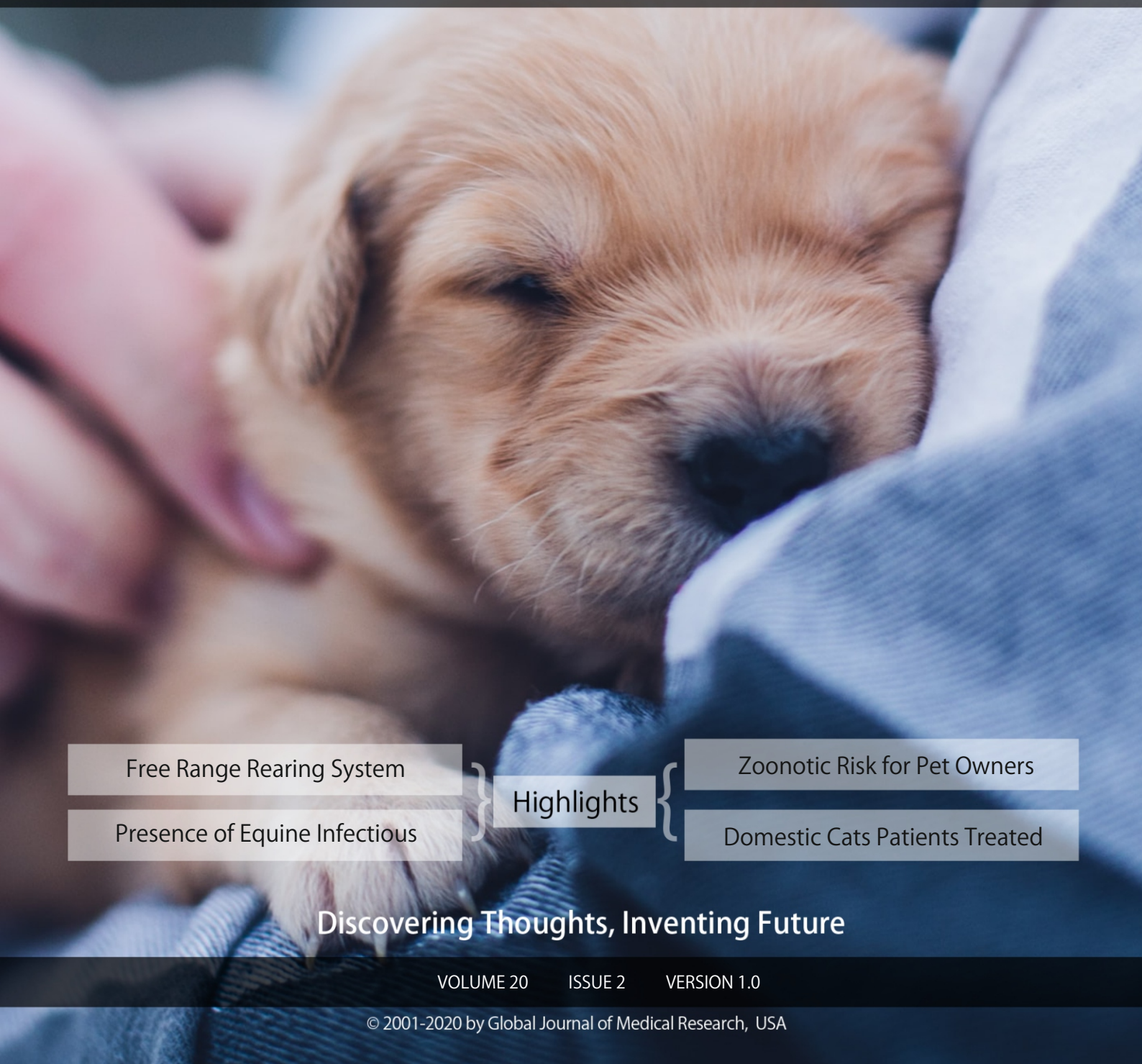


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Domestic Cats Patients Treated

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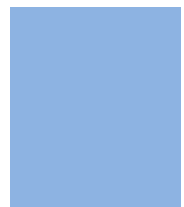
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## Presence of Equine Infectious Anemia in work Horses in San Andrés Itzapa, Chimaltenango, Guatemala

By García-García, Sandy Stephanie, Chávez-López, Juan José  
& Villatoro-Chacón, Daniela Mariel

*Universidad de San Carlos de Guatemala*

**Abstract-** Equine infectious anemia (IEA) is a worldwide distribution disease caused by a lentivirus associated with the presence of transmitting vectors of the gender *Tabanus* and equines by producing anemia and consequently hypoxia that produces a multi-organ deterioration. The presence of AIE in the working equine of San Andrés Itzapa, Chimaltenango was evaluated. This in order to assess the presence of the disease since this group of equines is part of the food security of the community. 100 blood samples were analyzed using the gel agar immunodiffusion test. The presence of the disease was found in 8% of the sampled animals. The prevalence was higher in females than in males. The mean age of the horses sampled was 5 years. The mean body condition of the study population was 2. The presence of the disease in the study population should be evaluated and considered as a starting point for the implementation of control and prevention measures given the vector spread of the disease. -age and the impact on the community due to the zootechnical function of the sampled animals.

**Keywords:** anemia, equine, immunodiffusion.

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



PRESENCE OF EQUINE INFECTIOUS ANEMIA IN WORK HORSES IN SAN ANDRÉS ITZAPA CHIMALTENANGO GUATEMALA

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# Presence of Equine Infectious Anemia in work Horses in San Andrés Itzapa, Chimaltenango, Guatemala

## Presencia De Anemia Infecciosa Equina En Caballos De Trabajo En San Andrés Itzapa, Chimaltenango, Guatemala

García-García, Sandy Stephanie <sup>α</sup>, Chávez-López, Juan José <sup>σ</sup> & Villatoro-Chacón, Daniela Mariel <sup>ρ</sup>

**Resumen-** La anemia infecciosa equina (AIE) es una enfermedad de distribución mundial causada por un lentivirus asociado a la presencia de vectores transmisores del género *Tabanus* y *Stomoxys*. Esta enfermedad tiene un impacto en la salud de los equinos por producir una anemia y consecuentemente hipoxia que produce un deterioro multiorgánico. Se evaluó la presencia de AIE en los equinos de trabajo de San Andrés Itzapa, Chimaltenango. Esto con el fin de evaluar la presencia de la enfermedad ya que este grupo de equinos forma parte de la seguridad alimentaria de la comunidad. Se analizaron 100 muestras de sangre utilizando la prueba de inmunodifusión en agar gel. Se encontró la presencia de la enfermedad en el 8% de los animales muestreados. La prevalencia fue mayor en hembras respecto a los machos. La edad media de los equinos muestreados fue de 5 años. La condición corporal media de la población de estudio fue de 2. La presencia de la enfermedad en la población de estudio debe evaluarse y ser considerada como punto de partida para la implementación de medidas de control y prevención dada la diseminación vectorial de la enfermedad y el impacto en la comunidad debido a la función zootécnica de los animales muestreados.

**Palabras clave:** anemia, equinos, inmunodifusión

**Abstract-** Equine infectious anemia (IEA) is a world wide distribution disease caused by a lentivirus associated with the presence of transmitting vectors of the gender *Tabanus* and *Stomoxys*. This disease has an impact on the health of the equines by producing anemia and consequently hypoxia that produces a multi-organ deterioration. The presence of AIE in the working equine of San Andrés Itzapa, Chimaltenango was evaluated. This in order to assess the presence of the disease since this group of equines is part of the food security of the community. 100 blood samples were analyzed using the gel agar immunodi-fusion test. The presence of the disease was found in 8% of the sampled animals. The prevalence was higher in females than in males. The mean age of the horses

sampled was 5 years. The mean body condition of the study population was 2. The presence of the disease in the study population should be evaluated and considered as a starting point for the implementation of control and prevention measures given the vector spread of the disease. -age and the impact on the community due to the zootechnical function of the sampled animals.

**Keywords:** anemia, equine, immunodiffusion.

### 1. INTRODUCCIÓN

Los equinos son requeridos en las fincas con alta demanda del sector ganadero. En Guatemala, el grupo de ganado caballar tiene una función zootécnica de trabajo principalmente en áreas rurales. Además, forma parte de la seguridad alimentaria del hogar. En las montañas, el caballo mueve diversos productos en la agricultura, como el café y leña (Gamarro, 2019). Según el Instituto Nacional de Estadística de Guatemala (INE), en el último censo equino realizado en el 2003, se estima una población de 218,675 équidos.

La anemia infecciosa equina (AIE) es una enfermedad de distribución mundial que afecta a los équidos (Sánchez et al., 2018). Es causada por un virus del género *Lentivirus* de la familia *Retroviridae*. Se ha reportado la presencia de la enfermedad en regiones con clima húmedo debido a la presencia de vectores transmisores del género *Tabanus* y *Stomoxys calcitrans* (Borges et. al., 2013).

La presentación clínica de la enfermedad suele ser crónica. Los animales infectados se convierten en portadores con signos inaparentes por lo que suele pasar inadvertida. Impacta negativamente tanto en la salud como el bienestar de los equinos, ocasionando pérdidas en el rendimiento y económicas en el ganado que es utilizado como fuerza de trabajo. (Garzón, 2015; Estrada, et al. 2018 y Sánchez, et al. 2018).

En Guatemala, el Ministerio de Agricultura, Ganadería y Alimentación (MAGA) registra proporciones de la enfermedad hasta de 18.54% entre los años 2005 al 2012. Por su parte Borja (2014) encontró 8.45% en un estudio retrospectivo sobre casos de AIE en Guatemala.

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Sin embargo, no todas las regiones del país han sido muestreadas en dichos estudios. En general, los equinos en Guatemala son una población poco estudiada, por lo que se desconoce el comportamiento epidemiológico de las enfermedades en el país. Borja (2014) menciona que avances relacionados a la salud equina del área en el año 2012 al 2013 son mínimos, y no se les brinda la atención necesaria lo que disminuye el tiempo de vida del equino.

San Andrés Itzapa es un municipio del Departamento de Chimaltenango cuya actividad económica depende del sector agrícola. La población estimada de équidos en el municipio de San Andrés Itzapa incluyendo sus aldeas y cantones es de 4,000 animales. Los équidos de trabajo en San Andrés Itzapa, representan una población aproximada de 1,100 (SABESA, 2019). Los equinos de San Andres Itzapa son ejemplares criollos en su mayoría. Son utilizados para el transporte en los caminos del área rural y trabajo de carga. Su alimentación se basa en el pastoreo, careciendo de suplementación y programas sanitarios (Mandrujano, 2017). Esto hace que muchos equinos sean utilizados como fuerza de trabajo, siendo un eje fundamental como colaborador en el trabajo diario para obtener el aporte económico para las familias.

El present estudio tiene la finalidad de encontrar la presencia de Anemia Infecciosa Equina en los equidos del municipio de San Andrés Itzapa, Chimaltenango. Esto con la finalidad de encontrar evidencia de la enfermedad y generar información respecto al comportamiento epidemiológico de la misma. De esta forma se podrán generar e implementar medidas de control y prevención en el área de estudio.

## II. MATERIALES Y MÉTODOS

### a) Área de estudio

El estudio se realizó en el municipio de San Andrés Itzapa, departamento de Chimaltenango; ubicado 65 km de la ciudad capital de Guatemala, cuyas coordenadas son 14°37'12" N, 90°50'39" O.

### b) Criterios de inclusión

En el estudio se incluyeron equinos de trabajo del municipio de San Andrés Itzapa, cuya función

zootécnica fuera de trabajo (carga de material para posterior venta). Además, se incluyeron animales mayores a seis meses de edad, no importando el sexo y condición corporal.

### c) Tamaño de muestra

El muestreo se realizó por conveniencia, en base a la previa autorización de los propietarios para participar en el estudio y coleccionar la muestra sanguínea de sus animales. De esta forma se obtuvo una muestra total de 100 equinos de trabajo.

### d) Toma de muestra

Las muestras de sangre fueron obtenidas por punción de la vena yugular utilizando la técnica descrita por OIE (2005) y PAHO (2017). Se coleccionaron 10 ml de sangre que fue colocada en tubos al vacío sin anticoagulante para su posterior extracción de suero. Las muestras fueron transportadas en hieleras, manteniendo una temperatura de 4° C hasta su llegada al Laboratorio de Sanidad Animal del VISAR del Ministerio de agricultura y Ganadería de la ciudad de Guatemala, en un periodo no mayor de 24 horas.

Se tomaron registros de los animales muestreados como sexo, edad, peso y condición corporal según escala de Carroll & Huntington (1988).

### e) Prueba diagnóstica

La prueba utilizada para la identificación de la Anemia Infecciosa Equina en los équidos fue inmunodifusión en agar gel según el protocolo indicado por la Organización mundial de sanidad animal (OIE, 2019).

### f) Análisis de datos

Los datos obtenidos fueron resumidos utilizando estadística descriptiva y tablas de contingencia, utilizando distribuciones de frecuencias, medias para las variables.

## III. RESULTADOS

Se encontró la presencia de Anemia Infecciosa Equina en la población de estudio utilizando la prueba de inmunodifusión en agar gel. La prevalencia de los animales muestreados fue del 8%. En el cuadro 1 se describen las prevalencias por sexo.

**Cuadro 1:** Distribución de frecuencias de equinos muestreados para Anemia infecciosa equina

Sexo	Positivos		Negativos		Total
	N	%	N	%	
Hembras	5	10	50	90	55
Machos	3	6.67	42	93.3	45

La edad media de los equinos muestreados fue de 5 años. En la Cuadro 2 se presenta la

categorización de las edades de los equinos muestreados.

**Cuadro 2:** Categorización de la edad de los equinos muestreados a AIE

Edad	Positivos		Negativos		Total N
	N	%	N	%	
6 meses a 2 años	1	2.86	34	97.14	35
3 a 6 años	2	5.71	33	94.29	35
7 a 9 años	1	7.69	12	92.31	13
Más de 10 años	4	23.53	13	76.47	17

La condición corporal de la población de estudio fue de 2. En el cuadro 3 se presenta la condición corporal de los animales muestreados utilizando la escala de Carroll & Huntington (1988).

**Cuadro 3:** Condición corporal de los equinos muestreados.

Condición corporal	Positivos		Negativos		Total
	N	%	N	%	
1	2	13.33	13	86.67	15
2	6	7.59	73	92.41	79
3	0	0	6	6	6
4	0	0	0	0	0
5	0	0	0	0	0

#### IV. DISCUSIÓN

La presencia de AIE en los equinos muestreados puede deberse a las condiciones climáticas del área de estudio. El municipio de San Andrés Itzapa es una zona de bosque húmedo tropical, las cuales son condiciones ambientales que permiten la distribución del vector. Según Craigo & Montelaro (2008) la incidencia de caballos infectados con AIE es la más alta en climas tropicales y subtropicales. Por lo tanto, el clima tropical y la presencia de humedales es favorable para el crecimiento de vectores mecánicos y la carga de los mismos puede ser mayor que en otros climas permitiendo así la constante exposición de équidos susceptibles a vectores hematófagos y équidos infectados (Borges et al., 2013). Además, debe de considerarse la transmisión puede ser iatrogénica por el mal uso de agujas o instrumentos contaminados y a través del calostro (Clabough, 1993).

En cuanto a la edad, la media de los equinos muestreados fue de 5 años siendo el 23.53% equinos mayores de 10 años. Borges et al. (2013), indica que los equinos de trabajo y equinos mayores de 10 años aumentan su probabilidad de exposición por vectores y a cualquier otro tipo de transmisión iatrogénica de la enfermedad.

La condición corporal de la población de estudio fue de 2, siendo un 7.59% corresponde a condición corporal 2 y un 13.33 en condición corporal 1.

Algunos factores externos relacionados a la finalidad laboral, horas de trabajo, alimentación y salud pueden ser reflejados en la condición.

Las prevalencias obtenidas en el presente estudio, son similares a los resultados obtenidos por Borga (2014). Sin embargo, difieren con los estudios realizados por el MAGA en los años 2005 al 2012. Esto puede deberse al tamaño de la muestra, las áreas y la amplitud del estudio. Garzón (2015) hace referencia a estudios que indican la prevalencia de AIE en América Latina que oscilan desde el 2% al 28% donde se evidencia la falta de investigación de la enfermedad principalmente en Centro América. Sin embargo, Centroamérica puede tener resultados similares a Nicaragua (8.07%) como sugiere Garcia (2015). Esto se debe a que en esta región predominan las zonas tropicales o subtropicales donde permite una fácil diseminación del virus por medio de los vectores mecánicos favorecidos por las condiciones climáticas y geográficas (Estrada et al, 2018; Sánchez et al, 2018; Garzón, 2015; García, 2015 y Morales, 2015). Por esta razón, es necesario llevar a cabo más investigaciones de la distribución de la enfermedad.



## V. AGRADECIMIENTOS

A los supervisores por las valiosas sugerencias. A los propietarios que permitieron las tomas de muestras en sus animales. A SABESA, MV Leonardo Montufar, Lic. Alejandro Salazar, Alejandra de Matta, René Porras y Wilson Azurdía por el material, transporte y apoyo brindado para la toma de muestras. A la Dra. Evelyn Godoy y al Laboratorio de Sanidad Animal del VISAR del Ministerio de Agricultura y Ganadería por las pruebas.

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## Zoonotic Risk for Pet Owners in Kathmandu, Nepal

By Kosh Bilash Bagale, Basanta Gaire & Ramesh Adhikari  
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**Abstract-** Pet keeping practices have a long history in human societies, but poor knowledge regarding pet keeping increases risk of several zoonoses. The aim of this study is to identify the risk of zoonoses based on knowledge, practices, and perception of threat among pet owners in Kathmandu. A cross-sectional questionnaire based study was conducted on 143 purposively selected pet owners from different three wards in Kathmandu metropolitan. Most of the respondents 86.7% (n=143) were keeping dog as a pet in their households. Feeding practices for pet was varied based on economic status where almost a fifth (18%) owners feeding readymade food. The purpose of pets keeping found diverse where most of them kept for purpose of household security (37.76%). All respondents were literate among them 88.11% of respondents had above secondary level qualifications. The majority of respondents received information from radio/television (92.30%)

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**GJMR-G Classification:** NLMC Code: WC 950



*Strictly as per the compliance and regulations of:*



# Zoonotic Risk for Pet Owners in Kathmandu, Nepal

Kosh Bilash Bagale <sup>α</sup>, Basanta Gaire <sup>σ</sup> & Ramesh Adhikari <sup>ρ</sup>

**Abstract-** Pet keeping practices have a long history in human societies, but poor knowledge regarding pet keeping increases risk of several zoonoses. The aim of this study is to identify the risk of zoonoses based on knowledge, practices, and perception of threat among pet owners in Kathmandu. A cross-sectional questionnaire based study was conducted on 143 purposively selected pet owners from different three wards in Kathmandu metropolitan. Most of the respondents 86.7% (n=143) were keeping dog as a pet in their households. Feeding practices for pet was varied based on economic status where almost a fifth (18%) owners feeding readymade food. The purpose of pet keeping found diverse where most of them kept for purpose of household security (37.76%). All respondents were literate among them 88.11% of respondents had above secondary level qualifications. The majority of respondents received information from radio/television (92.30%). Compare with knowledge on common zoonoses (like; salmonellosis (11.9%), toxoplasmosis (7.0%), pet-related allergy (41.3%)) found poor. But, most of the respondents (99.3%) had good knowledge about rabies, however, only half (52.44%) had knowledge about freely availability of anti-rabies vaccine (ARV) in government hospitals. The study area, almost four out of five (79 %) owners still practice open defecation for their pets. Data showed the association between level of education and threats perception on zoonoses. It is suggested that short orientation sessions about pet handling and zoonoses prevention practices should be provided to people interested to keep pets.

**Keywords:** zoonoses, pet keeping, education, kathmandu, perception, owners.

## 1. INTRODUCTION

Pet keeping practices have a long history in human societies. Mahabharata, which is the great Hindu epic, mentions that Yudhishtir, the great follower of the PATH OF DHARMA, had a dog as a pet (Mahabharata, MAHA- PRASTHANI PARVA). He had refused to pass the gate of heaven without his dog. Pet keeping practice is continuing in the modern age with diverse species of pets, and is now a symbol of modernization all over the world. In the context of Nepal, pet keeping practice is growing among the city dwellers. Dog, cat, different types of birds, reptiles are the common rearing pet in Nepal; however, it depends on people's passions and desires.

There are several benefits of pet keeping like; health benefits through increasing the opportunity to exercise; regular walking or playing with a pet can decrease blood pressure, emotional and social benefits (Stull, Brophy & Weese, 2015). Similarly pet can help manage loneliness and depression by giving us companionship as well as security to households and society by some kinds of pet, i.e., dog.

However, pets can be the source of several zoonotic diseases in different way. Pets can transmit infections to human either as a host (primary or secondary) or as healthy or asymptomatic carrier. Diseases which are transmitted between animal to human or vice versa are called zoonoses. World Health Organization (WHO) defines, 'Zoonoses are those diseases and infections which are naturally transmitted between vertebrate animals and human'. There are several zoonotic diseases prevalent in the world, often occurring as endemic, epidemic, or pandemic.

The three recent worldwide viral outbreaks, namely SARS (Severe Acute Respiratory syndromes), the bird flu (H5N1), and the swine flu (H1N1), are all examples of zoonoses. Even the HIV is believed to be transmitted from chimpanzee to humans in the last century (Basnyat, 2013). Likewise, ongoing pandemic of "Novel Corona Virus (CoVID-19) outbreaks from China and terrifies all over the world with many fatal outcomes. According to WHO daily press conference of last April 2020, more than two hundred countries of the world affected by the disease; however, USA, Spain, Italy, UK, China are badly affected by the disease until March 2020 and, still now it is spreading rapidly throughout the world. Although the source of COVID- 19 is not exactly determined by the epidemiologist, but they had concluded that this is from animal sources (zoonoses).

So based on facts and findings, most of the human diseases (more than 60 percent) come from animal sources (Niroula, 2016) and also due to illiteracy, poverty, or might be negligence –pet owners are exposed to the risk of zoonotic diseases. People who are in close contact with animals (pets) and have inadequate knowledge or poor practices are more susceptible to zoonoses. Livestock farmers, veterinarians, para veterinarians are close to animals due to their profession, and pet owners are also close to their pets. Similarly, most of them keep their pets inside the house and even in the same bed. Some people enjoy pets by kissing pets and playing with them.

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Due to the lack of proper studies, we have not actual data about incidence and prevalence of pet-related zoonoses in Nepal and also have not an idea about the knowledge, practices, and perception related to zoonoses among pet owners. So this study tries to identify the risk of zoonoses among pet owners in Kathmandu Nepal based on their existing knowledge, practices and perceived threats related to zoonoses and explore the factors associated with it.

## II. METHOD

This study was descriptive and cross-sectional study design. Data was collected from three randomly selected wards of Kathmandu metropolitan (ward no 3, 16, and 26). All the respondents who visited the park of these three wards were interviewed. A total of 143 pet owners were the sample of the study.

## III. DATA COLLECTION PROCEDURE

Respondents were interviewed with a semi-structure questionnaire on different aspects of pet management knowledge, practices, and perception on

zoonotic diseases. The questionnaire was prepared and pre -tested before the final data collection. Researchers visited the Pet owners on the respected wards (parks). Afterd is closing the purpose and methods to the respondents, the researcher started collecting the information from the respondents.

## IV. ETHICAL APPROVAL

No ethical approval was required for this study, as it was a survey-based study. However, verbal consent was taken during data collection process from all participants.

## V. RESULTS

After the interview every questionnaire was checked thoroughly. Questionnaires containing any obscure or misunderstood answered were excluded during the final data analysis. Data were entered into MS excel and exported into IBM SPSS 20 version for descriptive data analysis.

*Table 1:* Socio- demographic characteristics of the respondents

Description	Frequency (n= 143)	Percent
<b>Age in year</b>		
20 – 29	19	13.28
30 – 39	49	34.26
40 – 49	42	29.37
50 – 59	22	15.38
60 – 69	8	5.59
Above 70	3	2.097
<b>Ethnicity</b>		
Dalits	2	1.4
Janajati	65	45.5
Barmin/Chettri	75	52.4
Others	1	0.7
<b>Religion</b>		
Hindu	120	83.9
Buddies	19	13.3
Christian	2	1.4
Muslim	1	0.7
Others	1	0.7
<b>Education status</b>		
Basic Education (1-8)	16	11.18
Higher Secondary (9-12)	69	48.3
Above Bachelor	58	40.55
<b>Average monthly income</b>		
Below 50,000	108	75.52
Above 50,000	35	24.47
<b>Species of pet</b>		
Dog	124	86.7
Cat	18	12.58

Others (Tortoise)	1	0.7
<b>Purpose of pet keeping</b>		
Security	54	37.76
Self-hobby	43	30.06
Children pressure	33	23.07
Other	13	9.09

Table 1 reveals the socio-demographic status of the respondents. Among 143 respondents, a higher percentage were 30-39 years 49 (43.26%), and least 3 (2.09%) were above 70. The majority of the respondents, 75 (52.4%), were Brahmin/Chhettri. Likewise, Janajati were 65 (45.5%), and Dalits were 2 (1.4%). Basis of religion, most of the respondents 120 (83.9%) were Hindu, and 19 (13.3%) were Buddhists. Christian and Muslim were very negligible 1.4 and 0.7 percentages respectively. All respondents found literate and, most of

them, 126 (88.11%) had a more than higher secondary education. Economically most of the respondents 108 (75.52%) were below fifty thousand average monthly income (n=143). Most of the pet owners, 124 (86.7%) kept dogs, 18 (12.58%) kept cats, and only 1 (0.7%) kept tortoise as a pet in their households. Likewise, 54 (37.76%), 43 (30.06%), 33 (23.07%) respondents kept their pet for security, self-hobby and children pressure respectively, but 13 (9.09%) were not any specific purpose.

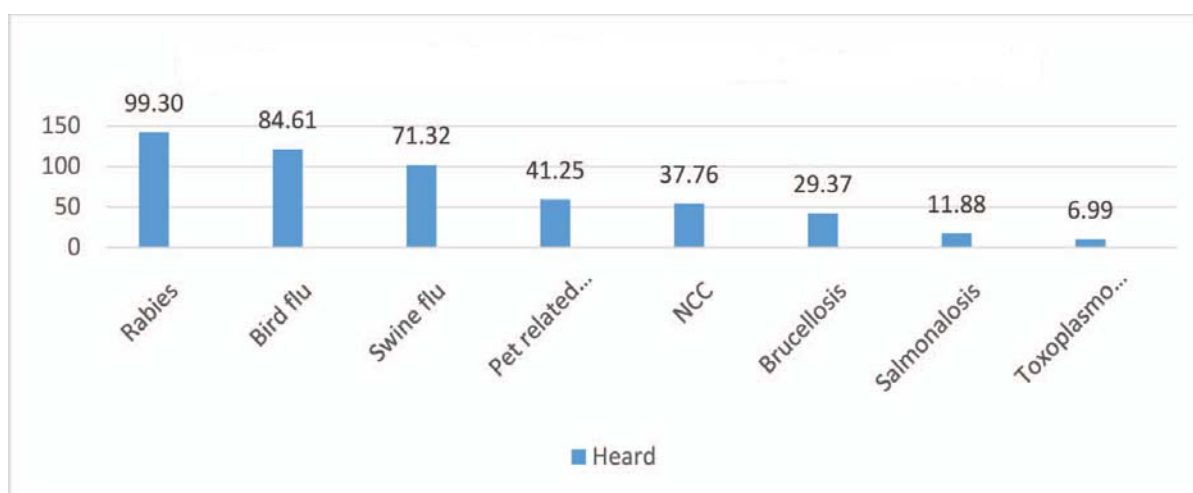


Figure 1: Heard about the Zoonotic Diseases n=143

Figure 1 shows that almost all 142 (99.3%) respondents had heard about rabies. Similarly, more than two-third of the respondents had heard about bird flu and swine flu, but still, only 41.25%, 37.76%, 29.37%, 11.88%, and 6.9% had heard about pet related allergy,

Neuro-cysticercosis (NCC), brucellosis, salmonella, and toxoplasmosis respectively. Similarly among 143 respondents, the majority (138, 96.50%) had agreed that their pets could be the source of zoonotic disease.

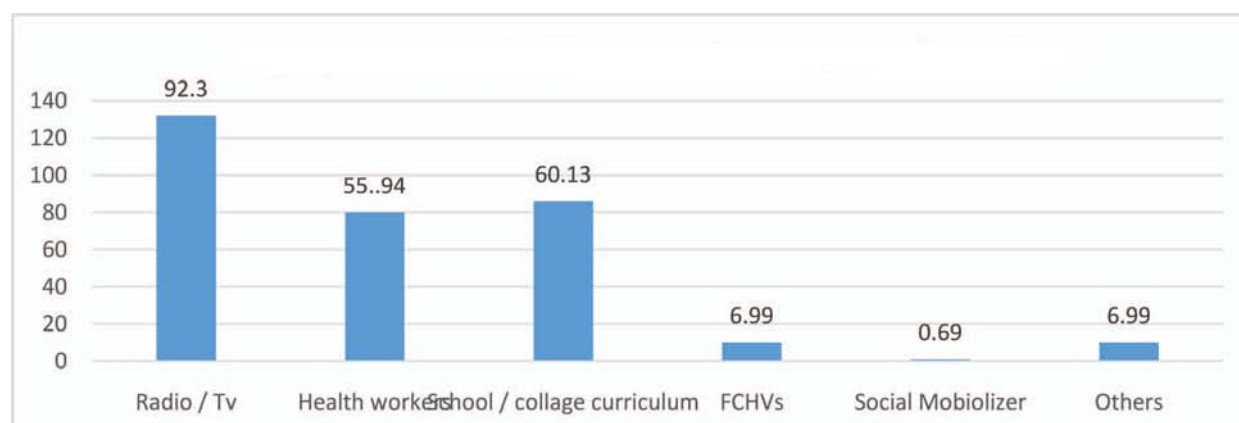


Figure 2: Sources of information about Zoonoses (n=143)

Figure 2 showed that pet owners obtained the information from more than one source. Most of the respondents, 132 (92.3%) said that their sources of knowledge about zoonoses was radio/television. Health workers were also found to be a remarkable source of information for zoonoses 80 (55.9%). 86 (60.1%) respondents learned that knowledge from the school/college curriculum. Female Community Health Volunteers (FCHVs) were sources of information for 10 (7.0%) respondents. But in contrast, social mobilizers

who are main agents of communication in societies were found to have a minimal role in disseminating information about zoonoses (1, 0.7%).

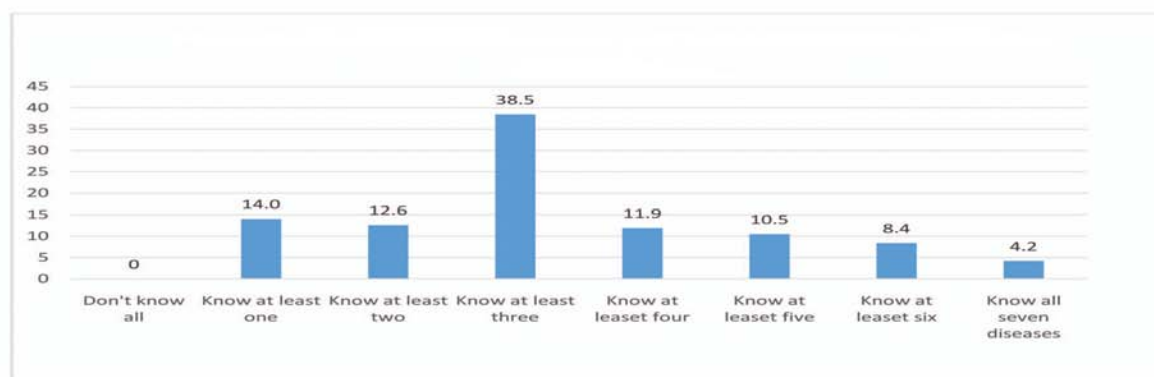
Results showed that most of the respondents (134, 93.7%) followed the recommended vaccination schedule. However, few (8, 5.6%) pet owners never provided vaccines for their pets. The reason for not immunizing pets was lack of information (6, 60%) and lack of time (2, 40%).

**Table 2:** Exposure of the respondents to potential risk factors associated with various activities and pet management practices

Exposure Description	Number	Frequency n= 143 Percentage
<b>Consultation during sickness of pet</b>		
Consult the Vet	130	90.90
Conservative therapy	13	9.1
<b>Place of pet keeping at night</b>		
Outside the door	21	14.68
Inside the door but separate	116	81.11
Same bed with owners or members	6	4.19
<b>Bathing schedule of the pet</b>		
Monthly	49	34.26
If necessary	94	65.73
<b>Place of defecation</b>		
Own house	30	20.97
Street/ park	113	79.03
<b>Food for pets</b>		
Left-over food	6	4.19
Readymade foods from market	27	18.88
Same food as family member	110	76.92

Table 2 showed that most of the pet owners followed the safety measures. Out of 143 respondents, 90.90% consult the vet during the sickness of their pets, but 9.0% of them still go for conservative practices. Similarly, 14.68% of respondents had kept their pets outside the living room, 81.11% had kept inside the room on separate places, but around 4.0% of respondents replied that keep their pets in their living room and sometimes in

the same bed. Likewise 79.03% of pet owners used public places (road or park) for defecation to their pets. However, all respondents who had cats as pets used to defecate pets inside their house. Feeding practices showed that 76.92% used the same food for family members and pets, 4.19% were giving leftover food, and 18.88% provided readymade food bought from the market.



**Figure 3:** Possible Causes of pets Died (n=18)

Figure 3 showed that majority of the pet owners 125 (87.41%) had no experience of pet died in their household however 18 (12.58%) had faced death incidence. Most common cause of death of pets were

aging (27.77%), scabies (16.66%), food poisoning (11.11%), heart attacks (11.11%), and injury (11.11%) while 22.22% of the respondents had killed their pets due to aggressive behaviour.

*Table 3:* Knowledge of particular zoonotic disease as reported by respondents

	Disease specific variables	Frequency n=143	Percentage
Rabies	Knowledge about mood of transmission:		
	Yes	142	99.30
	No	1	0.7
	Knowledge about sign and symptoms:		
	Yes	132	92.30
	No	11	7.70
	Knowledge about Preventive measure:		
	Yes	130	90.90
	No	13	9.10
	Government of Nepal provide ARV free in cost		
	Yes	75	52.44
	I don't know	45	31.46
	No	23	16.08
	Possible outcome of rabies:		
Swine flu	It is curable	16	11.18
	It is 100% fatal disease	102	71.46
	I don't know	25	17.48
	Knowledge about mood of transmission		
	Yes	102	71.32
	No	41	28.76
	Knowledge about sign and symptoms		
	Yes	84	58.74
Bird flu	No	59	41.25
	Knowledge about preventive measures:		
	Yes	86	60.13
	No	57	39.86
	Knowledge about mood of transmission		
	Yes	118	82.51
	No	25	17.48
	Knowledge about sign and symptoms		
Brucellosis	Yes	100	69.93
	No	43	30.06
	Knowledge about preventive measures:		
	Yes	100	69.93
	No	43	30.06
	Knowledge about mood of transmission		
	Yes	37	25.87
	No	106	74.12
Toxoplasmosis	Knowledge about sign and symptoms		
	Yes	30	20.97
	No	113	79.02
	Knowledge about preventive measures:		
	Yes	37	25.87
	No	106	74.12
	Knowledge about mood of transmission		
	Yes	8	5.59
	No	135	94.40
	Knowledge about sign and symptoms		
	Yes	30	20.97
	No	113	79.02
	Knowledge about preventive measures:		
	Yes	37	25.87
	No	106	74.12

Salmonellosis	Knowledge about mood of transmission	
	Yes	16
	No	127
	Knowledge about sign and symptoms	
	Yes	14
	No	129
	Knowledge about preventive measures:	
	Yes	16
NCC	Knowledge about mood of transmission	
	Yes	52
	No	91
	Knowledge about sign and symptoms	
	Yes	48
	No	95
	Knowledge about preventive measures:	
	Yes	50
	No	93

Table no 3 shows the disease based knowledge related to various aspects of zoonotic disease.

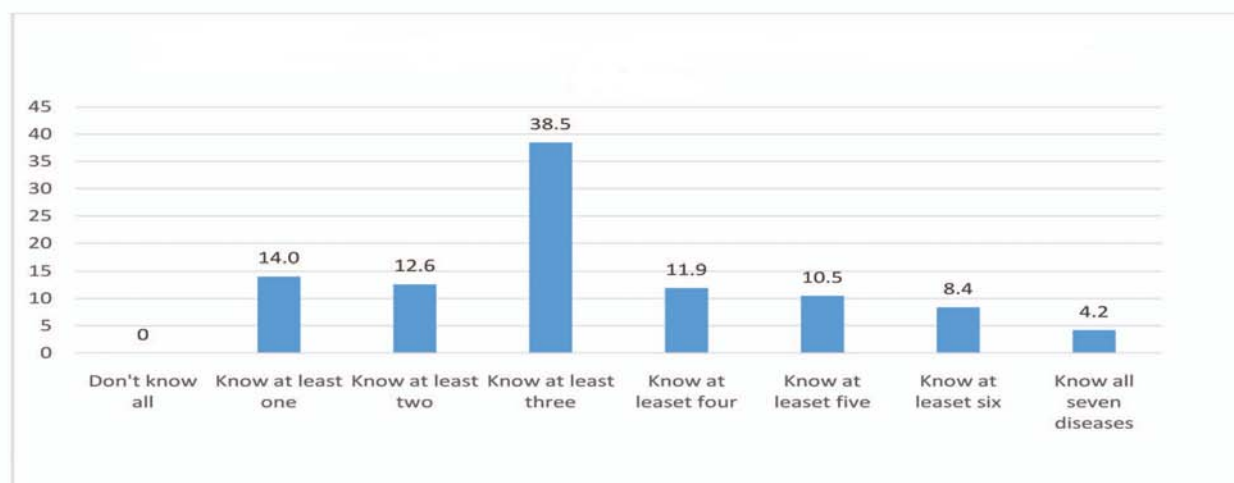


Figure 4: Knowledge about mode of transmission of zoonotic diseases

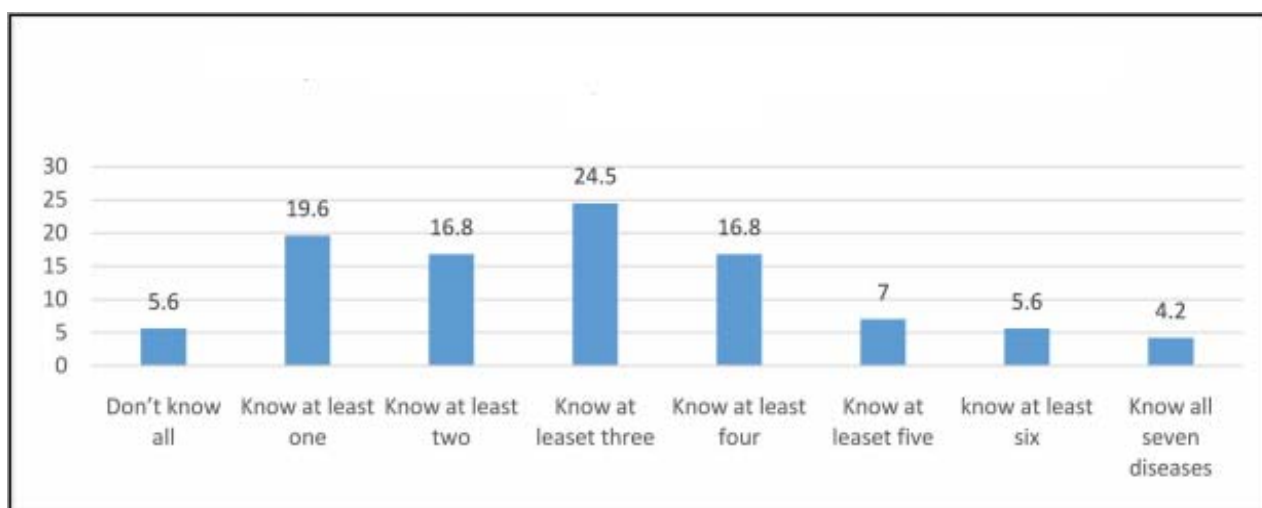


Figure 5: Knowledge about Sign/ symptoms of zoonotic diseases

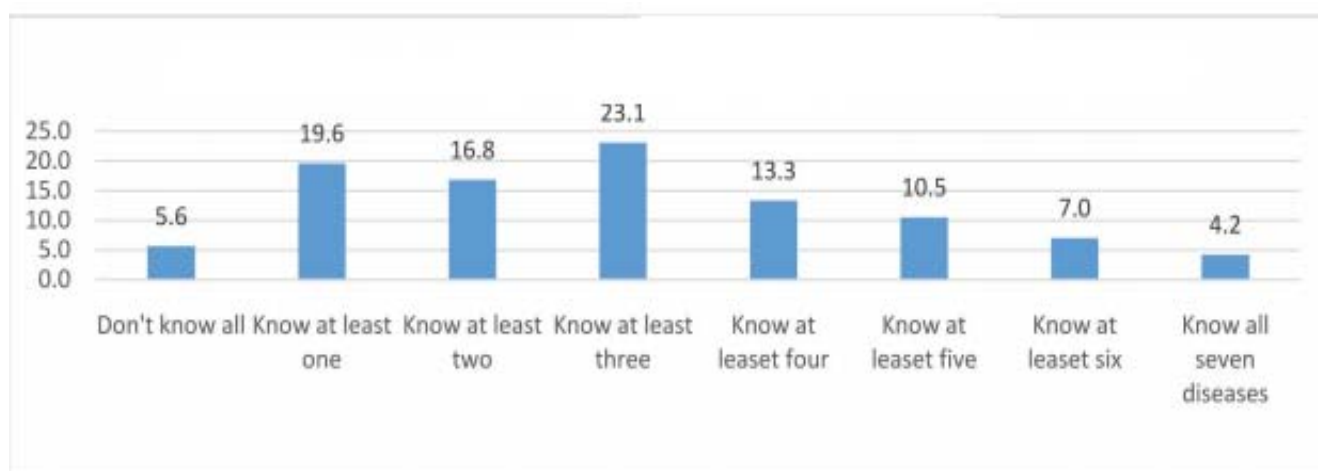


Figure 6: Knowledge about Prevention of zoonotic diseases

Figure no; 4, 5, and 6 showed overall knowledge of seven pet -related zoonotic diseases in the study. While analysing knowledge about three aspects (mode of transmission, sign and symptoms, and preventive measures) 4.2% of the respondents had good knowledge fall three aspects of studied seven diseases.

Table 4: Perception of zoonotic disease and related practices among the pet owners (n=143)

Description	Fully agree		Agree		Neutral		Disagree		Fully disagree	
	Number	%	Number	%	Number	%	Number	%	Number	%
Most of the Human diseases are zoonotic:	55	38.46	63	44.0	20	13.98	4	2.79	1	0.69
Raw meat/milk consumption are high risk for zoonoses:	51	35.66	77	53.84	14	9.79	1	0.69	0	0
It is necessary to wash hand with soap water after contact the pet:	98	63.53	44	33.56	1	0.69	0	0	0	0
Vaccination is necessary for pet to prevent communicable diseases:	107	74.82	29	20.27	6	4.19	1	0.69	0	0
Dogs are the main sources of human rabies:	88	61.53	40	27.97	14	9.79	1	0.69	0	0
Touches of pet faeces with bare hands is dangerous:	89	62.23	47	32.86	4	2.79	2	1.39	1	0.69
It is high risk for zoonotic transmission if pregnant women and children are close contact with livestock:	47	32.86	78	54.54	15	10.48	2	1.39	1	0.69

Table 4 showed that overall perception towards the pet -related zoonoses. Total of 143 respondents were rating in five scales Likert with different seven statements showed that the majority of respondents perceived positively.

## VI. DISCUSSION

Pets have been the potential sources of numerous human infectious diseases. However the situation of zoonotic diseases in developing countries like Nepal is not known due to lack of proper studies. It is estimated that nearly two -third of the emerging zoonoses that are viral or vector -borne raising from

birds, rodents, and pigs, will also rise from household pets (Smith & Whitfield, 2014). Several research findings have revealed that most of the pet owners have inadequate knowledge related to pet husbandry and infection control practices even in developed countries.

The majority of the respondents 49 (34%) were 30-39 years of age and only 3 (2.09%) were above 70 years who were visited in different parks in Kathmandu with their pets for recreation. Among the owners, most of them kept dogs 124 (86.7%), and 18 (12.58%) kept cats as pets. Only one (0.7%) respondent was found keeping tortoise in their household as a pet, which may represent the variety of pets species in Kathmandu. Most of the



households in Kathmandu keep dog as a pet but proportion of families with exotic pets was very low as compared to Canada where 56% homes have at least one dog or cat and other having fish (12%), birds (5%), rabbits or hamsters (each 2%), lizards, guinea pigs, snakes, frogs, turtles or gerbils (each 1%) in their family (Stull, Peregrine, Sargeant & Weese, 2013). Similarly, in USA more than fifty percent of households own at least one pet, and the number of exotic pet business are increasing. Over 72 million dogs and nearly 82 million cats, 40,000 primates, 4 million birds, 640,000 reptiles, and 350 million tropical fish are live traded world-wide each year (Smith & Whitfield, 2014).

In Kathmandu, the purpose of pet keeping found diverse. Out of 143 respondents, 54 (37.71%) kept for security purposes, 34 (30 %) for their hobby, 33 (23%) due to children's pressure, while 13 (9.09%) were keeping pets without any specific purpose. Nepali society has a very long history of pet keeping in their households, especially cats and dogs. But religion has not significant difference with pet keeping practices in Kathmandu. People of a different religions were found keeping pets, although the majority of them were Hindus and dogs were the most preferred pets (n=124, 86.7%).

Rabies, bird flu, and swine flu were the most familiar zoonotic diseases among respondents, which represent 99.3, 84.6, and 71.32%, respectively. On the other hand brucellosis, toxoplasmosis, salmonellosis, and NCC, which are also equally important pet associated zoonoses, pet owners were found to have poor knowledge. So they were vulnerable for zoonoses and also the risk for epidemic and pandemic transmission. Smith and Whitfield (2014) also state that household pets, such as cats, dogs, turtles, ornamental fish, baby chicks, gerbils, frogs and lizards have been associated with outbreaks of zoonotic disease in USA and Canada.

Radio, television were the main sources of information about the zoonotic disease. Although health workers and school/collage curriculum also contribute a pivotal role for knowledge transmission about zoonoses. Along with increment in school enrolment rate in Nepal, incorporation of information in the academic curriculum about the zoonotic disease may contribute to enhancing knowledge about zoonotic diseases. In this study most of the respondents consult vet during pet's sickness 130 (90%) but, 13 (10%) respondents in Kathmandu still seek conservative/traditional practices, and cat owners don't provide vaccines.

Data also showed that 116 (81.11%) n=143, pet owners kept their pets in separate places inside the house while 21 (14.68%) were keeping outdoor. However, 6 (4.19%) respondents allow easy access to their bed and slept with them, which may be recent and risky practices in Nepalese societies however most of them maintain the hygiene of their pet. These type of practices were also found in Canada, where children are

close contact with dogs and dogs were slept in child's bed (26%) or licked a child face (68%) sometimes or more frequently (Stull et al., 2013) which is most dangerous for zoonoses.

Out of 143 respondents, 12.38% experienced the death of a pet in their households with a variety of causes. 5 (27.77%) pets were found deceased due to aging. Food poisoning, heart attack, and injury -related death contribute equally to death (11.11 percentage). But 4 (22.22%) pets were killed by owners due to pets' aggressive behavior and 3 (26.66%) died due to disease like scabies.

110 (76.92%) respondents were feeding the same family food while 6 (4.19%) were feeding left-over food, but 27 (18.88%) respondents usually buy the readymade food from the market for their pets. Feeding practices of pets was associated with the economic status of the respondents. Stull et al. (2013) showed a similar finding is that developed countries like Canada 92.4% (n=244) owners feeding their pet commercial canned/dry food whose economic status found sound.

Another serious issue in Kathmandu is the open defecation of the pet animals (especially dog). Public defecation (park and street) practice was found among 113 (79.03%) pet owners, especially among dog owners which have raised public attention against it due to associated risk of zoonotic disease transmission. The study conducted in Denmark, which was observed at 72 puppies and kittens between 11 and 17 years of age, 29% of fecal samples from the puppies were positive for campylobacter, and many had C. Jejuni infection (O' Rourke, 2002). So the open defecation of pets is not only associated with the transmission of zoonoses but also distracts the beauties of the city.

Knowledge on different aspects of zoonoses found sound on rabies, bird flu, and swine flu. However, knowledge on brucellosis, toxoplasmosis, salmonellosis, and NCC, which are diseases equally risky by pets was found, poor respondents. Only 4.2% of respondents know about the mode of transmission, sign/symptoms, and preventive measures of all seven studied diseases. However, 38.5, 24.5, and 23.1% of the respondents had knowledge on the mode of transmission, sign/symptoms, and preventive measures respectively in any three zoonotic diseases and 5.6% respondents had not any idea of sign/symptoms and preventive measures of all seven diseases. Which indicate pet owners in Kathmandu had a poor knowledge of zoonoses. Majority of the respondents 102 (71.32%) said that rabies is a 100% fatal zoonotic disease but 41 (28.68%) respondents had poor knowledge about the possible outcome of rabies and only 75 (52.44%) respondents have knowledge that ARV is freely available up to district level government hospital in Nepal.

Educational status had positive association with perception about zoonoses. There were seven positive statements related to zoonoses prevention in which 69



to 95% of respondents were fully agreed or agreed in which 89% of respondents had secondary to higher level of education. But Smith and Whitfield (2014) mention that many pet owners in Canada often unaware of the risk of their pets, as a result, engage in husbandry and hygiene practices that increase the likelihood of acquiring diseases. However, they have sound education which found the reverse to compare with our study. This means only formal education may not be sufficient to create health –related awareness in the community.

Based on the study, few practices related to pet keeping were found satisfactory like consultation with veterinarian during pet sickness, place of pet keeping, and timely vaccination. However, more than two thirds (79.03%) of respondents were practicing open defecation to their pets, and sharing the same bed (4.19%) increased the susceptibility for zoonoses. Likewise, overall knowledge related to zoonoses found poor. 95% of the respondents are facing zoonotic risks either due to low knowledge of the mode of transmission, or sign/ symptoms, or on preventive methods, or by harmful pet keeping practice. But despite of poor knowledge they perceived zoonotic threats, which may be due to the influence of socio – cultural practices.

## VII. CONCLUSION

Pet contributes to human societies for physical as well as psycho-social well being; however, due to ignorance and illiteracy about zoonotic diseases associated with pets, owners are facing zoonotic risk. In this study, respondents were found to have low knowledge basically on common zoonoses (i.e., brucellosis, salmonellosis, toxoplasmosis, NCC, etc.) and free availability of ARV in government hospital even among respondent with higher education. Improper pet handling practices like open defecation, bed sharing and inadequate knowledge on pet -related zoonoses have been identified as primary risk factors for pet owners. Formal education alone is not sufficient to provide knowledge on healthy pet keeping practice, the government should make the registry of pets and make provision to provide necessary skills and knowledge for pet owners. Further studies are necessary to determine the impact of interventions targeted to zoonotic diseases and pet keeping practice. Policy and intervention gaps should be fulfilled with the collaboration of multi-sectoral agencies like a one health concept.

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## Characterization of Domestic Cats Patients Treated at the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechnics at the San Carlos of Guatemala University in 2019

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**Abstract-** The demand for pets to fill affective family spaces, the increased economic capacity of social classes and the displacement of rural populations to cities are influencing factors in increasing pet acquisition. This cohabitation can trigger epidemiological factors that affect human health. For this reason, it is necessary to know the diseases that affect domestic cats that live with humans and determine if there are zoonotic infectious agents that inflict on human health. In the present study, 103 medical records of domestic cats attended in 2019 at the Veterinary Hospital of the Faculty of Veterinary Medicine and Animal Zootechnics were studied. This in order to categorize the most frequent diseases in the species. 71.84% of the cats examined were sick. 55.41% were males, while the age range with the highest frequency was 0-1 years. The most frequent category according to the VITAMIN D System was Inflammatory/infectious.

**Keywords:** diseases, domestic cats, VITAMIND.

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



*Strictly as per the compliance and regulations of:*



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# Characterization of Domestic Cats Patients Treated at the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechnics at the San Carlos of Guatemala University in 2019

Caracterización de Gatos Domésticos Atendidos en el Hospital Veterinario de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad de San Carlos de Guatemala en el año 2019

Pérez-Archila Karla María<sup>α</sup>, Villatoro-Chacón Daniela Mariel<sup>σ</sup> & Arizandieta-Altán Carmen Grizelda<sup>ρ</sup>

**Resumen-** La demanda de mascotas para llenar espacios afectivos familiares, el aumento de la capacidad económica de las clases sociales y el desplazamiento de poblaciones rurales a las ciudades son factores influyentes en el incremento de la adquisición de mascotas. Esta cohabitación puede desencadenar factores epidemiológicos que afectan a la salud humana. Por esta razón, es necesario conocer las enfermedades que afectan a los gatos domésticos que conviven con el humano y determinar si existen agentes infecciosos zoonóticos que infieran en la salud humana. En el presente estudio se estudiaron 103 registros médicos de gatos domésticos atendidos en el año 2019 en el Hospital Veterinario de la Facultad de Medicina Veterinaria y Zootecnia. Esto con el fin de categorizar las enfermedades más frecuentes en la especie. El 71.84% de los gatos examinados estaban enfermos. El 55.41% fueron machos, mientras que el rango etario con mayor frecuencia fue de 0 -1 años. La categoría según el sistema VITAMIND con mayor frecuencia fue la inflamatoria/infecciosa (45.95%). Los datos obtenidos sugieren que esta especie debe ser estudiada dado que las enfermedades de tipo infeccioso pueden ser un eslabón epidemiológico para la población humana.

**Palabras clave:** enfermedades, felinos domésticos, VITAMIND.

**Abstract-** The demand for pets to fill affective family spaces, the increased economic capacity of social classes and the displacement of rural populations to cities are influencing factors in increasing pet acquisition. This cohabitation can trigger epidemiological factors that affect human health. For this reason, it is necessary to know the diseases that affect domestic cats that live with humans and determine if there are zoonotic infectious agents that inflict on human health. In the present study, 103 medical records of domestic cats attended in 2019 at the Veterinary Hospital of the Faculty of Veterinary Medicine and Animal Zootechnics were studied. This in order to categorize the most frequent diseases in the species. 71.84% of the cats examined were sick. 55.41% were males, while the age range with the highest frequency was 0-1 years. The most frequent category according to the VITAMIN D

System was Inflammatory/infectious. The data obtained suggests that this species should be studied since infectious diseases can be an epidemiological link for the human population.

**Keywords:** diseases, domestic cats, VITAMIND.

## I. INTRODUCCIÓN

Los felinos domésticos están dentro de los animales de compañía más comunes alrededor del mundo (1). En el 2006 la Asociación Americana de Medicina Veterinaria en Estados Unidos indicó que 6 de cada 10 familias poseían una mascota siendo el 54% perros, gatos o ambos (2). En Guatemala se estimó hace más de 30 años una población de 32,758 felinos, donde el índice habitante-gato fue de 20.98 a 1, sólo en la Ciudad de Guatemala (3). Sin embargo, se debe considerar que ambas poblaciones han aumentado con los años al igual que el valor adquisitivo y sentimental hacia las mascotas.

Desde el punto de vista sanitario los gatos son susceptibles a padecer enfermedades de todo tipo (4). Además, tanto el perro como el gato son causantes de provocar enfermedades de tipo zoonótico. Se estima que de 1,400 patógenos que afectan a humanos, alrededor del 58% son de origen zoonótico (5). La relación humano - animal hace susceptible a ambas especies a presentar enfermedades de origen infeccioso (6). Por esta razón, es importante conocer el comportamiento de las enfermedades en los gatos domésticos, para conocer el comportamiento epidemiológico de las mismas y tomar medidas preventivas.

En el presente estudio se evaluaron los registros clínicos de los gatos domésticos atendidos en el Hospital Veterinario de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad de San Carlos de Guatemala en el año 2019. Esto con el fin de categorizar las enfermedades que afectan a esta

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especie utilizando la regla nemotécnica VITAMIN D. Los resultados obtenidos son la primera fuente de información para investigaciones futuras respecto a la clínica diaria y el efecto epidemiológico en cuanto a la salud humana y animal.

## II. MATERIALES Y MÉTODOS

El estudio se realizó en el Departamento de Ayudas Diagnósticas, Hospital Veterinario, Facultad de Medicina Veterinaria y Zootecnia, de la Universidad de San Carlos de Guatemala, ubicado en la zona 12 de la Ciudad de Guatemala, cuyas coordenadas son: latitud 14.584154 y longitud -90.562422.

Se analizaron 103 expedientes de gatos domésticos atendidos en el Hospital Veterinario, durante el año 2019.

Se tomaron registros sobre las variables siguientes: sexo, edad, raza y se categorizaron las patologías que presentaba cada paciente de acuerdo a la regla nemotécnica VITAMIN D mediante su acrónimo: V= vascular; I= inflamatorio / infeccioso; T= traumático / tóxico; A=anómalo / alérgico / autoinmune; M = metabólico; I = idiopático; N = neoplásico / nutricional; D = degenerativo (7).

Los pacientes con patologías diagnosticadas fueron agrupados según su edad de acuerdo al rango etario siguiente: 0 a 1 año, 1 a 5 años, 5 a 10 años, 10 a 17 años y sin especificar.

Para el diagnóstico de las enfermedades en los pacientes felinos, se tomaron en cuenta exámenes de laboratorio como: hematología, bioquímica sanguínea, examen de orina, imágenes diagnósticas y en los casos donde no hubo ningún método diagnóstico se tomó como: sin diagnóstico.

Para el análisis de datos se utilizó estadística descriptiva utilizando distribuciones de frecuencias para las variables. Se utilizó el software estadístico SPSS versión 25.0.

## III. RESULTADOS

De los 103 pacientes atendidos durante el año 2019, un 71.84% (74/103) fueron diagnosticados con alguna enfermedad, un 22.33% (23/103) fueron clasificados como casos inconclusos por falta de pruebas para determinar el diagnóstico, un 5.83% (6/103) fueron pacientes sanos.

En cuanto al sexo, el 46.6% (48/103) de los gatos fueron hembras, el 52.43% (54/103) fueron machos y el 0.97% (1/103) no se especificó en el registro clínico.

En cuanto a la raza, todos los pacientes evaluados (100%) fueron sin raza definida (SRD). El promedio de edad de los pacientes fue de  $3.65 \pm 0.4$ . Sin embargo, se categorizó a los pacientes por rangos etarios (cuadro 1), siendo los pacientes entre 0 a 1 año con 44.6% con mayor prevalencia.

**Cuadro 1:** Rangos etarios de gatos domésticos atendidos en el Hospital Veterinario

Rango etario	N	%
0 a 1 año	33	44,59
1 a 5 años	21	28,38
5 a 10 años	10	13,51
10 a 17 años	8	10,81
Sin especificar	2	2,70

Según la regla nemotécnica VITAMIN D se encontró que el 45.95% (34/74) fueron diagnosticados con patologías de tipo inflamatorio/infeccioso, siendo la mayor casuística del estudio, seguida de las patologías

de tipo traumático/tóxico con un 31.08% (23/74) y de tipo neoplásico/nutricional con un 13.51% (10/74). En el cuadro 2 se describe la clasificación según VITAMIN D de los pacientes con diagnóstico clínico.

**Cuadro 2:** Clasificación de acuerdo a VITAMIND en gatos domésticos

VITAMIND	N	%
Vascular	1	1,35
Inflamatorio/ Infeccioso	34	45,95
Traumatico/ Tóxico	23	31,08
Anómalo/Alérgico/Autoinmune	2	2,70
Metabólico	2	2,70
Idiopático	1	1,35
Neoplásico/ Nutricional	10	13,51
Degenerativo	1	1,35

La enfermedad vascular los casos observados fueron únicamente efusión pleural.

Dentro de las enfermedades de tipo inflamatorio/infeccioso las patologías observadas fueron las siguientes: criptosporidiosis, giardiasis, coccidiosis, enteritis bacteriana, ectoparásitos (pulgas), gastritis, leucemia viral felina, sida felino, inflamación de miembro torácico anterior, cistitis idiopática, infección urinaria, dermatomicosis, lesión infectada por mordida, neumonía, pancreatitis y discoespondilitis.

Las enfermedades de tipo traumático/tóxico diagnosticadas fueron principalmente fracturas, hematomas en piel, contusión medular, hernia diafragmática, heridas post-quirúrgicas, subluxaciones y compresión medular.

Para las enfermedades de tipo anómalo /alérgico /autoinmune se presentó dermatitis alérgica por picadura de pulga.

Respecto a las enfermedades de tipo metabólico se encontró un caso de encefalopatía hepática e hipertiroidismo. La única enfermedad diagnosticada para el tipo idiopático fue megacolon.

En cuanto a las enfermedades neoplásicas/nutricionales se encontraron diagnósticos con síndrome paraneoplásico, neoplasias de tipo metastásicas pulmonares, urolitiasis asociada a dieta, neoplasias mamarias y linfoma.

Para el caso de las enfermedades de tipo degenerativo se observó un caso de cardiomiopatía hipertrófica.

#### IV. DISCUSIÓN

Los datos generados sugieren que las enfermedades más frecuentes en los gatos domésticos atendidos en el Hospital Veterinario son de origen inflamatorio/infeccioso, seguido de las traumáticas/tóxicas y neoplásicas/nutricionales. Estos datos son similares a los observados en perros por Alvarado-Pérez et al. (8) en la ciudad de Guatemala. Esto puede indicar que ambas especies pueden tener un factor epidemiológico o ambiental en común (9).

La efusión pleural fue la única patología observada en los trastornos vasculares. Se utilizó la radiografía como método diagnóstico. Algunos autores han encontrado que la efusión pleural afecta más a gatos que perros. Esto puede deberse a que las causas más frecuentes son el linfoma, peritonitis infecciosa felina, pitorax, quilotorax, mesotelioma, hemotorax y fallo cardíaco congestivo derecho (10).

Las enfermedades infecciosas/inflamatorias fueron las más frecuentes siendo las endo y ecto parasitosis las más reportadas. Esta prevalencia concuerda con estudios donde se observó que en el 61.46% de gatos evaluados presentaban algún tipo de parásito gastrointestinal, siendo parásitos potencialmente zoonóticos (11). Las enfermedades

virales como leucemia y el síndrome de inmunodeficiencia felina fueron la segunda patología observada en los pacientes en el estudio. Otros autores han reportado en el caso de leucemia viral felina una prevalencia entre el 1 al 8%; mientras que el virus de inmunodeficiencia felina presenta prevalencias hasta del 14% en los gatos sin signología clínica (12). Otra enfermedad inflamatoria observada fue la cistitis idiopática, la cual es considerada la principal enfermedad de tracto urinario bajo en gatos. Algunos autores han reportado prevalencias del 1.77% (13). La disco espondilitis también se observó en los pacientes del presente estudio. Esta enfermedad es poco diagnosticada en ésta especie y se han documentado solo 5 casos. Dentro de las causas de disco espondilitis más comunes están las infecciones de origen bacterianas siendo el 15% de la casuística total de disco espondilitis en gatos (14, 15). Existen diferentes formas de diagnóstico de esta patología, en el caso de éste paciente se utilizó radiografía simple en donde se observaron hallazgos radiológicos compatibles con discoespondilitis.

Para las patologías traumáticas/tóxicas, encontramos una prevalencia alta de fracturas de todo tipo, siendo las fracturas de huesos largos las más frecuentes. Los traumatismos por accidentes fueron el factor en común en estos pacientes. Además, se observó que algunos de ellos presentaban otras lesiones internas como neumotórax y contusión pulmonar (16). Las mielopatías evaluadas en la casuística del estudio fueron producidas principalmente por accidentes de auto además de las contusiones medulares y compresiones medulares. Pellegrino (2018), determinó que el trauma medular agudo producido por accidentes de autos es el trauma medular más frecuente en el gato.

La dermatitis alérgica a la pulga (DAPP), fue el único diagnóstico de la categoría alérgica/anómalo/autoinmune. La DAPP en gatos es la enfermedad alérgica más común en esta especie, manifestándose con alopecia inducida por el prurito (17), lo cual fue observado en los pacientes diagnosticados en el estudio.

La encefalopatía hepática fue uno de los trastornos metabólicos diagnosticados en el estudio. Este trastorno se presenta por alguna anomalía hepática en pequeños animales, causante de principalmente signos nerviosos, siendo una complicación neurológica, implica la translocación de toxinas del intestino al cerebro, es una patología de importancia clínica porque presenta entre sus signos, las convulsiones, que son un tema común de evaluación en la clínica diaria (18, 19). El hipertiroidismo se presentó en un paciente del estudio, publicaciones recientes, reportan que es la enfermedad endocrina más común en gatos, según Osorio y Matheus en 2012,





determinaron que en países en vías de desarrollo, en el cual se encuentra Guatemala, la enfermedad no está muy diagnosticada, pero con el paso de los años, la población con gatos como mascotas aumenta, por lo que es importante tener presente la enfermedad; afecta a poblaciones de gatos adultos o geriátricos, presenta pérdida de peso, anorexia, polidipsia, polifagia, vómitos y diarrea (20, 21).

El megacolon idiopático fue el único diagnóstico de trastorno idiopático en los felinos del estudio. Esta patología tiene una incidencia del 60% a 70%, es más frecuente en el gato que en el perro, puede ser primario o secundario, así como idiopático, donde no se encuentra lesión orgánica a la cual asociarla; produce como signología tenesmo, dificultad para defecar, depresión, anorexia, heces con sangre y moco, se presenta en edades variables, siendo la patología más frecuente en el gato relacionado a trastorno idiopático, es importante tomarla en cuenta como diferencial de patologías digestivas (22, 23).

Las neoplasias representan un porcentaje alto de motivo de consulta en la clínica diaria. Las neoplasias más frecuentes fueron metástasis pulmonar y neoplasias de glándula mamaria. La ocurrencia de tumores en gatos es de 158 a 470 de cada 100,000 animales y de éstos, cerca del 45% son tumores de piel y tejidos blandos. Para el caso de tumores mamarios, estos son los terceros más comunes después de los tumores hematopoyéticos y de piel (24). La urolitiasis en gatos, también fue parte del resultado del estudio. La causa más común de urolitos en gatos es la dieta que a su vez predispone a cálculos de estruvita. Se han observado prevalencias de hasta el 22% de urolitiasis en gatos de diferentes minerales, siendo los más comunes estruvita y fosfato de calcio (25, 26); El linfoma felino se presentó en un paciente, los signos presentados varían, dependiendo del lugar de ubicación de la neoplasia, los más comunes son de presentación digestiva, donde se observa vómitos, diarrea, y pérdida de peso, por lo que es importante tomarlo en cuenta al momento de realizar un diagnóstico diferencial. Existen los linfomas de tipo mediastínico, multicéntrico y extranodal (27, 28).

Para el caso de las enfermedades degenerativas, se observó un paciente con cardiomiopatía hipertrófica, caracterizada por la hipertrofia del corazón. Esta patología es la enfermedad más común diagnosticada por veterinarios, en muchos de los casos no se encuentra causa subyacente, por lo tanto, la mayoría son idiopáticos (29, 30).

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## Morphological and Molecular Identification of Hard Ticks (Acari: Ixodida) Infesting Herds of Cattle in Zaria, Northwestern Nigeria

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**Abstract-** Ticks are important vector arthropods of human and animal pathogens. Information on tick vectors as well as their distribution in Zaria and environs is very scanty. The study was aimed at identifying the various tick species infesting cattle in Zaria using a molecular technique. Ticks were collected from two hundred cattle (n=200) of which 193 (96.5%) were tick infested. The individual ticks were washed, crushed and incubated at 56°C overnight. The extracted DNA of each sample was extracted and amplified targeting the 16S rRNA portion. Ampli cons were sequenced using a capillary sequencer (ABI PRISMR 3130x1 Genetic Analyzer, Applied Biosystems). All sequences were subjected to a Basic Local Alignment Search Tool (BLAST) to determine their identities and assess their homologues and similarities to those in the Gen Bank.

**Keywords:** *morphological, molecular, hard ticks, herds, cattle, zaria.*

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



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# Morphological and Molecular Identification of Hard Ticks (Acari: Ixodida) Infesting Herds of Cattle in Zaria, Northwestern Nigeria

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& Sani, Dahiru <sup>§</sup>

**Abstract-** Ticks are important vector arthropods of human and animal pathogens. Information on tick vectors as well as their distribution in Zaria and environs is very scanty. The study was aimed at identifying the various tick species infesting cattle in Zaria using a molecular technique. Ticks were collected from two hundred cattle (n=200) of which 193 (96.5%) were tick infested. The individual ticks were washed, crushed and incubated at 56°C overnight. The extracted DNA of each sample was extracted and amplified targeting the 16S rRNA portion. Amplified products were sequenced using a capillary sequencer (ABI PRISM 3130x1 Genetic Analyzer, Applied Biosystems). All sequences were subjected to a Basic Local Alignment Search Tool (BLAST) to determine their identities and assess their homologues and similarities to those in the Gen Bank. Morphological and genetic data of individual specimens gathered in this study provide relevant information for future studies on tick population dynamics in the Zaria. The outcome of this study suggests that *Amblyomma variegatum* 82 (7.6%), *Rhipicephalus* (*Boophilus*) *decoloratus* 158 (14.7%), *Rhipicephalus* (*Boophilus*) *microplus* 55 (5.1%), *Rhipicephalus* *simus* Group 699 (62.3%), *Rhipicephalus* *sanguineus* 14 (1.3%), and *Hyalomma dromedarii* 96 (8.9%) are present on cattle in Zaria, northwestern Nigeria and pose a high risk of pathogens transmission and hence will contribute adversely in productivity losses to livestock owners.

**Keywords:** morphological, molecular, hard ticks, herds, cattle, zaria.

## I. INTRODUCTION

Ticks are destructive blood sucking ectoparasites of livestock and wild animal species causing huge economic losses, thus creating food insecurity (Natala et al., 2009; Habeeb, 2010), with an estimated global cost of control and productivity losses of 7 billion US-Dollar annually (Nchu et al., 2012). Their effects are diverse, including reduced growth, milk production, paralysis/toxicosis, and transmission of tick-borne pathogens that reduce production or cause mortality, extensive damage to body surfaces exposing animals to secondary attacks from other parasites and microbial

infections (Walker et al., 2003). Certain factors such as globalization and increased international trade, urbanization, climate change and increased travel and mobility of livestock have resulted in rapid extension of the zoo-geographical range for many tick species (Shaw et al., 2001; Kamani et al., 2010).

Morphological features are commonly used for the gross and microscopic identification of tick specimens; however, this method is not effective for damaged specimens, engorged female ticks, and the immature stages (Obadiah and Shekaro, 2012). To comprehend the epidemiology of tick-borne pathogens and develop effective strategies for controlling the diseases, accurate identification of the vector is very vital. Molecular analytical tools have proven valuable and complementary for overcoming this ineffectiveness associated with morphological identification of ticks and have been used to identify and differentiate tick species (Mohammed et al., 2016; Ogo et al., 2017).

There are currently little studies on the prevalence and epidemiology of ticks commonly affecting cattle production in Zaria, despite the fact that it is endowed with favorable weather condition suitable for the proliferation and multiplication of ticks as well as serving as a focal point of cattle concentration in the Northwestern Nigeria. Therefore, this necessitates the need for this study on the distribution of tick species, their morphological features as well as their genomic make up affecting cattle in Zaria, Kaduna State, Nigeria.

## II. MATERIALS AND METHODS

**Study area:** Zaria comprises of two Local Government Areas namely; Zaria and Sabon Gari. It is located between latitude 11°07' N and longitude 7°44' E within the Northern Guinea savanna zone. By the existing pattern of settlement; it is made up of a natural and stable ecosystem in the Northern Guinea Savannah zone, with a discontinuous layer of sparsely distributed short trees followed by relatively continuous layers of tall, medium and short grasses (Jatau et al., 2012; Obadiah and Shekaro, 2012). Zaria is an old commercial, administrative and academic town in Northern Nigeria. The mean annual rainfall in the area is 1100 mm lasting from May to October (816 mm/month).

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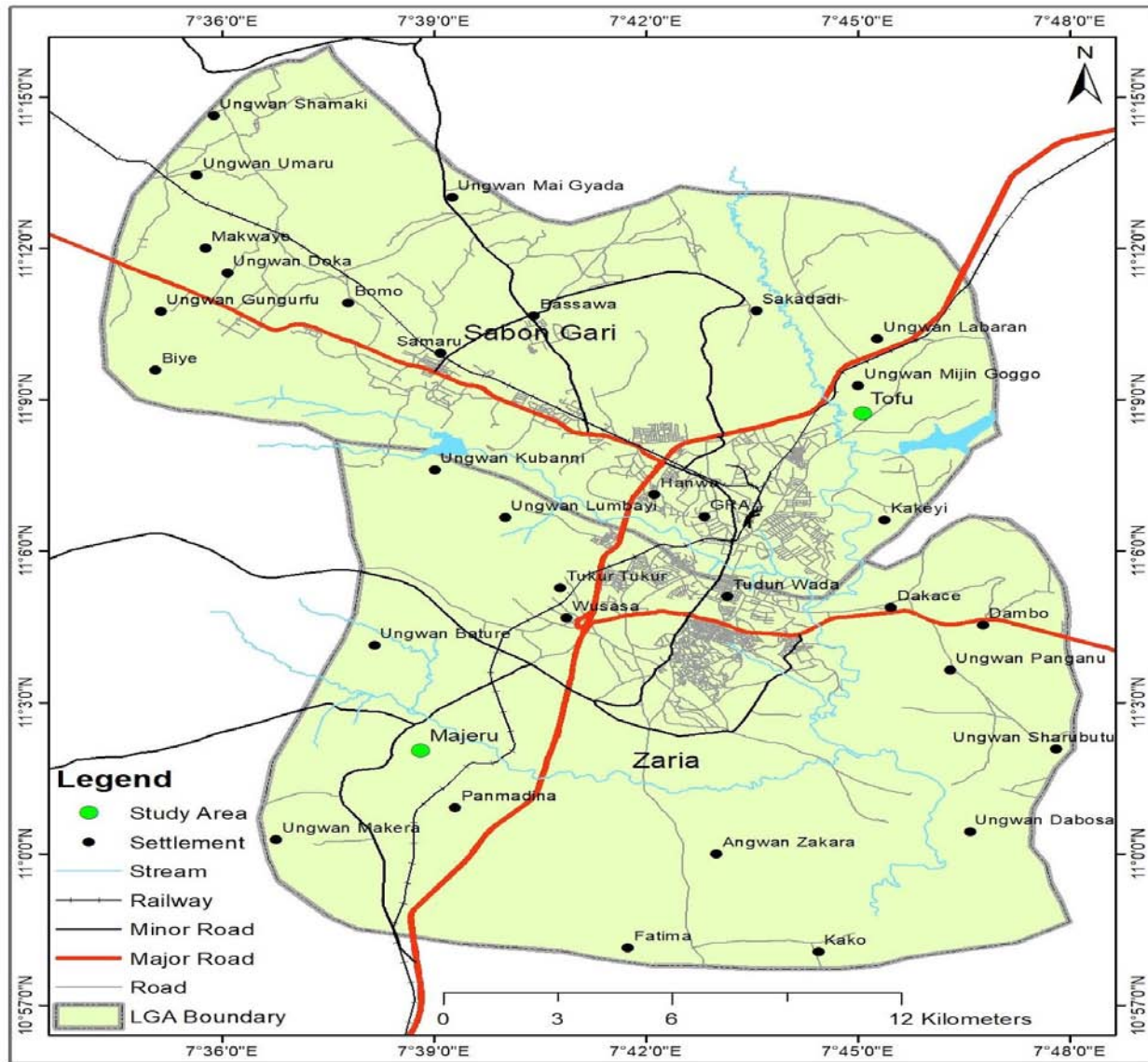
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Mean daily temperatures during the wet season are 25°C and mean relative humidity of 72%. The dry season lasts from November to April, the mean daily temperature ranging from 14 to 36°C and the relative humidity of 20-30% (Natala et al., 2009).

**Study design:** Ticks were collected from two (2) areas; Tofu district from Sabon Gari Local Government Area and Majeru district in Zaria Local Government Area. In each district, 10 herds were selected, and all

visible adult ticks were collected from 10 randomly selected cattle varying in age and sex, all belonging to the indigenous (*Bos indicus*) white Fulani breed from 10 herds in each sampling area. Age of the animals were estimated on the basis of the dentition score method developed for zebu cattle under a low plane of nutrition (Kikule, 1953) and on information provided by their owners.



Source: Adopted and modified map from <https://www.google.com/maps>

Figure 1: Map of study areas/locations

**Tick collection:** The collection was performed using blunt steel forceps, by thorough examination of the entire body surface of two hundred cattle ( $n=200$ ). Ticks were collected from different parts of the body including the neck/dewlap, eyes, ear, udder and external genitalia, inner thighs (ventrum), under the tail/perineum and legs/interdigital spaces by using forceps and hand gloves. Ticks from each animal were stored separately

in vials containing 70% ethanol, labelled with information on the host (i.e., sample number, age, and sex), village, and date of sampling as well as the site (s) collected.

**Ticks identification (morphological):** The experiment was carried out in the Research and Teaching laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. All collected ticks were



counted and identified to the genus and species level using a stereomicroscope (up to 100× magnification) and following the morphological key described by Walker et al., 2003. For those belonging to the genus *Rhipicephalus*, keys by (Walker et al., 2000; Madder et al., 2012) were also used.

**Molecular identification:** Generally, three ticks each from the same species were examined in one pooled sample. First, each tick was washed three times in sterile phosphate-buffered saline and then stored at  $-20^{\circ}\text{C}$ . The frozen ticks were mechanically crushed using pestle and mortar and transferred into 1.5 ml Eppendorf tube. Digestion with proteinase K (200  $\mu\text{g}/\text{ml}$ ; Roche, Mannheim, Germany) was performed by incubation at  $56^{\circ}\text{C}$  overnight.

DNA was isolated by phenol-chloroform extraction and followed by ethanol precipitation (Jain et al., 2017). DNA pellets was washed twice with ice-cold 70% ethanol, air dried, and re-suspended in 100  $\mu\text{l}$  elution buffer. The efficiency of the nucleic acid extraction was evaluated by electrophoresis in 1.5% (w/v) agarose gels containing ethidium bromide and visualized under ultraviolet light. The concentration of each DNA sample was determined in a Nano Drop 2000c spectrophotometer (Thermo Scientific, San Jose, CA, USA) for quality and quantity. The DNA was then stored in  $-200^{\circ}\text{C}$  until further use.

**PCR amplification:** Tick species identification was carried out using universal primers TQ16S+1F and TQ16S-2R targeting the 323bp fragment of 16S rRNA portion of the DNA (Halos et al., 2004) with the following nucleotide sequences (5'-3') CTGCTCAATGAT TTTTAAATTGCTGTG and ACGCTGTTATCCCTAGAG as the forward and reverse primers respectively. Five  $\mu\text{l}$  DNA extract from each tick sample was amplified in a 24  $\mu\text{l}$  reaction mixture containing; 13.65  $\mu\text{l}$  nuclease free water, 2.5  $\mu\text{l}$  of 10X PCR buffer, 1  $\mu\text{l}$  of 50mM  $\text{MgCl}_2$ , 0.5  $\mu\text{l}$  of 10mM Deoxynucleoside Triphosphate (dNTP), 0.25  $\mu\text{l}$  of DMSO, 1  $\mu\text{l}$  each of the forward and reverse primers (Halos et al., 2004), and 0.1  $\mu\text{l}$  of Taq polymerase following conditions by the manufacturer. Amplicons were visualized with ethidium bromide after electrophoresis in 1.5% agarose gels. Bands were detected from the gel by viewing and capturing under UV light with the aid of a Gel documentation system. Expected amplicon size was 323bp.

**Amplicon sequencing:** Prior to sequencing, the PCR product was purified by loading it into 1% agarose gel. Gel was run in the electrophoresis tank till bands were neatly separated. Bands of interest were excised using a scalpel blade under illumination for accuracy, with eye goggles worn to protect the eyes from the UV rays. Excised bands were kept in a 1.5 ml Eppendorf tubes. Sanger method was used to sequence the purified products. Amplicons were sequenced using a capillary

sequencer (ABI PRISM 3130x1 Genetic Analyzer, Applied Biosystems).

**Phylogenetic analysis:** BLAST search for previously reported sequences that are identical to the sequences in this study was done using the NCBI database (<http://www.ncbi.nlm.nih.gov/BLAST>). Phylogenetic tree was constructed using the Molecular Evolutionary Genetic Analysis (MEGA 7.0) software program (Kumar et al., 2016). The evolutionary distances were computed using the Maximum Composite Likelihood method and Neighbor-joining (NJ) algorithm was used to construct a phylogenetic tree (Saitou and Nei, 1987).

**Submission to Gen Bank for Ascension Numbers:** The sequences were assembled in notepad using Bankit method and then the corrected ticks' sequences were submitted in Gen Bank to obtain their ascension numbers.

**Data Analysis:** Data obtained were organized, edited and analyzed using statistical package for social sciences (SPSS) Version 20. Results generated from the investigation were expressed using descriptive statistics (mean  $\pm$  standard error of mean, percentage, and graph).

### III. RESULTS

**Tick species collected:** In this study, out of 200 cattle screened 193 (96.5%) were tick infested. A total of 1074 ticks were collected and of three genera *Amblyomma*, *Hyalomma*, *Rhipicephalus* and including the sub-genus *Rhipicephalus* (*Boophilus*). Six species of ticks were identified: *Amblyomma variegatum*, *Rhipicephalus* (*Boophilus*) *decoloratus*, *Rhipicephalus* (*Boophilus*) *microplus*, *Rhipicephalus simus* Group, *Rhipicephalus sanguineus*, and *Hyalomma impeltatum*. The frequency of occurrence in the study showed that *Rhipicephalus simus* Group 669 (62.3 %) was the commonest ticks observed in cattle in all the herds followed by *Rhipicephalus* (*Boophilus*) *decoloratus*, 158 (14.7%), *Hyalomma impeltatum* 96 (8.9 %), *Amblyomma variegatum* 82 (7.6%), *Rhipicephalus* (*Boophilus*) *microplus* 55 (5.1%) and *Rhipicephalus sanguineus* 14 (1.3 %) respectively. A relatively high number ( $n = 699$ ) of adult *Rhipicephalus simus* Group ticks were collected from cattle of all age groups.

**Tick distribution in relation to predilection sites:** The distribution described in percentage (%) of tick infestation in different body parts of cattle examined revealed that; tail (46.5%), ventrum (17.9%), neck/dewlap (14.1%), udder/scrotum (9.5%), prepuce/perineum (8.2%), head (1.9%), legs/interdigital space (1.1%) and dorsum (0.8%). It showed that the tail region/area (46.5%) is the most infested followed by the ventrum (17.9%), the neck (14.1%), while the dorsum (0.8%) is the least infested.

Molecular identification of tick species: DNA was isolated from five (5) tick samples (as in materials and methods), three (3) from each species of ticks. Following amplification of the 16S rRNA and gel

electrophoresis of the PCR products, all the five (5) tick species were positive and yielded products of approximately 323 bp. (see plate 1.0).



Figure 1: Gel image for amplified DNA of ticks

Figure 1.0: DNA extractions from five different tick species. Lanes: M; 100 bp ladder, 1. *Amblyomma variegatum*, 2. *Rhipicephalus decoloratus*, 3. *Rhipicephalus microplus*, 4. *Hyalomma impeltatum* 5. *Rhipicephalus simus* Group 6. Non-template control (NTC). Tick 16S rRNA gene, 1.5% agarose gel run for 35 mins at 65 V.

Gen Bank Ascension Numbers: In this study, we molecularly established the status of *Amblyomma variegatum* (MN044771), *Rhipicephalus* (*Boophilus*) *decoloratus* (MN044772), *Rhipicephalus* (*Boophilus*) *microplus* (MN044773), *Hyalomma dromedarii* (MN044774) and *Rhipicephalus simus* Group (MN044775) speciesticks from Zaria, Kaduna State, Nigeria, based on the partial sequence of 16S rRNA gene.

Phylogenetic tree for ticks collected on cattle: The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 2.46834081 is shown. (Next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 14763 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

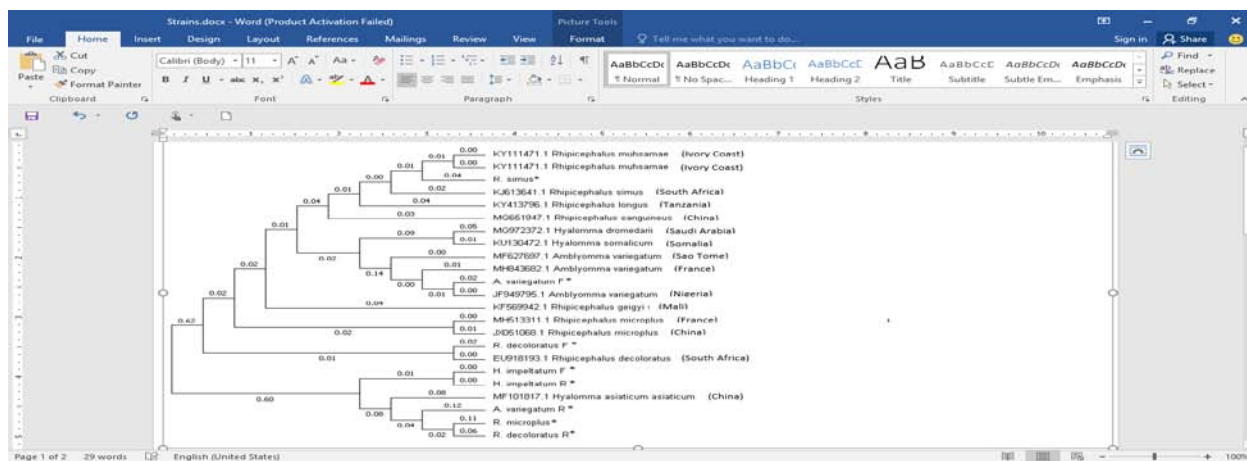


Figure 2: Phylogenetic tree for ticks. (Note: \* indicate the isolates analyzed in this work)



#### IV. DISCUSSION

Of the three taxa currently ascribed to the 'simus Group', only *Rhipicephalus muhsamae* (Morel and Vassiliades, 1965) was expected to be present in West Africa. However, in addition to the Group-specific punctuation pattern visible on the males' conscutum, a number of morphological features (e.g., female genital aperture and shape of adanal plates) of the specimens collected in this study appeared closely related to the East African taxon *Rhipicephalus praetextatus* (Unsworth, 1952). It was assumed these were the same specimens retrieved in the 1950s from several localities in central and northern Nigeria, identified as *Rhipicephalus simus muhsamae* 3.83% and 4.0% respectively (Mohammed, 1977; Lorusso et al., 2013). Usually found in regions with a savanna climate, the distribution of *Rhipicephalus simus* (or *Rhipicephalus. simus simus*) is believed to be restricted to southern Africa (Pegram et al., 1981), where the adults preferentially parasitize cattle, never reaching high loads (Lorusso et al., 2013). These findings are in contrast to studies carried out by Joseph et al., (2014) where he recorded *Rhipicephalus (Boophilus) microplus* (49.2%), *Amblyomma variegatum* (40.89%), *Rhipicephalus (Boophilus) annulatus* (7.39%) and *Amblyomma maculatum* (2.46%) respectively. Biu et al., (2012) recorded tick species infesting ruminants in University of Maiduguri, Nigeria and reported an overall high prevalence of 64% with 39 (68.01%) for cattle followed by 13 (20.13%) for sheep and 12 (18.75%) for goat. In their study, *Rhipicephalus (Boophilus)* species was observed to be most predominant with a prevalence of 56.1% followed by *Hyalomma* species (43.9%). Olabode et al., (2010) in a study of occurrence, species composition and economic impact of tick in Buruku market Jos-Plateau, Nigeria, observed that 12.5% of cattle were infested by ticks of which *Rhipicephalus (Boophilus)* spp were most prevalent with 7.5% followed by *Amblyomma* spp (4.5%) and *Hyalomma* spp (3.0%). Obadiah and Shekaro (2012) reported four species of ticks from Zaria, Nigeria and showed that *Rhipicephalus (Boophilus) decoloratus* was predominant with prevalence rate of (22.5%) followed by *Amblyomma variegatum* (17.7%), *Hyalomma* spp (6.7%) and *Rhipicephalus sanguineus* (3.3%). The differences in the results of the present and earlier studies might be due to variation in the geographical locations, climatic conditions of the experimental areas, region and method of study as well as sample size selection.

The distribution (%) of tick infestation in different body parts of cattle examined revealed that tail (46.5%), ventrum (17.9%) and neck (14.1%) were the most tick – infested sites in the body of examined animals and the dorsum (0.8%) was the least part infested. This further confirms that ticks prefer to attach and feed on some parts of the body of animals. This finding is not in

agreement with the work by Jajere et al., (2014) that recorded prevalence in predilection site as follows; udder and external genitalia (83.4%), inner thighs (79%), under the tail/perineum (69.8%), eyes (26.3%), neck and dewlap (14.6%) and ears (12.2%) respectively. Joseph et al., (2014) in his work suggested that ticks are widely distributed in different parts of the host body such as armpit, inner thigh, penis, udder, mammary gland, scrotum and vulva, of which inner thigh (26.66%) was most infected, while vulva (10.80%) was the least infected animal body part. These findings could be attributed to the fact that external genitals, perineum and inguinal/groin region of the body are highly supplied with blood and ticks usually prefer thinner and short hair skin for infestation. This helps in easy penetration of mouthparts of ticks into richly vascular area for feeding (Sajid, 2007). The higher number of *Rhipicephalus simus* Group (46.5%) recorded in the present study could be mainly attributed to the practice of hand-picking of ticks by the Fulanis, carried out up to three times a week during the wet season (Pullan et al., 1980; Lorusso et al., 2013). This control method mainly targets the most conspicuous *Amblyomma* adults, regarded as 'koti' (i.e., 'dangerous ticks' in Fulfulde language), by the local herdsmen, as opposed to the smaller *Rhipicephalus* and boophilid ticks that are consciously left attached, as they are believed to be 'miri' (i.e., 'less harmful') (Bayer and Maina, 1984; Lorusso et al., 2013).

The sequence of the amplified 16S rRNA gene fragment of *Amblyomma variegatum* was 99% identical from specimen from Nigeria JF949795.1 from ticks collected in Jos (Ogoh et al., 2012). And it shows 100% similarity from the sequence obtained from Sao Tome (MF627697.1) and 99% identical to MH843682.1 from France. The amplified 16S rRNA gene fragment of *Hyalomma impeltatum* revealed 92% identical to *Hyalomma asiaticum asiaticum* MF101817.1 from China. The sequence of the amplified 16S rRNA gene fragment of *Rhipicephalus simus* Group was 99% identical from specimen from *Rhipicephalus muhsamae* (KY111471.1) from Ivory Coast, and 98% similarity with *Rhipicephalus simus* (KJ613641.1) from South Africa. Also, the sequence of the amplified 16S rRNA gene fragment of *Rhipicephalus (Boophilus) decoloratus* was 98% identical from specimen from *Rhipicephalus (Boophilus) decoloratus* (EU918193.1) from South Africa. The sequence of the amplified 16S rRNA gene fragment of *Rhipicephalus (Boophilus) microplus* was 100% identical from specimen from *Rhipicephalus (Boophilus) microplus* (MH513311.1) from France and 99% identical from *Rhipicephalus (Boophilus) microplus* (JX051068.1) from China.

#### V. CONCLUSION

The demonstration of the presence of high number of *Rhipicephalus simus* Group (62.3%) from this

study poses a great danger to the environment as well as the cattle owners because of its affinity to rodent hosts and its ability to transmit anaplasmosis to cattle and humans. The presence of *Rhipicephalus (Boophilus) microplus* (5.1%) from this study is alarming because of their high fecundity which enable them to spread and establish fast in the environment. Also, *Rhipicephalus (Boophilus) microplus* are known with their acaricide resistance and hence lead to environment contamination due to abuse of acaricides by the pastoralists which leaves residues in the environment that may be toxic to other organisms. This makes transmission of ticks and tick-borne pathogens very easy and a serious problem to combat in Nigeria today.

## VI. CONFLICTS OF INTEREST

The authors unanimously confirm that there are no known conflicts of interest associated with this publication.

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## Seasonal Variations in Growth and Physiological Parameters Along with its Relationship with Various Haemato-Biochemical and Mineral Profiles in Black Bengal Goats in Free Range Rearing System

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**Abstract-** Determining animal growth and physiological parameters, their inter-relationship and correlation with various haemato-biochemical and mineral profile during summer and winter seasons in present climatic conditions are imperative for defining genetic potential and adaptation of black Bengal breed for higher meat production. The recorded body weight was found positively correlated with ( $P \leq 0.01$ ) height, heart girth and, linear length during both summer and winter season, indicating that they can be used to estimate body weight in black Bengal goats of different ages under field conditions where scales are not usually available. The pulse rate was found to be negatively correlated with ( $P \leq 0.01$ ) body weight during both the summer and winter season. Total erythrocyte count (TEC) was negatively correlated ( $P \leq 0.05$ ) with the weight during summer but positively ( $P \leq 0.01$ ) during the winter season.

**Keywords:** growth, free range system, weight, average weight gain, height, heart girth, linear length, season, summer, winter.

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



*Strictly as per the compliance and regulations of:*



# Seasonal Variations in Growth and Physiological Parameters Along with its Relationship with Various Haemato-Biochemical and Mineral Profiles in Black Bengal Goats in Free Range Rearing System

Shagufta Perveen <sup>α</sup>, Pradip Kumar Das <sup>σ</sup> & Syamal Naskar <sup>ρ</sup>

**Abstract-** Determining animal growth and physiological parameters, their inter-relationship and correlation with various haemato-biochemical and mineral profile during summer and winter seasons in present climatic conditions are imperative for defining genetic potential and adaptation of black Bengal breed for higher meat production. The recorded body weight was found positively correlated with ( $P \leq 0.01$ ) height, heart girth and, linear length during both summer and winter season, indicating that they can be used to estimate body weight in black Bengal goats of different ages under field conditions where scales are not usually available. The pulse rate was found to be negatively correlated with ( $P \leq 0.01$ ) body weight during both the summer and winter season. Total erythrocyte count (TEC) was negatively correlated ( $P \leq 0.05$ ) with the weight during summer but positively ( $P \leq 0.01$ ) during the winter season. Weight and body measurements were negatively correlated ( $P \leq 0.01$ ) with glucose and total cholesterol during both summer and winter season. A significantly negative correlation was observed between plasma Sodium and body weight gain features during both seasons.

**Keywords:** growth, free range system, weight, average weight gain, height, heart girth, linear length, season, summer, winter.

## 1. INTRODUCTION

The livestock production system is sensitive to climatic change and at the same time, itself a contributor to the phenomenon. Climate change has the potential to gradually become a more terrifying challenge to the development of the livestock sector in the future. Hence, improvements in productivity and adaptability of natural bio-resources, therefore, need to be achieved with a changing environment. Adaptation to climate change is unlikely to be attained with a single strategy (Hoffmann, 2010). Under field condition, multiple stresses like heat stress, nutritional stress, water stress, etc. occur together and simultaneously affecting

body weight, respiratory rate, pulse rate, rectal temperature, hemoglobin, packed cell volume, glucose, total protein, cortisol (Sejian *et al.*, 2013). High environmental temperature exerts a negative influence on the performance of the livestock population (Liu *et al.*, 2011).

In present changing climatic conditions, the study of growth dynamics is of significance as the demand for meat is always increasing. The growth determines the meat-producing ability up to a marketable age, i. e., six months. Goats are known for their wide adaptability, disease resistance (Banerjee, 2007). Goat is a valuable and promising livestock species mainly for meat production around the world (Barkley *et al.*, 2012). Due to its multifactorial abilities like low body mass, and low metabolic requirements, the goats, are regarded as an asset in climate-resilient agricultural system (Silanikove, 2000).

Hence, the study of growth dynamics up to puberty in goats is therefore vital because it can help to accurately and judge for the rate of response of young kids to the environment, particularly in present climatic conditions. Studies of different growth phases are expensive and exhaustive. But it can bring more information about the adaptation rate and or rate of response of breed under changed environmental conditions. Continuous and patient research is required to define mechanisms controlling the growth and production of animals to improve the efficiency of producing food from them. This type of research is the need of time as we can prepare our self for future challenges.

So, the present study aimed to access the growth pattern of kids during summer and winter season about its' physiological responses, haemato-biochemical and minerals parameters maintained in free-range rearing system and to use it as a base or platform for further study. The data generated will serve as a guide to the physiological characterization and helps in the interpretation of climatic influence on

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productivity. The attempt will help to refine strategies for goat improvement in the future.

## II. MATERIALS AND METHODS

### a) Description of the study area and period of study

The study was carried out at the Indian Council of Agriculture Research-Indian Veterinary Research Institute, Eastern Region Station at Kalyani Goat Farm of Nadia district, West Bengal, India. The place falls in the lower Gangetic Plain Region of India. Summer and Winter season were considered for the study. The environmental temperature was  $38.79 \pm 3.44$  °C in summer and  $14.98 \pm 4.64$  °C in winter. Relative humidity was 64.36% and 57.86% in summer and winter, respectively. Johanson *et al.* (1963) formula was used for Temperature Humidity Index (THI) calculation. The THI value was maximum during May (THI - 86.68) and minimum during January (THI - 67.96).

### b) Experimental animals and their management

Birth season was used for the selection of experimental kids [Summer born kids (n=50, male: 25, female: 25) and Winter born kids (n=50, male: 25, female: 25)]. The growth parameters like body weight, height, heart girth, and linear length in all these kids were recorded. To study blood parameters, twenty-four kids were selected [Summer born male kids (n=6), Summer female kids (n=6), Winter male kids (n=6), and Winter female kids (n=6)]. All kids were maintained under a free-range rearing system and were fed only with mother's milk up to weaning. Vaccination and deworming schedule followed in experimental animals were as per standard schedule. Each animal was turned out for natural grazing in the morning (8.00 am to 12.30 pm) and again in the afternoon (2.30 pm to 5.30 pm).

### c) Blood sample collection

Blood samples (5 ml/animal) were collected in heparinized vacutainer from the jugular vein at day 15<sup>th</sup>, 30<sup>th</sup> (1 Month), 60<sup>th</sup> (2 Month), 90<sup>th</sup> (3 Month), 120<sup>th</sup> (4 Month), 150<sup>th</sup> (5 Month) and 180<sup>th</sup> (6 Month) age considering day zero as the day on which the kid was born. The blood samples were put in an ice bucket and carried to the laboratory directly for further processing. The hematological analysis was immediately completed after blood sample collection and the rest portion was subjected to centrifugation at 3000 rpm for 30 minutes for separation of plasma. Frozen plasma was kept at  $-20^{\circ}\text{C}$  till further analysis.

### d) Growth Parameters

The weights of kids were measured as soon as the kids got cleaned and dried immediately after birth. Subsequently, body weights were recorded as per schedule from each animal in the morning before feeding and watering. Weighing machine (Balance Avery, Bombay, India) was used to record weight of the kids by subjecting them to stand individually. Weight

and weight gain were expressed respectively in Kg. and gram. Linear-length, heart girth, and height of the animal were measured by measuring tapes in centimetre.

### e) Physiological Parameters

Digital thermometer (Hicks Thermometer), was used for recording rectal temperature by placing the thermometer in contact of rectal mucosa until the reading stabilizes. The femoral artery was used for pulse rate per minute recording. Respiration rate per minute of each of the animals was recorded by visual observations of in ward, and outward abdominal movement. Counting of one inward and outward movement as one respiration was made and respiration rate was expressed in numbers per min.

### f) Hematological Parameters

For hematological study all standard procedures were followed (Hemoglobin, Packed Cell Volume, Total Erythrocyte Count, Total Leukocyte Count).

### g) Biochemical Parameters

All biochemical parameters (Total Protein, Albumin, Globulin, Total Cholesterol, Aspartate amino transferase and Alanine transaminase) were measured using commercially available kits specific for goats. For the determination of plasma NEFA, the Copper Soap Extraction Method modified by Shipe *et al.* (1980) and Good win (1968) method for Alpha-amino nitrogen was adopted.

### h) Mineral Parameters

The method of Fernandez and Kahn (1971) was used for Plasma electrolytes viz. Sodium, Potassium, Calcium, and Iron estimation in atomic absorption spectrophotometer (Thermo fisher Scientific, ICE 3000 Series).

### i) Statistical Analysis

Appropriate statistical analysis for the experimental data was adopted by using the method described by Snedecor and Cochran (1967) by using the software IBM-SPSS (version 20.0). For all variables under study, General Linear Model technique repeated over age-groups and seasons was considered.

## III. RESULTS AND DISCUSSION

### a) Weight gain features

The alterations in the growth parameters of the experimental kids of both seasons during different periods of pre-pubertal growth have been presented in Table 1. The kids born in winter achieved significantly ( $P \leq 0.01$ ) higher body weight ( $8.19 \pm 0.30$  Kg at 180 days) compared to the kids born in summer ( $7.95 \pm 0.32$  Kg at 180 days). The kids born in winter ( $39.64 \pm 2.46$  g / day) were having 12% more body weight gain compared to summer ( $35.32 \pm 2.07$  g/day). There was no or little variation between the heart girth and season.

No seasonal variation was observed in respect of height and linear length.

The weight gain featured in the study was close to the report of Singh *et al.* (2000) and more than that of Bera *et al.* (2008). Earlier Yusuff *et al.*, 1981; Khanal *et al.*, 2005 and Marai *et al.*, 2007 also reported the effect of season on the growth. In this investigation, the kids born in winter gained maximum body weight than that of the summer-born kids. It may be because of the fact that environmental temperature, as well as THI during summer, reduces the feed intake, which adversely affects the weight gain (Husain *et al.*, 1996; Khanal *et al.*, 2005 and Marai *et al.*, 2007). Sharma *et al.* (1998) also reported that factors such as forage availability, environmental stress such as heat and rain might modify the feeding pattern in goats.

#### b) Physiological Responses

Table – 2 present the mean of the rectal temperature, respiration rate, and pulse rate at different ages during summer and winter seasons. Rectal temperature was significantly ( $P \leq 0.01$ ) more in the kids born in summer ( $39.34 \pm 0.09^\circ\text{C}$ ) than winter ( $38.68 \pm 0.06^\circ\text{C}$ ). The respiration rate was significantly ( $P \leq 0.01$ ) more in the kids born in summer ( $19.20 \pm 0.20$  / min) than winter ( $12.96 \pm 0.20$  / min). The pulse rate was significantly ( $P \leq 0.01$ ) more in the kids born during summer ( $111.74 \pm 0.84$  / min) than winter ( $107.50 \pm 1.05$  / min).

Body temperature is one of the best indicators of heat tolerance, which represents the thermoregulatory mechanisms in terms of all heat gain and heat loss of the body. Rectal temperature is considered as an index of body temperature even though there is a considerable variation in different parts of the body core at several times of the day (Srikanda kumar *et al.*, 2003). The metabolic responses that occur during the transition from fetal to neonatal life present a change from a thermoregulatory state in which inhibitory stimuli dominate (Ball *et al.* 1995). Thermo regulatory process in terms of birth weight is reported in sheep, and they suggested that lighter lambs at birth have reduced the capability to sustain body temperature (Alexander, 1975; Dwyer, 2008).

In this investigation, we found that kids born in summer exhibited higher rectal temperature compared to the kids born in winter. The rectal temperature of goats elevated with high environmental temperature is reported by many workers (Devendra, 1987; Marai *et al.*, 2007). Stress-induced hyperthermia has been reported in goats (Bouwknicht *et al.*, 2007) and associated with an activation of the hypothalamic-pituitary-adrenal axis as well as the sym patho-adrenal system (Groenink *et al.*, 1994).

Also, it was found that the kids born in summer have a higher pulse rate compared to the kids born in winter. Ambient temperature has a significant

relationship with the respiratory and pulse rate fluctuations as it is a physiological mechanism against elevated heat load (Banerjee *et al.*, 2014). An increase in pulsation rate increases blood flow from the core to the surface as a result of it more heat is lost (Marai *et al.*, 2007). The increase in cardiac output and cutaneous blood flow by heat stress, due to blood redistribution from deep splanchnic to more peripheral body regions, have been implicated in goat (Silanikove, 2000)

In our study, kids born in summer exhibited a higher respiration rate compared to the kids born in winter. Respiration is the main route of evaporative heat loss of goat through the respiratory tract (Gall, 1991) as sweating is not a channel of heat loss in goat (Devendra and Burns, 1988). Therefore, an increase in respiratory rate aids in heat dissipation via evaporative cooling also reported with a rise in ambient temperature (Blackshaw and Blackshaw, 1994). Blight (1985) stated that a daily change in respiration rate per minute from the effect of environmental temperature might not be parallel with the change in body temperature and pulsation number.

## IV. RELATIONSHIP OF BODY WEIGHT GAIN FEATURES WITH HAEMATO-BIOCHEMICAL AND MINERAL PARAMETERS DURING SUMMER AND WINTER SEASON

#### a) Physiological parameters

Table – 3 & 4 present the correlation coefficients between body weight gain features and physiological responses of black Bengal goat during summer and winter. Height, heart girth, and linear length was positively correlated with Weight ( $P \leq 0.01$ ) during both summer and winter season. Positive correlation ( $P \leq 0.01$ ) was observed between respiration rate and body weight, body measurements, and rectal temperature only during the summer season. Whereas negative relation ( $P \leq 0.01$ ) of the pulse rate with body weight during both the summer and winter season was observed.

The results of the present study related to the correlation between pulse rate and post-natal growth are similar with the findings of Piccone *et al.* (2006) and Ocak *et al.* (2009). In the present investigation all the growth parameters viz. body weight, height, heart girth, and linear length were positively correlated among themselves which was in agreement with the earlier reports in cattle (Udeh *et al.*, 2011 and Olutogun *et al.*, 2003) and goats and sheep (Ozoje and Herbert, 1997; Ogungbayi *et al.*, 2003). Hence, these parameters can be used to measure the growth rate in goats. These parameters have a strong genetic correlation with body weight and is useful for genetic selection (Magnabosco *et al.*, 2002). Olutogun *et al.* (2003) also reported that body size and height were complementary, and the growth is a function of length, height, and circumference. Ocak *et al.* (2009) reported a positive

correlation between growth parameters and zero between growth and thermo-physiological parameters in Saanen kids.

#### b) Haematological Parameters

Weight and measurements were positively correlated ( $P \leq 0.01$ ) with hemoglobin in and packed cell volume (PCV) during both seasons. Total erythrocyte count (TEC) was negatively correlated ( $P \leq 0.05$ ) with weight during summer but positively ( $P \leq 0.01$ ) during the winter season.

The presence of positive correlations between body weight and hemoglobin, PCV, may be regarded as an outcome of a faster rate of erythrocyte production along with earlier rate of saturating them with hemoglobin. It be associated with the urgent need to enhance oxygen- carrying capacity, i. e., intensifying use of hemoglobin as a vehicle for oxygen transport for growing kids. High PCV hematocrit values indicate either an increase in the number of circulating RBC or a reduction in circulating plasma volume (Banerjee, 2007). Bentricks, 1974 reported that hematological parameters particularly, PCV and Hb, were associated with the nutritional status of the animal. However, the main functions of the erythrocyte are to serve as a carrier of hemoglobin. A positive relation of Hb and PCV values observed in this study might likely be a sign of healthier goats. The presence of positive correlation between TEC and body weight during the winter season may be associated with the improvement of the nutritional status of goats. At the same time the negative association between TEC and weight during summer may be related to a decrease of thyroid hormone secretion, which is related to declining the process of erythropoiesis.

#### c) Biochemical Parameters

Weight and body measurements were negatively correlated ( $P \leq 0.01$ ) with glucose and total cholesterol during both summer and winter season. The correlation pattern in cholesterol concentration may be due to the high intake of dietary fat provided by colostrum and milk.

Weight and body measurements were positively correlated ( $P \leq 0.01$ ) with Alanine Amino transferase, Aspartate Amino transferase,  $\alpha$ -Amino Nitrogen, and Non-esterified Fatty Acids during both summer and winter season. The increase in the activities of Alanine Amino transferase and Aspartate Amino transferase with growth parameters in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood (Shakoori *et al.*, 1994), which reflects active liver function with growing age. Blood  $\alpha$ -Amino Nitrogen is an indicator of the protein synthesis status of the animal. The findings of the present study are supported by Hornick *et al.*, 1996 and 1998; Mondal and Prakash, 2004. They also reported that plasma  $\alpha$ -Amino Nitrogen increases during growth and stabilizes or decreases after completion of an active phase of growth. Hornick *et*

*al.* (1998) reported that rapidly growing beef cattle had more plasma  $\alpha$ -Amino Nitrogen.

Many studies have shown a good correlation between energy balance and Non-esterified Fatty Acids concentration (Kartiarso *et al.*, 1989; Yelich *et al.*, 1996). The Concentration of Non-esterified Fatty Acids directly reflects the amount of adipose tissue breakdown in response to negative energy balance. Circulating Non-esterified Fatty Acids are absorbed and metabolized for energy by the liver and other tissues. Clinical experiences suggest serum Non-esterified Fatty Acid concentration be more sensitive to energy balance change compared with body scoring in growing situations (Van Saun, 2000). The physiological response, s and blood metabolites result of the kids during prepubertal growth and their energy balance showed that growth have a profound effect on some biochemical parameters (Nazifi *et al.*, 1999).

#### d) Plasma Minerals

The seasonal variation existed ( $P \leq 0.01$ ) in sodium, potassium, and calcium. The reduction of plasma potassium during summer may be due to loss of potassium in sweats. El-Nouty *et al.* (1980) also reported decline in plasma sodium and potassium level during heat stress. Orden *et al.* (1999) also informed that there was no significant difference with seasons in iron level in goat blood.

Plasma sodium and body weight gain features were significantly negative correlated during both seasons. The association among the minerals and body weight gain features can be inferred by different reasons. Blood Sodium concentrations in small ruminants is often used as an indicator of animal mineral status (McDowell, 2003). Calcium is involved in the synthesis of steroid hormones in ovaries and adrenal glands and the release of luteinizing hormone from the pituitary gland (Harvey *et al.*, 1987). Calcium is essential for bone formation in growing neonates, and the positive correlation is probably due to growth and milk intake (Herosimczyka *et al.*, 2011).

## V. SIMPLE REGRESSION EQUATIONS

The Simple Regression equations between age as the independent variable and other dependable variables during summer and winter season is presented in Table – 5 & 6, where also, negative relationship between blood glucose, total plasma cholesterol, plasma sodium, and age was found during both the seasons and pulse rate during winter only. At the same time all other parameters showed a positive relationship with the age during both summer and winter season.

Determined significant relationship of most parameters in blood of growing kids suggests an association between these indicators, particularly

metabolites involved in the metabolism of fats, proteins, and minerals during the entire process of the growth.

## VI. CONCLUSION

The findings of the present investigation not only substantiated the earlier findings but also help to assess the growth performance of black Bengal kid under free-range system during summer and winter season. Rapid growth during the pre-pubertal period can minimize the cost of rearing, thus providing more profit to the farmer. These data can be used in growth evaluation, improving management practices, nutrition, and health monitoring. The information on different growth parameters as an outcome of the investigation will undoubtedly help the stakeholder as well as planners to implement successful goat rearing practices.

## VII. DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Tables

**Table 1:** Mean with standard error of Growth parameters of Summer and Winter born black Bengal goats during pre-pubertal growth period

Parameters	Sex	G1	G2	G3	G4	G5	G6	G7	Overall	P value
Body Weight (Kg)	Summer	1.60 ± 0.06	2.21 ± 0.16	3.07 ± 0.22	4.59 ± 0.25	6.17 ± 0.36	6.99 ± 0.34	7.95 ± 0.32	4.65y ± 0.27	0.00** (between Season)
	Winter	1.70 ± 0.05	2.52 ± 0.18	3.38 ± 0.28	5.20 ± 0.21	6.49 ± 0.36	7.38 ± 0.32	8.19 ± 0.30	4.98x ± 0.27	
Weight Gain (gm/day)	Summer	34.83 ±2.97	20.44 ± 4.22	28.72 ± 4.43	50.61 ± 7.00	52.53 ± 4.76	28.19 ± 4.40	31.75 ± 3.13	35.32y ± 2.07	0.00** (between Season)
	Winter	55.94 ± 5.41	27.42 ± 4.81	29.53 ± 4.58	60.86 ± 6.46	44.39 ± 6.99	30.33 ± 5.72	29.03 ± 3.88	39.64x ± 2.46	
Height (cm)	Summer	24.58 ± 0.38	28.75 ± 0.66	38.42 ± 0.87	36.50 ± 0.52	36.42 ± 0.60	39.75 ± 0.80	40.67 ± 0.89	34.44 ± 0.65	0.14(between Season) and 0.00* (between Summer, Winter)
	Winter	27.00 ± 0.55	29.17 ± 1.02	32.17 ± 0.77	35.92 ± 0.50	39.00 ± 0.80	39.00 ± 0.75	41.25 ± 0.85	34.79 ± 0.62	
Heart Girth (cm)	Summer	26.08 ± 0.36	31.50 ± 0.49	39.50 ± 0.71	37.92 ± 0.68	40.67 ± 0.70	42.67 ± 0.86	45.25 ± 0.91	37.66x ± 0.72	0.03*(between Season) and 0.00* (between Summer, Winter)
	Winter	25.83 ± 0.59	31.92 ± 1.10	39.00 ± 0.80	39.67 ± 0.80	39.92 ± 0.60	41.00 ± 0.65	42.00 ± 0.97	37.05y ± 0.67	
Linear Length (cm)	Summer	26.83 ± 0.44	28.08 ± 1.13	33.00 ± 0.69	33.92 ± 0.58	34.92 ± 0.76	37.17 ± 0.79	38.75 ± 0.57	33.24 ± 0.52	0.77(between Season) and 0.00* (between Summer, Winter)
	Winter	24.42 ± 0.75	28.42 ± 1.74	33.83 ± 0.55	35.25 ± 0.39	34.25 ± 0.62	37.50 ± 0.95	38.50 ± 0.89	33.17 ± 0.62	

Means having different superscript in the last column (p, q) for the specific parameter differ significantly\*\*  $P \leq 0.01$ , \*  $P \leq 0.05$



**Table 2:** Mean with standard error of Physiological parameters of Summer and Winter born black Bengal goats during pre-pubertal growth period

Parameters	Sex	G1	G2	G3	G4	G5	G6	G7	Overall	P Value
Rectal Temperature (°C)	Summer	39.04 ±0.13	39.33 ±0.21	39.38 ±0.18	39.42 ±0.28	39.42 ±0.29	39.33 ±0.26	39.46 ±0.27	39.34x ±0.09	0.00** (between season)
	Winter	38.50 ±0.15	38.50 ±0.15	38.75 ±0.14	38.79 ±0.18	38.75 ±0.14	38.83 ±0.18	38.63 ±0.20	38.68y ±0.06	
Pulse rate (No./min)	Summer	115.17 ±0.75	110.67 ±1.06	115.67 ±1.08	107.33 ±1.9	106.08 ±2.06	103.75 ±1.50	108.67 ±1.49	109.62x ±0.70	0.00** (between season)
	Winter	111.08 0.58	111.75 ±0.68	111.08 ±0.70	103.92 ±1.94	106.50 ±1.38	97.58 ±1.77	97.17 ±1.48	105.58y ±0.80	
Respiratory rate (No./min)	Summer	17.08 ±0.47	19.08 ±0.56	19.92 ±0.47	19.75 ±0.46	19.92 ±0.43	19.08 ±0.48	19.58 ±0.47	19.20x ±0.20	0.00** (between season)
	Winter	13.67 ±0.47	13.58 ±0.53	12.17 ±0.51	13.17 ±0.59	12.83 ±0.58	12.75 ±0.52	12.58 ±0.53	12.96y ±0.20	

Means having different superscript in the last column (p, q) for the specific parameter differ significantly\*\*  $P \leq 0.01$

**Table 3:** The correlation coefficients between the Growth parameters and Physiological responses of black Bengal goat during Summer in pre-pubertal state

	Body weight	Average weight gain	Height	Heart girth	Linear length	Rectal Temperature	Pulse Rate
Average weight gain	0.264*						
Height	0.875**	0.145					
Heart girth	0.865**	0.112	0.942**				
Linear length	0.862**	0.171	0.897**	0.901**			
Rectal Temperature	0.159	0.091	0.182	0.231*	0.200		
Pulse Rate	-0.346**	-0.031	-0.269*	-0.173	-0.225*	0.120	
Respiration Rate	0.295**	0.074	0.490**	0.551**	0.480**	0.359**	0.166

\*\* Correlation is significant at the 0.01 level (2-tailed) \* Correlation is significant at the 0.05 level (2 tailed).

**Table 4:** The correlation coefficients between the Growth parameters and Physiological responses of black Bengal goat during Winter in pre-pubertal state

	Body weight	Average weight gain	Height	Heart girth	Linear length	Rectal Temperature	Pulse Rate
Average weight gain	-0.008						
Height	0.911**	-0.072					
Heart girth	0.760**	-0.069	0.843**				
Linear length	0.811**	-0.041	0.866**	0.867**			
Rectal Temperature	0.124	-0.093	0.131	0.208	0.116		
Pulse Rate	-0.617**	0.075	-0.549**	-0.523**	-0.495**	-0.206	
Respiration Rate	-0.185	-0.020	-0.096	-0.128	-0.205	0.006	0.092

\*\*Correlation is significant at the 0.01 level (2-tailed) \*Correlation is significant at the 0.05 level (2 tailed).

**Table 5:** Simple Regression equations between age as independent variable and other dependable variables studied for kid born in Summer season

Sl. No.	Equation		R <sup>2</sup> value
1	Body weight	= 1.13 Age + 0.12	0.99**
2	Height	= 2.58 Age + 24.12	0.91**
3	Heart Girth	= 2.89 Age + 26.08	0.88**
4	Linear Length	= 1.99 Age + 25.26	0.95**
5	Haemoglobin Concentration	= 0.43 Age + 8.83	0.68*
6	Blood Glucose	= - 5.08 Age + 72.93	0.91**
7	Total Protein	= 0.20 Age + 6.25	0.64*
8	Globulin	= 0.09 Age + 3.36	0.59*
9	Albumin	= 0.11 Age + 2.89	0.65*
10	Plasma Total Cholesterol	= - 10.21 Age + 144.36	0.81**
11	Aspartate Aminotransferase	= 4.15 Age + 64.63	0.63*
12	Alanine Aminotransferase	= 1.35 Age + 17.22	0.77**
13	α-Amino Nitrogen	= 4.81 Age + 8.28	0.96**
14	Non-esterified Fatty Acids	= 7.30 Age + 47.08	0.99**
15	Plasma Sodium	= - 2.49 Age + 136.97	0.89**
16	Plasma Calcium	= 0.36 Age + 8.69	0.94**
17	Plasma Iron	= 0.04 Age + 1.20	0.75**

\*\*P ≤ 0.01 and \*P ≤ 0.05

**Table 6:** Simple Regression equations between age as independent variable and other dependable variables studied for kid born in Winter season

Sl. No.	Equation		R <sup>2</sup> value
1	Body Weight	= 1.15 Age + 0.37	0.99**
2	Height	= 2.47 Age + 24.89	0.96**
3	Heart Girth	= 2.41 Age + 27.39	0.77**
4	Linear Length	= 2.17 Age + 24.48	0.87**
5	Pulse Rate	= - 2.67 Age + 116.25	0.84**
6	Haemoglobin Concentration	= 0.43 Age + 9.28	0.85**
7	Total Erythrocyte Count	= 0.27 Age + 12.47	0.69*
8	Blood Glucose	= - 4.69 Age + 75.00	0.75**
9	Total Protein	= 0.17 Age + 6.61	0.87**
10	Globulin	= 0.13 Age + 3.23	0.70*
11	Plasma Total Cholesterol	= - 9.59 Age + 144.58	0.76**
12	Aspartate Aminotransferase	= 3.94 Age + 68.46	0.63*
13	Alanine Aminotransferase	= 1.16 Age + 19.60	0.84**
14	α-Amino Nitrogen	= 4.96 Age + 8.49	0.97**
15	Non-esterified Fatty Acids	= 7.90 Age + 43.42	0.98**
16	Plasma Sodium	= - 2.63 Age + 140.58	0.99**
17	Plasma Potassium	= 0.08 Age + 6.77	0.76**
18	Plasma Calcium	= 0.22Age+9.54	0.70*
19	Plasma Iron	= 0.05Age+1.13	0.68*

\*\*P ≤ 0.01 and \*P ≤ 0.05

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

## **Declaration of Conflicts of Interest**

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

## POLICY ON PLAGIARISM

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Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



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

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

### Changes in Authorship

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

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### Appealing Decisions

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

### Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

### Declaration of funding sources

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## PREPARING YOUR MANUSCRIPT

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

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



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

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

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

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

A research paper must include:

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

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

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

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

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





## FORMAT STRUCTURE

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

All manuscripts submitted to Global Journals should include:

### **Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

### **Author details**

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

### **Abstract**

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

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

### **Keywords**

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

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

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

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

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

### **Numerical Methods**

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

### **Abbreviations**

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

### **Formulas and equations**

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

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

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



## Figures

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

### PREPARATION OF ELETRONIC FIGURES FOR PUBLICATION

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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

Color charges: Authors are advised 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. Also, you can email your editor to remove the color fee after acceptance of the paper.

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

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

**2. Think like evaluators:** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

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

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

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



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

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

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

**9. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

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

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

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

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

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

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

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

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

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

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

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



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

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

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

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

## INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

### Key points to remember:

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

### Final points:

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

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

### The discussion section:

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

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

### General style:

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

**To make a paper clear:** Adhere to recommended page limits.



### *Mistakes to avoid:*

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

### **Title page:**

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

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

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

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

*Reason for writing the article—theory, overall issue, purpose.*

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

### **Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

### **Introduction:**

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.





*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

#### **Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

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

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

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

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

#### **Materials:**

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

#### **Methods:**

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

#### **Approach:**

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

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

#### **What to keep away from:**

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



**Results:**

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

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

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

**Content:**

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

**What to stay away from:**

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

**Approach:**

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

**Figures and tables:**

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

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

#### **Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

### THE ADMINISTRATION RULES

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.*

*Segment draft and final research paper:* You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

*Written material:* You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.



CRITERION FOR GRADING A RESEARCH PAPER (COMPILATION)  
BY GLOBAL JOURNALS

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<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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