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Discovering Thoughts, Inventing Future

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Seroprevalence of Toxoplasma Gondii and *Neospora Caninum* Infection in Cattle in Grenada, West Indies

By Ravindra Sharma, Morgan Mcmillan, Keshaw Tiwari, Alfred Chikweto, Derek Thomas & Muhammad Iqbal Bhaiyat
St George's University, Grenada

Abstract- In view of the limited data on the seroprevalence of Toxoplasma gondii and Neospora caninum in the Caribbean region, this study aimed to estimate the seroprevalence of these parasites in cattle in Grenada, West Indies. In Total 148 serum samples were collected from the jugular veins of cattle from the six parishes in the country. The samples were surveyed for T. gondii and N. caninum antibody by an enzyme-linked immunosorbant assay (ELISA). The overall seroprevalence of T. gondii was 2.7% (4/148) and the seropositivity of N. caninum was 6.8% (10/148). The present results indicate exposure of cattle to T. gondii and N. caninum in Grenada.

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Seroprevalence of Toxoplasma Gondii and *Neospora Caninum* Infection in Cattle in Grenada, West Indies

Ravindra Sharma [α], Morgan Mcmillan [σ], Keshaw Tiwari [ρ], Alfred Chikweto [ω], Derek Thomas [¥] & Muhammad Iqbal Bhaiyat [§]

Abstract- In view of the limited data on the seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in the Caribbean region, this study aimed to estimate the seroprevalence of these parasites in cattle in Grenada, West Indies. In Total 148 serum samples were collected from the jugular veins of cattle from the six parishes in the country. The samples were surveyed for *T. gondii* and *N. caninum* antibody by an enzyme-linked immunosorbant assay (ELISA). The overall seroprevalence of *T. gondii* was 2.7% (4/148) and the seropositivity of *N. caninum* was 6.8% (10/148). The present results indicate exposure of cattle to *T. gondii* and *N. caninum* in Grenada.

I. Introduction

Toxoplasma gondii *(T. gondii)* is a zoonotic protozoan parasite causing infection in most warm blooded species of animals including humans (Dubey 2010). *T. gondii* causes major economic losses in livestock through abortions, stillbirths and neonatal losses (Dubey et al. 2007). Infection in humans and animals can occur by ingestion of *T. gondii* oocysts from cats which are the definitive hosts or by consuming raw uncooked meat containing *T. gondii* tissue cysts, which develop in infected animals (Dubey 2010). Cattle and other herbivorous animals contact the infection from grass and pastures contaminated with cats' feces (Jacek et al. 2007). In humans, *T. gondii* constitutes an important health problem in pregnant women because of the threat of fetal infection and in immuno-compromized patients, aggravates existing pathological conditions (Jacek *et al.* 2007, Dubey 2010). Apart from a survey in Grenada by Chikweto *et al.* (2011) who demonstrated a seroprevalence of 8.4% (10/119) tested by a modified agglutination test (MAT) in cattle, there is paucity of information on *T.gondii* infection in cattle in the Caribbean region.

Neosporosis caused by *N.caninum,* another coccidian parasite of animals morphologically similar to T.gondii, has emerged as a serious disease of cattle and dogs world wide (Dubey 2010). Antibodies to *N.caninum* have been demonstrated in many domestic and wild animals. *N. caninum* is a major cause of abortion and reproductive failure in both dairy and beef cattle (Vural et al. 2006, Dubey *et al.* 2007). Vertical transmission from dam to fetus and horizontal transmission through ingestion of oocysts voided by dogs are demonstrated modes of transmission in cattle Gavrea *et al*. 2009, Dubey 2010). In contrast to *T gondii,* infection of cattle with *N.caninum* has been reported from Argentina, Brazil, Chile, Paraguay, Peru and Uruguay, which are in vicinity of the Caribbean countries (Moore 2005). To our knowledge there is no information on *N. caninum* infection of cattle within the Caribbean region.

The objective of this study was to estimate the seroprevalence for *T. gondii* and *N. caninum* in cattle in Grenada.

II. Materials and Methods

For the present survey a 2 step (multistage cluster sampling) sampling procedure was adopted. Generally cattle herds in Grenada are small comprising 1-4 animals. In the first step, 35 herds consisting of 6% of (2500) estimated cattle population in Grenada, were selected randomly from all the six parishes in the country. All herds having less than 10 cattle were sampled. For a herd size greater than 10, 80% of the animals were sampled. A total of 148 cattle were sampled randomly. Two milliliter of blood from the jugular vein of each animal was obtained. Blood samples were centrifuged at 1500g for 15 minutes, and the serum was collected and stored at -20^{0}C until tested for antibodies to *T. gondii* and *N. caninum* using commercial ELISA kits (IDvet France). ELISA was performed following the instruction of the manufacturer.

III. Results

Out of 148 cattle tested 4 animals [2.7%, 95% confidence Interval (CI), 0.09% to 5.31%] were positive for *T. gondii* and 10 [6.8% 95% confidence interval (CI), 2.74% to 10.86%] for *C. caninum.* Results are presented in table 1.

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Table 1 : Antibodies to *Toxoplasma gondii* and *Neospora caninum* in cattle from Grenada, West Indies

Toxoplasma gondii			*Neospora caninum*		
Number of cattle tested	Number positive	Percent positivity	Number of cattle tested	Number positive	Percent positivity
148	4	2.7	148	10	6.8

IV. Discussion

In the present survey antibodies to *T. gondii* were detected in only 4 [2.7%, 95% CI, 0.09% to 5.31%] of the tested cattle. *Chikweto et al.* (2011) reported a seroprevalence of *T. gondii* (8.5%) in cattle using MAT. In both surveys antibodies to *T. gondii* in cattle was low. Cattle are considered to be a poor host for *T. gondii* because of its relative natural resistance to this parasite (Dubey and Thulliez 1994, Pita Gondim *et al,* 1999, Dubey 2010). Similar to our observations in Grenada, low seroprevalence has been reported from Iran1.6% (Raeghi *et al.* 2011 and 0% by Sharif *et al.* 2007) India 2.4% (Sharma *et al* 2008) Brazil 1.03% (Pita Gondim *et al.* 1999), USA 3.2% (Dubey 1985) and Malaysia 6.3% (Chandrawathani *et al* 2008). However, a high seroprevalence of *T. gondii* in cattle has been reported from many countries of the world; 32% in Sudan (Khalil and Elrayach, 2011); Tenter *et al.* (2000) in their paper reported 22% in the Czech Republic, 40% in Greece, 13-43% in Netherlands, 43% in Portugal, 40% in Spain, 69% in France, 92% in Italy and 66% in Turkey. Jacek *et al.* 2007 found 53% seroprevalence in Poland.

The variation in seroprevalence of *T. gondii* between various countries may be attributed to the difference in the rate of contamination of the environment with oocysts from cat, the definitive host and differences in management methods (Pet Gondim *et al.* 1999, Jacek *et al.* 2007). The low prevalence of *T. gondii* (2.7%) could be related to cattle production in Grenada. Cattle herds in Grenada are small compared to more intensely managed herds elsewhere in the world. Small herds get better hygienic conditions and are in less contact with infected cats. Further studies regarding the differences between management practices for cattle in Grenada could potentially shed more light on this topic.

This is the first report of *N. caninum* surveillance in cattle in Grenada. Antibodies to *N. caninum* was low [6.8%; 95% CI, 2.74% to 10.86%] in the tested cattle. Low prevalence has also been reported in Germany 4.1% (Conraths *et al.*1996), Canada 8.3% (Vanleeuwen *et al.* 2006), and Serbia 15% (Kuruca *et al.* 2013).

Variations in the seroprevalence of Neosporosis depending on the region, climate and type of serological tests have been reported (Dubey *et al.* 2007).

This variation amongst the different countries and regions of the world may be attributed to risk factors like dog density, climatic factors and management practices on the farm. Climatic factors influence the sporulation and survival of oocysts (Rinaldi *et al.* 2005). Variation in seroprevalence with respect to management practices could be attributed to the size of farm (Guimaraes et al. 2004; Rinaldi *et al.* 2005).

A positive relation between seropositivity of farm dogs and bovine neosporosis has been reported by previous researchers (Wouda *et al.*1999; Kacar *et al.* 2012)). Seroprevalence in cattle is lower where dogs are not present on the farm (Basso *et al.* 2001, Antony and Williamson 2003). In Grenada seropositivity for *N. caninum* in dogs has been demonstrated to be low varying from 1.2% in owned dog to1.6% in stray dogs (unpublished data). This low seropositivity in dogs correlates well with the lower seropositivity in cattle (6.8%).

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Prevalence of Bovine Trypanasomosis in Guto Gida District of East Wollega Zone, Oromia Regional State, Ethiopia

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Abstract- A cross sectional study was carried out to determine the prevalence of bovine trypanosomosis in five peasant associations of Guto Gida District of East Wollega Zone, Ethiopia from October 2013 to March 2014. From five peasant association, 384 cattle were randomly selected and examined for trypanosomosis. The overall prevalence of bovine trypanosomosis was 7.81% of which Trypanosoma congolense infection was 53.33%, Trypanosoma vivax infection was 30% and Trypanosoma brucei was 16.66% with statistically significant difference (P=0.00). A significant association was observed (P<0.05) between the disease positivity and body condition score. When the mean packed cell volume of trypanosome infected animals was compared with that of non- infected animals, it was significantly lower (P<0.05) in the infected animals. In conclusion, trypanosomosis caused by T. congolense, T. vivax and T.brucei with more prevalence of T. congolense remained the main constraint to animal production and agricultural development in study area.

Keywords: bovine, guto gida, PCV, prevalence, trypanosome.

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Prevalence of Bovine Trypanasomosis in Guto Gida District of East Wollega Zone, Oromia Regional State, Ethiopia

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Abstract- A cross sectional study was carried out to determine the prevalence of bovine trypanosomosis in five peasant associations of Guto Gida District of East Wollega Zone, Ethiopia from October 2013 to March 2014. From five peasant association, 384 cattle were randomly selected and examined for trypanosomosis. The overall prevalence of bovine trypanosomosis was 7.81% of which *Trypanosoma congolense* infection was 53.33%, *Trypanosoma vivax* infection was 30% and *Trypanosoma brucei* was 16.66% with statistically significant difference (P=0.00). A significant association was observed (P<0.05) between the disease positivity and body condition score. When the mean packed cell volume of trypanosome infected animals was compared with that of non- infected animals, it was significantly lower (P<0.05) in the infected animals. In conclusion, trypanosomosis caused by *T. congolense, T. vivax and T.brucei* with more prevalence of *T. congolense* remained the main constraint to animal production and agricultural development in study area.

Keywords: bovine, guto gida, PCV, prevalence, trypanosome.

I. Introduction

African animal trypanasomiasis (AAT) is a parasitic disease that causes serious economic losses in livestock from anemia, loss of condition and emaciation. Many untreated cases are fatal. AAT is found mainly in those regions of Africa where its biological vector (tsetse fly) exists (CFSPH, 2009). Bovine trypanasomosis continued to be the major constraints of livestock production in Sub-Saharan Africa, jeopardizing the lives of 55 million people. The risk of infection in humans as well as in domestic animals has greatly affected social, economical and agricultural development of communities within tsetse infested areas which roughly constitutes more than a third (10 million km^2) of Africa between 14°N and 29°S of the continent (FAO, 2002).

In Ethiopia, Trypanosomosis is widespread in domestic livestock in the Western, South and South-western lowland regions and the associated river systems (i.e. Abay, Ghibe, Omo and Baro/Akobo) (MoA, 1995). In (Afework *et al.*, 1998) and (Tewelde *et al.,* 2001) studies, farmers strongly recognized trypanosomosis as the primary problem for livestock productivity and agricultural development in the northwestern and western parts of Ethiopia, respectively.

Trypanosomosis in cattle locally referred, as "Gendi" is a serious constraint to livestock production in areas of the north and southwest Ethiopia at an altitude of below 2000 meters above sea level (masl). Currently about 220,000 Km^2 areas of the above mentioned regions are infested with five species of tsetse flies namely *Glossina pallidipes*, *G .morsitans, G. fuscipes, G. tachinoides and G. longipennis* (NTTIC, 2004).

Trypanosomosis is mainly restricted to areas in which the vector, tsetse fly (*Glossina* species) can survive. The disease is also found outside the tsetse belt areas transmitted mechanically by biting flies of the genus *Tabanus*, *Hematopota*, *Chrysops*, and *Stomxys*. A number of trypanosome species are important in bovine trypanosomiasis (*T. brucei brucei, T. congolense* and *T. vivax*) that differ from those causing the human form of the disease, sleeping sickness (*T. b. gambiense, T. b. rhodesiense)*. Economically the tsetse-transmitted trypanosomes (*Trypanosoma congolense, T. vivax*, and *T. brucei*) are most important in cattle with 14 million heads at risk in Ethiopia (Getachew , 2005). In Ethiopia, five species of trypanosomes are recorded and the most important trypanosomes in terms of economic loss in domestic livestock are tsetse transmitted species: *T.congolense*, *T.vivax* and *T. brucei* (Abebe, 2005).

Trypanasomosis control is a long-term fight and therefore requires the involvement of decision makers, researchers and farmers. Until now, the use of trypanocidal drugs to treat or to prevent susceptible livestock against trypanosomosis remains the only control measure for most of the farmers. Very limited trypanocidal compounds are available and they have been used for many years. This long-term use of the same molecules selected drug resistant strains of trypanosomes in many African countries (Geerts *et al.,* 2001).

In order to improve the welfare and security of rural communities, particularly Ethiopia, rapid method for assessing risk and diagnosing urgent problems are needed for the control of animal diseases. Although

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bovine trypanosomosis is considered an important livestock disease in Guto Gida District of East Wollega Zone, there is no information in the literature about the disease situation in the study area. The present study was, therefore, conducted in the district with objective of determining the prevalence of the disease, identifying the species of *Trypanosoma* and assessing of risk factors of the disease.

II. Material and Methods

a) Study Area

The study was conducted in Guto Gida District of East Wollega Zone, Oromia Regional State, Ethiopia. Guto Gida woreda is located at 331 Km West of Addis Ababa. It is situated at latitude and longitude of 9°5′N 36°33′E/9.083°N 36.550°E and at an altitude of 1350-2400 meters above sea level (Masl). The climatic condition of the area was highland (dega) (0.26%), midland (woyna dega) (46.74%) and lowland (bereha) (53%) with the mean annual rainfall range from 1800-2200 mm and average temperature 14-26^{0}c. The area receives bimodal rainfalls that were long rainy season (June to September) and short rainy season (March, April and May). The Guto Gida people practice mixed framing system that is crop production and livestock rearing and own large number of livestock. The livestock population in the area includes 86,724 cattle; 8,589 equine; 14,171 sheep; 11,821 goats and 57,695 poultry (CSA, 2009).

b) Study animals

The study animals were indigenous zebu cattle of all age group (*Bos indicus*). Animals were allowed to graze freely during the day and housed at night (extensively managed). The age of animals was determined by dentition (Delahunta and Hable, 1986) and categorized into three age groups. The body condition of animals was also grouped based on criteria described by (Nicholson and Butterworth,1986) but grouped in to two broad group good (G^+ to M) or poor (M^- To P^-).

c) Sampling method and Sample size

Random and purposive sampling methods were followed to select the study animals and study sites respectively. Since there was no previous study conducted in Guto Gida District to establish the prevalence, the sample size was determined by taking 50% expected prevalence of trypanasomosis using the formula given by (Thrusfield, 1995).

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

Where: n = required sample size
P_{exp} = expected prevalence = 50%
d = desired absolute precision = 5%
Hence, the sample size required as per the above formula was 384 heads of cattle.

d) Study Design

A cross sectional study was carried out to determine the prevalence of bovine trypanosomosis in five peasant association (Tolera, Eba, Muleta, Gari and Abdeta) of Guto Gida District of East Wollega Zone, Western Ethiopia from October 2013 to March 2014.

e) Study Methodology

i. Parasitological Study

A total of 384 blood samples were collected from ear veins of cattle. Samples were collected to heparanized capillary tube. During blood collection the necessary bio-data of each animal was recorded. The Buffy coat technique using phase contrast microscope was used for the detection of trypanosomes in the blood. Species identification was done by morphological examination of trypanosomes on Giemsa stained thin blood smears prepared from the positive animals and examined under a microscope using the oil immersion 100 × objectives (Murray *et al.,* 1977).

ii. Hematological Examination

Blood samples for packed cell volume (PCV) were collected from animals using heparinized capillary tubes. The packed cell volume (PCV) was measured after the heparinized capillary tubes containing blood were centrifuged for 5 min at 12,000 rpm in microhematocrit centrifuge and the results were observed using microhaematocrit reader following the standard procedure described by (Murray *et al.,* 1977).

f) Data Analysis and Management

Data collected were entered into Microsoft Excel spread sheet and descriptive statistics was applied to calculate the prevalence of trypanosomosis using SPSS version 16. ANOVA was used to determine the mean values of PCV and variation in the mean PCV between infected and non-infected animals was determined. The Percentages (%) were used to measure prevalence and chi-square (x^2) to measure significance of association among variables considered in this study. In all analysis, confidence level was held at 95% and $P < 0.05$ was set for significance.

III. Results

a) Parasitological Findings

From the total of 384 cattle examined with a Buffy coat technique, 30 were Positive for trypanosomes giving an overall prevalence of 7.81%. The prevalence of bovine trypanosomosis between different peasant associations (PA) was 11.39% in Abdeta, 9.89% in Gari, 6.52% in Muleta, 5.40% in Tolera and 5.31% in Eba with no statistically significant difference ($p>0.05$) (Table 1).

Trypnosoma congolense, Trypnosoma vivax, and *Trypnosoma brucie* were the *Trypnosoma* Species identified by Giemsa stained thin blood smear examination. Among the total of 30 cases of trypanosome infections detected 16(53.33%) of the

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infections were due to *T. Congolese, 9(30%)* were due to *T. Vivax* and the rest (16.66 %) were due to *T. brucie* with statistical significance difference (Table 2). Sex wise prevalence of trypanosome infection was slightly higher for female (8.37%) than for male (7.18%) animals (Table 3). However, statistical significant difference ($P > 0.05$) was not observed between sexes. With respect to body condition score, the prevalence was 2.65%, and 19.67% in good, and poor body condition score, respectively with a significant variation ($P < 0.05$) between them (Table 3). Age based prevalence was 9.21%, 7.42% and 3.33% for animal > 6 years, 1-6 years and < 1 year of age respectively. Although adult cattle have higher infection rate statistical significant difference ($P > 0.05$) was not observed between age group (Table 3).

b) Hematological Findings

The PCV of individual animals was measured for the assessment of degree of anemia. A mean PCV of 20.23% and 27.98% was found for infected animals and non-infected animals respectively (Table 4). The difference was statistically Significant ($P = 0.000$).

Table 1 : Origin based prevalence of bovine trypanasomosis

PA	Number of animal examined	Number of animal positive	Prevalence (%)	*T.congolense*	*T.vivax*	*T.brucie*	X^2 (P value
Tolera	74	4	5.40	2(2.70)	1(1.35)	1(1.35)	3.464 (0.44)
Eba	94	5	5.31	3(3.19)	1(1.06)	1(1.06)	
Muleta	46	3	6.52	2(4.34)	1(2.17)		
Gari	91	9	9.89	5(5.49)	3(3.29)	1(1.09)	
Abdeta	79	9	11.39	4(4.16)	3(3.78)	2(1.30)	
Total	384	30	7.81	16(4.17)	9(2.34)	5(1.30)	

Table 2 : Species based prevalence of bovine trypanasomosis

Species	Number of animal positive	Prevalence (%)	X^2	P- value
T.congolense	16	53.33	384	0.00
T.vivax	9	30		
T.brucie	5	16.66		
Total	30	100		

Table 3 : Prevalence of trypanosomosis infection with different potential risk factors

Potential risk factors	Nomber of animals examined	Infected animals (prevalence)	X^2	P- Value
Age			1.29	0.178
< 1year	30	1(3.33)		
1-6year	202	15(7.42)		
> 6year	152	14(9.21)		
sex			1.92	0.405
Male	181	13(7.18)		
Female	203	17(8.37)		
Body condition			34.92	0.000
Good	262	6(2.65)		
Poor	122	24 (19.67)		
Total	384	30(7.81)		

Table 4 : Mean PCV of infected and non – infected animals in the study sites

Animal	Number of animal	Mean PCV (%)	X^2	p-value
Infected	30	21.23	110.51	P=0.001
Non-infected	354	27.98		
Total	384	27.45		

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IV. Discussion

The distribution of the most common species of trypanasomes infesting cattle in Ethiopia varies greatly from one area to another. Considering this the present study revealed the overall prevalence of 7.81% in the study area, this prevalence of trypanosomes concord with prevalence of 8.55% of Sasiga and Diga district of East Wellega (Tefese *et al*., 2012) and 5.85%, in Diga District of Eastern Wollega (Dinsa *et al*., 2012). The similarity of prevalence between these studies might be due to similarity in altitude. In contrast, the result is low when compared with previous reports, 40% in the Wolyta and Dawero zones of southern Ethiopia (Miruk *et al.,* 2008), (24.7%) in Maokomo special district of Benshangul Gumz regional state (Daud and Molalegn, 2011) and 25.7% in the tsetse-infested zones of the Amhara region of northwestern Ethiopia (Cherenet *et al.,* 2006). The relatively low prevalence of trypanosomosis in this report may be due to the differences in agro ecology, which less favors tsetse flies growth and multiplication. And also prevalence rate of 29% along the escarpment of the Upper Didessa Valley (NTTICC, 1998), 25% in Gawo Dale district of Kelem Wollega zone (NTTICC, 2004) were reported.

The associations of the disease with different peasant associations were also assessed. No significance association was observed between prevalence of the disease among the different peasant associations (Table1). This may be due to the result of uncontrolled animal movements between the areas. The sex wise prevalence of trypanosome infection was 7.18% in male and 8.37% in female.Though prevalence a slightly higher among the females, statistically there was no significant difference. Daya and Abebe, (2008), Tefese *et al*. (2012) report similar results where they observed no significant difference in trypanosome infection between males and females. Onyiah, (1997) and Quadeer *et al.* (2008), in separate studies added that no statistically significant difference in the prevalence bovine trypanosomosis between sex groups. Therefore, they have equal chance of coming in contact with the flies and allowed in the same ecology having comparable degree to acquire infection.

T.vivax and *T. congolense* and *T.brucei* were the species detected from infected animal with statistically significant difference in the prevalence of trypanosome species (P=0.00) (Table 2). This result agreed with work of (Abebe and Jobre, 1996) who reported an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in Southwest Ethiopia, which is similar with current situation in Guto Gida District. The dominant trypanosomes species in the present study was *T. congolense*. This agreed with work of Tewelde *et al*., (2001) and (Afewerk, 1998) who reported a prevalence rate of 17.2% and 21% in Upper Didessa of tsetse infested region and in Metekel district respectively. The dominant species was *T. congolense* which is similar with the current result in Guto Gida District. Additionally, 71.8% prevalence of *T. congolense* in the Gawo Dale district was reported (Waktole *et al*., 2008). The predominance of *T. congolense* infection in cattle may be due to the high number of serodams of cattle as compared to *T. vivax* and development of better immune response to *T. vivax* by the infected animal (Leak *et al*., 1999). Langridge *et al*., (1976) also reported, *G. pallidipes* and *G.m. Sub-morsitans* are efficient in the transmission of *T. congolense* than *T. vivax* in Africa that support the present study in Guto Gida District. In contrast, in areas of East Wollega Zone (Sibu Sire) the respective ratios between *T.congolense (*36%) and *T.vivax* (64%) infections were reported (Shimelis and Sisay, 2011), because of the abundance of mechanical vectors also known to be effective transmitters of *T. vivax* (Desquesnes and Dia, 2004).

The association of the disease with age was also assessed. No significance difference was observed with respect to age. The result agreed with report of (Daud and Molalegne, 2011) in Mao-komo Special District of Benishangul Gumuz Regional State, (Molalegne *et al.,* 2010) in Jabi Tehenan district of West Gojjam Amhara regional state (Tefese *et al*., 2012) in Sasiga and Diga District of western Oromia region, (Efrem *et al*., 2013) in Lalo kile District of kelem Wollega. Similar findings were also reported by (Cherenet *et al.,* 2006) and (Habtamu, 2009), in tsetse infested region of Amhara and in the Jawi district of the Amhara region respectively. This can be associated to the fact that adult animals travel long distance for feed and water as well as for drought to tsetse high challenge areas. There is also evidence that *T. congolense* infection was chronic diseases that increase infection rates with age, (McDermott *et al*., 2003). According to (Torr *et al.,* 2000), tsetse flies are attracted significantly more by odor of large animals. Rowlands *et al.,* (2001) in Ghibe valley indicated that suckling calves did not go out with their dams but graze at home until weaned off. Additionally young animals are naturally protected to some extent by maternal antibodies (Fimmen *et al*., 1982). These could be the reason for lower prevalence of trypanosomosis that was observed in calves.

We also tried to assess the relationship of infection with body condition score of sampled animals (Table 3). In this study, there was a significant difference in the prevalence of trypanosomosis between animals with good and poor body conditions. This is in agreement with (Mussa, 2002) and (Molalegne *et al.,* 2010). This may be related to the debilitating nature of the disease (Radostits *et al.,* 2007). However, it would be difficult to conclude either poor body condition predispose to trypanosome infection or trypanosome infection cause loss of body condition based on such

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cross-sectional study (Dohoo *et al.,* 2003) and it should be verified by using a longitudinal study designs. The disease itself results in progressive emaciation of the infected animals; nevertheless, non–infected animals under good body condition have well developed immune status that can respond to any foreign protein better than those non–infected cattle with poor body condition which can be immune compromised due to other diseases or malnutrition, since malnutrition and concurrent infections depress the immune responsive-ness in some cases (Collins, 1994).

A significant decrease in PCV was observed in the trypanosome infected animals signifying anemia to be one of the important consequence of infection (Table 4). It was in agreement to the work done by Tafese *et al.*, (2012) mean PCV value of infected animals (21.45%) was significantly lower ($P < 0.05$) than that of non-infected animals (26.6%). Daud and Molalegne, (2011); Molalegne *et al.* (2010) also reported lower mean PCV value in infected animals than the non-infected animals. Rowlands *et al*. (2001) in also reported in an increase in PCV value, the proportion of positivity decreases and hence mean PCV was a good indicator for the health status of herds in an endemic area.

V. Conclusion

Trypanosomosis caused by *T. congolense, T. vivax and T.brucei* with more prevalence of *T. congolense* was remains the main constraint to animal production and agricultural development in Guto Gida woreda. This dominance of *T.congolense* suggest presence of biologically (tsetse fly) transmitted trypanosome and the presence of *T. vivax* in the area indicated the importance of mechanically transmitted trypanosome in the study area. The observed association between reduction in PCV and body condition with infection showed the impact of the disease on productivity of infected animals. Nevertheless, trypanocidal drugs remain the main control tools used by livestock owners.

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Synchroniztion of Estrus in Sub Estrus Murrah Buffaloes by Single Injection of PGF2α Analog in Low Breeding Season

By Madhu Shivhare & Dr. M. S. Thakur
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Abstract- Cent percent induction and fertility fine percent fertility was obtained following single injection of prostaglandin F2**α** analog inj. Clostenol(500mg) in 24 murrah buffaloes in private dairy farm, Jabalpur (M.P.). The result are promising but accurate diagnosis of corpus lutem in susceptible prone of PGF2**α** analog is required with heat detection for a period of 5 days.

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Synchroniztion of Estrus in Sub Estrus Murrah Buffaloes by Single Injection of PGF2α Analog in Low Breeding Season

Madhu Shivhare [α] & Dr. M. S. Thakur [σ]

Abstract- **Cent percent induction and fertility fine percent fertility was obtained following single injection of prostaglandin F2α analog inj. Clostenol(500mg) in 24 murrah buffaloes in private dairy farm, Jabalpur (M.P.). The result are promising but accurate diagnosis of corpus lutem in susceptible prone of PGF2α analog is required with heat detection for a period of 5 days.**

I. Introduction

About 40 percent buffaloes are usually sub estrous in summer season even in well arranged dairy farm. Prostaglandin F2α and its analog are effective in causing luteolysis leading to estrus with acceptable fertility. The present experiment was conducted to evaluate efficacy of single injection of Prostaglandin analog in induction, Synchroniztion of estrous and conception during summer in an organized farm.

II. Material and Methods

120 buffaloes are examined per rectly to determined the reproductive status during april–june 2012, at Choubey Dairy Farm, having a herd strength of 700 breading murrah buffaloes the dairy farm had water sprinklers on asbestos roof to keep the dairy farm cool , besides this all animals were bathed in morning and evening after milking.

The Experiment was conducted on 24 murrah buffaloes in a group of six animals having palpable corpus luteum (Supposed to be sub estrus) were injected 2ml Clostenol (500mg) I/m in a group of six animal murrah buffaloes during the period. Estrus detection was done from 2nd day of injection – 5th day in morning and evening in farm. Animals detected in estrus were served naturally by difficult bulls of known fertility. One group of 6 animals as control groups

III. Result and Discussions

The result obtained with single injection of prostaglandin F2α analog in sub estrus buffaloes which is tabulated below.

Response of Sub estrus murrah buffaloes to single injection of PGF2α analog

No. of animals treated	Interval between induction of estrus	Induction percent	Conception percent
a)24 experiment murrah buffaloes	77.66±0.003	100	75
b)6 control animals	-	-	-

All the sub estrus buffaloes treated with a single injection of Clostenol responded to treatment on an average interval of 77.66±88 hrs with maximum responding at 72 hrs. post injection (66.6) with fertility rate of 75 percent.

The result obtained in this study are higher than 90 percent induction report by PGF2α (Jha , 2011) in 72 – 96 hrs., however , the percent findings are close to induction interval of 85 ±4.4 hrs in breeding season with 500 mg inj. Clostenol in buffaloes (Chohor,1998) but are higher in conception rate. The high induction and synchronization rate obtained in present study may be due to good nutrition and managemental practices at the farm particularly provision of water sprinkle at roof of shed might have saved buffaloes from heat stress which resulted in excellent response in form of synchronization of estrus and high fertility more over selection of animals with corpus luteum, who were in susceptible prone and rigorous heat detection by the bull from 2nd – 5th day appear to be main factor in obtaining high induction and fertility in treated buffaloes.

IV. Conclusion

It is concluded that the Inj. Clostenol in dose of 500 mg is fully effective in synchronization of estrus in low breeding season, sub estrus murrah buffaloes with 75% fertility, however detection of corpus luteum in susceptible phase of prostaglandin is required for best result.

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Cryptosporidium Infection in Pre-Weaned Ruminants and Pigs in Southwestern Nigeria

By Akinkuotu Olufemi Ambrose & Fagbemi Benjamin Olakunle
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Abstract- The study was carried out to detect Cryptosporidium coproantigens in pre-weaned ruminants and pigs in Ogun State, southwestern Nigeria. Faecal samples of 186 pre-weaned animals comprising calves (n=32), lambs (n=47), goat kids (n=36) and piglets (n=71) were collected and examined for Cryptosporidium antigens by the use of an enzyme-linked immunosorbent assay (ELISA). 60.2% (112/186) of the samples were positive for Cryptosporidium antigens with infection rates of 78.1% (25/32), 51.1% (24/47), 83.3% (30/36) and 46.5% (33/71) for calves, lambs, goat kids and piglets respectively. The infection rates among animal species sampled were significantly different ($p<0.05$) from one another. The rate of infection in neonates, 73.5%, was significantly higher ($p<0.05$) than the rate recorded in other pre-weaned age group (>1 month-3 months). Furthermore, the infection rates, 67.3% and 68.6%, observed in females and diarrhoeic animals respectively were significantly higher ($p<0.05$) than those recorded in males (51.8%) and non-diarrhoeic animals (44.6%). This study demonstrates that Cryptosporidium infection is prevalent among pre-weaned category of ruminants and pigs in Ogun State, Nigeria.

Keywords: cryptosporidium, ELISA, nigeria, pre-weaned animals.

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Cryptosporidium Infection in Pre-Weaned Ruminants and Pigs in Southwestern Nigeria

Akinkuotu Olufemi Ambrose[α] & Fagbemi Benjamin Olakunle[σ]

Abstract- The study was carried out to detect *Cryptosporidium* coproantigens in pre-weaned ruminants and pigs in Ogun State, southwestern Nigeria. Faecal samples of 186 pre-weaned animals comprising calves (n=32), lambs (n=47), goat kids (n=36) and piglets (n=71) were collected and examined for *Cryptosporidium* antigens by the use of an enzyme-linked immunosorbent assay (ELISA). 60.2% (112/186) of the samples were positive for *Cryptosporidium* antigens with infection rates of 78.1% (25/32), 51.1% (24/47), 83.3% (30/36) and 46.5% (33/71) for calves, lambs, goat kids and piglets respectively. The infection rates among animal species sampled were significantly different ($p<0.05$) from one another. The rate of infection in neonates, 73.5%, was significantly higher ($p<0.05$) than the rate recorded in other pre-weaned age group (>1 month-3 months). Furthermore, the infection rates, 67.3% and 68.6%, observed in females and diarrhoeic animals respectively were significantly higher ($p<0.05$) than those recorded in males (51.8%) and non-diarrhoeic animals (44.6%). This study demonstrates that *Cryptosporidium* infection is prevalent among pre-weaned category of ruminants and pigs in Ogun State, Nigeria.

Keywords: cryptosporidium, ELISA, nigeria, pre-weaned animals.

I. Introduction

Cryptosporidium is a protozoan parasite that causes enteric infection in several animal species and humans (Quilez et al., 2008). Various species of *Cryptosporidium* infect mammals and humans with *C. parvum* being the most common zoonotic species (Quilez et al., 2008; Chako et al., 2010; Xiao, 2010). While several studies have revealed that cattle are important reservoirs of *C. parvum* (Xiao and Fayer, 2008; Xiao, 2010), sheep have been reported to be more frequently infected by other host-adapted *Cryptosporidium* genotypes, mostly *C. bovis* and the *Cryptosporidium* Cervine genotype and rarely habours the zoonotic *C. parvum* (Elwin and Chalmers et al., 2008; Mueller-Doblies et al., 2008). The most frequently encountered *Cryptosporidium* species in cattle are *C. parvum*, *C. andersoni*, *C. bovis* and *C. ryanae* (Amer et al., 2013; Couto et al., 2014). Sheep and goats are also commonly infected with *C. hominis*, *C. xiaoi*, *C. andersoni*, *C. fayeri*, *Cryptosporidium* Pig genotype II (Diaz et al., 2010; Fiuza et al., 2011). Pigs however are naturally infected with *C. muris*, *C. suis*, *C. srofarum*, *Cryptosporidium* Pig genotype II, *Cryptosporidium* Mouse genotype I and rarely the zoonotic *C. parvum* (Hamnes et al., 2007; Budu-Amoako et al., 2012; Zhang et al., 2013).

The parasite is considered as a major enteric pathogen associated with neonatal diarrhoea in calves (Xiao, 2010; Bhat et al., 2013), lambs and goat kids (Wang et al., 2010; Giadinis et al., 2012; Maurya et al., 2013) and piglets (Maikai et al., 2009; Zhang et al., 2013; Yui et al., 2014). The severity of the diarrhoea seen in cryptosporidiosis in pre-weaned animals is usually high and may ultimately lead to severe loss of condition and death (Ayinmode and Fagbemi, 2010).

The results obtained from several prevalence studies have been largely dependent on the sensitivity and specificity of the screening methods used and the age categories of the animals studied (Brook et al., 2008; Maikai et al., 2009; Ayinmode et al., 2010; Zhang et al., 2013; Yui et al., 2014). The diagnostic methods employed in studies on cryptosporidiosis include microscopic examination of acid-fast stained faecal smears (Maikai et al., 2009; Ayinmode and Fagbemi, 2010), the more sensitive immunoassays such as the enzyme-linked immunosorbent assay and immune-fluorescence assay (Ayinmode and Fagbemi, 2011; Cho et al., 2012; Giadinis et al., 2012) and the highly sensitive molecular techniques such as PCR which is used to differentiate and characterize the *Cryptosporidium* genotypes found in animals and humans (Ayinmode et al., 2010; Zhang et al., 2013).

This study was conducted to determine the rate of infection of *Cryptosporidium* in pre-weaned age category of ruminants and pigs in a southwestern state of Nigeria by the use of an enzyme-linked immune-sorbent assay.

II. Materials and Methods

a) Sample collection

The study was carried out in Ogun State, southwestern Nigeria. Samples were collected from five farms for each animal species considered. Faecal samples were collected from 186 randomly selected pre-weaned animals comprising calves (n=32), lambs (n=47), goat kids (n=36) and piglets (n=71). Each animal species were categorized into neonates (1 day to 1 month) and other pre-weaned age group (>1 month to 3 months).

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Stool samples were collected directly from the rectum of each animal. For animals in which rectal sampling was not possible, such as neonates, freshly voided faeces were collected by the use of wooden tongue depressors which were used to scoop up the superficial layer of faeces without contacting the floor. The faeces were then dropped into individual universal sample bottles and labeled appropriately. The stool samples were conducted to the laboratory in cold packs, where they were catalogued, processed and analyzed. The stool samples were stored under a temperature of 4°C until they were processed.

b) Detection of Cryptosporidium spp. antigens by ELISA

The detection of *Cryptosporidium* spp. coproantigens in the samples was done using a commercially available ELISA kit for faecal samples (RIDASCREEN® *Cryptosporidium*; R-Biopharm AG, Germany). The procedure was carried out according to manufacturer's instructions.

The optical densities (OD) of the samples were read at 450nm using an ELISA reader (BIOTEX; Model: ELx800, Biotex Instruments, USA). Samples were analyzed using the manufacturer's cut-off calculations in the instruction manual. The cut-off was calculated as shown below:

Cut-off = Extinction of the negative control + 0.15

Samples were considered positive if their extinction is more than 10% above the calculated cut off but considered negative if their extinction was more than 10% below the calculated cut-off. Samples were however considered as equivocal and repeated if their extinction was within the range 10% above to 10% below the cut-off.

III. Statistical Analysis

Data was collated and analyzed with Statistical Package for Social Sciences (SPSS) on Windows 7. Chi-square test was used to compare the differences in occurrence of *Cryptosporidium* spp. coproantigens between the categories, sexes and stool consistencies of all animal species at 5% level of significance.

IV. Results

Cryptosporidium spp. antigens were detected in all species of animals studied. 60.2% (112/186) of all pre-weaned animals examined were positive for *Cryptosporidium* coproantigens. The rates of infection were 78.1%, 51.1%, 83.3% and 46.5% for calves, lambs, goat kids and piglets respectively.

The infection rate observed in neonates of all animal species, 73.5% (72/98), was significantly higher ($p<0.05$) than the rate recorded in the other pre-weaned age group (>1 month to 3 months).

The rates of infection in females were higher in lambs, goat kids and piglets while the males recorded higher infection rate in calves. The overall infection rate in females, 67.3%, was significantly higher ($p<0.05$) than the rate observed in the males, 51.8%.

In all the animal species considered, the infection rates were higher in diarrhoeic animals than those without diarrhoea. The occurrence rate of *Cryptosporidium* infection was significantly higher ($p<0.05$) in pre-weaned animals with diarrhoea (68.6%) than the rate observed in those with formed stools (44.6%) (Table 1).

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Table 1 : Occurrence of *Cryptosporidium* spp. coproantigens in pre-weaned ruminants and pigs in Ogun State

Animal Species	Infected/Sampled	Occurrence (%)	Age Categories		Gender		Stool Consistency	
			1day -1month	>1month -3months	Male	Female	Diarrhoeic	Non -diarrhoeic
Calves	25/32	78.1	94.4% (17/18)	57.1% (8/14)	82.4% (14/17)	73.3% (11/15)	81.0% (17/21)	72.7% (8/11)
Lambs	24/47	51.1	65.2% (15/23)	37.5% (9/24)	37.5% (9/24)	65.2% (15/23)	62.1% (18/29)	33.3% (6/18)
Goat kids	30/36	83.3	85.0% (17/20)	81.2% (13/16)	66.7% (8/12)	91.7% (22/24)	92.0% (23/25)	63.6% (7/11)
Piglets	33/71	46.5	62.2% (23/37)	29.4% (10/34)	40.6% (13/32)	51.3% (20/39)	54.3% (25/46)	32.0% (8/25)
Total	112/186	60.2	73.5% (72/98)	45.5% (40/88)	51.8% (44/85)	67.3% (68/101)	68.6% (83/121)	44.6% (26/65)

V. Discussion

The detection of *Cryptosporidium* infection in ruminants and pigs within 3 months of age supports several reports carried out in Nigeria (Maikai et al., 2009; Ayinmode and Fagbemi, 2010; Faleke et al., 2013; Pam et al., 2013) and in other countries (Zhang et al., 2013; Yui et al., 2014). Furthermore, the rate of infection (60.2%) observed in this age category of ruminants and pigs was higher than previous reports of 9.3% in Nigeria (Pam et al., 2013), 28.0% in UK (Brook et al., 2008), 24.5% in Australia (Yang et al., 2009) and 30.2% in Egypt (Amer et al., 2010). Differences in prevalence rates of cryptosporidiosis have been suggested to depend on the age category of animals studied, method of detection and season in which sampling was done (Brook et al., 2008; Yang et al., 2009; Yui et al., 2014). The high infection rate observed in this study may therefore result from the higher risk of infection reportedly possessed by pre-weaned animals (Chen et al., 2011; Zhang et al., 2013; Yui et al., 2014) which may be associated with their underdeveloped immune system and/or the husbandry practice on farms in Nigeria in which animals, irrespective of their ages, are grazed together thereby facilitating neonatal transmission of the infection (Ayinmode and Fagbemi, 2010; Fuiza et al., 2011; Bhat et al., 2013). Furthermore, the ELISA used in the study may also contribute to the higher rate of detection of the parasite as this method has been reported to possess higher sensitivity than the acid-fast staining method utilized in majority of previous investigations in Nigeria (Yilmaz et al., 2008).

The specie-specific occurrence rates observed in this study were higher than previous reports of 27.4%, 33.0% and 25.0% among calves in Oyo State (Ayinmode and Fagbemi, 2010), Sokoto State (Faleke et al., 2013) and Sokoto State (Pam et al., 2013) respectively. They were also higher than 16.0% recorded among goat kids in Plateau State (Pam et al., 2013), 15.7% and 13.6% among piglets in Kaduna State (Kwaga et al., 1988; Maikai et al., 2009) and 24.5% among lambs in Australia (Yang et al., 2009). This observation may therefore imply that *Cryptosporidium* oocysts survive for longer periods in the study area, possibly due to the high relative humidity and rainfall, thereby leading to higher risks of transmission to animals (Yu and Seo, 2004).

The lowest occurrence rate recorded among piglets corroborates the submissions of Maddox-Hyttel et al. (2006) and Hamnes et al. (2007) suggesting that *Cryptosporidium*-infected piglets shed lower numbers of oocysts when compared to calves and other animals as these infected piglets shed oocysts intermittently for only 4 weeks.

The higher rate of infection seen in neonates supports the findings of several studies (Maikai et al., 2009; Yang et al., 2009; Ayinmode and Fagbemi, 2010; Budu-Amoako et al., 2012; Zhang et al., 2013). This therefore suggests that neonates of ruminants and pigs are important reservoirs of infection and are mainly responsible for contamination of water with oocysts of *Cryptosporidium*.

Our observation of higher infection in diarrhoeic animals is in tandem with reports of Maurya et al. (2013) and Caccio et al. (2013). The diarrhoea observed in cryptosporidiosis may be associated with the pathogenesis of the parasite and concurrent infection with other enteric pathogens such as *Salmonella*, *Escherichia coli*, *Eimeria*, Rotavirus and *Giardia* (Thompson et al., 2007; Ayinmode and Fagbemi, 2010). This observation therefore suggests that pre-weaned animals having diarrhoea should be routinely screened for *Cryptosporidium*.

The results of this study indicates that *Cryptosporidium* infection is highly prevalent in pre-weaned ruminants and pigs with neonates and diarrhoeic animals being at higher risk of infection and may also serve as important sources of infection to other animals and humans in the study area. The ELISA employed in this study, though rapid, easy to perform and interpret, is less sensitive in detecting the species of *Cryptosporidium* infecting the animals in the study. This has been associated with the cross-reactivity of ELISA-positive *C. parvum* with other species identified by PCR-based molecular techniques (Ayinmode and Fagbemi, 2011). The need to know the species of *Cryptosporidium* affecting pre-weaned ruminants and pigs and their zoonotic potential therefore necessitates the use of molecular techniques in the detection and genotyping of

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Cryptosporidium species found in these animals (Amer et al., 2013).

Ethical consideration

The manuscript does not contain clinical studies or patient data.

Conflict of interest

The authors declare that they have no conflict of interest.

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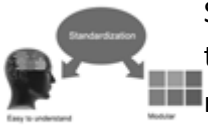

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1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. 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.

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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.

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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.
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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.

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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.

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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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