Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: G

Veterinary Science & Veterinary Medicine

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VOLUME 19 ISSUE 2 VERSION 1.0

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine

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Veterinary Science and Veterinary Medicine
Volume 19 Issue 2 (Ver. 1.0)

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USA Tax ID: 98-0673427

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Contents of the Issue

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 19 Issue 2 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Keratinophilic and Saprophytes Fungi Isolated in Canines of Veterinary Hospital of San Carlos of Guatemala University in 2016

By Álvarez-Muñoz, Verónica María, Villatoro-Chacón, Daniela Mariel & Arizandieta-Altán, Carmen Grizelda
San Carlos of Guatemala University

Abstract- Keratinophilic and saprophytic fungi are microor- ganisms that have been isolated in hairs and nails in small species. They have a high zoonotic potential with immune compromised patients being the population with the highest risk. In the present study, the presence of keratinophilic fungi and saprophytes was evaluated in dogs with dermatological lesions examined in the Veterinary Hospital of San Carlos of Guatemala University in 2016. A total of 1,457 patients were evaluated, of which 195 presented dermatological lesions. The potassium hydroxide (KOH) test was performed on the fur of patients with dermatological lesions. KOH-positive patients underwent mycological culture with dextrose sabourad agar and selective agar for pathogenic fungi with cycloheximide. 13.38% of the patients presented dermatological lesions. 18.46% were positive to the KOH test. 16.7% obtained growth to keratinophilic and saprophytic fungi.

Keywords: *mycoses, dermatomycoses, fur, culture media.*

GJMR-G Classification: *NLMC Code: WY 130*

Strictly as per the compliance and regulations of:

Keratinophilic and Saprophytes Fungi Isolated in Canines of Veterinary Hospital of San Carlos of Guatemala University in 2016

Hongos queratinofílicos y saprófitos aislados en caninos del Hospital Veterinario de la Universidad de San Carlos de Guatemala en el año 2016

Álvarez-Muñoz, Verónica María [α], Villatoro-Chacón, Daniela Mariel [σ] & Arizandieta-Altán, Carmen Grizelda [ρ]

Abstract- Keratinophilic and saprophytic fungi are microorganisms that have been isolated in hairs and nails in small species. They have a high zoonotic potential with immune compromised patients being the population with the highest risk. In the present study, the presence of keratinophilic fungi and saprophytes was evaluated in dogs with dermatological lesions examined in the Veterinary Hospital of San Carlos of Guatemala University in 2016. A total of 1,457 patients were evaluated, of which 195 presented dermatological lesions. The potassium hydroxide (KOH) test was performed on the fur of patients with dermatological lesions. KOH-positive patients underwent mycological culture with dextrose sabourad agar and selective agar for pathogenic fungi with cycloheximide. 13.38% of the patients presented dermatological lesions. 18.46% were positive to the KOH test. 16.7% obtained growth to keratinophilic and saprophytic fungi. Four genera were isolated: Mucor spp., Aspergillus spp., Penicillium spp. and Alternaria spp. Of these 50% of the cases was Mucor spp.; being 16.7% respectively for the rest of the pathogens. The data obtained indicate the presence of keratinophilic and saprophytic fungi in the canine population.

Keywords: mycoses, dermatomycoses, fur, culture media.

Resumen- Los hongos queratinofilicos y saprófitos son microorganismos que se han aislado en pelos y uñas en pequeñas especies. Poseen un alto potencial zoonótico siendo los pacientes inmunocomprometidos la población con mayor riesgo. En el presente estudio se evaluó la presencia de hongos queratinofilicos y saprofitos en caninos con lesiones dermatológicas atendidos en el Hospital Veterinario de la Universidad de San Carlos de Guatemala en el año 2016. Se evaluaron 1,457 pacientes de los cuales 195 presentaron lesiones dermatológicas. Se realizó la prueba de hidróxido de potasio (KOH) al pelaje de los pacientes con lesiones dermatológicas. Los pacientes positivos a KOH se les realizó cultivo micológico con agar sabourad dextrosado y agar selectivo para hongos patógenos con cicloheximida. El 13.38% de los pacientes presentó lesiones dermatológicas. El 18.46% fueron positivos a la prueba de KOH. El 16.7% obtuvo crecimiento a hongos queratinofilicos. Se aislaron cuatro géneros: *Mucor spp., Aspergillus spp., Penicillium spp. y Alternaria spp.* De éstos el 50% de los casos fue *Mucor spp.*; siendo el 16.7% respectivamente para el resto de los patógenos. Los datos obtenidos indican la presencia de hongos queratinofilicos y saprófitos en la población canina.

Palabras clave: micosis, dermatomicosis, pelaje, medios de cultivo.

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I. Introducción

Los problemas dermatológicos en pequeñas especies han ido incrementando en los últimos años. Dentro de los patógenos que afectan la piel de los animales, los dermatofitos son considerados uno de los más comunes. Estos se clasifican como hongos que afectan la capa córnea de la piel, pelos y uñas (Stanchi, 2007).

Según el Centro de Seguridad Alimentaria y Pública de la Universidad Estatal de IOWA (2005) la dermatofitosis en caninos se ve con mayor frecuencia en cachorros y en adultos inmunodeprimidos. Las lesiones pueden aparecer sobre cualquier parte del cuerpo y en general se presentan como áreas alopécicas, con descamación, costras, eritema y prurito. En el inicio de una infección se pueden observar vesículas y pústulas. También puede presentarse una forma nodular focal (querion), caracterizada por una inflamación grave localizada, que contiene material purulento, el cual le da un aspecto esponjoso. Sin embargo, en la mayoría de los pacientes suele ser autolimitante.

El diagnóstico de las infecciones fúngicas suele ser un problema si el médico veterinario se basa sólo en las características clínicas. La dermatofitosis se diagnostica en exceso o, en su defecto se obvian casos que verdaderamente lo son. Por esta razón, es necesario confirmar una dermatofitosis obteniendo muestras para su aislamiento e identificación (Betancourt et al., 2009). En la práctica clínica la prueba de hidróxido de potasio (KOH) es considerada como prueba tamiz para el diagnóstico micológico, ya puede revelar la presencia de hifas o conidias. Sin embargo, el diagnóstico definitivo suele realizarse mediante cultivo micológico (Centro de seguridad alimenticia y salud pública de la Universidad estatal de IOWA; 2005).

La finalidad del estudio fue determinar la presencia de hongos queratinofilicos y saprófitos en caninos con lesiones dermatológicas. Esto con el fin de generar información sobre la prevalencia de la enfermedad en la población de estudio y determinar los posibles agentes infecciosos y su potencial zoonótico.

II. Materiales y Métodos

a) Área de studio

El estudio se llevó a cabo en el Hospital Veterinario de Animales de Compañía, Laboratorio Clínico y Laboratorio de Microbiología de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad de San Carlos de Guatemala (FMVZ-USAC), cuyas coordenadas son 14°34′58″N 90°33′10″O.

b) Criterios de inclusión

Se evaluaron clínicamente 1,457 pacientes caninos no importando edad, sexo o raza, en el periodo de febrero a noviembre del año 2016. Se incluyeron únicamente los pacientes que presentaron lesiones dermatológicas siguiendo los criterios descritos por Foil (2013). La muestra total fue de 195 pacientes.

c) Toma de muestra

Se obtuvieron muestras de pelo y escama de los 195 pacientes con lesiones dermatológicas. Estas fueron colectadas, transportadas y enviadas al Laboratorio Clínico y Microbiológico de la Facultad de Medicina Veterinaria de la Universidad de San Carlos de Guatemala, siguiendo los criterios de Gadea, Cuenca, Martín, Pontón y Rodríguez (2006). A estos pacientes se les realizó la prueba de Hidróxido de potasio (KOH) en el Laboratorio Clínico y cultivo micológico en el Laboratorio Microbiológico respectivamente.

d) Selección de muestra para KOH

Se colectaron muestras de 195 pacientes con lesiones dermatológicas. Se realizó la prueba de KOH (prueba tamiz) con muestras de pelo y escama según la técnica descrita por Llovo y Pontón (2007).

e) Selección de muestra para micocultivo

A los pacientes positivos a la prueba tamiz, se les realizó cultivo micológico, por lo que se enviaron las muestras de pelo al Laboratorio de Microbiología. Se realizó el cultivo microbiológico utilizando la técnica descrita por Llovo y Pontón (2007). Se realizaron dos siembras en dos diferentes medios de cultivo. Los medios de cultivo utilizados fueron agar Sabouraud dextrosado al 4% y agar selectivo para hongos patógenos con cicloheximida. Las muestras fueron incubadas a temperatura ambiente (28 a 37° C) por lapso de 30 a 45 días.

f) Análisis estadístico

Los datos fueron resumidos utilizando estadística descriptiva (Sokal y Rolf, 1995). Se utilizaron paquetes estadísticos de R® y SPSS 2.4® para el análisis de los datos.

III. Resultados

a) Prevalencia

La prevalencia de pacientes con lesiones dermatológicas fue del 13.38%. En cuanto a los pacientes positivos a KOH la prevalencia observada fue de 18.46%. Sin embargo, la prevalencia de pacientes positivos a crecimiento de hongos saprófitos fue del 16.7% (Tabla 1).

Tabla 1: Muestras positivas y negativas a prueba de KOH y cultivo micológico de perros domésticos.

	KOH	%	Cultivo	%
Positivos	36	18.46	6	16.7
Negativos	159	81.54	30	83.3
Total	195	100	36	100

b) Patógenos aislados

Se aislaron cuatro géneros: *Mucor sp., Aspergillus sp., Penicillium sp. y Alternaria sp.* De éstos el 50% de los casos fue *Mucor sp.*; y el resto de patógenos aislados corresponden al 16.7% (Tabla 2).

c) Prevalencia según sexo, edad y raza respecto al género del patógeno aislado

Se observó que tanto hembras y machos se ven afectados de igual manera (50%). En cuanto a la edad, se categorizó a los pacientes en dos grupos (0-3 años y de 4-6 años). El grupo de pacientes entre los 0-3 años fue el más afectado (67.7%). Respecto a la raza, se aislaron patógenos en 4 categorias: sin raza definida (SRD), poodle, cocker spaniel y Husky siberiano. Los pacientes SRD fueron la mitad de los casos (50%) positivos (Tabla 2).

Tabla 2: Hongos queratinofílicos potencialmente patógenos y saprófitos aislados de perros domésticos.

	Mucor spp.	Aspergillus spp.	Penicillium spp.	Alternaria spp.	Total N (%)
Sexo					
Hembra	3				3 (50)
Macho		1	1	1	3 (50)
Edad					
0-3 años	2		1	1	4 (66.7)
4-6 años	1	1			2 (33.3)
Raza					
SRD	2		1		3 (50)
Poodle	1				1 (16.7)
Cocker		1			
Spaniel					1 (16.7)
Husky Siberiano				1	1 (16.7)
Total					
N	3	1	1	1	6
%	50	16.7	16.7	16.7	100

IV. Discusión

La prevalencia de hongos queratinofílicos y saprófitos observada en el presente estudio es similar a las obtenidas por Arias (2013); Mattei, et al. (2014) y Josa, Quijano y Urias (2017). Esta datos sugieren que la prevalencia de la enfermedad puede verse influenciada por el clima, temperatura, humedad relativa y precipitación de las diferentes zonas geográficas, así como de los reservorios naturales. Por tal razón, las condiciones ambientales pueden haber sido similares a los estudios mencionados.

Los hongos filamentosos encontrados en el estudio pueden ser considerados como posibles patógenos. Esto se debe a que se consideran causantes de infecciones micóticas superficiales que afectan al estrato corneo de la piel, pelo y uñas. Por lo general son oportunistas causando infecciones en pacientes susceptibles con patologías como diabetes, cáncer o cualquier otra enfermedad crónico-debilitante (Maldonado, 2012; Giusiano, 2017, Curutchet, 2010 e Instituto nacional de seguridad e higiene en el trabajo, 2016).

En cuanto a las especies encontradas, se ha determinado que tanto perros y gatos albergan numerosos mohos y levaduras saprófitos en el pelaje y tegumento. Los géneros aislados con mayor frecuencia corresponden a *Alternaria, Aspergillus, Aureobasidium, Chrysosporium, Cladosporium, Mucor, Penicillum y Rhyzopus* (Domínguez y Sanz, 2010). Por otra parte, algunos hongos no dermatofitos pueden ser transmitidos de perros y gatos al ser humano. Los géneros de hongos oportunistas que algunos autores reconocen con carácter zoonótico son *Aspergillus spp*. y *Candida spp*., ambos asociadas a diferentes cuadros clínicos. Los géneros de hongos *Aspergillus, Penicillium* y *Mucor* corresponden a hongos miceliados saprófitos, donde algunas de estas especies se pueden comportar como patógenos oportunistas (Méndez et al, 2013). Además Groll y Walsh (2001), han determinado que *Aspergillus spp. y Candida spp.* provocan infecciones fúngicas de invasión profunda con riesgo de muerte. Esto se debe a las tendencias epidemiológicas de la última década sugieren un cambio hacia las infecciones por *Aspergillus* y otros hongos oportunistas poco comunes como *Penicillum spp*.

La mayor prevalencia de casos aislados fue del género *Mucor spp*. Este es un Zygomiceto, caracterizado por un crecimiento rápido, que se encuentra ubicuo en la naturaleza, por lo que es muy común que contamine medios de cultivo en el laboratorio, y que puede llegar a producir infecciones en seres humanos inmunocomprometidos. Las cepas de *Mucor spp*. no crecen por lo regular a 37°C, pero las cepas patógenas que afectan al ser humano son termotolerantes, por lo que crecen a mayor temperatura que la mencionada anteriormente (Rodríguez, 2016).

Las infecciones de hongos filamentosos oportunistas de perros son raramente reportadas. Las infecciones reportadas son causadas por *Aspergillus spp*. (Langlois et.al., 2014). Jimenez (2010) aisló *Aspergillus spp.*en perros callejeros con lesiones dérmicas encontrando altas prevalencias. Esto sugiere el cuidado que debe tenerse en el manejo de perros callejeros con lesiones en la piel con alopecia parcial.

Por su parte, pacientes afectados por especies de *Penicillum spp.* son poco comunes en perros. Sin embargo, la prognosis es pobre en los casos en los que se presenta ya que la mayoría de perros sucumben ante la enfermedad o se realiza eutanasia poco después del diagnóstico (Langlois et.al., 2014).

Los casos presentados en perros con *Alternaria spp.* y *Mucor spp*. son muy escasos. Ambos hongos afectan principalmente a sujetos inmuno-comprometidos. Las patologías causadas por *Alternaria spp*. tienen una resolución rápida y sin complicaciones (Dedola, 2010 y Instituto nacional de seguridad e higiene en el trabajo, 2014). Las afecciones causadas

por *Mucor spp*, se caracterizan por invasión y necrosis vascular aguda, rápidamente progresiva en humanos al contrario de lo que ocasiona en perros. Estos últimos se ven afectados por este hongo, a menos que presenten enfermedades como diabetes mellitus, tumores como linfoma y leucemia o cualquier condición inmunosupresora (Revankar, 2018).

V. Conclusión

Los datos generados en el estudio sugieren la presencia de hongos queratinofilicos y saprofitos en la población canina. Por esta razón, deben realizarse más estudios en esta línea de investigación, para evaluar su potencial patógeno y zoonótico.

Agradecimientos

Al personal del Departamento de Microbiología de la Universidad de San Carlos de Guatemala por su aporte en dicha investigación.

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Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 19 Issue 2 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Laxative Activity of *Trigonella Foenum- Graecum* Seed on Loperamide Induced Constipation in Rats

By R. Chandrasekar, G.G.P. Madhuri, T. Bhavya, K. Keerthi, S. Aishwarya, Y. Saipavan & M. Niranjan Babu

Abstract- *Trigonella foenum-graecum* L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. The genus name Trigonella means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name foenum-graecum means 'Greek hay'. It is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Fabaceae. It is used as a functional food, traditional food and as a nutraceutical, as well as its physiological utilization such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant and anti-diabetic agent.

The main objective of the study was to extract phytoconstituents from fenugreek seeds. The present study will be helpful in determining the quality and purity of a crude drug and laying down pharmacopoeial standards for *Trigonella foenum-graecum*. Fenugreek seed was extracted with water using hot continuous percolation method and the aqueous extract was used for determining the laxative activity.

Keywords: *Fenugreek Seeds, Laxative activity, Constipa- tion, Trigonella foenum-graecum.*

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

Strictly as per the compliance and regulations of:

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Abstract- ***Trigonella foenum-graecum*** L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. The genus name Trigonella means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name foenum-graecum means 'Greek hay'. It is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Fabaceae. It is used as a functional food, traditional food and as a nutraceutical, as well as its physiological utilization such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant and anti-diabetic agent.

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Phytochemical screening of the extract revealed the presence of alkaloids, amino acids, carbohydrates, proteins, saponins, tannins, terpenoids, and vitamins. The present study was carried out to investigate the laxative activity of fenugreek seeds in Albino Wistar rats. Rats were divided into four groups of 5 animals each, the first group as a control; the second group served as standard (sodium picosulfate) while group 3 and four were treated with aqueous seed extract of *Trigonella foenum-graecum* at doses of 250 and 500 mg/kg body weight (b.w.), *p.o.* Respectively. The laxative activity was determined based on the weight of the fecal matter.

The results revealed that the aqueous extract of fenugreek seed has shown significant laxative activity and supports its traditional claim in herbal medicine. From the available literature, it's evident that there is a need for the development of proper medication and dosage form for the treatment of constipation.

Keywords: Fenugreek Seeds, Laxative activity, Constipation, Trigonella foenum-graecum.

I. Introduction

Constipation affects all ages; it affects elderly people at the age of 60 and above, adults and children. Constipation can be classified into different categories which include common constipation, occasional constipation, chronic constipation, travel-related constipation, age-related constipation, pregnancy-related constipation, chronic idiopathic constipation and functional constipation. This condition may be due to consumption of low fiber-rich food, improper time in consuming food, lack of exercise, life style habits, less intake of fluids, etc. Constipation may end in restlessness, tired, indigestion, discomfort, vomiting, and accumulation of fecal matter in the intestine. Laxatives commonly bulk laxatives can relieve constipation in smoothening the walls of the intestine, colon and rectum and ease the fecal matter out of the rectum.

Fenugreek belonging to *Fabaceae* family is one of the well-known spices in food. Its seeds and green leaves are used in food as well as its medicinal value in the treatment of various diseases. It provides natural food fiber and other nutrients required to the human body. [1] Fenugreek has a strong spicy and a sweet flavor. [2] Aromatic and flavouring fenugreek is a popular spice and is widely used for well recognized culinary and medicinal properties. [3] "*Kasuri Methi*" is very famous for its appetizing fragrance and it is used for culinary preparations. [4] In a recent trend, fenugreek is also used as spice adjunct. [5] India is a major producer of fenugreek and also a major consumer of it for its culinary uses and medicinal application. It is used in functional food, traditional food, nutraceuticals as well as its pharmacological activities such as an antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant, and anti-diabetic agent. It has a beneficial influence on digestion and also can modify food texture.

The mechanisms of action of laxatives include enhancement of fluid retention by hydrophilic or osmotic mechanisms, decreasing net absorption of fluid through effects on the fluid and electrolyte transport in the small or large intestines, and finally an alteration of motility by inhibiting nonpropulsive contractions or stimulating propulsive contractions. Laxatives are often classified into four categories that include: bulk-forming laxatives, osmotic laxatives, stimulant laxatives, and stool softeners or surfactant laxatives. [6]

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Fenugreek is a mild bulk-forming laxative that's best suited for long-term use in people with constipation. The laxatives most frequently used world-wide come from plants. Herbal laxatives are either bulk-forming or stimulating. Bulk-forming laxatives come from plants with a high fiber and mucilage content that expand when they come in contact with water; examples include psyllium, flaxseed, and fenugreek. As the volume in the bowel increases, a reflex muscular contraction occurs, stimulating a bowel movement. These mild laxatives are best suited for long-term use in people with constipation. [7] Fenugreek fiber could be useful for treating constipation and hinder the development of diverticulosis and diverticulitis. Fenugreek fiber promotes the normal location due to imperfect fermentation in the large intestine. It can make the waste bulky, soften the stool by holding water and minimize the transit time through the intestine; hence, it helps to keep constant and steady stool time. Fenugreek is a mild bulk-forming laxative that's best suited for long-term use in people with constipation. [1]

II. Materials and Methods

a) Collection of plant material

The seeds of *Trigonella foenum-graecum* were collected from departmental stores, Tirupati. They were identified and verified taxonomically and authenticated in the Department of Botany, S. V. University, Tirupati. The seeds were coarsely powdered by using a mixer grinder and the powder was stored in airtight plastic containers. The preserved powder was used for physicochemical analysis.

Figure 1: Fenugreek Seeds

b) Determination of physicochemical parameters

Determination of physicochemical parameters such as total ash, acid insoluble ash, water-soluble ash, extractive values such as water soluble extractive value, ethanol soluble extractive value and ether soluble extractive value of the crude drug was determined according to WHO guidelines on quality control methods for medicinal plant materials (WHO, 1992). [8]

c) Preparation of extracts

The collected plant material was washed and dried at room temperature for seven days and was subjected to size reduction. The prepared powder was used for extract preparation. The aqueous extract was prepared by the Soxhlet extraction method.

Figure 2: Soxhlet Extraction Method

d) Phytochemical Evaluation

The freshly prepared aqueous extract of *Trigonella foenum-graecum* was qualitatively analyzed for the presence of major phytochemical constituents. [9]

e) Pharmacological studies

i. *Experimental animals*

The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 5°C), humidity (55 ± 5%) and maintained on a 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water and libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee CPCSEA Reg. No. (1995/PO/RE/S/17/ CPCSEA)

f) Laxative Screening

i. *Animals*

Wistar rats of either sex with an average weight of 150-200 g were obtained from Bangalore. The animals were housed in clean cages placed in a well ventilated house. They were acclimatized to the animal house condition for seven days during which they were allowed free access to commercial pelleted rat chow. All experimental procedures were performed in compliance with international policies governing the Institutional Animal Ethical Committee for the treatment of experimental animals.

g) Acute toxicity studies

Acute oral toxicity study for the test extract of the *Trigonella foenum-graecum* was carried out as per the guidelines set by Organization for Economic Co-operation and, revised draft (OECD) 425 and by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The study revealed that the administration of aqueous seed extract of *Trigonella foenum-graecum* was safe up to a dose of 2000 mg/kg. No death was observed up to this dose, and the experimental animals were physically active. Hence 1/4th (250 mg/kg) and 1/8th (500 mg/kg), were selected as working doses for the present study. [10]

h) Experimental design

Animals were divided into four groups, each group containing five animals.

- Group I - Normal control, control rats - received normal saline (negative control)
- Group II - Received standard drug sodium picosulfate (positive control) 5mg/kg p.o.
- Group III - Aqueous extract of *Trigonella foenum-graecum* seed at a dose of 250 mg/kg body weight p. o. (Test –I)
- Group IV - Aqueous extract of *Trigonella foenum-graecum* seed at a dose of 500 mg/kg body weight p. o. (Test –II)

i) Evaluation of laxative activity

i. *Laxative activity of aqueous extract of Trigonella foenum-graecum in rats*

The animals were fasted for 12 hours before the experiment, but provided with water ad libitium. The animals were divided into four groups of five in each and were placed individually in cages lined with clean filter paper. Group I treated as control, (2 ml p.o. Normal Saline), group II received sodium picosulfate (1ml/kg p.o) served as standard and Group III received *Trigonella foenum-graecum* seed aqueous extract (250 mg/kg p.o.) and Group IV received *Trigonella foenum-graecum* seed aqueous extract (500 mg/kg p.o.) respectively. Immediately after dosing, the animals were separately placed in cages suitable for collection of faces. After 8 hours of drug administration, the feces were collected and weighed. Food and water were given to all rats and fecal outputs were weighed after 16 hours. After 8-16 hrs test drug exhibited an increase in fecal output. The extract showed a dose dependant increase in fecal output of rats when compared to the control group (Table 4). The effects of *Trigonella foenum-graecum* increased significantly fecal output at doses of 250 and 500 mg/kg (p.o.) of rats compared to control group ($p < 0.05$ and $p<-0.01$ respectively). The method of Capasso *et al*. [11] was followed for this activity. All oral administration was done using metal oropharyngeal cannula. The water and feed intake and the number of fecal pellets of all the rats were recorded during the experimental period.

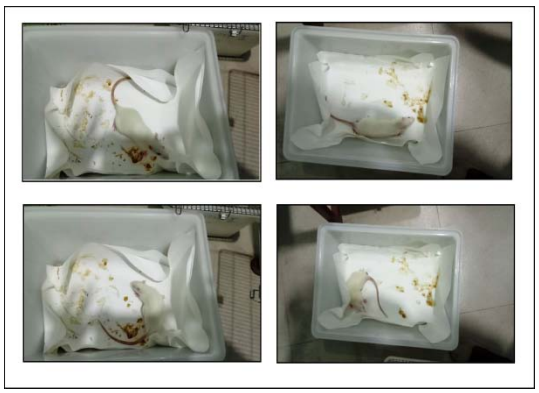

Figure 3: Pictorial representation of fecal output

ii. *Determination of total number, dry weight, and water content of fecal pellets*

The excreted fecal pellets of individual rats were collected at 10.00 h throughout the experiment. The total number, weight and water content of the pellets were determined. The water content was calculated as the difference between the wet and dry weights of the pellet. [12] The water content of feces was calculated as: fecal water content (%) = (feces weight before dried - feces weight after dried)/feces weight before dried × 100. [13, 14]

The laxative activity of the *Trigonella foenum-graecum* seed aqueous extract was evaluated by observing the stool consistency parameters like normal pellet stool, soft-formed stool, watery stool and mucus stool. The onset and duration of feces was recorded. *Trigonella foenum-graecum* seed aqueous extract at a dose 500 mg/kg P.O. showed a significant Laxative Activity $p< 0.05$.

iii. *Effect of Trigonella foenum-graecum seed aqueous extract on Loperamide induced constipation in rat*

The laxative activity of *Trigonella foenum-graecum* seed aqueous extract was evaluated by observing the fecal output. Rats were allowed to fast for 18 hours and divided into four groups of five animals each. Rats were placed individually in cages lined with clean filter paper. Group I received *Trigonella foenum-graecum* seed aqueous extract (250 mg/kg p.o.) and Group II received *Trigonella foenum-graecum* seed aqueous extract (500 mg/kg p.o.) respectively. Group II treated as control, (2 ml p.o. Normal Saline), group IV received sodium picosulfate (5mg/kg p.o) served as standard. After one h, all the animals received Loperamide (5 mg/kg, *p.o.*) by gavage. It was observed that after 8 hours of treatment. Extract effect at the higher dose of *Trigonella foenum-graecum* seed aqueous extract 500 mg/kg (p.o.) was similar to that of the standard drug sodium picosulfate (5 mg/kg, p.o.).

The reduction of Loperamide-induced constipation at 500 mg/kg (p.o.) of *Trigonella foenum-graecum* seed aqueous extract treatment was found to be almost comparable with that of treatment by 5 mg/kg of sodium picosulfate. Hence *Trigonella foenum-graecum* seed aqueous extract showed significant laxative activity ($P<0.05$) at 500 mg/kg dose level when compared to standard. The feces production (total number) in all groups was monitored for eight h. This study was carried out, as described by Takahara *et al*. [15, 16]

j) Data analysis

The data obtained by the various parameters were statistically evaluated by one-way analysis of variance (ANOVA). The mean values ± SEM were calculated for each parameter. $P< 0.05$ was considered significant.

III. Results and Discussion

a) Pharmacognostical and Phytochemical Evaluation

To establish the quality and purity of the raw material used for the various physiochemical parameters such as ash values and extractive values were evaluated and reported in table 1 & 2.

The results revealed that the plant *Trigonella foenum-graecum* shows the percentage of total ash as well as extractive values.

Table 1: Different Ash values of *Trigonella foenum-graecum*

S. No.	Ash Values	Ash Values in Percentage
		Trigonella foenum-graecum
1	Total ash	4.76
2	Water soluble ash	1.2
3	Acid insoluble ash	2.1

Table 2: Different Extractive Values of *Trigonella foenum-graecum* seeds

S. No.	Extractive Values	Extractive values in Percentage
		Trigonella foenum-graecum
1	Water soluble extractive value	6.3
2	Alcohol soluble extractive value	6.1
3	Ether soluble extractive value	5.15

b) Phytochemical Evaluation

The extracts are subjected to various qualitative phytochemical tests and reports are shown in table no.3. The results reveal the presence of alkaloids, carbohydrates, flavonoids, proteins and saponins, etc.

Table 3: Phytochemical analysis of *Trigonella foenum-graecum*

S. No.	Phytochemical analysis	*Trigonella Foenum-Graecum* AE
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Proteins	+
4.	Amino acids	+
5.	Glycosides	-
6.	Steroids & sterols	-
7.	Flavonoids	+
8.	Tannins	+
9.	Phenolic compounds	-
10.	Terpenoids	+
11.	Saponins	+
12.	Fats and oils	-
13.	Gum and Mucilage	+
14.	Vitamins	+

(+) Present (-) Not Present

Table 4: Laxative activity of aqueous extract of *Trigonella Foenum-Graecum* in rats

Groups	Treatment	Dose	Faeces output (g)	
			0-8 hrs	8-16 hrs
GP I	Control	(5 ml/kg)	0.4975±0.04975	0.56±0.056
GP II	Sodium Picosulfate	(5 mg/kg)	3.4875±1.4874**	3.85±0.45**
GP III	Test I TFG AQ Ex p. o.	(250 mg / kg)	2.49875±0.74875*	2.66125±0.91125*
GP IV	Test II TFG AQ Ex p. o.	(500 mg / kg)	3.05±0.55**	3.4875±0.4875**

Values are expressed as mean ± S.E.M (n = 5); * $p < 0.05$ compared to control group; and ** $p < 0.01$ compared to control group.

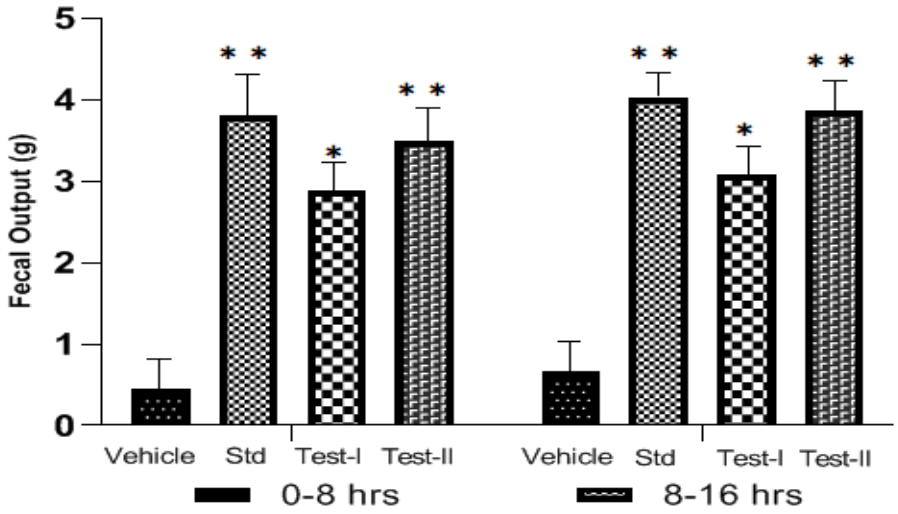

Figure 5: Time interval of fecal output of aqueous extract of *Trigonella Foenum-Graecum* in rats

Table 5: Loperamide induced constipation on aqueous extract of *Trigonella Foenum-Graecum* in rats

Groups	Treatment	Dose	Weight of faeces (g)
GP I	Control	(5 ml/kg)	0.4375±0.1685
GP II	Sodium Picosulfate	(5 mg/kg)	4.45±0.6141**
GP III	TFG AQ Ex	(250 mg / kg)	2.4±0.5043*
GP IV	TFG AQ Ex	(500 mg / kg)	4.07±0.49**

Values are expressed as mean ± S.E.M (n = 5); * p < 0.05 compared to control group; and **p < 0.01 compared to control group.

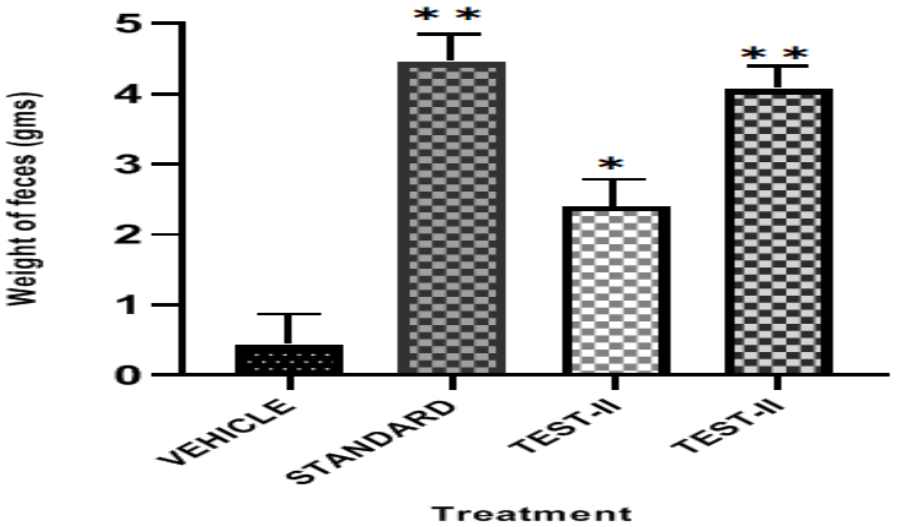

Figure 6: Loperamide induced constipation of *Trigonella Foenum-Graecum* seed aqueous extract

IV. Discussion

Trigonella foenum-graecum was subjected to systematic physicochemical evaluation and phytochemical screening by extracting with aqueous solvent to determine the soluble constituents present in a given amount of the plant material. The present work was helpful in determining the quality and purity of a crude drug. In this study the parameters used for the evaluation of *Trigonella foenum-graecum* were, Extractive values by different solvents (includes water, ethanol and petroleum ether) ash values (total ash, water soluble and acid insoluble ash). On incineration, drugs leave an ash which consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful for detecting the adulterants, exhausted drugs,

low-grade products and excess of sandy matter which is especially applicable to powdered drugs. [17]

Phytochemical analysis was performed on the aqueous extracts of *Trigonella foenum-graecum*. The aqueous extract contains carbohydrates, proteins and amino acids, glycosides, alkaloids, flavonoids, phenolic compounds, phytosterols, and tannins. The present study was carried out to investigate the laxative activity of fenugreek seeds in albino Wistar rats. Rats were divided in 4 groups of 5 animals each, first group as control, second group served as standard (sodium picosulfate) while group 3 and 4 were treated with aqueous seed extract of *Trigonella foenum-graecum* at doses of 250 mg/kg and 500 mg/kg body weight (b.w.), *per oral* respectively. The laxative activity was determined based on the weight of the fecal matter. The results showed that the aqueous extract of *fenugreek* has a significant laxative activity and supports its traditional claim in herbal medicine.

Fecal output depends on the dietary fiber, water-electrolyte balance, the rate of absorption and secretion from the lumen. Many laxatives have common mechanism of action in increasing water electrolyte secretion, decreasing its absorption in the colon. The presence of terpenoids, flavonoids, sterols, phenolic compounds can be responsible for the laxative activity of the plant. Although the Phytochemical screening revealed the presence of terpenoids, flavonoids like components. The laxative activity of *Trigonella foenum-graecum* seed aqueous *extract was* studied in rats. Oral administration of extract showed the significant and dose-dependent increase in fecal output of rats in regards to the accumulation of water in the intestine.

V. Conclusion

The plant *Trigonella foenum-graecum* has shown a higher percentage of total ash as well as alcohol- soluble extractive values. Qualitative Phytochemical screening of the plant extract *Trigonella foenum-graecum* reveals the presence of alkaloids, amino acids, carbohydrates, proteins and saponins. *Trigonella foenum-graecum* extract was prepared by Soxhlet extraction. Form the available literature it was found that *Trigonella foenum-graecum* contains more number of proteins, amino acids, vitamins, minerals, and flavonoids. The *Trigonella foenum-graecum* seed aqueous extract has shown better laxative activity indicating the additive property of the herbs. *Trigonella foenum-graecum* seed has a tremendous scope on further studies mainly as a Nutraceuticals, and dietary supplements; because it contains many amino acids, carbohydrates, fatty acids, vitamins and minerals, etc., therefore further research work to be carried out on this plant towards enhancing the medicinal claims. More research work is recommended on the plant for isolation and characterization of bioactive compounds that may be active against many diseases.

Acknowledgements

The authors are thankful and grateful to the Management of Seven Hills College of Pharmacy, Tirupati for providing us with the required facilities to carry out this research work.

Conflict of Interest

The authors do not have any conflict of interest.

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Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 19 Issue 2 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Aflatoxin Risk in Dairy Production: Assessment of Dairy Cattle Feed Contamination by *Aspergillus Flavus* and *A. Parasiticus* in both Conventional and Traditional Dairies

By G. K. Omeiza, J. Kabir, J. K. P. Kwaga, C. N. Kwanashie, M. Mwanza & L. Ngoma
University of Abuja

Abstract- Contamination of dairy cattle feeds by aflatoxigenic Aspergillus group, poses public health challenges as a result of high chances of aflatoxicosis. In this study, therefore, dairy cattle feed samples (n=144) collected from both conventional and traditional dairies were examined for the presence of aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* using microbiological and molecular techniques. Fungal Colonial counts (CFU) were determined, and the mean CFU/g of the feed samples was 3.8 ± 0.47. A Significant number of the feeds, 86 (59.7%) showed positive contamination level, out of which 55.8% and 18.6% (representing 33.3% and 8.3% of the total dairy cattle feed samples collected) were contaminations due to *Aspergillus flavus* and *Aspergillus parasiticus* respectively. All the 64 isolates of the *A. flavus* and *A. parasiticus* were examined for aflatoxin producing abilities under a long UV light (365 nm). Aflatoxin production levels were quantitatively determined using ELISA technique and 16 isolates representing 25.0% of the total isolates; in the ratio of 3:1 respectively, showed a varied level of production of aflatoxins.

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

Strictly as per the compliance and regulations of:

Aflatoxin Risk in Dairy Production: Assessment of Dairy Cattle Feed Contamination by *Aspergillus Flavus* and *A. Parasiticus* in both Conventional and Traditional Dairies

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Abstract- Contamination of dairy cattle feeds by aflatoxigenic Aspergillus group, poses public health challenges as a result of high chances of aflatoxicosis. In this study, therefore, dairy cattle feed samples (n=144) collected from both conventional and traditional dairies were examined for the presence of aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* using microbiological and molecular techniques. Fungal Colonial counts (CFU) were determined, and the mean CFU/g of the feed samples was 3.8 ± 0.47. A Significant number of the feeds, 86 (59.7%) showed positive contamination level, out of which 55.8% and 18.6% (representing 33.3% and 8.3% of the total dairy cattle feed samples collected) were contaminations due to *Aspergillus flavus* and *Aspergillus parasiticus* respectively. All the 64 isolates of the *A. flavus* and *A. parasiticus* were examined for aflatoxin producing abilities under a long UV light (365 nm). Aflatoxin production levels were quantitatively determined using ELISA technique and 16 isolates representing 25.0% of the total isolates; in the ratio of 3:1 respectively, showed a varied level of production of aflatoxins. Distribution of the aflatoxigenic strains was highest amongst the feeds collected from the traditional Fulani dairy herds showing a prevalence of 8 (50.0%) of the total identified aflatoxigenic strains and lowest, 2 (12.5%) among the conventional dairies. The observed effect of the *aflR* gene, suggests that it was capable of suppressing other structural genes such as O-methyltransferase *(omt),* vericocysteine *(ver)* and norsolorinic *(nor),* involved in the biosynthesis of aflatoxins. Both microbiological and molecular studies identified intermediary and potential aflatoxin-producing (IPAP) strains of the *Aspergillus flavus* and *Aspergillus parasiticus*. The occurrence of *Aspergillus flavus* and *Aspergillus parasiticus* in dairy cattle feeds was significantly higher ($p < 0.05$) compared with the low level of their corresponding aflatoxigenic strains. Traditional dairies were found to constitute public health risk about the presence of the significant number of the aflatoxigenic strains as feed contaminants.

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I. Introduction

Aflatoxins are toxic and carcinogenic metabolites produced by some strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius.* These species of fungi are commonly found in the environment, foodstuffs and animal feed, but its population increases during hot-humid weather (Hedayati *et al*., 2007). Aflatoxins (AF) are found in grains that have been produced under stressed conditions (Naidoo *et al*., 2002). *A. flavus* grows and develops faster under a relative humidity of 85%, a moisture content of the surface of about 30%, temperature of 25°C and a suitable substrate (Tvrtković, 2006). Thus, drought, heat, insect, nematode and fertilizer stress, all promote high levels of AF production. However, the mere presence of these fungi, may not depict toxigenicity as not all strains can produce aflatoxins, and this explains the need for ascertaining their aflatoxin producing abilities.

In Nigeria, Traditional Fulani Dairy Herds (TFHs) are responsible for about 80% of the total milk production (Ajala, 2004). These products are frequently purchased in significant quantities to boost the low level of production by the few available conventional dairy farms. Feeds from the traditional Fulani dairies may suffer heavy contamination by *Aspergillus flavus* due to poor husbandry practices. Such complementary practices between the conventional dairies and TFHs may pose serious public health risks particularly in situations where the status of the contaminating fungi is uncertain. The above critical issues have informed the basic needs to evaluate the aflatoxin-producing capabilities of the isolates of the *A. flavus* and *A. parasiticus* commonly associated with the dairy cattle feeds.

II. Materials and Methods

a) Feed sampling

Feed samples were obtained as fresh and preserved (stored) samples (where applicable) from 6 selected commercial and institutional farms on the one hand, and four traditional Fulani cooperative herds on

the other. Polytene bags and metal probes were purchased and sterilized for sample collections from troughs and stores respectively. In the case of stored samples, systematic random sampling technique was adopted. Assuming an imaginary diagonal line, bags of feed were randomly selected at intervals of three for probing at different points to pool an estimated representative sample of averagely 40g each. In the case of unpreserved feed, two feeding troughs containing the feed were examined among others in the milking parlor. Collected feed samples were pooled to make one representative feed sample per farm/herd. Sampling was carried out at weekly intervals until a total pooled feed samples reached 144. For the ethical reason, names of farms and cooperative herds used in this study were identified as Farm A (NP), Farm B (DC), Farm C (YS), Farm D (CG), Farm E (JM), Farm F (GG). Other farms comprising of Traditional Fulani dairy cooperatives (FH) were also identified as EM, JN, AL, and JE.

b) *Cultural isolation of Aspergillus flavus and Aspergillus parasiticus*

Feed sample preparation was carried out in line with the methods reported by Makun *et al*., (2010) and Udom *et al*., (2012). Forty gram of feed sample was collected from each farm and homogenized out of which 1g was taken and prepared as one fold dilution in a test tube using 9 ml sterile water. Using a sterile syringe, 1 ml of the feed suspension was dispensed on to a sterile Saboraud Dextrose Agar (SDA) medium. A sterile spreader was employed to gently and evenly spread the dispensed feed suspension. The preparation was incubated at an ambient temperature of 28°C in a relatively dark place for 3-5 days. Colonies which appeared greenish yellow with powdery texture having the reverse side pale to yellow were treated as suspects (Mycology-Critique, 2004). Suspected colonies of *Aspergillus* spp were counted and presented as Log10 CFU/gram of feed according to the method of Udom *et al*. (2012). Pure cultures of the colonies were obtained after repeated isolation and maintained as stock cultures in water culture technique and kept at ambient temperature according to the method reported by Larone (1995).

c) *Identification of the A. flavus and A. parasiticus using phenotypic techniques*

Identification of *Aspergillus flavus* was carried out according to the method reported by James and Natalie (2001) and Bandh *et al*. (2012) using microscopic and macroscopic morphologic techniques respectively. Primary macroscopic morphological studies were carried out on SDA while Czapek Dox Agar and Rose Bengal agar served as differential media. Aflatoxigenic potential of the *Aspergillus* spp under study utilized desiccated coconut impregnated neutral red agar, sometimes referred to as, *neutral red desiccated coconut agar (NRDCA)* as described by Atanda *et al*., (2011). Fluorescence characteristics of produced aflatoxin around each colony of *Aspergillus* were observed and categorized into very strong fluorescence, strong fluorescence, weak fluorescence and non-fluorescence (negative samples). Microscopic studies, on the other hand, were carried out using lactophenol staining as previously described (James and Natalie 2001; Ibrahim and Rahma, 2009).

d) *Quantitative determination of the associated aflatoxin B1 on NRDCA using HPLC*

Aflatoxin B1 Content: About 2 g of the positive NRDCA agar sample was extracted and homogenized simultaneously for 10 min in a homogenizer with 6 ml of methanol-water mixture (4+1). The mixture was spinned by centrifugation for ten minutes at 3500 round per minute. An aliquot of 100 µl of the supernatant was diluted with 700 µl of phosphate buffer, and the resultant solution used for the determination of AFB1.

AFB1 Clean-up procedure and determination

A 5ml aliquot of the extract was added to 14 ml of phosphate buffered saline (1 x PBS) solution (8.0g NaCl, 1.2 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2g KCl, dissolved in 990 ml purified water) and pH adjusted to 7.0 with HCl. The diluted filtrate (19 ml) which is equivalent to 1gram of the sample was passed through the Aflatest® IAC at a flow rate of 2 ml per minute to enable the aflatoxin captured by the antibodies present in the column. After that, the column was cleansed with 20ml of 1 x PBS at a flow rate of 5ml per minute to remove the unbound material, until air passed through the column. Eluate-containing aflatoxins, was eluted from the column with 1 ml of 100% methanol at a flow rate of 1 drop per second and 1 ml of water passed through the column and collected in the same vial to give a total of 2 ml. The eluate (AFs extract) collected in the amber vials, was evaporated to dryness with stream of nitrogen gas at 50°C and stored at +4˚C. The resulting dry extracts were subsequently dissolved in 500 µl of HPLC grade acetonitrile. The sample extracts were analyzed at a flow-rate of 1 ml per minute (min^{-1}) retention times. The analysis of the extract of aflatoxin involved the coupling to a detector a coring cell (CoBrA cell) (Dr. Weber Consulting, Germany) as an electrochemical cell for the derivatization of aflatoxins; using methanol/Acetonitrile/ Water (20/20/60, v/v/v) containing 119 mg of potassium bromide (KBr) and 350ul of nitric acid (4M HNO_3) as mobile phase.

e) *Polymerase Chain Reaction Methods Used to Detect the Strains of Aspergillus flavus*

Specific PCR was carried out to increase the sensitivity of gene identification. All the molecular methods used in this study were harmoniously carried out under the same conditions of PCR.

i. *Fungal DNA extraction*

The fungal genomic DNA was extracted using Fungal/Bacterial DNA extraction kit (Zymo Research Corporation, Southern California, USA) according to the manufacturer's instructions. The DNA of 5-day old cultures of the isolates grown on PDA plates was extracted. A sterile wire loop was employed to harvest the fungal mycelia, by scrapping the agar surface using sterile wire loop to obtain about 200 mg of mycelia.

ii. *Fungal identification to the strain level*

The internal transcribed spacer (*ITS*) region homologous to fungi was amplified by PCR using the primer set: FF2; 5'-GGT TCT ATT TTG TTG GTT TCT A-3' (forward) and FR1; 5'-CTC TCA ATC TGT CAA TCC TTA TT-3' (reverse) designed by Zhou *et al*. (2000) which encodes an amplicon size of 674bp. The identification of the genus Aspergillus was achieved through amplification of the intergenic spacer region (IGS) of the fungal DNA homologous to the genus *Aspergillus* using a primer set: *Asp*-F, 5'-CGGC CCTTAAATAGCCCGGTC-3'; *Asp*-R, 5'-ACCCCCCTGAGCCAGTCCG-3' encoding an amplicon size of 500 bp described by Willem *et al*. (1994). The IGS is located between V7 and V9 regions of the *18S rRNA* (White *et al*., 1990; Willem *et al*., 1994; Latha *et al*., 2008). Identification of *Aspergillus flavus* utilized specific primer (*Fla*-F., 5' -GT A GGG TTC CT A GCG AGCC-3'; *Fla*-R., 5'-GGA AAA AGA TTG ATT TGCG-3') encoding an amplicon size of 500bp, described by Gonzalez-Salgado *et al*. (2008) to identify certain flanking gene fragment *(Fla)* specific to *A. flavus,* located within the highly variable portion of the internally transcribed spacer regions, *ITS*.

iii. *PCR Reactions*

Individual PCR reactions contained 4µl of DNA (12-116 ng /µl) template which was mixed with 25 µl master mix (Taq DNA polymerase (Fermentas Life Science, Lithuania), dNTPs, $MgCl_2$ and reaction buffers), 1 µl of the primer i.e. Reverse (0.5 µl), Forward (0.5 µl) and 20 µl of nuclease-free water to make up a reaction volume of 50 µl. A negative control was also prepared to contain all the reagents except the DNA. The PCR experiment was carried out in eppendorf tubes placed in a C1000 Touch™ thermocycler (Bio-Rad, USA) with the following reaction conditions: initial denaturation temperature of 95°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute. Primer reannealing temperature was held at 58°C for 45 seconds and extension at 72°C for 1.5 minute .The PCR was finally extended for 10 minutes at 72°C and held at 4°C until samples were retrieved.

iv. *Molecular differentiation between A. flavus and A. parasiticus*

The IGS, *aflJ-aflR,* enclosing the aflatoxin biosynthetic gene was amplified using the primer sequence: IGSF,5′AAGGAATTCAGGAATTCTCAATTG3′; IGSR,5′GTCCACCGGCAAATCGCCGTGCG-3′ previously reported (Ehrlich *et al*., 2003, 2007) that correspond to a PCR product of 674 bp which discriminates between *A. flavus* and *A. parasiticus*. Restriction site analysis (PCR-RFLP) of the PCR products of the IGS was carried out to achieve this. The amplified PCR products were subjected to endonuclease restriction enzyme digestion using *Bg III* (Zymo Research Corporation, Southern California, USA) in a total reaction volume of 40 µl containing 15 units of enzyme, 4 µl of buffer, 15 µl of PCR product, and Ultrapure water up to 40 µl. The reaction mixture was incubated at 37°C for 3 h. Then the resulting fragments were separated by electrophoresis on a 2% w/v agarose gel for 1 h 45 min at 100 V.

v. *In vitro detection of genes that encode aflatoxin production*

In this study, the effects of 3 structural and 1 regulatory gene were studied to evaluate aflatoxin-producing capabilities among the isolated *Aspergillus flavus.* These include: Norsolorinic reductase *(nor),* O-methyl transferase *(omt),* Vesicolorin dehydrogenase *(ver)* and Aflatoxin regulated gene *(aflR).* The Primers used have been previously described (Geisen, 1996; Criseo *et al*., 2001; Gonzalez-Salgado *et al*., 2008; *Latha el al.,* 2008; Rashid et a1., 2008).

vi. *Gel electrophoresis of PCR products*

Agarose gel DNA electrophoresis was performed according to the method previously described (Saghai-Maroof *et al*., 1984). Briefly, the PCR product (8 µl) mixed with 6 µl of loading dye was pipetted slowly into each of the wells in the gel with a sterile micropipette. Care was taken not to cross-contaminate the wells. A 6 µl of the molecular marker also referred to as Gene Ruler (1-kilo base (kb) DNA ladder (Fermentas Life Science, Lithuania) was pipetted into the first and last wells. The chamber was closed and ran at 400 V, and 100 mA for 30 minutes and DNA fragments were viewed by removing the gel slab from the tray and placed on a UV transilluminator, the Geldoc™ MP imaging system (Bio-Rad Laboratories, California, USA).

f) Statistics

Data generated were subjected to Fisher's Exact Test using SPSS statistical software of version 20.0. Null hypotheses were analyzed and the statistical level of significance was fixed at p-value less than 0.05.

III. Results

a) The Occurrence of Aspergillus species in dairy cattle feed

Out of the 144 dairy cattle feed samples collected across different dairy farms and herds and tested, 86 (59.7%) yielded *Aspergillus* species. Of these 86 isolates of *Aspergillus* spp from dairy feeds, 48 (55.8%) and 16 (18.6.7%) representing 33.3% and 8.3%

were *Aspergillus flavus* and *A. parasiticus* respectively as shown in Table 1. Out of the 64 isolates of *A. flavus* and *A. parasiticus* isolated, 16 (25.0%) were aflatoxigenic in the ratio of 3: 1 respectively (Table 1). Other *Aspergillus* species isolated in this study were distributed in the following proportions: *Aspergillus fumigatus* (7.9%), *A. tamarii* (1.8%), *A. niger* (11.4%) and *A. vesicolor* (4.5%). The occurrences of these are presented in Table 2 as Log_{10} CFU (logarithmic value for colony forming unit) per gram of analyzed feed samples in accordance to WHO pattern of reporting as reported by Udom *et al.* (2012). A mean colonial count of (μ=3.8) Log_{10} CFU/g was determined. There was no statistically significant difference ($P>0.05$) in Aspergillus counts (Log_{10} CFU/g) between fresh and stored feed samples, even though, apparently higher CFU values were noticed among the stored feeds of all dairy feed types analyzed in this study. *Aspergillus flavus* was found predominant (4.5 CFU/g of feed) among the feeds fortified with concentrates than any other feed types (Table 2).

b) Contamination of dairy cattle feed by aflatoxigenic strains of A. flavus

The 64 isolates of *A. flavus* and *A. parasiticus* were examined for aflatoxin production under a long UV light (365 nm), and 16 (25.0%) comprising of 12 (18.75%) *A. flavus* and 4 (6.25%) *A. parasiticus* representing 8.3% and 2.8% respectively (Table 1), showed varying degrees of aflatoxin production (Table 3). The remaining 48 (75.0%) isolates of *A. flavus* and *A. parasiticus* were found to be non-aflatoxigenic. The toxigenic properties of the aflatoxin-producing isolates of *A. flavus* and *A. parasiticus* were also studied and categorized by their fluorescence strength as shown in Table 3. Feed samples collected from the traditional Fulani dairy herds and institutional farms with low commercial activities showed higher occurrences (50.0% and 31.3%) of the aflatoxigenic strains of *A. flavus* and *A. parasiticus* respectively (Table 1).

Table 1: The occurrence and distribution of aflatoxigenic *A. flavus* and *A. parasiticus* along the different dairy settlements

Type of dairy settlement	Level of commercial activity	No. of feed samples tested	No. of samples +ve for *Aspergillus* spp	*A. flavus* + *A. parasiticus* +ve samples	*A. flavus* +ve samples	Aflatoxigenic +ve samples for	
						A. Flavus	*A. parasiticus*
Institutional	High	15	5	4	3	1	0
	Low	45	23	18	13	4	1
Commercial	High	15	7	5	3	1	0
	Low	15	9	6	5	1	0
Traditional Fulani dairy cooperatives	Mixed commercial activities	54	42	31	24	5	3
Total	-	144	86 (59.7%)	64 (44.4%)	48 (33.3%)	[a]12 (8.3%)	[b]4 (2.8%)

Table 2: The occurrence of *Aspergillus* spp in fresh and stored feeds among the different feed types

Feed type	Storage cond. of feed	N	Log_{10} CFU	% *A. flavus*	% *A. parasiticus*	% *A. fumigatus*	% *A. niger*	% *A. vesicolor*	% *A. tamarii*
Feed + concentrates	Fresh	24	4.1	42.0	8.0	14.0	24.0	11.0	1.0
	Stored	24	4.5	55.8	18.6	7.9	11.4	4.5	1.8
Feeds of grain origin	Fresh	24	3.2	44.0	10.0	0.0	29.0	17.0	0.0
	Stored	24	3.8	42.0	13.0	12.0	18.0	9.0	6.0
Dry pasture only	Fresh	24	3.4	76.0	0.0	0.0	22.0	2.0	0.0
	Stored	24	3.8	76.0	0.0	0.0	12.0	4.0	8.0
Total mean	-	-	3.8	55.9	8.3	5.7	19.4	7.9	2.8

Table 3: The distribution of *A. flavus* and *A. parasiticus* isolates by fluorescence characteristics

Aspergillus spp	Total No. of isolates (%)	No. of non-aflatoxigenic isolates (%)	No. of aflatoxigenic isolates (%)	Fluorescence strength of the aflatoxigenic isolates			Total
				+	++	+++	
A. flavus	48 (55.8)	36 (41.9)	12 (13.9)	3	8	1	12
A. parasiticus	16 (18.6)	12 (13.9)	4 (4.6)	0	4	0	4
Other *Aspergillus* spp	22 (25.6)	22 (25.6)	0	0	0	0	0
Total	86(100.0)	70 (81.4)	16 (18.6)	3	12	1	16

Table 4: Quantitative determination of AFB1 amongst the fluorescence-positive isolates

S/No.	Isolate ID	Fluorescent strength of AF produced on NRDCA in 48 hrs	Quantitative Analysis of produced (AFB1) μgKg^{-1}	Mean concentration of AFB1 (μgKg^{-1}) produced by each fluorescence group
1	A	+++	22.45	22.45[a]
2	B	++	12.02	10.51[b]
3	C	++	14.97	
4	D	++	10.21	
5	E	++	13.10	
6	F	++	12.41	
7	G	++	12.01	
8	H	++	8.22	
9	I	++	11.80	
10	J	++	12.36	
11	K	++	10.24	
12	L	++	9.01	
13	M	++	11.81	
14	N	+	3.42	3.41[c]
15	O	+	4.61	
16	P	+	2.19	

A significant difference ($P<0.05$) exists between a, b and c

c) *PCR-based identification of fungal isolates from dairy cattle feeds*

All the suspected fungi based on the conventional microbiological methods represented by letters A-Z, yielded the expected IGS amplicon sizes of 674 bp as shown in Plate I. However, the negative control samples, the *E. coli* standard organisms EC1 and EC2 showed no amplicons (Plate 1).

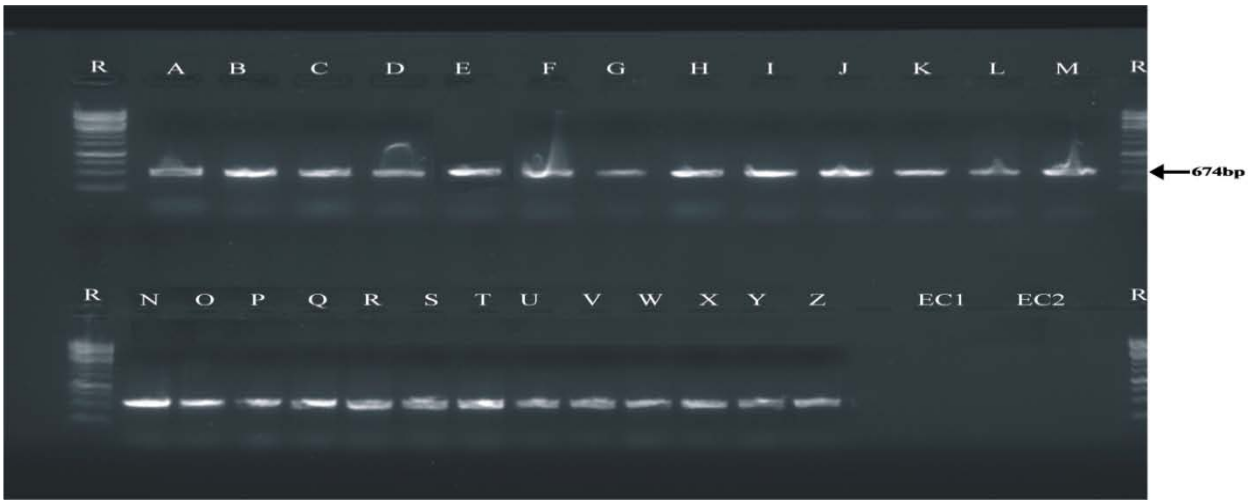

Plate I: Agarose gel electrophoresis of PCR product of IGS homologous to Fungi. Lane R (Pharmacia 1000 bp ladder), Lanes: A ('very strong' aflatoxigenic strain), B to M ('strong' aflatoxigenic strains), N to P (weak aflatoxigenic strains), Q to Z (atoxigenic strains) and EC1 and EC2 (strains of *E. coli* for –ve controls).

d) *PCR-based identification of Aspergillus spp among the identified fungal isolates*

The result of the primer set, *Asp1,* and *Asp2,* designed from V7 and V9 regions of *18S rRNA* confirmed and identified all the 86 isolates as *Aspergillus* species with the expected amplicon size of 500 bp as presented in Plate II. All the 16 UV-detected aflatoxigenic strains of Aspergillus yielded the expected amplicons (Plate II: A, B to M and N-P). Both *Fusarium* sp and *Rhizopus* sp used as standard and negative control organisms showed no amplicons (Plate II).

Plate II: Agarose gel electrophoresis of PCR product of *18S rRNA* gene. Lane R (Pharmacia 1000 bp ladder), Lanes: A-P (represent *Aspergillus* spp) and Rz and Fs (represent standard *Rhizopus* sp and *Fusarium* sp respectively as –ve controls)

e) *PCR-based Detection and Identification of Aspergillus flavus strains that encode the specific gene, Fla*

The PCR-based detection and identification clearly showed a distinction between *A. flavus* and *A. parasiticus*. All the *A. flavus* yielded the expected amplicon size of 500 bp (Plate III). Among the 16 aflatoxigenic strains of *Aspergillus* section *Flavi* detected in this study, 4(25%) were negative for *A. flavus* specific amplicons as shown in Plate III. Partial sequencing of the amplified *IGS* regions successfully identified common strains of *A. flavus* in dairy feeds. The most common strain identified was *A. flavus* EGY1. Other less common strains identified include: *A. flavus* ITD-G11, *A. flavus* MJ49, A. *flavus* HKF30, *A. flavus* HKF13, *A. flavus* HKF49 and *A. flavus* 1985.

Plate III: Agarose gel electrophoresis of *fla* gene amplicon. Lane R (molecular size markers (Pharmacia 3000 bp ladder) Lanes E, F and K, L represent *A. parasiticus;* while other lanes represent *A. flavus*

f) *Restriction fragment analysis (PCR-RFLP) of A. flavus and A. parasiticus*

Further to the above molecular based differentiation between *A. flavus* and *A. parasiticus,* an enzyme based restriction analysis was carried out to differentiate the two species. Findings from agarose electrophoresis showed that in *A. flavus* strains, the PCR product was digested into three fragments of amplicon sizes 102bp, 210bp and 362bp (Plate IV). *A. parasiticus* showed one restriction site with two fragments of amplicon sizes 311bp and 362bp (Plate IV).

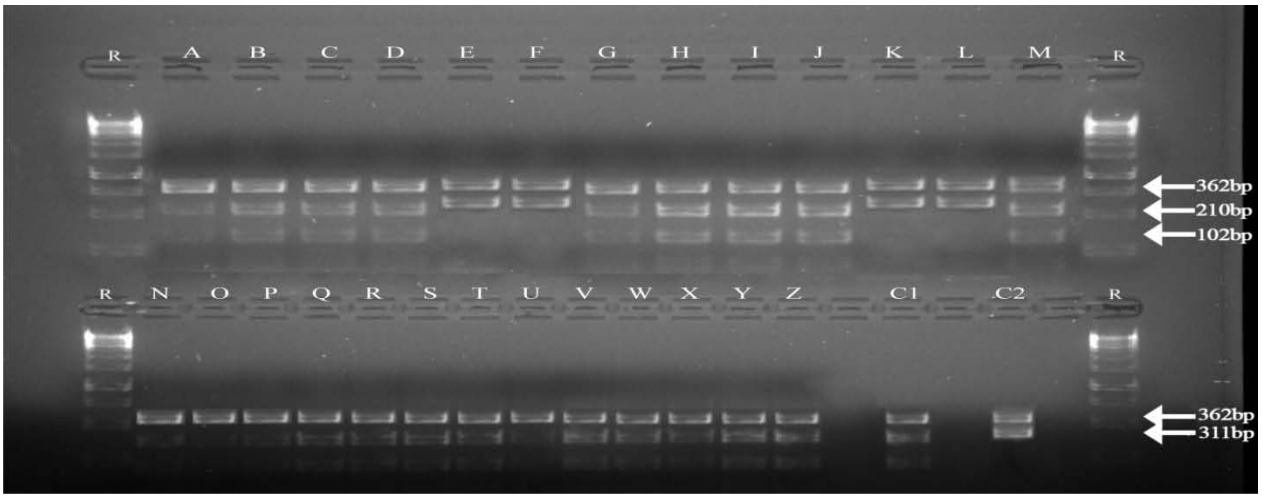

Plate IV: Agarose gel electrophoresis of *Bg III* digested PCR products of the *ITS* region of *A. flavus*. Lane R (Pharmacia 1000 bp ladder), Lanes E, F and K, L represent *A. parasiticus* while other lanes represent *A. flavus*. C1 and C2 (represent standard organisms of *A. flavus* and *A. parasiticus* used as positive controls)

g) Genes that encode aflatoxin biosynthesis

From the *in-vitro* study on the toxigenicity profile carried out to examine genes that encode aflatoxin biosynthesis, one particular isolate (A) presented a pronounced fluorescence. This isolate was earlier found to display a mono amplicon pattern for *O-methyl transferase* gene instead of the usual quadruplet amplicons (Plate VI), some other 12 strains (lanes B-M) showed strong fluorescence characteristics and three others (lanes N-P) showed weak fluorescence characteristics as summarily presented in Tables 3, 4. The non-aflatoxigenic strains had different amplicon patterns with few showing incomplete quadruplet amplicons for *aflR, omt, ver and nor* genes as presented accordingly in Plates V, VI, VII and VIII.

h) Determined level of AFB1 during the active growth on NRDCA

Concentrations of AFB1 produced during the active growth phase of the aflatoxigenic strains of *A. flavus,* and *A. parasiticus* as determined by the HPLC method are shown in Table 4. The analysis by the HPLC method confirmed the various degrees of fluorescence produced under a long wavelength UV. Mean concentrations of the different fluorescence groups showed a significant difference ($p < 0.05$) between them (Table 4).

IV. Discussion

Isolation and proper identification of aflatoxigenic strains of *A. flavus* require characterization and differentiation between aflatoxigenic and non-aflatoxigenic strains of the organism. Such differentiation was achieved in this study by demonstrating a link between produced aflatoxin on coconut-based agar medium and the presence of the corresponding aflatoxin biosynthetic genes. The strain differentiation of the organism in the present study is partly in agreement with the work of Latha *et al*. (2008) who differentiated aflatoxin-producing strains of *A. flavus* from the non-producing strains using *Aspergillus* Differential Media (ADM). Part of the current finding, however adopts the use of a locally prepared coconut agar medium in determining aflatoxin production under a long wavelength UV light (365 nm) in line with the report of Atanda *et al*. (2011).

The occurrence of *A. flavus* was observed to be higher in dairy cattle feeds fortified with concentrates than in any other feed types. This finding may be of relevance in the management of feed composition and preservation. Improved feeding is recommended for increased and quality yield; this depends on fortification of feeds with improved concentrates. Accensi *et al*. (2004) in a separate study also showed that *A. flavus* was the predominant species amongst the *Aspergillus* section *Flavi* isolated from mixed feeds. It, therefore, implies that a quality source of feed coupled with adequate preservation are fundamental requirements for achieving safety in animal production particularly dairy. The mean total count of *Aspergillus* species in this study was however found to be below the maximum limit recommended (5.0 $\log_{10}$ CFU/g of feed) for poor feed quality (Udom *et al*., 2012). Thus, comparing the current findings of 4.5 $\log_{10}$ CFU/g of feed with the previous works of 4.1 $\log_{10}$ CFU/g of feeds (Accensi *et al*., 2004; Udom *et al*, 2012), parts of which were conducted in Nigeria, may arouse safety questions about the quality of feeds fed to dairy cattle if contamination is unchecked. Such increasing trend in the level of fungal contamination as depicted by the rising Aspergillus colonial counts, portends serious public health concern.

The relatively low occurrence of aflatoxigenic strains of *Aspergillus* section *Flavi* (>11%) amidst high incidence rates of unclassified *Aspergillus* section *Flavi* (>59.0%) in the current study, may be tentatively

considered as non-significant, but the instability and the genetic diversity shrouding the biosynthesis of aflatoxins may raise safety concerns. Udom *et al*. (2012) had, in one of his reports, articulated a correlation between the lower incidence of aflatoxigenic strains of *Aspergillus flavus* and the unpredictable high levels of AFB1.

About 50.0% of the total aflatoxigenic strains identified in this study was from the traditional Fulani dairy herds. It, therefore, implies public health risk, especially, in situation where many big institutional and commercial dairy farms depend on these traditional dairy herds for their production boosts. Such interrelationship between the conventional and the traditional dairy herds emphasizes the importance of the traditional dairies, thereby signifying the urgency of promulgating an act or strengthening the existing ones, if any, for effective management of both traditional and conventional dairy industries.

In the current study, the concurrent application of conventional microbiological and molecular methods identified a potential aflatoxin-producing (PAP) strains. The identification of the PAP strains is considered an important public health issue. Some genes have been found to play complementary roles in aflatoxin biosynthesis. In this study, *A. flavus* isolates were examined for the presence of the full amplicons of the quadruplet genes. The genes consist of 3 structural genes, *omt, ver,* and *nor* and one aflatoxin regulatory gene, *aflR,* involved in the biosynthesis of the aflatoxins, which also suggest full aflatoxigenic potential. Findings in this study showed that all the 16 isolates of the *A. flavus* and *A. parasiticus* identified as aflatoxin-producers by the stream of UV light, yielded a complete quadruplet amplicons. This PCR-based method of identification of the genes that encode aflatoxin production further stressed the capability and reliance of the microbiological method used for the preliminary aflatoxigenicity screening. Also, a quadruplet amplicon pattern was found amongst some of the observed non-aflatoxin producing strains detected during UV examination. This finding further elucidates the intrinsic and inherent aflatoxin-producing potential in the seeming non-producer of aflatoxins, should the conventional microbiological methods were used alone. The finding has also improved on the previous understanding that quadruplet amplicons are mostly associated with potential aflatoxin-producing strains. Genetic modification could occur in some of the strains during their growth phase on culture media. This genetic diversity may modify or even hamper the biosynthesis of aflatoxins among the potential aflatoxin-producing strains. This explanation is in agreement with the work of Abarca *et al*. (1988) which reported that certain instability of aflatoxin production may occur in aflatoxigenic strains growing on culture media. Such instability might be as a result of simple genomic drift as seen in the case of substitution of bases, leading to the formation of non-functional products (Criseo *et al*., 2001; Latha *et al*., 2008).

Findings in the present study also showed that all the aflatoxigenic and non-aflatoxigenic strains of *Aspergillus flavus* group examined possessed aflatoxin regulatory gene, except one isolate in the aflatoxigenic group. The isolate showed a distinct amplicon band for only *omt* gene. This strain, at the level of UV identification, displayed a very strong fluorescence (perhaps the strongest) characteristic under a long wavelength UV light (365 nm) indicating an apparently high level of aflatoxin production. Further confirmation showed that the amount of aflatoxin B1 produced by the strain was quite higher when compared with the other strains. One of the possible explanations is the omission of a particular gene, the aflatoxin regulatory gene, *aflR,* which may explain the strongest fluorescence characteristic associated with the strain, since *aflR* regulates the activities of the other structural genes. This finding may suggest the possible roles of gene alleles or alternative genes, not detected by the PCR method used. In previous studies, the *aflR* gene was shown to play a role in aflatoxin biosynthesis pathway by regulating the activities of the structural genes such as *omt, ver* and *nor* (Chang *et al*., 1992; Liu and Chu, 1998). Criseo *et al*. (2001) and Latha *et al*. (2008) had demonstrated the significant interdependent roles played between *omt* (a structural gene) and *aflR* (a regulatory gene) in biosynthesis of aflatoxins. Liu and Chu (1998) also demonstrated, through a hybridization technique that, *AFLR*, a product of *aflR* gene, regulates the expression of *omt*, a structural gene in the aflatoxin biosynthetic pathway.

Over expression of the structural gene, *omt,* may be caused by a state of relative inexpression of *AFLR.* This fact may be responsible for the 'very strong' fluorescence seen during the conventional examination of one of the aflatoxigenic isolates. Aflatoxin production is controlled by a mechanism of regulation of structural gene transcription in which the *aflR* plays a role. Liu and Chu (1998) also demonstrated the interdependent role of *AFLR,* a product of *aflR* and *Omt* genes in the final steps of aflatoxin biosynthesis. This may explain the inexpression of one or other structural genes amongst the non-aflatoxigenic strains isolated in the current study.

The instability associated with the aflatoxigenic *A. flavus* and *A. parasiticus* so far established in the preceding findings, has raised safety concerns about the use of non-producers of aflatoxins in the biological control of mycotoxin contamination of crops. The works of Dorner *et al*. (1999), Dorner and Cole (2002) and Abass *et al*. (2006) had advocated for the use of non-toxigenic strains of *Aspergillus flavus* for biological control of mycotoxin contamination of crops. The observed instability in the capacity of aflatoxin production among the different strains of *Aspergillus*

section *Flavi* may be dependent on both climatic and environmental conditions. This aspect of the discussion may further explain the previous reports (Criseo *et al*., 2001; Latha *et al*., 2008).

The study identified the genus *Aspergillus* to species level. Two different species of *Aspergillus, A. flavus* and *A. parasiticus* were identified with *A. flavus* predominantly found to contaminate feed substances. This finding agrees with that of White *et al*. (1990), Gonzalez-Salgado (2008) and Latha *et al*. (2008) which demonstrated the use of primers in targeting DNA regions, *18S rRNA* and *Fla,* to identify the genus *Aspergillus* and *A. flavus* with the expected amplicon size of 674bp. A restriction fragment analysis of the PCR product of *aflR-aflJ* intergenic region using restriction endonuclease; *Bg III,* further affirmed the specificity of *Fla* gene in differentiating *A. flavus* from *A. parasiticus.* The 2 restriction sites at which the PCR product was cleaved into three and two fragments of 362, 210 and 102 bp and 362 and 311 bp confirmed *A. flavus* and *A. parasiticus* respectively which are in agreement with the findings of Somashekar *et al*. (2004).

This strain diversity found in the study agrees with the reports of early workers (Dorner *et al*., 1999; Abass *et al*., 2006) who also implicated many diverse strains of *A. flavus* in aflatoxin production. Successful aflatoxin control programs in any country may need a perfect understanding of strain specifics as regards aflatoxin production. The diverse toxigenic strains identified from cattle feed in this study may be a reflection of poor management of international trade amongst the neighboring African nations, thereby posing a wide range of public health risks to the communities concerned. Sequenced intergenic spacer region failed to differentiate between the strains of *A. flavus* and *A. parasiticus.* The close genomic similarity that exists between the two species may be responsible for this. Expanded whole genomic sequencing may, therefore be a better option towards resolving this complexity.

It was concluded that the occurrence of-*Aspergillus flavus* in dairy cattle feeds was high; the aflatoxigenic strains of *A. flavus* was however relatively low. Traditional dairies constitute the major public health risks as majority of the identified aflatoxigenic strains were associated with them. A potential aflatoxin-producing (PAP) strains and the role of aflatoxin regulatory gene on aflatoxin biosynthesis were established in the current study. This aspect of the work may be explored to biotechnologically raise strains of *A. flavus* with a modulated *aflR* function for biological control of aflatoxin biosynthesis in feed marketed for animal production in Nigeria and the world over.

Acknowledgments

The authors appreciate the technical assistance of the laboratory staff of Animal Health Department, North West University, South Africa and the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.

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Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 19 Issue 2 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Prevalence and Risk Factors of Bovine Trypanosomiasis in Khartoum State, Sudan, April-July 2012

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Abstract- A cross-sectional study was conducted in the dry season from April to July 2012 in Khartoum State, Sudan to: estimate the prevalence of bovine trypanosomiasis (BT), identify the prevailing species of trypanosomes and investigate the associated potential risk factors of the disease. Blood samples were collected from 271 randomly selected cattle of the eight study areas and evaluated through standard parasitological methods (Haematocrit Centrifugation Technique (HCT) and thin blood smear). Also, the packed cell volume (PCV) of each animal was measured using Haematocrit Techniques for anemia estimation. The results indicated that the overall prevalence of BT was 4.8% (13/271). All the trypanosomes encountered in cattle belonged to a single species of Trypanosoma vivax. For investigation of associated risk factors of the disease, a univariate analysis using the Chi-square (χ^2) test identified 15 risk factors statistically significantly associated with BT ($p \leq 0.25$). These 15 risk factors were entered to the multivariate analysis using logistic regression for further analysis. The significant level of association in the multivariate analysis was set at ($p \leq 0.05$).

Keywords: *prevalence, risk factors, cattle, trypanosome- vivax, Khartoum state, sudan.*

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

Strictly as per the compliance and regulations of:

Prevalence and Risk Factors of Bovine Trypanosomiasis in Khartoum State, Sudan, April-July 2012

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Keywords: prevalence, risk factors, cattle, trypanosome-vivax, Khartoum state, sudan.

I. Introduction

African animal trypanosomiasis is indisputably a great problem on the African scene, but the perspective alters when it is examined against a global background, even when taking into account mechanically and venereally transmitted African trypanosomes which have spread to other continents (*Trypanosoma evansi* causing surra, mechanically transmitted *T. vivax* and *T. equiperdum* the causal agent of the venereal disease dourine) (Uilenberg 1998). Trypanosomiasis is a parasitic disease caused by species of flagellated protozoa belonging to the genus *Trypanosoma* which inhabit the blood plasma, various body tissues and fluids of vertebrate host. The disease is transmitted cyclically by tsetse flies (*Glossina* species), and non-cyclically by other biting flies (Tadesse et al. 2011). Trypanosomiasis induces loss of body condition in pregnant animals leading to birth of offspring with low birth weights, fetal and neonatal losses, besides production losses in lactating animals. The consequences of trypanosomiasis are less severe in better-nourished animals but good nutrition does not by itself provide protection. Adequate energy, protein and vitamin nutrition enhances the ability of trypanosome-infected animals to withstand the adverse effects of infection (Pathak 2009). *T. vivax* is enzootic in wide areas of Sudan more than 2,000 km away from the known tsetse belt of the country. Previous outbreaks of bovine trypanosomiasis (BT) reported to be due to *T. congolense* were reported as far north as Kosti area along the White Nile, more than 1,000 km north of the Tsetse belts. The transmission of the disease is due to the enormous populations of *Tabanidae* and biting flies (Rahman 2005). *Trypanosoma* infection restricts the animal production and causes economic losses by the clinical signs of the infection such as restricted growth, abortion, anemia, treatment cost, and death of the affected animals. Hence, the objectives of this study were to estimate the prevalence of BT in Khartoum State, to identify the prevailing species of trypanosomes and to investigate the potential risk factors associated with the disease.

II. Materials and Methods

a) Study area

Khartoum is the political capital of the Sudan. The state lies between longitudes 31.5°-34° east and latitude 15°-16° north in an area about 28.165 km^2. Geographically, Khartoum is divided into three

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governorates (first governorate Khartoum (Jabal Awlia and Khartoum localities), second governorate Khartoum North (East Nile and Baharri localities) and third governorate Omdurman (Omdurman, Ombeda and Karari localities) (Khartoum state website (2012). www.krt.gov.sd). Diurnal and annual temperature variations are very wide. The highest mean maxima are between March and May, ranging from 35° to 43 °C and the lowest mean minima is between December and February ranging from 15° to 28 °C.

The population of animals in Khartoum state is 6300000 for birds, 513000 for sheep, 19000 of goats, 6585 camels and 240003 cattle. The number of cattle distributed in Khartoum state is 138067 in East Nile, 28016 in Bahri, 13578 in Ombeda, 13901 in Karari, 20455 in Omdurman, 20360 in Jabal Awlia and 5626 in Khartoum localities (Khartoum state census, Khartoum, Ministry of agriculture and animals' resources and irrigation, Khartoum state, Sudan, 2008).

b) Study design

A cross sectional survey was conducted from April to July 2012 to estimate the prevalence of BT, to identify the prevailing species of trypanosomes and to investigate potential risk factors associated with the disease.

c) Sampling Method

A multistage random sampling method was followed to select the study animals based on state, governorates (Khartoum, Khartoum North, and Omdurman), localities (Jabal Awlia, East Nile, Omdurman and Ombeda), and areas (Ombeda, Felstine, Almoilih, Aldekhinat, Edbabekir, Alozozab, Alkeriab and Suba). Finally, farms and animals were conveniently selected.

i. *Sample size determination*

The sample size was determined using the following formula (Thrusfield 2005):

$$n = (1.96)^2 \times P^{\wedge} \times Q^{\wedge} / L^2$$

Where:
n = the sample size.
$(1.96)^2$= constant.
P^ = expected prevalence of BT, which was 4.4% (Abdalla et al. 2008).
Q^ = 1- P^
L^2= allowable error (5%).
n= $(1.96)^2 \times 0.044 \times 0.956 / 0.0025$= 64 samples.

To increase the precision of the study; the sample size was multiplied by 4 (Thrusfield 2005), so the number of samples became 256 samples. Finally, the investigators were able to collect samples from 271 animals.

ii. *Samples collection and laboratory tests*

Blood samples were collected from each animal by puncture of the jugular vein using a sterile needle (Abenga et al. 2004, Adam et al. 2011, Mogona et al. 2011, Batista et al. 2012). Blood was obtained in dry clean sterile heparinized tube 5 milliliters (containing EDTA (ethylene diamine tetra acetic acid, as anticoagulant) and was put in cold box with ice and transported to the laboratory (Central Veterinary Research Institute Laboratories, Soba, Khartoum) as soon as possible for diagnosis.

iii. *Diagnostic techniques*

For diagnosis, parasitological methods (Haematocrit Centrifugation Technique (HCT) and thin blood smear stained with Giemsa stain) was used to identify the parasite, also packed cell volume (PCV) of each animal was measured for anemia estimation.

iv. *Investigation of potential risk factors*

A pre-tested questionnaire was completed in an interview with the farms owners and/or workers to collect information data about selected potential risk factors on the occurrence of BT.

d) Statistical analysis

The overall prevalence was calculated based on positive results divided by the total number of animals tested. Potential risk factors (independent variables) and the laboratory test outcome (dependent variable) were analyzed using SPSS (version 16.0). The Chi-square test (χ^2) was used for univariate analysis; the significance level was set at $p \leq 0.25$. In order to control for confounding, risk factors found significant at $p \leq 0.25$ were entered to the multivariate analysis using logistic regression, the significance level of association was set at $p \leq 0.05$. The strength of association was measured by the odds ratio (OR) accompanied by the 95% confidence interval (CI) for OR.

III. Results

a) Prevalence and trypanosome species

Out of the total 271 animals tested 13 were positive. The overall prevalence of BT in Khartoum state, Sudan was 4.8% (13/271).The prevalent trypanosome species in the study was *Trypanosoma vivax*.

b) Univariate analysis

The Chi-square test (χ^2) showed that there were 15 out of 33 potential risk factors statistically significantly associated with BT (P-value ≤ 0.25). These were: locality (p = 0.084), area (p = 0.000), sex (p = 0.219), body condition (p = 0.009), herd size (p = 0.094), treatment of sick animal (p = 0.003), presence of other species of animals in the farm (p = 0.000), farm hygiene (p = 0.148), veterinary care (p = 0.000), surgical operation or wound in the animal's body (p = 0.108), location of livestock market (p = 0.002), presence of insects in the farm (p = 0.108), species of insect found in the farm (p = 0.106), presence of ticks in the farm (p = 0.099) and farmer awareness about the disease (p = 0.065) (Table1).

c) *Multivariate analysis*

The multivariate analysis using the logistic regression showed that there were four potential risk factors statistically significantly associated with BT ($p \leq 0.05$). These were: treatment of sick animals ($p = 0.012$), presence of other animal species in the farm ($p = 0.003$), veterinary care ($p = 0.023$) and location of livestock market ($p = 0.004$).

IV. Discussion

Trypanosomiasis is a major constraint that affects the health and productivity of livestock. The major consequences of infection include mortality, loss of body condition and abortion (Delafosse et al. 2006). Among all species of animals, the diseasehas been regarded as one of the most serious animal disease problem in Sudan. *Trypanosoma vivax* occurs in most parts of the country (Rahman 2005).

In our study, the prevalence and risk factors of BT in Khartoum state, Sudan was investigated. *T. vivax* was the only trypanosome species found during the study period. This is in agreement with previous studies (Tadesse et al. 2011, Rahman 2005, Abdalla et al. 2008, Batista et al. 2012, Delafosse et al. 2006) which reported that *T. vivax* constituted the majority of infection in the tsetse free zone.

The prevalence in our study was 4.8%. This result is not different from another study carried out in the Blue Nile area between Addamazin and Khartoum where the prevalence was 1% in the dry season and 6% in the rainy season (Rahman 2005), and in Sinnar, Sudan the prevalence was 4.4% (Abdalla et al. 2008). Another study carried out in Lake Chad showed a prevalence of 1.6% (Delafosse et al. 2006). Also, in another study in South Western Ethiopia the prevalence was 4.4% (Tadesse and Tsegaye 2010). However, the prevalence in our study was very low compared with other studies in Sudan and different countries which was 50.3% in Sinnar, Sudan where there is no tsetse fly in the area (Abdalla et al. 2005), 43% in Blue Nile State, Sudan (Salim et al. 2011), and 70% in Western Kenya (Thumbi et al. 2010).The difference in BT prevalence between our study and different studies may be due to different factors associated with the disease like: Khartoum state is out of the tsetse belt and the disease is transmitted mechanically, environmental factors which affect the vector, control strategy applied in the areas, variation on climatic conditions and dry season which affects the vectors breeding and low parasitaemia.

Distribution of the prevalence of BT in the localities showed significant association ($p = 0.084$). The higher prevalence in Ombeda might be attributed to the proximity of the area to livestock market.

A significant association between the disease and areas was observed ($p = 0.000$). This association could be due to the presence of irrigation canals which extended in the area as well as good vegetation making a suitable environment for vector breeding.

This study showed a prevalence of 5.3% in females. There was a significant association in the univariate analysis between BT and sex ($p = 0.219$). This result is in agreement with a previous study (Mogona et al. 2011) which reported that a female is at risk two times higher than a male. Females have a higher prevalence than males, because females remain longer for production purposes, and also due to physiological stress like estrous cycle, pregnancy and lactation, so the disease has more chance to develop.

The significant association of body condition with the disease in this study ($p = 0.009$) agrees with other studies (Tadesse and Tsegaye 2010, Begna et al. 2011, Bitew et al. 2011, Mulaw et al. 2011) which found that the prevalence in poor body condition animals was significantly higher than good body condition animals ($p \leq 0.05$). This could be due to the chronic nature of the disease that resulted in anemia, poor body condition and emaciation.

There was a significant association between the disease and small herd size ($p = 0.094$). This could be attributed to the fact that most of < 30 groups, in this study, were located in areas with high density of insects, thus more fly attacks to the herd of small size than large size one.

The current study investigated the use of drugs for treatment of animals. There was significant association between the disease and treatment of sick animals ($p = 0.003$). The association of treatment of animals with the disease was also significant in the multivariate analysis ($p = 0.012$). These results agree with a previous study (Kidanemariam et al. 2002) which reported that chemotherapy and chemoprophylaxis is important against trypanosomiasis. Logically, treatment of sick animals restricts spread of the disease.

There was significant association between BT and the presence of other species of animals in the farm ($p = 0.000$). This risk factor was also significant in the multivariate analysis ($p = 0.003$). This result agrees with Mustafa (2004) who reported that sheep can act as a potential reservoir in mixed herd. Thus, presence of other species of animals in the farm may act as a source of the disease.

Also, the study investigated the farm hygiene as a risk factor possibly associated with the disease and there was a significant association between the disease and farm hygiene ($p = 0.148$). This could be attributed to the fact that poor farm hygiene provides a suitable environment for breeding of insects.

For veterinary care the study showed a significant association between BT and the availability of veterinary care in the univariate analysis ($p = 0.000$). Also, veterinary care had a significant association with BT ($p = 0.000$) in the multivariate analysis with a

protective effect. Logically, veterinary care plays a role in restricting the disease by intervention if any clinical sings appear.

Surgical operation and wounds in the animal's body were investigated and a significant association between BT and surgical operation or wounds in the animal's body was observed (p = 0.108). That is logical because surgical operation or wounds in the animal's body invite insects to have their blood meal.

There was a significant association between the disease and location of livestock market (p = 0.002). Also, location of livestock market had a significant association with the disease (p = 0.004) in the multivariate analysis. These findings are consistent with a previous study (Abdelkarim 1991) which reported that Trypanosoma infected animals in the livestock market can act as a source of infection to the neighboring farms. Logically, if there is one animal with the disease in the market all the area around may be at risk when the vector is present.

Also, the study investigated presence of insects in the farms. There was a significant association between the disease and presence of insects in the farm (p = 0.108). Furthermore, another related risk factor investigated was insects' species found in the farm. There was a significant association between the disease and insects' species found in the farm (p = 0.106). This result agrees with a previous study (Rahman 2005). These insect species may act as a mechanical vector for the disease.

Furthermore, presence of ticks in the farm was investigated in our study. There was a significant association between the disease and presence of ticks in the farm (p = 0.099). Ticks may predispose animals to infection with BT by infection with other tick-borne diseases which reduce the animal immunity.

Farmer awareness about the disease was investigated as a risk factor possibly associated with the disease. There was a significant association between the disease and farmer awareness (p = 0.065). Logically, aware farmer could apply control methods to control the disease and vectors.

In this study, the univariate analysis showed that 15 risk factors were statistically significantly associated with BT ($p \leq 0.25$). However, in the multivariate analysis, there were only four risk factors that were found statistically significantly associated with BT ($p \leq 0.05$), a result which indicates a presence of confounding between these risk factors.

V. Conclusion

The current study confirmed that BT is widely distributed in Khartoum state farms with an overall prevalence of 4.8%. Furthermore, the study indicated that *T. vivax* is the predominant species implicated in BT in Khartoum state.

The presence of various biting flies and the absence of tsetse flies in this investigation indicated that BT in Khartoum state is caused by mechanical transmission mediated by these biting flies.

The results of the multivariate analysis showed that four risk factors had statistically significant associations with BT ($p \leq 0.05$). These risk factors were: treatment of sick animals (p = 0.012), presence of other species of animals in the farm (p = 0.003), veterinary care (p = 0.023) and location of livestock market (p = 0.004). These risk factors should be considered as predictors for the disease and should be taken into account when planning for control programs of the disease.

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Table 1: Univariate analysis of association between bovine trypanosomiasis and potential risk factors in 271 cattle in Khartoum state, Sudan, using the Chi-square (χ^2) test

Risk factors	No. tested	No. +ve (%)	d. f.	χ^2	p-value
1. Locality					
Ombeda	22	3 (13.6)			
Omdurman	22	0	3	6.648	0.084
JabalAwlia	34	0			
East Nile	193	10 (5.2)			
2. Area					
Felstine	22	3 (13.6)			
Almoilh	22	0			
Aldkhinate	34	0			
Alozozab	42	1 (2.4)	7	27.293	0.000
Eidbabeker	67	1 (1.5)			
Sudan university farm	8	0			
Alkeriab	45	8 (17.8)			
Soba	31	0			
3. Sex					
Male	27	0	1	1.511	0.219
Female	244	13 (5.3)			
4. Body condition					
Poor	119	11 (9.2)			
Good	122	2 (1.6)	2	9.327	0.009
Very good	30	0			
5. Herd size (# of animals)					
<30	195	12(6.2)	1	2.803	0.094
≥30	76	1(1.3)			
6. Treatment of sick animals					
Not treated	72	8 (11.1)	1	8.559	0.003
Treated	199	5 (2.5)			

7. Presence of other animal species in the farm			1	26.319	0.000
Present	67	11 (16.4)			
Not present	204	2 (1.0)			
8. Farm hygiene			1	2.095	0.148
Good	115	3 (2.6)			
Poor	156	10 (6.4)			
9. Veterinary care			1	12.550	0.000
Not available	121	12 (9.9)			
Available	150	1 (0.7)			
10. Surgical operation or wound in animal's body			1	2.578	0.108
Yes	5	1 (20.0)			
No	266	12 (4.5)			
11. Location of livestock market			1	9.944	0.002
Close to the farm	67	8 (11.9)			
Away from the farm	204	5 (2.5)			
12. Presence of insects in the farm			1	2.578	0.108
Present	5	1 (20.0)			
Not present	266	12 (4.5)			
13. Insects species found in the farm			3	6.122	0.106
Tabanus	136	9 (6.6)			
Tabanus+ *Stomoxys*	16	1 (6.2)			
Stomoxys	17	2 (11.8)			
Other species of insects	102	1 (1.0)			
14. Presence of ticks in the farm			1	2.79	0.099
Not present	45	0			
Present	226	13 (5.8)			
15. Farmer awareness about the disease			1	3.398	0.065
Not aware	217	13 (6.0)			
Aware	54	0			

Table 2: Multivariate analysis of association between bovine trypanosomiasis and potential risk factors in 271 cattle in Khartoum state, Sudan, using logistic regression

Risk factors	No. tested	No. +ve (%)	OR	95% CI for OR	p-value
1. Treatment of sick animals			ref		
Treated	199	5 (2.5)	5.536	1.4-21.06	0.012
Not treated	72	8 (11.1)			
2. Presence of other animal species in the farm			ref		
Not present	204	2 (1.0)	.076	0.014-0.418	0.003
Present	67	11(16.4)			
3. Veterinary care			ref		
Available	150	1 (0.7)	.092	0.012 -0.724	0.023
Not available	121	12 (9.9)			
4. Location of livestock market			ref		
Away from the farm	204	5 (2.5)	.118	0.028-0.497	0.004
Close to the farm	67	8 (11.9)			

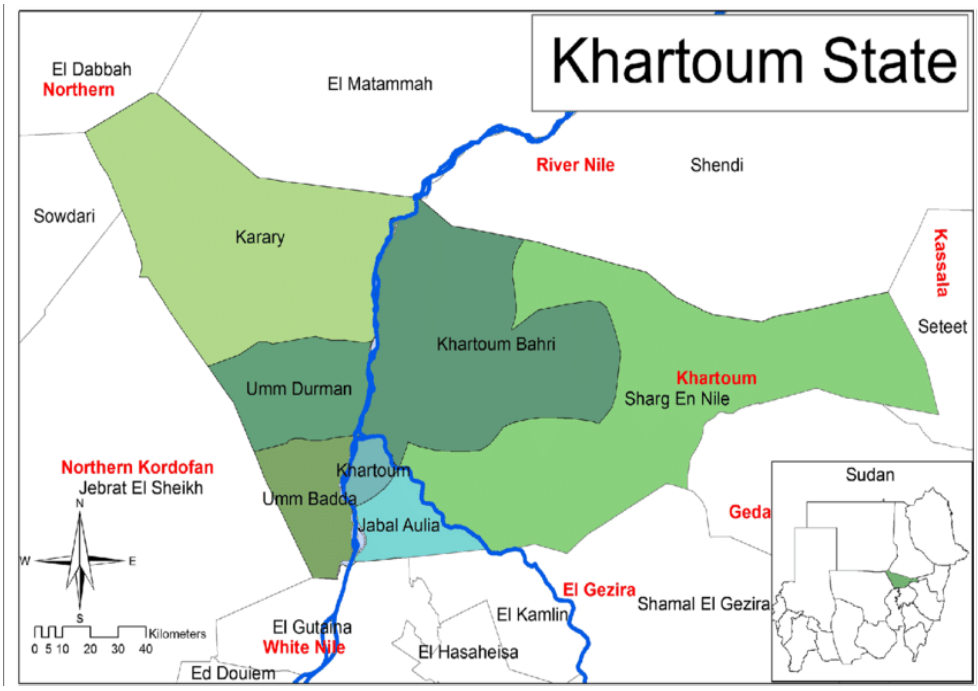

Figure 1: Map of Sudan and Khartoum state showing the study areas for bovine trypanosomiasis

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Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 19 Issue 2 Version 1.0 Year 2019
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Caracterización de Pacientes Caninos de Raza Pura Con Diagnóstico de Enfermedad Metabólica Atendidos en el Hospital Veterinario de la Universidad de San Carlos de Guatemala

By López-Rivera, Sofía Isabel, Laparra-Galindo, Julia Patricia, Chávez–López, Juan José & Villatoro-Chacón, Daniela Mariel

Resumen- Los perros de raza pura han brindado información sobre la morfología, el comportamiento y las enfermedades caninas siendo la diversificación de las mismas lo que ha llevado al desarrollo de enfermedades metabólicas, las cuales son causadas por anormalidades en sistemas enzimáticos. El presente estudio fue realizado en el Hospital Veterinario de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad de San Carlos de Guatemala en el cual se evaluaron 406 fichas clínicas de pacientes caninos de raza pura de las cuales 48 eran correspondientes con un diagnóstico definitivo de enfermedad metabólica. De los pacientes evaluados, las cuatro razas de mayor presencia fue el Labrador Retriever 17%, Husky Siberiano13%, French Poodle 10% y Schnauzer 8%.

Palabras clave: *canino, raza pura, genética, metabolismo, obesidad, hipotiroidismo, urolitiasis, diabetes mellitus.*

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

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El propósito del estudio fue obtener una perspectiva general de la predisposición racial de desórdenes metabólicos en caninos de raza pura así como la relación clínica que estos tienen con otros factores tales como la condición corporal, estado reproductivo y sexo.

Palabras clave: canino, raza pura, genética, metabolismo, obesidad, hipotiroidismo, urolitiasis, diabetes mellitus.

Abstract- Purebred dogs have provided information on the morphology, behavior and canine diseases being the diversification of them which has led to the development of metabolic diseases, which are caused by abnormalities in enzymatic systems. The present study was carried out in the Veterinary Hospital of the Faculty of Veterinary Medicine and Zootechnics of the University of San Carlos of Guatemala in which 406 clinical records of purebred dogs were evaluated, of which 48 were for a definitive diagnosis of disease metabolic Of the patients evaluated, the four races with the greatest presence were the Labrador Retriever 17%, Siberian Husky 13%, French Poodle 10% and Schnauzer 8%. Among the metabolic diseases observed in the study were: obesity and hypothyroidism in 27% each, kidney disease 25%, urolithiasis 17% and diabetes mellitus 4%.

The purpose of the study was to obtain a general perspective of the racial predisposition of metabolic disorders in dogs of the breed, as well as the clinical relationship with other factors such as body condition, reproductive status and sex.

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I. Introducción

Los seres humanos han ejercido distintas selecciones en los perros desde su domesticación como lobos hasta la diversidad de razas que conocemos actualmente. A lo largo de esto, ha intervenido la selección natural durante su adaptación a un estilo de vida domesticado seguido de una intensa selección artificial durante la formación de razas modernas (Deane, Chu, Slavney, Boyko, & Sams, 2018). Los perros de raza pura han brindado información sobre la morfología, el comportamiento y las enfermedades, siendo la diversificación de las mismas lo que ha llevado al desarrollo de trastornos. (Sutter & Ostrande, 2004).

Análisis realizados en 2017 indican las diferencias metabólicas entre los tamaños corporales de distintos caninos. Estas diferencias reflejan no solo la variabilidad inducida morfológicamente sino también las diferencias genéticas metabólicas específicas asociadas con la creación de razas debido a la selección artificial (Middleton et al,. 2017). Las enfermedades metabólicas son aquellas patologías causadas por anormalidades en sistemas enzimáticos implicados en el metabolismo. Las anormalidades congénitas son producidas por alteraciones genéticas que van a dar lugar a enzimas defectuosas (errores congénitos del metabolismo), mientras que las adquiridas son debidas a enfermedades de órganos endócrinos o al fallo de órganos metabólicamente actives (Mayor & Cascales, 2006). Actualmente algunas de las enfermedades metabólicas que afectan a los caninos son la obesidad, insuficiencia renal crónica, diabetes mellitus, hipotiroidismo, desórdenes en la glándula paratiroides, hipertiroidismo, hiperadreno-corticismo, entre otras (Aillón & Enríquez, 2013; Catherin, Moncrieff & Guptyll, 2010). En Guatemala no se poseen estudios publicados sobre las enfermedades metabólicas que afectan a los caninos específicamente según la raza que estos sean.

El presente estudio se realizó en elHospital Veterinario de la Facultad de Medicina Veterinaria y

Zootecnia (HVAC) de la Universidad de San Carlos siendo éste el único hospital universitario veterinario, con atención al público en el cual se reciben pacientes con todo tipo de afecciones, entre las cuales se encuentran las de origen metabólico. El propósito del estudio fue obtener una perspectiva general de la predisposición racial de ciertos desórdenes metabólicos en caninos así como la relación clínica que estos tienen con la condición corporal, estado reproductivo y sexo. Así mismo generar una fuente de información que puede ser útil para mejorar los registros del hospital y contribuir a en el ámbito de la investigación.Es importante tomar en cuenta que en el estudio la mayor parte de los caninos pertenecían a zona 12 y Mixco, zona en la que se encuentra ubicado el hospital y que forman parte de la ciudad capital respectivamente.

II. Métodos

El presente estudio fue realizado en el Hospital Veterinario de la Facultad de Medicina Veterinaria y Zootecnica de la Universidad de San Carlos de Guatemala ubicado en la zona 12 de la ciudad capital con coordenadas UTM al Este 763330 y Norte 1613701.

Dentro de los parámetros a tomar en consideración se incluyó que fueran caninos de raza pura, su estado reproductivo, condición corporal y sexo. Se excluyó cualquier dato que no cumpliera con los parámetros antes mencionados.

Los datos fueron tomadas de todas las fichas clínicas de los pacientes correspondientes al mes de julio 2017 - enero 2018. Las mismas debían pertenecer a pacientes con diagnóstico definitivo de enfermedad metabólica siendo éstos corroborados con exámenes complementarios de laboratorio realizados en dicho centro de estudio así como el respaldo de su ficha clínica.

Para el análisis se realizó un estudio retrospectivo evaluando 406 fichas clínicas de pacientes caninos, de las cuales 48 correspondían a pacientes con un diagnóstico definitivo de enfermedad metabólica.

El análisis de datos se utilizó estadística descriptiva en la cual se clasificaron de acuerdo a raza, sexo, estado reproductivo, condición corporal y diagnóstico final.

Las pruebas diagnósticas a utilizar según la enfermedad fueron las siguientes:

Enfermedad	Ayuda Diagnóstica											
	Hemograma	GPT	Urianálisis	Glucosa	Creatinina	Urea	BUN	Colesterol	T4 libre	US	Ex. Fecal	Urocultivo
Hipotiroidismo	X	X	X					X	X			
Enfermedad Renal Crónica	X	X	X		X	X	X			X		X
Diabetes Mellitus	X	X	X	X	X		X	X				
Urolitiasis	X		X		X	X	X			X		

III. Resultados

Tabla 1: Numero de Pacientes con Enfermedad Metabólica Según Raza

Raza	No.	%
Alaska Malamute	1	2%
Bichón Habanero	1	2%
Bull terrier inglés	1	2%
Collie	1	2%
Dálmata	1	2%
Dobermann	1	2%
Pastor alemán	1	2%
Rottweiler	1	2%
Samoyedo	1	2%
Bulldog Inglés	2	4%
Dachshund	2	4%
Shihtzu	2	4%
CockerSpaniel Inglés	3	6%
Golden retriever	3	6%
SRD	4	8%
Schnauzer	4	8%
French Poodle (Caniche)	5	10%
Husky siberiano	6	13%
Labrador retriever	8	17%
	48	100%

Tabla 2: Desórdenes Metabólicos en el Hvac

Enfermedad	No.	%
Obesidad	13	27%
Hipotiroidismo	13	27%
Enfermedad Renal Crónica	12	25%
Urolitiasis	8	17%
Diabetes	2	4%
	48	100%

Tabla 3: Porcentaje de Caninos con Diagnóstico de Enfermedad Metabólica Según Sexo

Sexo	Pacientes	Esterilizados	No esterilizados	FI	Porcentaje
Hembras	27	11	13	1	56.25%
Machos	21	7	12	3	43.75%
Total	48	18	25	5	100%

Tabla 4: Enfermedades Metabólicas Según Raza

Enfermedad según raza	
Labrador Retriever	Obesidad
	Hipotiroidismo
	Diabetes Mellitus
	Urolitiasis
	Enfermedad Renal Crónica
Husky Siberiano	Obesidad
	Hipotiroidismo
	Urolitiasis
	Enfermedad Renal Crónica
	Enfermedad Renal Crónica
Frencha Poodle	Obesidad
	Hipotiroidismo
	Diabetes Mellitus
	Urolitiasis
	Enfermedad Renal Crónica
Schnauzer	Obesidad
	Hipotiroidismo

Tabla 5: Cantidad y Porcentaje de Caninos Según Condición Corporal

Condición Corporal	Cantidad	%
1	2	4%
2	2	4%
3	4	8%
4	11	23%
5	5	10%
6	2	4%
7	5	10%
8	14	29%
9	2	4%
FI	1	2%
	48	100%

Tabla 6: Cantidad de Pacientes con Enfermedad Metabólica y Su Localidad

Localidad	Pacientes
Zona 12	7
Zona 13	4
Zona 7	4
Zona 8	1
Zona 10	1
Zona 11	4
Zona 17	2
Zona 18	1
Zona 19	2
Zona 21	1
Zona 5	1
Mixco	7
Villa Nueva	2
Villa Hermosa	1
Villa Canales	1
San José Pinula	1
Carretera a Fraijanes	2
Carretera San Lucas Sacatepéquez	1
Chinautla	2
Escuintla	1
Jalapa	1
Sacatepéquez	1
Total	48

IV. Discusión

El grupo de estudio se conformó por 406 pacientes del área de Medicina Interna del HVAC, de los cuales 48 tuvieron un diagnóstico final de enfermedad metabólica. Entre las dificultades para la obtención del diagnóstico final estuvo la situación económica por parte de los propietarios, la cual no permitió en algunos casos la realización de ayudas diagnósticas complementarias así como el seguimiento clínico del paciente.

De acuerdo a Gought y Thomas (2004)la mayoría de las enfermedades genéticas reconocidas del perro se heredan de forma autosómica recesiva. Esto puede ser debido a la endogamia, pero también se debe a la dificultad de identificar y eliminar los rasgos recesivos en los programas de mejoramiento. Es común observar que perros cruzados son más sanos que los de raza pura,relacionándose con la endogamia, la cual tiende a enmascarar el efecto de muchos genes recesivos.

La fijación del fenotipo y el apareamiento de individuos relacionados han dado lugar a patrones de enfermedad específicos de raza y variaciones en la esperanza de vida de éstos. (Fleischer, Sharkey, Maeley, Ostrander & Martínez, 2008). Por esto mismo la presencia de enfermedades metabólicas en ciertas razas puras cada vez es más caracterizable y presente en Guatemala.

Dentro del estudio se determinó la condición corporal de los distintos caninos atendidos en el HVAC ya que la obesidad además de ser considerada una enfermedad metabólica se relaciona con el desarrollo de otras afecciones de este tipo. En los pacientes se pudo determinar que solamente el 33% mostraba una condición corporal adecuada en rangos entre 4 – 5 de 9 y un 47% presentó una condición corporal en rangos entre 6 -9 de 9, siendo éstos últimos ya considerados con sobrepeso y con mayor exposición a un síndrome metabólico. La obesidad canina se asocia con el desarrollo de resistencia a la insulina, perfiles lipídicos alterados e hipertensión leve, que se alivian con la pérdida de peso. Además, los perros con sobrepeso son más propensos a padecer diabetes mellitus, mientras que la sobrealimentación de por vida conduce a un sobrepeso, trastornos metabólicos y una disminución de la esperanza de vida. (Gought & Thomas, 2004) (Mori et al., 2010). Estudios realizados en Estados Unidos indican que el sobrepeso y la obesidad afectan aproximadamente a 1 de cada 5 perros, habiendo aumentado su prevalencia a un 37% en perros desde 2007. En perros, el 62% con sobrepeso presentan hipotiroidismo (García, 2012) y 5.18 veces más tendientes a padecer insuficiencia renal (Aillón & Enríquez, 2013).

Aunque el número de caninos esterilizados resultó ser menor que el de no esterilizados es importante mencionar que los estrógenos regulan la saciedad a nivel del sistema nervioso central, de modo que tras la castración, sobre todo en las hembras, se produce un aumento del apetito y reducción del índice metabólico, siendo los animales castrados los de mayor probabilidad de padecer sobrepeso u obesidad y por ende una enfermedad metabólica (González & Serrano, 2017).

Dentro del estudio se observó que las hembras tuvieron un porcentaje superior que los machos a padecer una enfermedad metabólica correlacionándose con estudios de medicina humana, en los cuales las endocrinopatías se presentan con mayor frecuencia en mujeres (González, 2015). Por ejemplo, entre las endocrinopatías con mayor predisposición en las hembras se encuentra el hipotiroidismo. (Malaga Vet Summit, 2017).

Según los resultados el hipotiroidismo y la obesidad resultaron ser losdesórdenes de mayor frecuencia en el HVAC con un 27% cada una.El hipotiroidismo es una enfermedad con un rango de aparecimiento medio, usualmente afectando entre los 2-6 años (Gought & Thomas, 2004). Entre las razas con mayor predisposición a la enfermedad y de mayor presencia presentadas en el HVAC se encontraron elLabrador Retriever, Husky Siberiano, Schnauzer y French Poodle.

El protocolo diagnóstico de Ettinger para el hipotiroidismo (Catherin, Moncrieff & Guptyll, 2010) difiere con el HVAC solamente en que no se miden electrolitos ni TSH, debido a que no se encuentra como parte del protocolo la medición de los mismos y no existe dicha prueba en el país. Así mismose realizael urianálisis con el fin de no incluir como enfermedad principal la enfermedad de Cushing, la cual presenta signos similares al hipotiroidismo pero densidades urinarias bajas en el 85% de los pacientes (DiBartola, 2010).

De acuerdo a Mori et al. (2010) los Schnauzer miniatura son la primera raza canina reportada en Estados Unidos que suele padecer de hiperlipidemia primaria presentando niveles elevados de triglicéridos plasmáticos y colesterol total. Ambos, signos de hiperlipidemia la cual puede ser provocada por el hipotiroidismo. En el HVAC se presentó un 8% de caninos raza Schnauzer entre los cuales existía diagnóstico de hipotiroidismo. Dentro de las pruebas a realizarse en dicho hospital son los niveles de colesterol, más no de triglicéridos por lo cual el diagnóstico de hiperlipidemia puede llegar a ser inconcluso.Por esta razón sería recomendable agregar como protocolo la medición de estos en todos los animales con énfasis en los Schnauzer. Según un estudio de la red de hospitales estadounidenses Banfield Pet Hospital, el hipotiroidismo se da en 1 de cada 200 perros, además, el 61% de los perros hipotiroideos padecen también sobrepeso u obesidad. (Aillón & Enríquez, 2013).

Seguido del hipotiroidismo, la enfermedad renal crónica resultó ser la segunda enfermedad de mayor aparecimiento con un 25%. Las nefropatías crónicas son las enfermedades renales más frecuentes en la clínica de los pequeños animales y una de las principales causas de mortalidad en pacientes de edad avanzada. Ocurre en perros de todas las edades, sexos y razas (Suárez, 2007). De acuerdo con los resultados del HVAC tanto el Husky Siberiano, Labrador Retriever y French Poodle fueron razas que presentaron dicha afección. Según estudios, en esta enfermedad existe una prevalencia general que oscila entre el 0.5 y el 7% en los perros. (Suárez, 2007).De acuerdo al protocolo deEttinger (DiBartola, 2010) el HVAC difiere en la medición de la presión sanguínea, la cual no se realiza en el mismo debido a la falta de equipo específico. Esta es aconsejable realizarla ya que la hipertensión suele ser una secuela del fallo renal. Estudios demuestran que la incidencia de hipertensión en perros con fallo renal suele ser del 30 – 93% siendo aquellos con afección glomerular los que en mayor riesgo se encuentran (DiBartola, 2010).

Luego la urolitiasisresultó ser la tercera enfermedad de mayor prevalencia en el HVAC la cual puede estar asociada a distintos factores tales como raza, sexo, edad y dieta. SegúnRobles (Robles, 2016)dentro de las razas de mayor tendencia a padecer esta afección se encuentran el Labrador Retriever, Schnauzery Poodle, mismas presentadas en el estudio con mayor presentación en el HVAC y algunas de ellas con diagnóstico final de urolitiasis. De acuerdo a Robles (2016) estudios anteriores indican que dicha enfermedad tiende a afectar a las razas más pequeñas con mayor frecuencia que las grandes pudiendo relacionarse esto con su menor volumen de orina, y el menor número de micciones, por lo tanto mayor concentración de minerales. La predisposición racial a tipos específicos de minerales sugiere una base genética. (Robles, 2016). Las principales pruebas diagnósticas realizadas en el HVAC cuando se sospecha de urolitiasis son ultrasonido abdominal, urianálisis para determinar qué tipo de cristales se encuentran, urocultivo y análisis del urolito Seguido de éstas se realizan algunas pruebas básicas como medición de creatinina sanguínea y perfiles proteicos para descartar cualquier tipo de falla renal asociada al urolito. Muchas veces el análisis del urolito es realizado sólo si el dueño puede pagarlo y algunas veces se limita a las pruebas mencionadas anteriormente. El análisis del mismo es para saber su composición ya que dependiendo de ella puede detectarse otro tipo de afecciones tales como lasalteraciones hepáticas e hipercalciuria.De mismo modo a partir del análisis de éste se puede saber qué manejo dietético debe seguirse y así evitar la recurrencia de la enfermedad.

En cuanto a la diabetes mellitus se encuentra ampliamente distribuida a nivel de razas de perros tendientes a padecerla. Según los pacientes del HVAC tanto la raza Labrador Retriever como French Poodle resultaron con un diagnóstico concluyente de DM. De acuerdo a Fall et al. (2007)las diferencias significativas de raza, sexo y edad indican que la variación genética podría hacer que las razas sean más o menos susceptibles a diferentes tipos de DM. Se requiere de

mayor investigación, principalmente sobre las diferencias entre las distintas características, y los estudios específicos de raza. Así mismo ese mismo estudio realizado en Estados Unidos concluyó que los samoyedos (y todas aquellas razas nórdicas como el Husky Siberiano), los Schnauzers miniatura y el caniche miniatura tenían un alto riesgo de padecer DM; razas presentes dentro de éste mismo estudio. También dentro de ésta enfermedad algunas hembras de raza específica presentan mayor riesgo de sufrirla, sin embargo razas como el Labrador Retriever no poseen preferencia de sexo en cuanto a padecer dicha afección. (Fall, Hansson, Hedhammar, Kampe & Egenvall, 2007)

Dentro del diagnóstico de diabetes mellitus realizado en el HVAC se encuentra la presencia de signos clínicos clásicos como la poliuria, polidipsia, polifagia y pérdida de peso. Una vez encontrados estos se procede a la medición de glucosa tanto en orina como en sangre. Así mismo se mide la densidad urinaria, hemograma, GPT, perfil renal, creatinina y colesterol en sangre. La batería de pruebas se hace con el fin de descartar cualquier tipo de afección que pueda estar causándola o incrementando su presencia. De acuerdo a Ettinger (Nelson, 2010) otras pruebas a realizar son medición de lipasa pancreática, insulina, concentración de progesterona sérica en hembras sin esterilizar y urocultivo. Sin embargo éstas últimas generalmente no se realizan en el HVAC ya que con las pruebas básicas es posible determinar en gran medida la enfermedad así como por razones económicas antes mencionadas.

Abreviaturas

HVAC: Hospital Veterinario de la Facultad de Medicina Veterinaria y Zootecnia
GPT: Transaminasa glutámica pirúvica
BUN: Nitrógeno ureico en sangre
US: Ultrasonido
SRD: Sin raza definida
Fi: Final inconcluso

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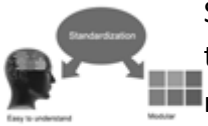

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Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and 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 though 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

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

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

INDEX

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ISSN 9755896