious Diseases of Livestock Second Edition, Volume 2, pp.1277-81. Oxford University Press Southern Africa, Capetown.
3. Rao ,T.V. and Bandyopadhyay, S.K. (2000): A comprehensive review of goat pox and sheep pox and their diagnosis. Anim Health Res Rev.;1(2): 127-36.
4. Kallesh, D.J.; Hosamani, M.; Balamurugan, V.; Bhanuprakash, V.; Yadav, V. and Singh, R.K. (2009): Quantitative PCR: A quality control assay for estimation of viable virus content in live attenuated goat pox vaccine. *Ind. J. Exper. Biol.;* 47: 911-915.
5. Bhanuprakash, V.; Indrani, B.K.; Hosamani, M. and Singh, R.K. (2006): The current status of sheep pox disease. Comp. Immuno. Microbio. and Infect. Dis.; 29(1): 27-60.
6. Alan, D.T.B. and Lawrence, R.S. (2009): Vaccines for biodefense and emerging and neglected diseases. Elsevier inc.
7. Bhanuprakash V, Venkatesan G, Balamurugan V (2010): Pox outbreaks in Sheep and Goats at Makhdoom (Uttar Pradesh), India: Evidence of Sheeppox Virus Infection in Goats. Transboundary and Emerging Diseases, 57: 375-382.

- CLEVB

8. Kitching, R.P. (1986): The control of sheep and goat pox. Rev Sci Tech.;5:503–11.
9. Mirzaie Kamran, Seyed Mohammad Barani and Saied Bokaie (2015): A review of sheep pox and goat pox: perspective of their control and eradication in Iran. J. Adv. Vet. Anim. Res., 2(4): 373-381.

10. OIE Terrestrial Manual (2010): Chapter 2.7.14 Sheep pox and goat pox in manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 1-12.
11. Tuppurainen, E. S. M. ;Venter, E. H.; Shisler, J. L.; Gari, G. ; Mekonnen,G. A.; Juleff, N.; Lyons, N. A.;K. De Clercq, Upton, C.; Bowden, T. R. ; Babiuk, S. and. Babiuk, L. A. (2017): Review: Capripoxvirus Diseases: Current Status and Opportunities for Control.Transboundary and Emerging Diseases, 64(3): 729–745.
12. Stokes, W. S.k; Kulpa-Eddy , J .; Brown, K.; Srinivas, G and McFarland, R. (2012) : Recent Progress and Future Directions for Reduction, Refinement, and Replacement of Animal Use in Veterinary Vaccine Potency and Safety Testing. Dev Biol (Basel) 134, 9-21. 2012.
13. Aparna Madhavan, G. Venkatesan and Amit Kumar (2016): Capripoxviruses of Small Ruminants: Current Updates and Future Perspectives.Asian J.Anim.Vet.Adv.,11(12):757-770.
14. Jungbäck. C. (2012): Potency Testing of Veterinary Vaccines for Animals: The Way From in Vivo to in Vitro. International Alliance for Biological Standardization Dev Biol (Basel). ; 134:149-52.
15. Gibert, R .; Alberti, M.; Poirier, B.; Jallet, C.;Tordo, N. and Morgeaux S.(2013): A relevant in vitro ELISA test in alternative to the in vivo NIH test for human rabies vaccine batch release.Vaccine 5; 31(50):6022-9.
16. Horvath, E.; Czifra, G.; Nagy, E.; Engstrom, B. and Merza M.(1999): Potency test of inactivated Newcastle disease vaccines by monoclonal antibody blocking ELISA. Vaccine 1999; 17: 2969-73.
17. Kubiak, V.(2012): In vitro antigen measurement and potency tests: challenges encountered during method development...and lessons learned. International Alliance for Biological Standardization Dev Biol (Basel). ;134:83-91.
18. Maas , P.A.; Winter, M.P.M.; Venema,S. ; Oei, H.L. and. Claassen, I.J.T M (2000): Antigen quantification as in vitro alternative for potency testing of inactivated viral poultry vaccines, Veterinary Quarterly, 22:4, 223-227.
19. Kamphuis, E.; Krämer,B.; Schildger,H.; and Duchow,K.(2012):Potency Testing of Inactivated Rabies Vaccines Using a Serological Method Dev Biol (Basel) ;134:23-7.
20. Sabban, M.S. (1960): Sheep pox and its control in Egypt. *Bull. Off. Int. Epiz.,* 53: 1527-1539.
21. Rai-El-Balhaa, C.; Pellerin, J.L.; Bodin, G.; Abdullah, A. and Hiron, H. (1985): Lymphocyte transformation assay of sheep peripheral blood lymphocyte a new rapid assay to read technique. Commp. Immunol., Microbiol. Infect. Dis., 8(3/9): 311-318.
22. Alvero, O.; Parlak, C. and Senyel, M. (2006): FT-IR and NMR investigation of 1- phenylpiperazine: Acombined experimental and theoretical study. *Spectochim Acta A Mol. Biomol. Spectrosc.*
23. Rossiter P.B., Jessett D.M& Taylor W.P (1985): Micro neutralization systems for use with different strains of PPR virus and rinderpest virus. Trop.Anim.Hlth.prad, 17, 2, 75-81.
24. Martin W.B., Erhan M & Onar B (1975): Studies on sheep pox vaccine, serum – virus neutralization test. Pendik Veteriner Kontrol Ve Arastirm Institusu Dergisi, 8(1):26-47.
25. Anderson J., Rowe L.W., Taylor W.P & Crowther J.B (1982): An enzyme linked immunosorbant assay for the detection of IgG, IgA and IgE antibodies. Res. Vet. Sci., 32 (2):242-247.
26. Kalra S.B. & Sharma U.R. (1984): Cell-mediated and humoral immune response of sheep to attenuated sheep pox-virus. Indian J. Anima Sci. 54: 450-456.
27. Amitha, R. Gomes; Raveendra Hegde, Byregowda, S.M.; Vijayashree Varadarajan, Yeshwant , S.L.; Giridhar, P. and Renukaprasad, C. (2010): of Evaluation of humoral immune response to sheep pox vaccine. J. Veterinary & Animal Sciences 6 (5) 236-238.
28. Romberg, J. ; Lang, S.; Balks, E.; Kamphuis, E.; Duchow, K.; Loos, D.;Rau, H.; Motitschke. A. and Jungbäck, C.(2012): Potency testing of veterinary vaccines: the way from in vivo to in vitro. Biologicals.;40(1):100-6.
29. Lewis, C.E .; Fry, A.M.; Hermann, J.R.; Siev, D.; Dusek, D.M and Gatewood, D.M.(2012): Potency testing of veterinary rabies vaccines: replacement of challenge by in vitro testing: considerations for development of alternative assays. Dev Biol (Basel). ;134:29-33.
30. Code of Regulation of Egypt (CLEVB) (2017): Published by Central Laboratory for Evaluation of Veterinary Biologics.
31. OIE Terrestrial Manual (2017): Chapter 2.7.13 Sheep pox and goat pox in manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 1-12.
32. Mahmood, M. A., A. Sajid, M. Ahmad, Z. Hassni and M. S. Khan (1989): Preparation of live goat pox tissue culture vaccine. Pakistan. J. Vet. Res. 2: 31-37.
33. Zineb Boumart; Samira Daouam; Imane Belkourati; Lamya Rafi; Eeva Tuppurainen; Khalid Omari Tadlaoui, and Mehdi El Harrak(2016): Comparative innocuity and efficacy of live and inactivated sheeppox vaccines. BMC Vet Res.; 12: 133.
34. Yogisharadhya,R.; Bhanuprakash ,V.; Hosamani,M.; Venkatesan ,G.; Balamurugan ,V.; Bora,D.P.; Bhanot,V.; Prabhu,M. and Singh,R.K. (2011): Comparative efficacy of live replicating sheeppox vaccine strains in Ovines *Biologicals, 39: 417-423.*
35. Carn V.M (1993): Control of capripox viruses infections. Vaccine, 11: 1275- 1279.

36. Bachh A.S., G.C.Ram., J. Hopkins & M.P Bansal (1997): Observation on cellular response in experimentally sheep pox – infected lambs. Indian. J. Anim .Sci, 64:263-266.
37. Mohamed A.A., Mukhtar M.M., Elhussein A.M., Tageldin A.M.N & Fadol M.A (2007): Immune response of sheep vaccinated with capripox vaccine. Vet.Res. 1(1): 12-16.
38. Olfat, E. Nakhla; Samir, S.S.; Manal, Awad; Soad, M. Soliman and Daoud, A.M. (2002): Studies on cell mediated immune response of Goats vaccinated with Goat pox vaccine. 6th *Vet. Med. Zag. Conference.*, 139-151.
39. Heba A. Khafagy, Mohamed G. Abdelwahab, Abdelmoneim M. Mustafa, Mohamed A.Saad (2016): Preparation and field evaluation of live attenuated sheep pox vaccine for protection of calves against lumpy skin disease. Benha Vet. Med. J., 31(2):1-7.
40. Agag, B.I., Mousa, S., Hassan, H.B., Saber, M.S., El-Deghidy, N.S., Abdel Aziz, A.M., (1992): Clinical serological and biochemical studies on LSD. Lumpy Skin Veterinary Sciences 33, 58-66.
41. Rashwan, S.M.; Al Khalaf, H.N.; Al Hammad, M.I. and Abdel-Baky, M.H.(2001): Determination of sheep pox circulating neutralizing antibodies in immunized sheep and goats in the north region of the kingdom of Saudi Arabia. J. Appl. Anim. Res. 19: 233-235.
42. Zeidan S.M, Namaa A. Mohamed, HananM.S.ELZawahry, Saad M.A.A, Olfat E. Nakhla, Afaf A.Abdel-wahab, Abeer A. Tammam, El-Dakhly A.T(2016): Preliminary study for preparation of cmbined attenuated vaccine against sheep pox and PPR viruses. International Journal of Advanced Research, 4 (4) , 212-219.
43. Fakri,F.; Ghzal,F.; .Daouam,S.; Elarkam,A.; Douieb,L.; Zouheir,Y.; Tadlaoui,K. and Fassi-Fihri,O.;(2015): Development and field application of a new combined vaccine against Peste des Petits Ruminants and Sheep Pox. Trials in vaccinology, Volume 4, 33-37.

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- The "FARSM" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall,Ph.D., FARSS or William Walldroff, M.S., FARSM.

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

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

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

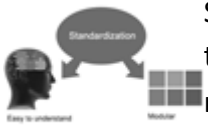
Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it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

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

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSM can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

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



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

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

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

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The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

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

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

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

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

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

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

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

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

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

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

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

It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

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

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

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

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

The Institute will be entitled to following benefits:

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

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

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

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

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

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

The following entitlements are applicable to individual Fellows:

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

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

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

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

Other:

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

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

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

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

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

(II) Choose corresponding Journal.

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

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

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

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

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Page Size: 8.27" X 11'"

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

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

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

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

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2. ETHICAL GUIDELINES

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

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

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

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

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

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

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Please mention proper reference and appropriate acknowledgements wherever expected.

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3. SUBMISSION OF MANUSCRIPTS

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

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

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

4. MANUSCRIPT'S CATEGORY

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

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

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

Research articles: These are handled with small investigation and applications

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

5.STRUCTURE AND FORMAT OF MANUSCRIPT

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

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

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

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

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

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

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

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

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

(h) Brief Acknowledgements.

(i) References in the proper form.

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

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

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

Format

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

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

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

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

Structure

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

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

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

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

Key Words

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

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

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

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

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

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

Acknowledgements: Please make these as concise as possible.

References

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

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

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

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

Tables, Figures and Figure Legends

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

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

Preparation of Electronic Figures for Publication

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

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

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

6. AFTER ACCEPTANCE

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

6.1 Proof Corrections

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

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

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

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

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

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

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

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6.4 Author Material Archive Policy

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

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final Points:

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

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

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

General style:

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

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

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

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

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

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

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

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

Approach:

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

Introduction:

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

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

Approach:

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

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose 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 sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

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

Methods:

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

Approach:

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

What to keep away from

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

Results:

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

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

Content

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

What to stay away from

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

Approach

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

Figures and tables

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

Discussion:

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

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

Approach:

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

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

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

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

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

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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