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BACTERIAL CONTAMINATION OF STETHOSCOPIES AT A TERTIARY CARE HOSPITAL IN SOUTHWESTERN NIGERIA

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ABSTRACT

Hospital acquired infections are a recognized cause of morbidity and mortality all over the world. They are frequently caused by organisms residing in healthcare environment, including contaminated medical equipment like stethoscopes. There is limited awareness of health workers of the contribution of contaminated hospital equipment to nosocomial infections. Hence we aimed to determine the level of bacterial contamination and bacterial profile of the isolates from stethoscopes at our centre- a tertiary care hospital in Abeokuta, Southwest Nigeria.

To achieve this, 2 stethoscopes were selected from each of the clinical care units in the hospital and studied. Specimens were collected using moistened sterile cotton swab from the ear pieces and diaphragms of each stethoscope and processed following standard microbiological techniques. In all, 26 stethoscopes were studied. 46.2% (12) of the diaphragms cultured yielded growth of bacteria while only 11.5% (3) of the ear pieces cultured yielded a growth of bacteria . *Staphylococcus aureus* (58.3%) was the most commonly cultured organism. Other organisms cultured included: Diphtheroids, *Proteus species* and *Escherichia coli*.83.3% of the isolated organisms were gram positive while 16.7% were gram negative organisms.In conclusion,the study shows that there is a high level of contamination of stethoscopes in use by health care workers. Disinfection of stethoscopes before and after use is advocated to reduce the spread of infections.

Keywords: Stethoscopes, Contaminated medical equipment, Disinfection, Hospital acquired infections, Health care workers, Bacterial profile.

CONTAMINATION BACTÉRIENNE DES STÉTHOSCOPIES À UN HÔPITAL DE SOINS TERTIAIRES DANS LE SUD-OUEST DU NIGERIA

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ABSTRAIT

Contexte: Les infections acquises dans les hôpitaux sont une cause reconnue de morbidité et de mortalité dans le monde entier. Ils sont fréquemment causés par des organismes résidant dans l'environnement des soins de santé, y compris l'équipement médical contaminé comme les stéthoscopes.Objectif: Déterminer le niveau de contamination bactérienne et le profil bactériologique des isolats provenant des stéthoscopes dans un hôpital de soins tertiaires à Abeokuta, dans le sud-ouest du Nigeria.

Méthodologie: Deux stéthoscopes ont été sélectionnés dans chacune des unités de soins cliniques de l'Hôpital et étudiés. Les échantillons ont été prélevés à l'aide d'un tampon de coton stérile humidifié des morceaux d'oreille et des diaphragmes de chaque stéthoscope et traités selon des techniques microbiologiques standard. Résultats: 26 stéthoscopes ont été étudiés. 46,2% (12) des diaphragmes cultivés ont produit une croissance de bactéries alors que seulement 11,3% (3) des morceaux d'oreille cultivés ont produit une croissance de bactéries. *Staphylococcus aureus* (58,3%) était l'organisme le plus couramment cultivé. D'autres organismes cultivés comprenaient: les diphtéroïdes, les espèces *Proteus* et

Escherichia coli. 83,3% des organismes isolés étaient Gram positif tandis que 16,7% étaient Gram négatif organismes. Conclusion: L'étude montre qu'il ya un niveau élevé de contamination des stéthoscopes utilisés par les travailleurs de la santé. La désinfection des stéthoscopes avant et après usage est recommandée pour réduire la propagation des infections.

Mots clés : Stéthoscopes, Equipement médical contaminé, Désinfection, Infections hospitalières, Travailleurs de la santé, Profil bactérien.

INTRODUCTION

Hospital acquired infections continue to be a challenge to physicians and patients worldwide. It contributes to prolonged stay on admission, increase in hospital bills and occasionally may result in deaths (1). In the United States more than 2 million people a year, contract a health care-associated infection, resulting in 90,000 deaths and economic costs of \$4,500 to \$5,700 million(1). In a developing country like Mexico, approximately 30 deaths per 100,000 inhabitants were attributed to hospital-acquired infections; and were the fourth commonest cause of death in the country(2). Whereas, there is no comparable data from Nigeria on the quantity of losses in terms of expenditure and mortality, available studies suggest that the prevalence of hospital acquired infections ranges from 2.6 –4.2% in Nigerian tertiary care hospitals (3,4,5).

Among sources of hospital acquired infections, contaminated fomites such as stethoscopes and other commonly used hospital equipment such as electronic thermometers, blood pressure cuffs and latex gloves have been implicated (6,7,8). Instruments such as stethoscopes are constantly in contact with patients and may become contaminated with pathogenic microorganisms, they are however, seldom included in disinfection protocols(9).

Virulent organisms may be transferred by stethoscopes from carriers (asymptomatic and symptomatic patients) to other patients who may have impaired immunity and may be unable to resist the infection(9). Other category of patients at risk is those with open wounds like burns or tracheostomies which may be contaminated (10).

Our extensive search of English medical literature revealed that there are very few studies in sub Saharan Africa on the role of stethoscopes in the spread of nosocomial infections with none previously carried out in the South western part of Nigeria (the most densely populated part of the country). We aimed to investigate the role of stethoscopes as potential fomites for potentially pathogenic microorganisms in our hospital – a referral hospital serving residents of Abeokuta and neighboring towns in Southwest, Nigeria. Our specific objectives were to determine the prevalence of bacterial contamination

and bacterial profile of the isolates from stethoscopes used by healthcare workers. In addition we aimed to evaluate the disinfection protocol for stethoscopes in the various clinical units within the hospital.

METHODS

This was a cross-sectional study conducted in September 2015 at our institution, a Federal Government funded tertiary care hospital located in Abeokuta, South West, Nigeria. The hospital has 250 beds located in 20 wards/ clinical care units including the Intensive care Unit and Neonatal care unit. It serves Abeokuta and adjoining towns- an estimated population of about 1.5million people.

Sample Size and Sampling Technique

Two stethoscopes, used by the hospital staff in clinical care of patients, was randomly selected from each of the ward/clinical care units of the hospital and studied. Ethical clearance was obtained from the Hospital Ethics committee before commencement of the study. A pre-structured questionnaire was used to interview a senior nurse on each ward on the condition of anonymity, regarding the disinfection protocol on each ward including -frequency of cleaning and type of disinfectant used to clean the stethoscope. An identification number was assigned to each ward/ clinical care area, and anonymity was maintained for all participants and wards by substituting random numbers in place of wards in each survey distributed.

Specimen Collection and Identification of Pathogen

Cultures from stethoscopes were obtained by swabbing the diaphragm and the bell of the stethoscope with a sterile swab moistened with saline. Subsequently the ear pieces were swabbed with separate moistened saline swabs. These swabs were immediately streaked onto blood agar plates and incubated in air at 37°C for 48 hours. Cultures were identified by colony morphologic characteristics, Gram stain characteristics, and standardized microbiological biochemical tests.(11).

Statistical analysis

Data was analyzed using SPSS version 16.0 computer software. P-value of <0.05 was considered indicative of a statistically significant difference.

RESULTS

26 stethoscopes regularly used stethoscopes were selected in total from the 20 clinical care units in the hospital. 46.2% (12) of the diaphragms cultured yielded growth of organisms while only 11.5% (3) of the ear pieces cultured yielded a growth of organisms (Table I). *Staphylococcus aureus* was the most

TABLE 1: ISOLATES FROM CULTURES OF DIAPHRAGMS AND STETHOSCOPIES

Isolated organisms	no growth	Total
Diaphragm 12 (46.2%)	14 (53.8%)	26
Ear piece 3 (11.5%)	23 (88.5%)	26

TABLE II: MICROORGANISMS ISOLATED FROM DIAPHRAGMS OF STETHOSCOPIES

Microorganism	Gram stain	Number	%
<i>Staphylococcus aureus</i>	positive	75	8.3
Diphtheroids	positive	21	6.6
<i>Proteussp</i>	negative	1	8.3
<i>Staphylococcus epidermidis</i>	positive	1	8.3
<i>Escherichia coli</i>	negative	1	8.3

TABLE III: MICROORGANISMS ISOLATED FROM EAR PIECES OF STETHOSCOPIES

Microorganism	gram stain	Number	%
<i>Staphylococcus aureus</i>	positive	2	66.6
<i>Staphylococcus epidermidis</i>	positive	1	33.3

TABLE IV: FREQUENCY OF CLEANING BY RESPONDENTS

Frequency	number	%
Before and after use	7	35
Three times a day	1	5
Daily	9	45
Rarely	3	15

commonly cultured organism from the ear piece and the diaphragm (Table II and III). Other organisms cultured include: Diphtheroids, *Proteus species* and *Escherichia coli*. 83.3% (13) of the isolated organisms were gram positive while 16.7% (2) were gram negative organisms.

TABLE V: FREQUENCY OF CLEANING FROM RESPONDENTS COMPARED WITH CULTURE RESULTS

Frequency of cleaning	Total	no growth	contaminated (rate %)
Before and after use	7	2	5(71.4 %)
Three times a day	11	0	(0.0 %)
Daily	95	4	(45.0 %)
Rarely	3	2	1 (33.3 %)

Regarding the disinfection practice of stethoscopes - 35% of wards clean the stethoscope parts before and after use while about 50% have various sub-optimal cleaning routines with 15% having no protocol for cleaning (Table IV). All respondents use methylated spirit (containing 70% isopropyl alcohol) in cleaning the ward stethoscopes. There was no association between the frequency of cleaning of the stethoscopes and contamination rate (Table V).

DISCUSSION

Stethoscopes because of their utility are the perfect fomite for transmission of hospital acquired infection in the hospital environment. Due to the load of patients on admission and in busy outpatient clinics, very few clinicians have the time to appropriately disinfect their stethoscope, as they proceed from one patient to the other.

This present study shows that almost half (46.2%) of the stethoscopes used by physicians and nurses in providing care for admitted patients at our institution are contaminated with virulent organisms. Our findings are in keeping with other international studies which demonstrated that 71% to 100% of stethoscopes analyzed were colonized by various species of bacteria (7-10,12-19). Previous African studies in Enugu, Nigeria and Ethiopia also recorded higher levels of contamination- 79% and 90% respectively (10,20).

Ten of the twelve isolates on the diaphragms are potential pathogens(excluding diphtheroids), 83.3% were gram positive while 16.7% are gram negative organisms. This was in keeping with previous studies and might be because of the direct contact of the stethoscope to human skin flora, which contains mostly gram-positive bacteria. Moreover, the lifespan of gram-negative bacteria is not more than six hours *in vitro*(21). Excessive bacterial colonization on stethoscope diaphragm, however, enables them to remain alive for a longer period exceeding eight hours whereas, gram-positive bacteria could remain alive for a longer period, even up to months (22).

Like other studies, *Staphylococcus aureus* was the most commonly isolated organism over other pathogenic organisms (10,20).

Another remarkable finding from this study is the level of contamination of the ear pieces of the stethoscopes used by health workers in the centre. Our value of 11.3% is lower than 20.8% reported by Whittington et al (23) in an intensive care unit in Hammersmith, United Kingdom but indicates a high risk of transmission of ear infections among health workers that share this equipment without disinfection. The lower rate of contamination compared to the diaphragms suggests that users probably clean the ear pieces before use.

Lack of an appropriate disinfection protocol and use of inappropriate disinfectants have been identified as being responsible for the high level bacterial contamination of stethoscopes in previous studies (10,20). Only 35 % of the ward respondents claimed to clean their stethoscopes before and after use. However, there was no positive correlation between frequency of cleaning claimed by the health workers and the level of contamination of stethoscopes determined from our study. This contradicts findings by other workers who found a significant correlation between frequency of cleaning and level of contamination (10, 20). This suggests non-adherence to the protocol or use of inappropriate disinfectant solutions by respondents.

Various agents have been used in cleaning stethoscopes including alcohol swabs, non-ionic detergent, and antiseptic soap. Previous studies have shown that regular cleansing before and after use

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with isopropyl alcohol will eliminate all contaminants from stethoscopes. In 2008, the Centres for Disease Control (CDC) recommended appropriate disinfection of all reusable equipment before use on another patient. Cleaning of stethoscopes with 70% ethyl or isopropyl alcohol after every use is recommended by CDC (24).

There is a need to educate health workers on the need for regular cleaning of their stethoscopes. The recent findings of Melanson *et al* (25) in this direction are encouraging. They evaluated the short- and long-term effect of an educational intervention on the contamination rate of physicians' stethoscopes. They found that a 30-minute lecture addressing the importance of stethoscope cleaning significantly decreased the contamination rate of physicians' stethoscopes at three weeks and the effect was maintained after a 6-month period (25).

CONCLUSION

This study shows that there is a high level of contamination of the diaphragms and ear pieces of stethoscopes used by health-care workers at our centre. There is no effective cleaning protocol in place. There is thus an urgent need to educate all clinicians and health workers on the need to carry out regular disinfection of stethoscopes and other diagnostic equipment used on patients to decrease the incidence of nosocomial infections.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

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ETHICAL APPROVAL

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HAND WASHING PRACTICES AND THE OCCURRENCE OF ENTEROPATHOGENIC BACTERIA AMONG RESIDENTS OF A NIGERIAN UNIVERSITY

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ABSTRACT

Hand washing is known to be an important preventive strategy and a major step in infection control. However, compliance is low in most communities. The present work investigated the relationship between the levels of compliance to hand washing and related this to the occurrence of infectious bacteria in the test population. A questionnaire which contained information on bio-demographic characteristics and hand hygiene practices was applied to 100 individuals in the study population. Microbiological samples were obtained, Total Colony Counts was done and the isolates were identified using standard bacteriological methods. The results showed that 46% of the respondents wash their hands before eating food; 40% of the test population washes their hands after using the toilet; while none of the respondents wash their hands after handling money. The highest bacterial load was found in the 0-15 years age group. The most highly occurring isolate was *Salmonella enterica* (23.7%). These results confirm the low level of compliance to hand hygiene in the test population and underscores the need to effectively break the fecal-oral transmission route via hands through effective interventions such as hand washing with soap and water.

Key Words: enteropathogenic bacteria, hand washing, compliance

LES PRATIQUES DE LAVAGE DES MAINS ET L'APPARITION DE BACTERIES ENTEROPATHOGENES PARMIS LES RESIDENTS D'UNE UNIVERSITE NIGERIANE.

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RESUME

Le lavage des mains est connu pour être une stratégie préventive importante dans le contrôle des infections. Néanmoins, la conformité est faible dans la plupart des communautés. Le présent travail a étudié les niveaux de conformité au lavage des mains et a rapporté cela à la présence de bactéries infectieuses dans la population d'essai. Un questionnaire contenant des informations sur les caractéristiques bio - démographiques et les pratiques d'hygiène des mains a été appliqué à 100 individus de la population étudiée. Les échantillons microbiologiques ont été recueillis, les dénombrements totaux de colonies ont été effectués et les isolats ont été identifiés en utilisant de méthodes bactériologiques standard. Les résultats ont montré que 46% des personnes interrogées se lavent les mains avant de manger de la nourriture; 40% de la population d'essai se lavent les mains après avoir utilisé les toilettes ; alors qu'aucun des répondants ne se lave les mains après avoir touché de l'argent. Le charge bactérienne la plus élevée a été observée dans le groupe d'âge 0 - 15 ans. L'isolat le plus fréquent était *Salmonella enterica* (23,7%). Ces résultats confirment faible niveau de conformité à l'hygiène des mains dans la population d'essai et souligne la nécessité de rompre efficacement la voie de transmission fécale - orale via les mains par des interventions efficaces telles que le lavage des mains avec du savon et de l'eau.

Mots clés: Bactérie entéropathogène, lavage de main, conformité.

INTRODUCTION

Hand washing, defined as the vigorous, brief rubbing together of all surfaces of lathered hands, followed by rinsing under a stream of water in order to remove dirt and infectious microorganisms (1) is perhaps the single most important preventive strategy and a major step in infection control (2). Studies have shown that the basic control of fecal-oral route of spreading potentially pathogenic microorganisms

through food by food handlers may be achieved thorough hand-washing, particularly at critical points in the food dispensing process (3-5). Moreover, optimal hand hygiene behavior is considered to be the cornerstone of healthcare associated infection (HCAI) prevention (6-8). This is because healthcare workers (HCWs) are known to play a major role in the propagation of micro-organisms within the healthcare environment and may spread these to

patients and ultimately from one patient to another (6, 9).

Bacteria, particularly those belonging to the family *Enterobacteriaceae* have been found to be associated with a large percentage of human diseases and these have been grouped, depending on the mode of transmission under different classifications such as those transmitted in healthcare settings (nosocomial), airborne, soil transmitted etc. Some members of the bacterial family *Enterobacteriaceae* produce endotoxins that, when released into the bloodstream following cell lysis, cause a systemic inflammatory and vasodilatory response such as endotoxic shock which can be rapidly fatal (10). Regardless of the method of transmission; bacterial infections may be controlled to a large extent through hand hygiene (11).

There are two principal types of skin flora associated with the hand, namely, resident and transient flora. These microbial and viral floras play a major role in the epidemiology of infections (12). Resident floras are permanent inhabitants of the skin and are found mainly on the surface of the skin. These are non-pathogenic on intact skin but are capable of causing infections on non-intact skin. Examples include bacteria such as *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Propionibacteria*, *Micrococci* and few species of fungi such as *Pityrosporum* (13). These organisms often serve the protective function of protecting against infection by transient organisms through microbial antagonism and the competition for nutrients in the ecosystem (13). Transient floras on the other hand, are microorganisms found only at times on the hand and are easily removed by hand washing. Transient flora organisms usually do not multiply on the skin but survive and occasionally multiply and cause disease. These are acquired from infected persons and/ or inanimate surfaces (fomites) that contain deposits of causative microbes. The transmissibility of transient flora depends on the species of microorganism, its ability to survive on the skin, the population on the hand and the dermal water content.

The simple practice of hand washing is known to reduce the risk of microbial transmission greatly from one person to another as well as limit transmission from a contaminated site to a clean one (2). However, the level of hand hygiene compliance remains low worldwide (14); the lack of appropriate infrastructure (such as water supply in resource poor countries where potable water is frequently inaccessible) and equipment to enable hand hygiene performance (poor location of hand washing kit), the cultural background, and even religious beliefs can play important roles in hindering good hand hygiene practices (15, 16, 17). Moreover, individual cognitive factors such as perception and knowledge of the

transmission risk, social pressure, conviction of hand hygiene efficacy in preventing the spread of diseases, little or no idea on the proper way to wash hands, personal evaluation of perceived benefits against the existing barriers have been identified as reasons for non-compliance with hand hygiene practices (6,18,19).

Hand hygiene behavior appears to be homogenous and has been classified into two types of practices namely, the inherent hygiene practice which occurs when hands are visibly soiled, gritty or sticky. On the other hand, elective hand hygiene practice occur when hand cleansing is performed when hands are not obviously dirty but common social interactions such as shaking of hands, touching of a patient (e.g. taking a pulse or taking blood pressure) by a HCW or having contact with an inanimate object in an infected person's surroundings (9). According to behavioral theories, the elective hand hygiene practice is the component most likely to be omitted and is responsible for most compliance issues in hand hygiene practice particularly among HCWs (20).

The World Health Organization (WHO) has set guidelines on the proper way to conduct hand-washing as follows: the hands must be wetted with clean water (this is important in order to enable the soap make better contact with the hand surface); then lather behind the hands and between the fingers and under the finger nails by rubbing together with the soap, ensuring that the soap gets to every corner; scrub for at least 20 seconds (to ensure that all the germs on the hands are eradicated); rinse thoroughly with clean water, to take away the soap and finally dry with clean piece of cloth, to avoid the transfer of germs (21).

There are other hand hygiene techniques such as wearing of hand gloves and using alcohol or non alcohol-based hand rubs. Alcohol and non-alcohol based hand rubs are considered to be the gold standard for hand hygiene in most clinical situations. This hand hygiene technique is promoted and recommended by the CDC and the WHO and embraced by many national hand hygiene guidelines, based on the evidence of better microbiological efficacy, less time required to achieve the desired effect, point of patient care accessibility and a better skin tolerance profile (6,22,23). However, there has been a lot of concern regarding their lack of efficacy against spore-forming pathogens. Apart from iodophors used at concentrations remarkably higher than the one used in antiseptics, no hand hygiene agent (including alcohols, chlorhexidine, hexachlorophene, chloroxylenol and triclosan) is reliably sporicidal against *Clostridium* or *Bacillus spp* (6, 21). Mechanical friction while washing hands with soap and water is perhaps the only effective intervention against spore forming bacteria as the

spores are physically removed from the surface of contaminated hands (24).

With the foregoing, the present study was aimed at investigating the relationship between the level of awareness and compliance to hand washing as a personal hygiene technique at the Redeemer's University. Moreover, the study was focused on determining whether or not any relationship exists between poor hand hygiene and the occurrence of bacteria of the family *Enterobacteriaceae* as a predictor of risk for potentially serious infections in the test population.

MATERIALS AND METHODS

The Study population, Experimental Design and Collection of Samples

The study population consisted of members of the Redeemer's University community from various walks of life including University students, staff (academic and non-academic), staff children, construction workers, laborers and traders. Demographically, these individuals were of different age brackets and of different levels of education. A 12 item questionnaire which contained information on bio-demographic characteristics and hand hygiene practices was applied to 100 individuals in the study population. A sample size of 91 by simple random cluster sampling technique considering $P=0.5$ as the estimated proportion of hand contamination in the study population, $d=0.09$ was calculated as the desired level of precision, at a confidence level of 95%. The actual sample size was extended to 100 in anticipation of unexpected circumstances in the course of the study. Microbiological samples were obtained from every individual that completed the questionnaire using sterile swabs dipped in saline solution across the palms and fingers of the individuals. Data collected were entered and analyzed using SPSS-16 statistical software. Proportions were compared using Chi-square test and 'p' value less than 0.05 was considered statistically significant.

Microbiological analyses

Swab sticks pre-moistened in sterile normal saline used for collecting samples were dipped in 10ml sterile normal saline and thoroughly stirred using a vortex. Serial dilution was performed into dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} . 1 ml of dilutions 10^{-1} , 10^{-3} , 10^{-5} was inoculated in duplicates onto Eosine Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. The colonies were then counted and the pure colonies were sub-cultured on nutrient agar. EMB agar was used to screen for members of the family *Enterobacteriaceae*, the bacterial contaminants of interest. The bacteria isolates were identified based on shape, colony, color, and Gram's staining reactions

and biochemical tests such as methyl red, Vogues-Praskauer, Citrate, Urease, Indole, Motility, Catalase, Oxidase, Lysine decarboxylase and Sugar fermentation tests. The Duncan's Multiple Range Test ($p \leq 0.05$) was used to compare the mean Total Colony Counts for the demographic groups.

RESULTS

There were one hundred (100) participants in the present study. There were 38 males and 62 females giving a male to female ratio of 1:1.5. A majority of these individuals (76%) have a high school education or higher and were above sixteen years of age (Table 1). The questionnaire on the level of awareness and compliance to hand washing as a personal hygiene technique among residents of Redeemer's University, Ede, Nigeria was completed by all of the 100 persons from whom swab samples were taken for microbiological evaluation. Tables 2 and 3 show the results of the questionnaire items designed to evaluate the level of awareness and compliance to hand washing as a personal hygiene technique in the test population. The results show that all the male respondents washed their hands at least once a day, whereas 2% of the females do not wash their hands at least once daily after taking their bath in the morning (Table 2a). On the other hand, the proportion of women that wash their hands at least more than once daily was more than those recorded for the males; the number of females that wash their hands at least three times daily was twice the number of men that washed their hands three times a day (Table 2a).

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF THE SAMPLE POPULATION

Variables	Frequency/ Percentage
Age	
0-15	39
16-21	32
22 and above	29
Total	100
Gender	
Male	38
Female	62
Total	100
Level of Education	
Preschool/ Primary	22
High School	17
Undergraduate	56
Post graduate	5
Total	100

TABLE 2A: HAND HYGIENE PRACTICES WITHIN THE SAMPLE POPULATION: FREQUENCY OF HAND WASHING ON A DAILY BASIS

Questionnaire item: How frequently do you wash your hands daily?	0	1	2	3
Male	0	12	16	10
Female	2	14	26	20
Frequency	2	26	42	30
Percentage	2	26	42	30
Total	100			

Key: 0= None; 1= Once; 2= Twice; 3= 3 or more times

TABLE 2B: HAND HYGIENE PRACTICES WITHIN THE SAMPLE POPULATION: EVENTS THAT TRIGGER HAND HYGIENE COMPLIANCE

Questionnaire item: When do you wash your hands daily?	0	1	2	3	4	5
Male	0	8	12	0	18	0
Female	1	4	28	0	28	0
Frequency	1	12	40	0	46	0
Percentage	2	12	40	0	46	0
Total	100					

Key: 1= Before, during and after preparing food; 2= After using the toilet; 3= After taking care of sick people; 4= Before eating food; 5= After handling money

In order to determine the intrinsic motivators for compliance in the test population, respondents were presented with options on the daily events that would motivate them to wash their hands. The results showed that forty six percent of the respondents (46%) wash their hands before eating food, followed by 40% of the test population who wash their hands after using the toilet. Only 12% of the respondents wash their hands before, during and after preparing food, 2% of the test population does not wash their hands before during or after any of the listed activities, while none of the respondents wash their hands after handling money (Table 2b). Table 3a shows the results when the respondents were asked to indicate what hand hygiene technique they routinely used. This was in order to serve as a predictor of the effectiveness of the hand washing method adopted by the respondents. The results showed that a majority of the subjects (76%) washed their hands with soap and water, 10% used soap, water and hand sanitizer afterwards, 8% washed their

hands with water only, and 4% used hand sanitizer only while 2% of the respondents did not wash their hands at all. Table 3b shows the results of the questionnaire item designed in order to establish the reasons for non-compliance with hand hygiene practice within the test population. 2% of the respondents listed nonchalance as their reason for non compliance, 6% lacked the awareness of the health significance of hand washing, none of the respondents indicated “little or no idea on the proper way to wash hands” as their reason for non compliance. However, a majority listed “laziness” (46%) and “lack of availability of soap and water” (46%). To investigate further whether there is awareness in this population of the proper way to wash hands as recommended by the WHO respondents were asked if of their awareness of “WHO’s recommended way to wash hands”; the results show that 50% of the respondents were unaware of the WHO’s recommended way to wash hands (Table 3c).

TABLE 3A: HAND HYGIENE PRACTICES WITHIN THE SAMPLE POPULATION: HAND HYGIENE TECHNIQUE USED

Questionnaire item: What do you wash your with?	0	1	2	3	4
Male	0	8	28	0	2
Female	2	0	48	4	8
Frequency	2	8	76	4	10
Percentage	2	8	76	4	10
Total	100				

Key: 0= None; 1= Water only; 2= Soap and water; 3= Hand sanitizer only; 4= Soap, water and hand sanitizer afterwards

TABLE 3B: HAND HYGIENE PRACTICES WITHIN THE SAMPLE POPULATION: REASONS FOR NON-COMPLIANCE WITH HAND HYGIENE PRACTICE

Questionnaire item: Reasons for non compliance	0	1	2	3	4
Male	0	4	0	18	16
Female	2	2	0	28	30
Frequency	2	6	0	46	46
Percentage	2	6	0	46	46
Total	100				

Key: 0= Nonchalant; 1= Lack of awareness of the health significance of hand washing; 2= Little or no idea on the proper way to wash hands; 3= Laziness; 4= Lack of availability of soap and water

TABLE 3C: HAND HYGIENE PRACTICES WITHIN THE SAMPLE POPULATION: AWARENESS OF WHO STANDARD OF WASHING HANDS

Questionnaire item: I am aware of WHO's recommended way to wash hands	Yes	No
Male	18	20
Female	32	30
Frequency	50	50
Percentage	50	50
Total	100	

As shown on Table 4, a total of 118 distinct bacterial isolates were obtained from the entire study and these were separated into eight (8) groups based on differences in their cultural characteristics. Biochemical tests were then applied to the representative isolates in order to identify and characterize these isolates. Eight (8) distinct

organisms were identified, namely, *Klebsiella oxytoca*, *Proteus vulgaris*, *Shigella sonneri*, *Morganella morganii*, *Salmonella enteric*, *Serratia marcescens*, *Proteus mirabilis*, *Proteus penneri*. As shown on Table 5, the most highly occurring of these was *Salmonella enterica* (23.7%), followed in descending order by *Shigella sonnei* (16.9%); *Proteus vulgaris* (15.3%); *Klebsiella oxytoca* (13.6%); *Morganella morganii* (10.2%); *Proteus mirabilis* (8.5%); *Proteus penneri* (6.7%) and *Serratia marcescens* (5.1%). Table 6 shows that the highest bacterial load was found in the 0-15 years age group from where 76 distinct isolates, representing the age group from which the highest number of the bacteria were isolated. This number represents more than half the total number of isolates obtained from the entire study (64.4%; Table 6). This was followed in descending order by respondents that were in the 16-21 years and respondents 22 years and older at 28.8% and 6.8% respectively.

TABLE 4: IDENTIFICATION TABLE OF BACTERIAL ISOLATES FROM THE HANDS OF RANDOMLY SELECTED MEMBERS THE REDEEMER'S UNIVERSITY COMMUNITY.

Representative Isolates	Gram Staining	Cell Shape	Oxidase	Catalase	Citrate	Methyl Red	Voges - Praskauer	Motility	Orthinine Decarboxylase	H ₂ S	Lactose	Indole	Suspected organisms
A	-	R	-	-	+	-	+	+	-	-	+	+	<i>Klebsiella oxytoca</i>
B	-	C	-	+	+	-	-	+	-	+	-	+	<i>Proteus vulgaris</i>
C	-	R	-	+	+	+	-	-	+	-	-	-	<i>Shigella sonnei</i> .
D	-	R	-	+	+	+	-	+	-	-	-	+	<i>Morganella morgani</i>
E	-	R	+	+	+	+	-	+	-	+	-	+	<i>Salmonella enterica</i>
F	-	R	-	+	+	-	-	+	-	-	-	+	<i>Serratia marcescens</i>
G	-	R	-	+	+	+	-	+	+	-	-	-	<i>Proteus mirabilis</i>
H	-	R	-	+	+	+	-	+	-	-	-	+	<i>Proteus penneri</i>

Key: C=cocci; R= Rod; + = positive; - = negative

TABLE 5: PERCENTAGE OCCURRENCE OF THE ISOLATES FROM THE ENTIRE STUDY

S/N	Group number	Identified organisms	Frequency of Isolates	Percentage
1	A	<i>Klebsiella oxytoca</i>	16	13.6
2	B	<i>Proteus vulgaris</i>	18	15.3
3	C	<i>Shigella sonnei</i>	20	16.9
4	D	<i>Morganella morgani</i>	12	10.2
5	E	<i>Salmonella enteric</i>	28	23.7
6	F	<i>Serratia marcescens</i>	6	5.1
7	G	<i>Proteus mirabilis</i>	10	8.5
8	H	<i>Proteus penneri</i>	8	6.7
		Total	118	100

TABLE 6: PERCENTAGE OCCURRENCE OF THE ISOLATES AMONG THE AGE GROUPS

Age group (years)	Identities of bacterial isolates isolated from subjects/ respondents								
	<i>Klebsiella oxytoca</i>	<i>Proteus vulgaris</i>	<i>Shigella sonnei</i>	<i>Morganella morgani</i>	<i>Salmonella enteric</i>	<i>Serratia marcescens</i>	<i>Proteus mirabilis</i>	<i>Proteus penneri</i>	Total (%)
0-15	10	4	14	8	28	4	4	4	76(64.4)
16-21	4	12	4	4	0	0	6	4	34(28.8)
22 and above	2	2	2	0	0	2	0	0	8(6.8)
Total (%)	16 (13.6)	18(15.3)	20(16.9)	12(10.2)	28(23.7)	6(5.1)	10(8.5)	8(6.7)	100(100)

DISCUSSION

The fact that hand washing contributes to keeping the individual healthy and free from microbial infection is well established in literature. For example, Aiello et al (25) in a survey carried out at the University of Michigan, USA reported that hand wash hygiene could reduce the spread of flu-like symptoms by up to 75%. Moreover, numerous surveys carried out with the objective of linking hand washing to the reduction of microbial infection particularly in healthcare settings have concluded after series of investigations

that hand washing reduces the transmission of pathogenic organisms from individual carriers (patients) to health care workers and visitors (6, 9, 26).

In addition, an example of how hand washing may serve as a preventive measure against microbial infection is the recent Ebola virus outbreak in Nigeria. When on the 20th of July 2014, Ebola found its way down to Lagos, Nigeria through a traveler from Liberia (a diplomat who went by the name Patrick Sawyer) infected with the virus. Nigerian authorities

were caught unawares, so he was able to infect several other people including health care workers in the hospital where he was taken to for treatment. The number of people infected with Ebola virus in Nigeria as at October 2014 was recorded to be twenty and eight deaths which involved health care workers and innocent victims. However, the rapid interventions in the quarantine of sick individuals by Nigerian HCW, WHO and CDC together with the compliance of citizens to hand hygiene by a combination of hand washing with soap and water and the use of sanitizers especially in public places like banks, airports, schools and so on, led to the removal of the disease from Nigeria. In October 2014, Nigeria was declared free from Ebola (27, 28).

In spite of the obvious advantage of hand hygiene in stemming the spread of infectious diseases, compliance is low even among the enlightened and educated particularly among health workers who should know about the importance of hand washing in personal health and the spread of diseases. In a study conducted to evaluate hand washing practices among medical personnel at the University of Port Harcourt, Nigeria, it was found that only 37.6% washed their hands regularly after interacting with their patients while 33.9% did so only after the days work. 58.3% and 58.9% washed hands before meals and after defecating respectively (29). In the present study, compliance rate to hand hygiene as a means of personal hygiene is equally low as the results showed that only 46% of the respondents wash their hands before eating food, followed by 40% of the test population who wash their hands after using the toilet. Only 12% of the respondents wash their hands before, during and after preparing food, 2% of the test population does not wash their hands before during or after any of the listed activities, while none of the respondents wash their hands after handling money (Table 2b).

The results of the Opara et al, (29) and present studies underscore the fact that the level of education and the awareness of the importance of hand washing to prevention of infection and the spread of disease does not necessarily translate to good compliance. Moreover, the motivators for compliance to hand washing may differ depending on the population of interest. These findings suggest that intrinsic behavioral (e.g., role modeling) and socioeconomic factors such as accessibility or acceptability of soap may play greater roles in the use of soap and other hygiene practices. This is especially important in the case of elective compliance to hand washing when hands are not visibly dirty but may have been exposed to infectious disease causing agents.

The results from the present study indicating that the highest bacterial load (64.4%; Table 6) was found among the children (age 0-15 years old) in the test population is quite worrisome. However, it further underscores the importance of intrinsic behavioral factors such as modeling in order to commit to and comply with good hand hygiene practices. In a related direct observation study conducted in Zimbabwe on 23 caregiver-infant pairs for 130 hours and recorded wash-related behaviors to identify pathways of fecal-oral transmission of bacteria among infants. It was discovered that hand washing with soap was not common and drinking water was contaminated with *Escherichia coli* in half (12 of 22) of the households (30). In another related study conducted in Tanzania, half of the caregivers' dominant hands were positive for *E. coli* in a context where hand washing with soap after fecal contact was rarely practiced (31). Moreover, even in Healthcare settings, studies have shown that compliance to hand washing increased when hand washing is actively supported and promoted by senior administrators and senior physicians (20). In addition, studies have shown that HCWs have a higher likelihood of practicing hand washing when senior members of staff were present (20, 23). These results suggest that children and young adults are less likely to practice hand hygiene when there are no good role models to help them commit to good hygiene practices. Furthermore, the fact that 46% of the respondents in the present study indicated that their non compliance to hand hygiene practice was due to "lack of availability of soap and water" underscores the importance of provision of necessary amenities such as sinks, potable flowing water, soap and clean towels in order to encourage compliance with hand hygiene in the test population.

Although there has been no outbreak of enteric diseases within the test population of this study, the present results showing that eight distinct bacterial organisms from the family Enterobacteriaceae, members of which are associated with fecal contamination is a serious observation and are predictive of possible outbreaks of enteric diseases.

The most occurring of the isolated organisms was *Salmonella enterica*, an organism known to be responsible for causing salmonellosis. *S. enterica* causes four different clinical manifestations: gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state. There is an infectious dose (between 10^3 to 10^5 bacilli by ingestion) at which this organism is able to cause disease which varies with the serotype; young children, patients with depressed cell-mediated immunity, or who are elderly may become infected with at a lower infectious dose (32). As reported by Solomons (33) environmental

enteropathy, a chronic subclinical intestinal pathology typically a feature of populations infected with less than 'infectious dose' of enteric pathogens, is common among infants in low-income countries and has been proposed as a cause of childhood stunting (33). Environmental enteropathy, may be a more important cause of poor growth in children than diarrhea because it is characterized by reduced intestinal barrier function and chronic systemic inflammation (34).

Findings from the present study confirms the low level of compliance to hand hygiene in the test population and further underscores the need to effectively break the fecal-oral transmission route via hands through effective interventions such as hand washing with soap and water. Provision of necessary amenities such as sinks, potable flowing water, soap and clean towels coupled with promotion of hand hygiene practice among opinion leaders such as crèche care givers, school teachers, University lecturers and administrators is expected to increase

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the participation of residents of the Redeemer's University Campus, Ede in hand hygiene practice. This in turn is expected to reduce the risk for outbreak of enteropathogenic diseases and may serve as an index case for the entire community within Osun State of Nigeria.

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MULTIPLE ANTIBIOTIC RESISTANCE INDICES OF *AEROMONAS HYDROPHILA* ISOLATES OF MUSCLE OF CATFISH (*CLARIAS GARIEPINUS*, BURCHELL 1822) FROM SELECTED MARKETS IN IBADAN, NIGERIA

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ABSTRACT

The extensive use and misuse of antimicrobials for treatment and prophylaxis in livestock production generally and aquaculture in particular is of great concern to environmental and public health. In Nigeria, regulation and monitoring of aquaculture and other livestock production activities at best is lax. Drug resistance pathogens have therefore been consistently reported in Nigeria.

Ninety-eight adult live fishes weighing an average of 684.88 ± 141.73 g were purchased at random from different live-fish selling points fortnightly over a fourteen-week period. Fish were anaesthetized using Tricaine Methane Sulfonate (MS222) and 15g of muscle excised and processed according to standard methods. Growth, isolation and characterization of *Aeromonas hydrophila* was accomplished using Rimler-Shotts agar medium which had been infused with ampicillin supplement for 24 hours and incubated at 37°C and appropriate biochemical tests.

Ten positive isolates (AH1-AH10) were subjected to culture and sensitivity test using the disc diffusion method on nutrient agar. Zones of growth inhibition around the colonies were observed, measured and characterized as sensitive, intermediate and resistant based on the Manual of Antimicrobial Susceptibility Testing method. All the isolates had MAR >0.2. Isolate AH9 had the highest MAR index (1). Three of the isolates (AH3, AH5 and AH8) had MAR indices of 0.89, while AH2, AH4 and AH7 had MAR indices of 0.67. This study established the resistance of *Aeromonas hydrophila* isolates from fish muscle to a wide range of antibiotic. The detection of high MAR *A. hydrophila* in muscle of fish intended for consumption is significant and could act as a potential source of resistant bacteria for humans. Further investigation into antimicrobial resistance is recommended.

PLUSIEURS INDICES DE RÉSIDENCE AUX ANTIBIOTIQUES DE *AEROMONASHYDROPHILA* ISOLE OF MUSCLE POISSON-CHAT (*CLARIAS GARIEPINUS*, BURCHELL 1822) DE CERTAINS MARCHÉS À IBADAN (NIGÉRIA)

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RÉSUMÉ

L'utilisation intensive, la mauvaise utilisation des antimicrobiens pour le traitement et la prophylaxie en élevage en général et de l'aquaculture en particulier est de grande préoccupation pour l'environnement et la santé publique. Au Nigeria, réglementation et surveillance de l'aquaculture et des autres activités de production de bétail au mieux est laxiste. Pathogènes de résistance de drogue ont donc été toujours déclarés au Nigeria.

Quatre-vingt-dix-huit des poissons vivants adultes pesant en moyenne 684.88 ± 141.73 g ont été achetées au hasard à différents points de vente de poissons vivent toutes les deux semaines sur une période de quatorze semaines. Poissons ont été anesthésiés à l'aide de méthane-Sulfonate de tricaine (MS222) et 15g de muscle excisé et préparée selon les méthodes standard. Croissance, l'isolement et caractérisation des *Aeromonashydrophila* a été réalisée à l'aide de milieu gélosé Rimler-Shotts qui avait été imprégné de supplément ampicilline pendant 24 heures et incubé à 37 ° c et des tests biochimiques appropriés.

Dix isolats positifs (AH1-AH10) ont été soumis à essai de culture et antibiogramme à l'aide de la méthode de diffusion des disques sur la gélose nutritive. Zones d'inhibition de croissance autour des colonies ont été observées, mesurés et caractérisés comme sensible, intermédiaire et résistant basé sur la méthode du manuel des tests de sensibilité aux antimicrobiens. Tous les isolats avaient MAR > 0,2. AH9 isolat avait le plus fort indice de MAR (1). Trois des isolats (AH3, AH5 et AH8) avaient des indices de MAR de 0,89, tandis que AH2, AH4 et AH7 avaient MAR indices de 0,67. Cette étude a établi que la résistance de *Aeromonashydrophila* isole du muscle de poisson à une vaste gamme d'antibiotique. La détection de haute MAR *A. hydrophila* dans le muscle de poisson destinés à la consommation est importante et pourrait agir comme

une source potentielle de bactéries résistantes pour les humains. Enquête plus approfondie sur des gènes résistants aux antimicrobiens provenant de cette source est recommandée par les présentes.

INTRODUCTION

Aeromonas hydrophila is an ubiquitous organism ranked as the most common bacteria in freshwater habitats all over the world. It is also represented as a part of the microflora of the intestine of healthy fish. However, these bacteria frequently cause disease among cultured and feral fishes as soon as there is an imbalance in a pond set-up or an introduction of any of the stress factors. Members of this group exhibit diverse genetic, biochemical and antigenic heterogeneity and therefore forms a complex of disease organisms that are associated with bacterial hemorrhagic septicemias and other ulcerative conditions in fishes and humans-making it zoonotic.

Motile aeromonad septicemia has been demonstrated in both fresh- and brackish-water fish species, marine fishing grounds and raw and processed products of marine fish [1, 2, 3, 4, 5].

Aeromonas hydrophila occurs as a Gram-negative, motile, straight rod (0.3-1.0x 1.0-3.5µm). It forms white to buff, circular, convex colonies within 24 hours at 22- 28oC. Rimmler-shotts infused with ampicillin or novobiocin has been found useful for the isolation and presumptive diagnosis of *A. hydrophila*. It is a cytochrome oxidase-positive organism resistant to the vibriostatic agent O/129. It ferments glucose with or without the production of gas and this differentiates it from *Pseudomonas* [6, 7].

Its possession of two classes of adhesins permits it to bind to specific receptors on cell surfaces and these are described as adhesins associated with filaments and those associated with proteins. The filaments are also involved in phage-binding biofilm formation as well as twitching motility and are distributed in different proportion on the different strains of aeromonads. The variation in the distribution of the pili is suspected to be [18] causing enteric infections such as traveller's diarrhoea, severe diarrheal disease of short duration or chronic loose stools in children, the elderly, or immuno-compromised individuals [19]. Dermal infections such as cellulitis contracted from fish handling [20], septicemia sustained due to immunodeficiency from other protracted illness, urinary tract infections, surgical wound infections, meningitis, peritonitis, and endocarditis have been associated with *Aeromonas hydrophila* infection in humans [21]. It is important to note that *Aeromonas* species have also been reported to have the inherent capability to be resistant to chlorination [22]. *Aeromonas hydrophilais* listed in the Contaminant Candidate List, and Environmental Protection Agency Method 1605 has further validated its detection and enumeration in drinking water system [23].

responsible for the behaviours of the bacteria such as seen when motile aeromonads taken from lesions on diseased fish shows a greater chemotactic response to skin mucus than isolates that were obtained as free-living organisms from pond water [8]. [9] also indicated that *A. hydrophila* had adhesive agglutination characteristics which facilitated attachment to eukaryotic cells. [10] showed that the presence of a 52kD surface protein in the S-layer has been responsible for an increase in their cellular hydrophobicity which enhances resistance of the bacterium to serum lysis and phagocytosis by leukocytes. [11] and [12] have documented that many of the aeromonads possessed hemorrhagic factors and lethal toxins at different concentrations. Further interest in the virulence of *A. hydrophila* was elicited when it was revealed that enterotoxins, haemolysins, proteases, haemagglutinins, and endotoxins produced by bacterial organisms exhibit synergism in inducing clinical pathology [13, 14, 15].

In the acute or severe form of the disease, morbidity and mortality is high and sudden, clinical signs include exophthalmia, reddening or darkening of the skin, and fluid accumulation in the scale pockets [16]. There may be ascites and the scales may bristle out from the skin to give a "washboard" appearance. The gills may show varying degree of haemorrhage and ulcers may develop on the dermis. Dermal ulcers form shallow necrotic lesions, internal organs are swollen and congested with haemorrhages over the viscera. The kidney and swollen spleen usually contain a semi-fluid which may drip out.

In humans, *Aeromonas* species have been listed as a pathogen for various diseases in man [17]. It has been shown as food and water borne pathogen of significance

Aeromonas hydrophila and antimicrobial use

Antimicrobials have been in use to control the incidence of bacterial diseases in aquaculture for a long time. In the 1980s, Norway experienced huge losses in its fish industry due to bacterial diseases and a total crash in the industry was only prevented by the use of vast quantity of antibiotics [24].

The use of antimicrobial agents in aquaculture and its potentials to contribute to antimicrobial resistance raises important issues relating to public health, food safety, animal health and production. Due to non-existence of rules curbing the indiscriminate use of antimicrobials in aquaculture [25, 26, 27] and other livestock production [28, 29, 30] in developing countries or the lack of enforcement in places where such rules exist; antimicrobial are used without caution or

consideration of the adverse effect on public health with regards to development of drug resistance. This study was designed to determine Multiple Antibiotic Resistance (MAR) indices of *Aeromonas hydrophila* isolates in the muscle of African sharp-tooth catfish (*Clarias gariepinus*, Burchell 1822) from selected live-fish markets in Ibadan, Nigeria.

MATERIAL AND METHODS

To ensure fishes were not from the same source in Ibadan metropolis, ninety-eight adult live fishes weighing 684.88±141.73g at the average were purchased at random from seven live-fish selling points fortnightly over a fourteen-week period. Fish were anaesthetized using Tricaine Methane Sulfonate (MS222), then 15g of muscle was excised from the mid-region and homogenized with 30ml of alkaline peptone solution (Oxoid, UK). Homogenates were left at room temperature for 24 hours after which aliquots were taken and inoculated in Rimler-Shotts agar medium which had been infused with ampicillin supplement for 24 hours and incubated at 37°C. Bacterial growth was harvested and subjected to biochemical tests to identify and isolate *Aeromonas hydrophila* from the samples.

Culture and sensitivity test was carried out using the disc diffusion method on nutrient agar. Zones of growth inhibition around the colonies were observed and measured and then characterized based on the Manual of Antimicrobial Susceptibility Testing method as sensitive, intermediate and

resistant. The sensitivity disc used was from Abtek Biologicals UK, LOT QE06/PB/P. The choice of antibiotic was based on the frequently used antibiotics for treatments of infection in local clinics [31] as well as antibiotics of choice used by farmers during fish rearing [27].

Multiple Antibiotic Resistance Indexing

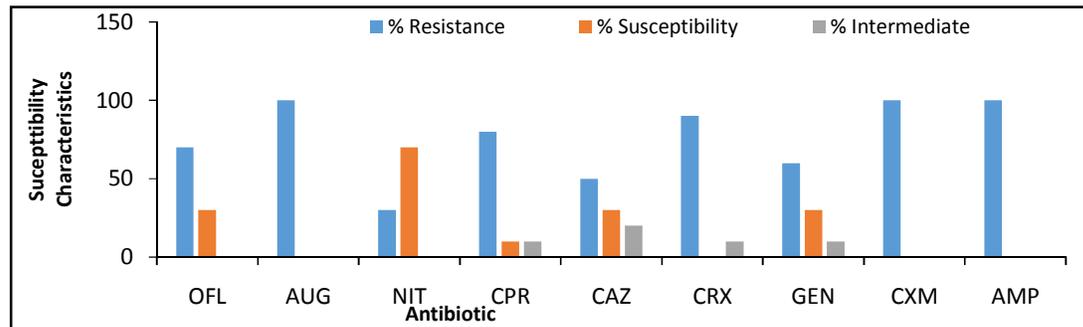
The MAR index in individual isolates was calculated as a/b according to [32], where:

- a represents the number of antibiotics to which the isolate was resistant to
- b represents the number of antibiotics to which the isolate was exposed.

An MAR index higher than 0.2 was considered to be high-risk, with possible exposure to high doses of antibiotics over a period of time. While a MAR index less than or equal to 0.2 was considered as an original strain resistance to the antibiotic without previous exposure to such antibiotic.

RESULTS

A total of ten *Aeromonas hydrophila* isolates (AH1-AH10) were obtained and tested for antibiotic susceptibility during the sampling period. All the isolates showed varying degree of multiple resistant pattern to more than one antibiotic. All the isolates showed 100% resistance to Ampicillin, Cefixime and Augmentin, resistance to ciprofloxacin was 80% while it was 90% for cefuroxime. Nitrofurantoin had the highest susceptibility at 70%, while (30%) were susceptible to Ofloxacin, Ceftazidime and Gentamycin.



OFL- Ofloxacin, AUG- Augmentin, NIT- Nitrofurantoin, CPR- Ciprofloxacin, CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamycin, CXM- Cefixime, AMP- Ampicillin

FIG. 1: SHOWING THE SUSCEPTIBILITY CHARACTERISTICS OF SOME AEROMONASHYDROPHILA TO SELECTED ANTIBIOTICS

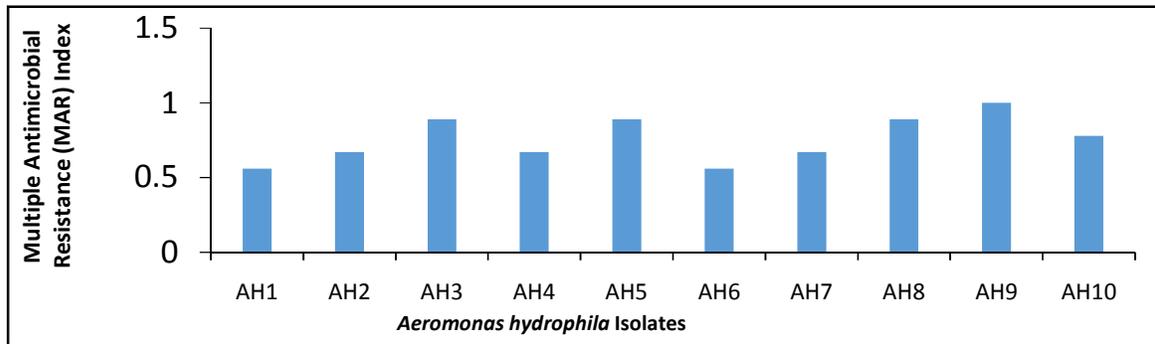


FIGURE 2: MULTIPLE ANTIMICROBIAL RESISTANCE (MAR) INDICES OF AEROMONASHYDROPHILA ISOLATES FROM CLARIASGARIEPINUS' MUSCLES

DISCUSSION

In this study, all the isolates (100%) had multiple antibiotic resistance (MAR) indices >0.2 (Figure 2). Isolate AH9 had the highest MAR index (Figure 1). Three of the isolates (AH3, AH5 and AH8) had MAR indices of 0.89, while AH2, AH4 and AH7 had MAR indices of 0.67. This agrees with some studies where the capabilities of these bacteria to develop resistance and transmit such effectively have been established [33, 34]. Ofloxacin is a fluoroquinolone which is supposed to be active against skin infection such as cellulitis caused by *Aeromonas hydrophila* in humans but no report of its potency against *Aeromonas hydrophila* has been documented. It is further categorized as 'of high regulatory concern' by the FDA in the United States and its use to treat any food animal is regarded as illegal and completely irresponsible [35], yet the level of resistance is highly significant in *Aeromonas hydrophila* from this study. The resistance to augmentin (100%) recorded in this study agrees with records of [36] who also recorded 100% resistance to Ticarcillin/clavulanic acid and 92.6% to Amoxicillin/Subactam (AMX-SUL) by Trifamox IBL (Laboratories Bagó, Buenos Aires, Argentina). Nitrofurantoin demonstrated the highest susceptibility (70%) in all isolates tested, this is close to a previously report of 100% by [34] but contrary to that of [37] who reported 42% susceptibility to nitrofurantoin. Such differences may be clearly demonstrates the kinetic ability of bacterial organisms in developing resistance to antimicrobials. The nitrofurans, which include nitrofurantoin, nitrofurazone, furanace, and furazolidone, are frequently used to treat pet and ornamental fishes in developed nations; it is strictly forbidden for use in food fish by the FDA in the US [35]. However, the same cannot be said of Nigeria where there is indiscriminate use of antibiotics in food animals and fishes because of the inadequate regulations and lack of enforcement of existing regulations. Antimicrobials are dispensed without proper diagnosis and prescription by veterinary personnel [38, 39, 40] reported that nitrofurans are not readily absorbed into the body in gilthead sea bream (*Sparusaurata*) and tilapia (*Oreochromis mossambicus*); therefore, their efficacy is seen more

in superficial infections. Nitrofurans are also known to be reactive to light therefore there is the need to administer it under the cover of some form of darkness to achieve the most effective result. Therefore even the recorded susceptibility is not so good news for food fishes.

Records show that aminoglycosides such as kanamycin and gentamycin have broad spectrum activity against Gram negative and gram positive bacterial organisms. However, it is to be noted that the reported 100% efficacy of gentamycin were from those administered via injection and it has also been reported that such administration predisposes to kidney damage in fishes [35]. Gentamycin resistance (60%) and susceptibility (30%) in this work therefore calls for concern because aeromonads has been largely reported to be sensitive to gentamycin although there are reports of resistance as well [41].

Presence of antibiotics in aquatic bodies starts with natural production. The presence of the group of Actinomycetes which includes Streptomycetes produces antibiotics, several antibiotics such as some β -lactams, streptomycins, aminoglycosides and others are produced by soil bacteria [42]. This antibiotic activity from local soil samples is variable and as such naturally occurring bacterial presence is seen more in tropical soil and by extension aquatic bodies as tropical soil are known to possess more bacteria producing antibiotics especially for tetracycline [42].

However, antibiotics usage has been largely responsive for the development of resistance in the bacterial pathogens. Extensive usage in human and indiscriminate usage in veterinary practise especially for the purpose of preventing (prophylaxis) or treating microbial infections. International usage of antibiotics is also not harmonised therefore antibiotics prohibited in some countries are permitted in others such as the use of streptomycin in fruit growing in the USA meanwhile this is prohibited in Germany. Utilization as growth promoters also has its contribution to bacterial resistance. Of the 10,200

tons of antibiotics utilized in the EU in 1996, 50% was applied in veterinary medicine and as growth promoters. Data such as that of the European Federation of Animal Health [43], revealed that a total of 13,216 ton of antibiotics were used in the European Union and Switzerland, 65% of which was applied in human medicine in 1999 [42]. Interestingly, [44] and [45] revealed that contrary to expectation, hospitals are not the main source of pharmaceuticals in municipal sewage. Rather, community use is largely responsible supported by records such as 70% in the UK [46], 75% in the US [47] and 75% in Germany [48].

Metabolism of these antibiotics in human and animals vary a lot [49, 48]. Some compounds are metabolized by 90% or more, while others are metabolized by only 10% or even less. The excretion rates for the unchanged active compound for most of the antibiotics also remains in the same range of between 10%–90%. [48] recorded an average of 30% metabolic rate for all antibiotics used leaving 70% of the used antibiotics excreted unchanged into waste water with every likelihood that the metabolites are more water soluble than the parent compounds. It is also common practise to flush unused drugs including antibiotics down into the toilet.

In the developed nations, attempts are made to remove these antibiotics from the environment during sewage treatment but this only reduces the volume on such antibiotics and partially eliminates them. Therefore these antibiotics end up in the environment and precisely in aquatic bodies.

Conclusion

This study has established that all the strains tested showed a MAR index value greater than 0.2, thus indicating that the isolates are from high risk sources, such as sewage, animal husbandry waste, faecal contaminated drinking water. Organic feeding of chicken waste, chicken entrails as well as blood meal has been implicated in the transmission of such antimicrobial exposed aeromonads to fish. The detection of *A. hydrophila* in muscle of fish intended for consumption is significant and grave as it could act as a potential source of resistant bacteria for humans. Similar studies all over the world have consistently documented multiple **antibiotic resistance** to *A. hydrophila* [50, 36, 37, 51,34]. This study also further corroborates the reports of excessive and uncontrolled use of antimicrobials in Nigerian aquaculture [27] and other livestock industries [28, 29, 30] leading to the development of antimicrobial resistant pathogens.

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ANTIMICROBIAL ACTIVITY OF *LYCOPERDON PERLATUM* WHOLE FRUIT BODY ON COMMON PATHOGENIC BACTERIA AND FUNGI

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ABSTRACT

Antimicrobial activities of extracts of fruit bodies of *Lycoperdon perlatum* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Candida glabrata* were investigated. Antimicrobial components from the mushrooms were extracted using ethanol, methanol and water. The antimicrobial activities were examined by agar well diffusion method. The MIC, MBC and MFC were evaluated for each extract of the mushroom. The aqueous extract of *Lycoperdon perlatum* inhibited the growth of all the tested pathogenic organisms except *P. aeruginosa* while the methanol and ethanol extracts inhibited all the tested organisms. The phytochemical analysis revealed the presence of varying levels of bioactive compounds. Flavonoids, saponins, protein and carbohydrate were detected in all the extracts while glycosides, alkaloids and tannins were found in some. The results obtained from this study suggest that *Lycoperdon perlatum* has broad-spectrum of activity against microbial isolates used.

Key words: *Lycoperdon perlatum*, antimicrobial, phytochemicals, well diffusion

ACTIVITÉ ANTIMICROBIENNE DES LYCOPERDON PERLATUM ANTOFLES CORPS SUR LES CHAMPIGNONS ET LES BACTÉRIES PATHOGÈNES COMMUNS

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RÉSUMÉ

Les auteurs ont étudié les activités antimicrobiennes des extraits des fructifications de *Lycoperdon perlatum* contre *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* et *Candida glabrata*. Les composants antimicrobiens de champignons ont été extraits à l'aide de l'éthanol, le méthanol et l'eau. Les activités antimicrobiennes ont été examinées par la méthode de diffusion en puits d'agar. Le MIC, la MBC et la MFC ont été évaluées pour chaque extrait du champignon. L'extrait aqueux de *Lycoperdon perlatum* inhibe la croissance de tous les organismes pathogènes testés à l'exception de *P. aeruginosa* alors que le méthanol et l'éthanol extraits inhibent tous les organismes testés. L'analyse phytochimique a révélé la présence de différents niveaux de composés bioactifs. Flavonoïdes, saponines, protéines et hydrates de carbone ont été détectés dans tous les extraits en glycosides, alcaloïdes et tannins trouvées dans certains. Les résultats de cette étude suggèrent que *Lycoperdon perlatum* a large spectre d'activité contre les isolats microbiens utilisés.

Mots clés : *Lycoperdon perlatum*, antimicrobiennes, phytochimiques, diffusion en puits.

INTRODUCTION

Time immemorial, mushrooms have been used as a part of regular diet due to their nutritional and medicinal values. Mushrooms have been found to contain minerals, vitamins and nutritive compounds, proteins, polysaccharide and a low fat content (1). A number of medicinal mushroom genera such as *Aleurodiscus*, *Coprinus*, *Clitocybe*, *Daedalea*, *Marasmius*, *Merulius*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella*, and *Tricholoma* spp., are rich sources of β -glucan, lectin, phenolic compounds, flavonoids, polysaccharides,

triterpenoids, dietary fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthenes, coumarins, alkaloid, purin, purimidin, kinon, fenil propanoid, kalvasin, volvotoksin, flammutoksin, porisin, eryngeolysin etc (2).

Mushrooms are also rich sources of natural antibiotics. Their cell wall glucans have been known to poses immunomodulatory properties with many of their secondary metabolites combating bacteria, fungi

and viruses (3, 4, 5, 6, 7, 8, 9). Prior to the discovery of their high medicinal value, mushrooms have been used for hundreds of years in traditional medicine for curing various types of diseases such as antimicrobial, antioxidant, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular diseases, immunomodulating, central activities (10, 11, 12, 13).

Lycoperdon perlatum, popularly known as the common puffball, warted puffball, gem-studded puffball, or the devil's snuff-box, is a species of puffball fungus in the family Agaricaceae. A widespread species with a cosmopolitan distribution, it is a medium-sized puffball with a round fruit body tapering to a wide stalk, and dimensions of 1.5 to 6 cm wide by 3 to 7 cm tall. It is off-white with a top covered in short spiny bumps or "jewels", which are easily rubbed off to leave a netlike pattern on the surface. When mature it becomes brown, and a hole in the top opens to release spores in a burst when the body is compressed by touch or falling raindrops (14). A saprobic species, *Lycoperdon perlatum* grows solitarily, scattered, or in groups or clusters on the ground. It can also grow in fairy rings. Typical habitats include woods, grassy areas, and along roads (15). It is edible when young and the internal flesh is completely white, although care must be taken to avoid confusion with immature fruit bodies of poisonous *Amanita* species. *L. perlatum* can usually be distinguished from other similar puffballs by differences in surface texture. Due to the dearth in literature on the dual value of *Lycoperdon perlatum* as food and its antimicrobial efficacy, this study was designed.

MATERIALS AND METHODS

Collection and Identification of Materials

Lycoperdon perlatum was collected from different sources of Umuahia North Local Government area, Abia state and identified by a botanist in the Department of botany, University of Nigeria, Nsukka.

Test	Organisms	Used
	Pure cultures of <i>Escherichia coli</i> JCM 20135 and <i>Bacillus cereus</i> IFO 13804 were obtained from Department of Microbiology, University of Nigeria Nsukka while pure cultures of <i>Staphylococcus aureus</i> ATCC 25923, <i>Candida albicans</i> ATCC 10231, <i>Pseudomonas aeruginosa</i> ATCC 25783 and <i>Candida glabrata</i> ATCC 22018 were obtained from Spectramedics Laboratories No. 2 Adebayo close, Araromi Offiri Ikenne Road, Sagamu, Ogun State.	

Standard	Antimicrobials
Tetracycline (5 µg/ml), Ampicillin (5 µg/ml),	Gentamycin (5 µg/ml), Oxacillin (5 µg/ml),

Fluconazole (5 µg/ml) and Nystatin (20 µg/ml) Oxoid disk were used as positive standards.

Sample preparation and extraction

Fresh *L. perlatum* mushrooms were thoroughly washed with clean water, cut into pieces, air-dried at room temperature and pulverized using manual grinder. Fifty grams of each of the ground samples was soaked in 300 ml ethanol, cold water, and methanol for 24 hours with intermittent shaking. Each sample was filtered using Whatman No1 filter paper. The filtrate was poured into a crucible and allowed to dry under steady air current in order to obtain the extract which was scooped and poured into well-labeled sample bottles and stored at 4°C (16).

Inoculum preparation

Pure cultures of *Escherichia coli* JCM 20135 and *Bacillus cereus* IFO 13804 were obtained from the Department of Microbiology, University of Nigeria Nsukka while pure cultures of *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 25783 and *Candida glabrata* ATCC 22018 were obtained from Spectramedics Laboratories, Sagamu, Ogun State, Nigeria. Inoculum was prepared by emulsifying overnight colonies from an agar medium. A 0.5 McFarland standard (equivalent to approximately 10⁸cfu/ml) was used. Media plates were inoculated within 30 minutes of standardizing the inoculum to avoid changes in inoculum density.

Determination of antimicrobial activity of mushroom extracts

Antimicrobial activity of mushroom extracts was determined according to the National Committee of Clinical Laboratory Standards (17). Agar well diffusion method on Sabouraud dextrose agar (SDA) and Muller-Hinton agar were used for fungi and bacteria respectively. Up to 100 µl of the inoculum was poured onto the agar plate and spread with glass rod under sterile conditions. Wells (6mm diameter) were bored into the agar using sterile cork-borer and 0.1 ml of different concentrations of the extracts (500, 250, 125, 62.5, 31.25, 15.63 and 7.81 mg/ml) was applied into each well. Negative control wells were filled with dilute dimethylsulfoxide while positive controls were antibiotic discs of tetracycline (10 µg/ml); ampicillin (10 µg/ml) for Gram negative bacteria isolates and oxacillin (5 µg/ml); gentamicin (10 µg/ml) for Gram positive bacteria isolates. Antifungal discs of fluconazole (25 µg/ml) and nystatin (20 µg/ml) (Oxoid, United Kingdom) were used as positive controls for fungal isolates. This procedure was done in triplicate for the entire test organisms, allowed to stand for 30 minutes on the bench and incubated for 24 hours at 37±2 °C for bacteria and 72 hours at 28±2 °C for yeast. After incubation, the inhibition zone diameters produced

by the different concentrations of the crude extracts were measured (in millimeter) and recorded. Antimicrobial activities were expressed in terms of the mean value of the inhibition zone produced by the mushroom extracts.

Determination of minimum inhibitory concentrations (MICs) of the mushroom extracts

The MIC of the extracts was determined for the test organisms in triplicates at varying concentrations (250, 125, 62.5, 31.25, 15.62, 7.80 and 3.90 mg/ml). To obtain these concentrations, 1.0 ml of varying concentrations of the extracts with double strength (500, 250, 125, 62.5, 31.25, 15.62 and 7.80 mg/ml) were constituted in different test tubes. About 1.0 ml of Mueller-Hinton broth (for bacteria) and Sabouraud dextrose broth (for fungi) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. Controls of Mueller-Hinton broth and Sabouraud dextrose broth without the mushroom extract were set up. All the bacterial cultures were incubated at $37 \pm 2^\circ\text{C}$ for 24 hours and yeast culture incubated at $28 \pm 2^\circ\text{C}$ for 72 hours. After incubation each tube was examined for microbial growth. The lowest concentration of the extract that inhibited the growth of the test organisms as detected by lack of visual turbidity was designated the MIC (18).

Determination of minimum bactericidal concentrations (MBCs) of the mushroom extracts

MBC was determined by selecting tubes that showed no bacterial growth during the MIC determination. A loopful from each of the tubes was sub-cultured on the Mueller Hinton Agar and incubated for 24 hours at $37^\circ\text{C} \pm 2^\circ\text{C}$. The MBC was determined as the least concentration that showed no visible growth on the plate (17).

Determination of minimum fungicidal concentrations (MFCs) of the mushroom extracts

MFC was determined by selecting tubes that showed no fungal growth during MIC determination. A loopful from each of the test tubes was sub-cultured on Potato Dextrose agar. The plates were incubated for 72 hours at $28 \pm 2^\circ\text{C}$. The MFC was determined as the least concentration that showed no visible growth on the plate (17).

Statistical analysis

Experimental values were given as means \pm standard deviation (SD). Statistical significance of data were analyzed at $P \leq 0.05$ (ANOVA) using statistical package for social sciences (SPSS, Armonk, NY, USA) version 20.

RESULTS

The antimicrobial activity of *Lycoperdon perlatum* was determined by agar well diffusion method against six pathogenic isolates.

Table 1 shows the result of the average MIC and MBC of the ethanolic, methanolic and aqueous extracts of *L. perlatum* on test organisms. The MIC of ethanolic extract varied between 15.63 and 125 mg/ml with MBC of 31.25 to 125 mg/ml, MIC of methanolic extract varied between 15.63 and 62.5 mg/ml with MBC of 31.25 to 125 mg/ml while the MIC of aqueous extract varied between 31.25 and 125 mg/ml with MBC of 62.5 to 250 mg/ml.

TABLE 1: THE MIC AND MBC OF CRUDE EXTRACT OF *L. PERLATUM*

Extract	Test organism	MIC (mg/ml)	MBC (mg/ml)
Ethanolic	<i>B. cereus</i>	125	125
	<i>S.aureus</i>	15.63	31.25
	<i>P. aeruginosa</i>	125	125
	<i>E.coli</i>	31.25	62.5
Methanolic	<i>B. cereus</i>	62.5	125
	<i>S.aureus</i>	31.25	31.25
	<i>P. aeruginosa</i>	31.25	62.5
	<i>E.coli</i>	15.63	31.25
Aqueous	<i>B. cereus</i>	31.25	62.5
	<i>S.aureus</i>	62.5	62.5
	<i>P. aeruginosa</i>	ND	ND
	<i>E.coli</i>	125	250

ND = NOT DETERMINED

Table 2 shows the result of the average MIC and MFC of the ethanolic, methanolic and aqueous extracts of *L. perlatum* on test organisms. The MIC of ethanolic extract of *L. perlatum* showed 62.5 mg/ml with MFC of 125 mg/ml for *C. albicans* and MIC of 7.81 mg/ml with MFC of 15.63 mg/ml for *C. glabrata*, the MIC of methanolic extract of *L. perlatum* showed 7.81 mg/ml with MFC of 15.63 mg/ml for *C. albicans* and MIC of 31.25 mg/ml with MFC of 62.5 mg/ml for *C. glabrata*, the aqueous extract of *L. perlatum* showed MIC of 62.5 mg/ml with MFC of 62.5 mg/ml for *C. albicans* and MIC of 62.5 mg/ml with MFC of 125 mg/ml for *C. glabrata*.

TABLE 2: THE MIC AND MFC OF THE CRUDE EXTRACT OF *L. PERLATUM*

Extract	Test organism	MIC (mg/ml)	MFC (mg/ml)
Ethanol	<i>C.albicans</i>	62.5	125
	<i>C.glabata</i>	7.81	15.63
Methanol	<i>C.albicans</i>	7.81	15.63
	<i>C.glabata</i>	31.25	62.5
Aqueous	<i>C.albicans</i>	62.5	62.5
	<i>C.glabata</i>	62.5	125

TABLE 3: PHYTOCHEMICAL ANALYSIS OF *LYCOPERDON PERLATUM* IN DIFFERENT SOLVENT

Solvents	Methanol	Ethanol
Aqueous		
Saponin	++	++
Tannins	-	-
Flavonoid	+	+
Alkaloid	+	-
Proteins	+	++
Glycosides	++	++
Carbohydrates	+++	+++

Table 3 showed the qualitative phytochemistry of *Polyporus alveolaris* using different solvents (ethanol, methanol and aqueous). The phytochemical analysis revealed the presence of bioactive compounds which were present at varying levels. Saponins, protein and carbohydrate were detected in all the extracts while glycosides, alkaloids, tannins and flavonoids were found in some.

KEY: - Not present; + present in low conc. ++ moderate conc.; +++ high conc.

Figure 1 shows the result obtained for the antimicrobial activity of *Lycoperdon perlatum* methanol extract. This extract exhibited a broad spectrum activity, inhibiting all the tested organisms including *P. aeruginosa* that was resistant to other crude extracts. However, the mean inhibition zone diameter of *P. aeruginosa* was significantly ($p < 0.05$) lower than that of other inhibited organisms.

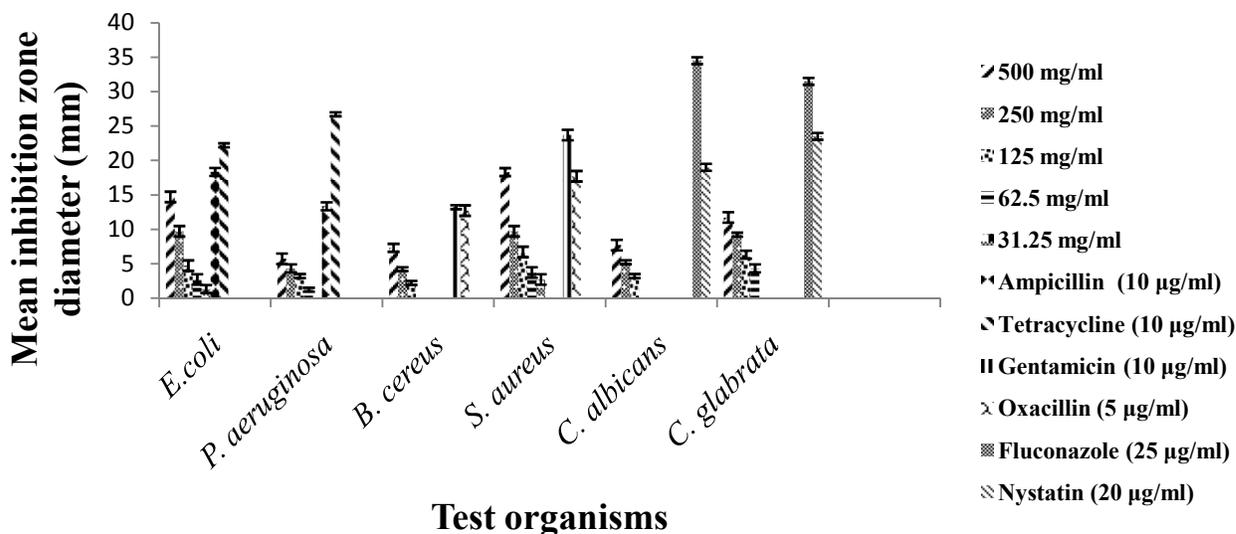


FIGURE 1: THE ANTIMICROBIAL ACTIVITY OF *LYCOPERDON PERLATUM* METHANOL EXTRACT ON THE TEST ORGANISMS

Figure 2 shows the result obtained for the antimicrobial activity of *Lycoperdon perlatum* ethanol extract. This extract exhibited a broad spectrum of activity, inhibiting all the tested organisms including

P. aeruginosa that was resistant to other crude extracts. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

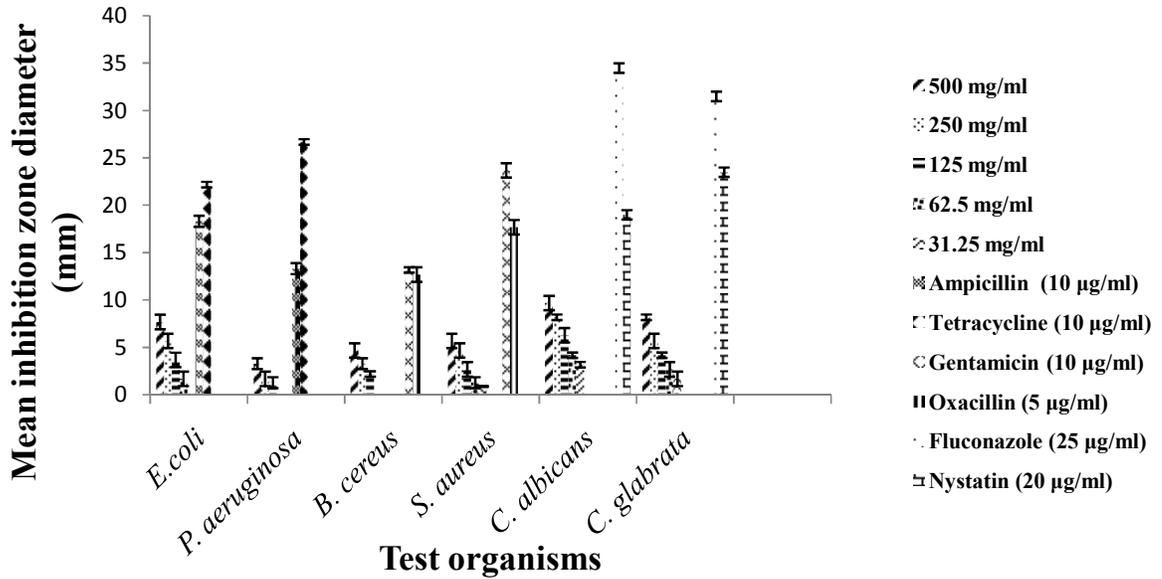


FIGURE 2: THE ANTIMICROBIAL ACTIVITY OF *LYCOPERDOM PERLATUM* ETHANOL EXTRACT ON THE TEST ORGANISMS

Figure 3 presents the antimicrobial activity of *Lycoperdom perlatum* aqueous extract. The different test microorganisms showed varied susceptibility to the extract. *B. cereus*, *S. aureus*, *C. glabrata* and *C. albicans* were well inhibited by the extract. *E. coli* was only inhibited at concentrations of 500 mg/ml

and 250 mg/ml while *P. aeruginosa* was not inhibited even at the highest tested concentration of 500 mg/ml. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

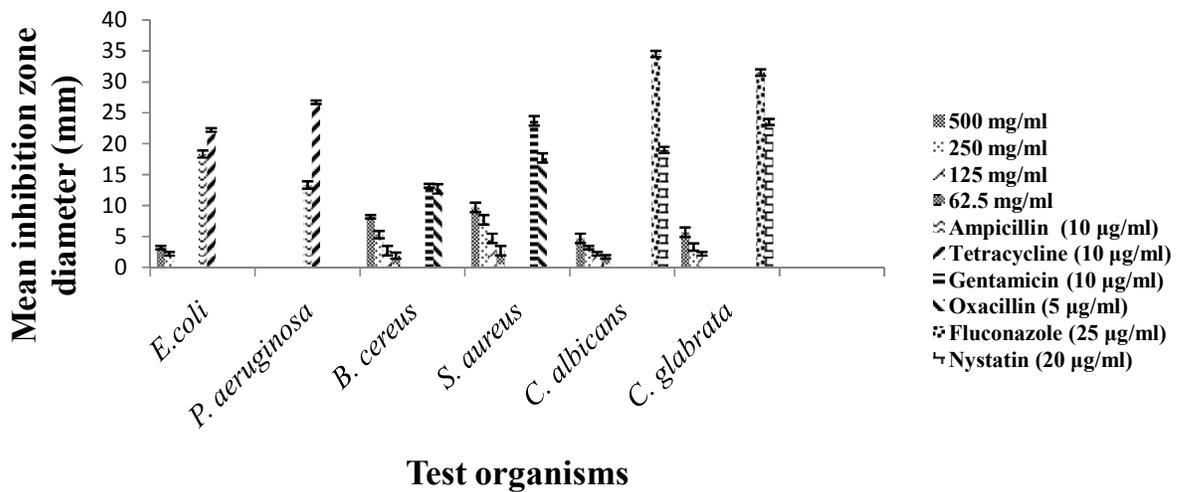


FIGURE 3: THE ANTIMICROBIAL ACTIVITY OF *LYCOPERDOM PERLATUM* AQUEOUS EXTRACT ON THE TEST ORGANISMS

DISCUSSION

The first antimicrobial agent (antibiotic) to be produced was Penicillin, and it was discovered through the sheer serendipity of Alexander Fleming in 1928. This was derived from the ascomycetous fungus *Penicillium notatum*. The antibiotic was put into mass production and large scale therapeutic use because of the scale up work subsequently carried out by Howard Florey and Ernst Chain in the 1940s, and this work was supported by the necessity to cure wounded soldiers of infections during the II world war (19, 20). Antimicrobial activity of the crude extract of *Lycoperdon perlatum* as well as phytochemical characteristics were studied. The specific zone of inhibition against various types of pathogenic bacteria and fungus was shown in Figure 1, 2 and 3. The results indicated that extracts from mushroom have antimicrobial properties as reported by Nwachukwu and Uzoeto (16). Mushrooms produce various antiviral, antifungal compounds to survive in the wild against competing or pathogenic agents (21, 22). Also observed in this study is that there were variations in the degree of antimicrobial activities of mushrooms. The sensitivity of isolates to the mushroom extracts implies that intrinsic substance in the extracts is unknown to the microorganisms which made it impossible for them to resist. The variations in the antimicrobial activities of *Lycoperdon perlatum* extracts may be due

to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients (23).

The results of the present study strengthened the outcomes of earlier works done by others that showed mushrooms produced a great variety of antimicrobial agents. For instance, it is known that the extract from fruit bodies of several *Lactarius* sp. (24, 25); *Fomitopsis* sp. (26); *Boletus* sp. (27); *Cortinarius* sp. (28); *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* (29); *Pleurotus tuberregium* (30); *Amanita caesarae*, *Armillaria mellea*, *Chroogomphus rutilus*, *Clavariadelphus truncates*, *Clitocybe geotropa*, *Ganoderma* sp., *Ganoderma carnosum*, *Hydnum repandum*, *Hygrophorus agathosmus*, *Lenzites betulina*, *Leucoagaricus pudicus*, *Paxillus involutus*, *Polyporus arcularius*, *Rhizopogon roseo*, *Sarcodon imbricatus*, *Suillus collitinus*, *Trametes versicolor*, *Tricholoma auratum*, *Tricholoma fracticum* (31); *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum* (32); *Russula delica* (33); *Pleurotus eryngii* var. *ferulae* (34); *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius deliciosus* and *Phellinus hartigii* (35); *Lactarius indigo* (36); *Trametes hirsuta* (20) and *Stereum ostrea* (37) contain a wide range of antimicrobial activity. The potential of developing antimicrobials from mushroom appears a rewarding expedition worthy of spending time and other currencies on.

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MOLECULAR DIAGNOSTICS BY PCR OF POXVIRUSES (*ORTHOPOXVIRUS (OPV) AND MOLLUSCUM CONTAGIOSUM VIRUS (MCV)*) IN CÔTE D'IVOIRE WEST AFRICA

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ABSTRACT

The *Orthopoxvirus (OPV)* and the *Molluscum contagiosum virus (MCV)* are Poxviruses involved in viruses skin lesions in humans. *OPV* infects many vertebrates and *MCV* mainly infects humans. A diagnostic confusion is often observed between the clinical lesions due to the different Poxviruses firstly and secondly with other viruses like the virus of the chickenpox. In Côte d'Ivoire, the diagnosis of *MCV* remains essentially clinical and that of *OPV* is non-existent despite the risk of circulation of the virus. This study aims to implement the molecular detection of the *OPV* and the *MVC* in Côte d'Ivoire. Material and method: *Cowpoxvirus* DNA and 21 DNA extracts from suspicious cutaneous lesions of the *MCV* were analyzed by conventional PCR. The consensus primers (EACP1, EACP2) designed from the surface hemagglutinin gene were used for the detection of the *OPVs* and the primers (MCV1, MCV2) targeting the K fragment of the *MCV* were used for the *MCV's* detection . A growing dilution series of the *Cowpoxvirus* DNA and the *MCV* allowed the study of the method's sensitivity used. The DNAs of *S.aureus*, *M. ulcerans*, *VZV*, *HSV*, the *Measles virus* and *Varicella virus* were used for the specificity tests. Results: The detection of the *OPV* from the *Cowpoxvirus* viral strain was positive with a positivity threshold at 10⁻¹ dilution. That of the *MCV* DNA from the suspected *MCV's* lesion was positive with a positivity threshold of up to 10⁻⁶ dilution. No non-specific amplification was observed with the DNAs of the other pathogens responsible for lesions Cutaneous. The clinical diagnosis of the *MCV* was confirmed by PCR in 18 out of the 21 patients, ie 85.71%. On the 3 patients with a negative *MCV* PCR, 2 were positive for the *OPV* PCR , reflecting the risk of confusion between clinical lesions due to Poxviruses.

Keywords: Molecular diagnostic, Poxviruses, West Africa

MISE AU POINT DU DIAGNOSTIC MOLECULAIRE PAR PCR DES POXVIRUS (*ORTHOPOXVIRUS (OPV) ET MOLLUSCUM CONTAGIOSUM VIRUS (MCV)*) EN COTE D'IVOIRE AFRIQUE DE L'OUEST.

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Résumé

Justification : *Orthopoxvirus (OPV)* et *Molluscum contagiosum virus (MCV)* sont des *Poxvirus* impliqués dans les lésions cutanées d'origine virale chez l'homme. *OPV* infecte de nombreux vertébrés et le *MCV* infecte essentiellement l'Homme. La confusion est souvent observée entre les lésions cliniques dues aux différents *Poxvirus* d'une part et d'autre part avec d'autres virus comme le virus de la varicelle. En Côte d'Ivoire, le diagnostic du *MCV* reste essentiellement clinique et celui des *OPV* est inexistant malgré le risque de circulation du virus. Cette étude vise à mettre au point la détection moléculaire des *OPV* et du *MCV* en Côte d'Ivoire. Matériel et méthode : ADN de *Cowpoxvirus* et 21 extraits d'ADN issus de lésions cutanées suspectes de *MCV* ont été analysés par PCR classique. Les amorces consensus (EACP1, EACP2) issus du gène de l'hémagglutinine de surface ont été utilisées pour la détection des *OPV* et les amorces (MCV1, MCV2) ciblant le fragment K de l'ADN du *MCV* ont été utilisées. Une série dilution croissante de l'ADN du *Cowpoxvirus* et du *MCV* ont permis l'étude de la sensibilité de la méthode utilisée. Les ADN de *S aureus*, de *M. ulcerans*, du *VZV*, du *HSV*, du Virus de la rougeole et du virus de la varicelle ont été utilisés pour les tests de spécificités. Résultats : La détection de l'*OPV* à partir de la souche virale *Cowpoxvirus* était positive avec un seuil de positivité à la dilution 10⁻¹. Celle du *MCV* à partir de l'ADN de lésion suspecte de *MCV* était positive avec un seuil de positivité pouvant aller jusqu'à la dilution 10⁻⁶. Aucune amplification non spécifique n'a été observée avec les ADN des autres pathogènes responsables de lésions cutanées. Le diagnostic clinique à *MCV* a été confirmé par la PCR chez 18 des 21 patients soit 85,71%. Sur les 3 patients à résultat PCR *MCV* négatif, 2 étaient positifs pour la PCR *OPV* traduisant le risque de confusion entre les lésions cliniques dues aux *Poxvirus*.

Mots clefs : Diagnostic moléculaire – PCR – Poxvirus – Afrique de l'ouest

INTRODUCTION

Poxviruses are double-stranded DNA with cytoplasmic multiplication. They have a number of autonomous elements allowing this intracytoplasmic multiplication unlike the majority of other viruses [1]. Several genes of this family of viruses including Orthopoxviruses (OPV) and Molluscum contagiosum virus (MCV) are involved in skin infections in humans and other vertebrates.

Since the 1980s, the smallpox virus belonging to the Orthopox virus group has been eradicated [2]. However, viruses such as Monkeypox virus, Cowpox virus and other OPVs continue to infect humans accidentally but with a less severe degree of virulence than the smallpox virus [3]. Their eradication is problematic because they have many reservoirs. The emergence of Monkeypox virus, especially in Central Africa, with the epidemiological characteristics of the epidemic of 1996 - 1997 in Zaire [3] and the appearance of the virus in 2003 in the USA makes it a global concern. Clinically, confusion has already been made between certain oxic infections and chickenpox according to the literature [5, 6]. Côte d'Ivoire is a probable zone of virus circulation [7, 8]. Despite this fact, diagnostic methods for OPVs in general and Monkeypox virus in particular are non-existent.

The MCV is increasingly encountered with HIV infection. It is often involved in skin lesions in

children. To date, in Côte d'Ivoire, the diagnosis of this virus remains essentially clinical whereas atypical clinical forms were encountered with HIV infection [9, 10].

In order to monitor the emergence of this group of viruses in the population in Côte d'Ivoire, it could be necessary to set up molecular diagnostic tools to detect a certain number of viruses of this family.

MATERIALS AND METHODS:

This is an experimental study of the analytical type carried out at the Pasteur Institute of Côte d'Ivoire on the site of Adiopodoumé to the platform of molecular biology in 2016.

Biological material

In this study, the following biological products were used: strains of Cowpox virus derived from Cowpox virus culture on a Vero cell supplied by the Pasteur Institute of Bangui for the technical development of the detection of the Orthopoxviruses. Cutaneous lesions of 21 patients suspected of MCV infection for the detection of the Molluscum contagiosum virus. Microorganisms involved in cutaneous infections in Côte d'Ivoire from various biological products of patients were used for primer specificity tests (Table 1).

TABLE 1: PATHOGENS TESTED IN THE SPECIFICITY TEST OF PCR FOR THE ORTHOPOXVIRUS AND THE MOLLUSCUM C VIRUS

Number	Pathogens	Sample type	Laboratory where the pathogens used were isolated
Souche KN	<i>S. aureus</i>	Pus	UBY
258UB	<i>Mycobacterium ulcerans</i>	Cutaneous lesion	GER-Buruli
017 HSV	<i>Herpes simplex virus</i>	Cerebrospinal fluid	DVE
014 VZV	<i>Virus de la varicelle</i>	Cerebrospinal fluid	DVE
258 G	<i>Virus de la rougeole</i>	Oropharyngeal secretion	DVE

DVE: Department of epidemic viruses of the Pasteur Institute of Côte d'Ivoire, GER-Buruli: Research group on Buruli ulcer of the Pasteur Institute of Côte d'Ivoire, UBY: bacteriology unit of Hospital University Center of Yopougon

METHODS

Pre-treatment of samples
The cutaneous lesions were ground with mortar and dissolved in 2 ml of 1X PBS. They were then stored at -20 ° C until extraction of the DNA.

DNA extraction

The DNAs of the different microorganisms were extracted using the Nuclisens magnetic extraction protocol (Biomerieux). Briefly 400 µl samples were added to 800 µl of lysis buffer and incubated for 10 min. Then 40 µl of magnetic silica was added to the mix and incubated for 10 minutes at laboratory temperature. The solution was centrifuged for 30 s at 13000 rpm. A series of washing was carried out on the silica-DNA complex using the Minimag (Biomerieux). An elution buffer was used after washing to collect 25 µl of DNA.

Amplification and revelation

A PCR using consensus primers of *Orthopoxviruses* designed from the virus surface membrane HA gene were used (Forward: EACP1: 5 'ATG ACA CGA TTG CCA ATA C 3', Reverse: EACP2: 5 'CTA GAC TTT GTT TTC TG 3 '); the desired PCR product band size being 942 bp[11]. Amplification conditions were as following for the detection of *Orthopoxviruses*: 94 ° C 5 min (1 cycle), 94 ° C 30 sec, 48 ° C 1 min, 72 ° C 1 Min) (36 cycles), 72 ° C 8 min (1 cycle). Concerning the *Molluscum contagiosum virus*, primers targeting the fragment K gene of the virus DNA were used (MCV Primer1: 5'CCGATCTTTGCGAGCGTTCCTAA 3' 'MCV Primer 2: 5'TCCCATACAGCGAGGACAGCATA 3'), the desired PCR product being 167 bp size[12]. Amplification conditions: 94 ° C 5 min (1 cycle), 94 °

C sec, 65 ° C C 1 min, 72 ° C 1 min) (36 cycles), 72 ° C 8 min (1 cycle)

The GoTaq G2 Flexi DNA polymerase kit (Promega Corporation, USA) was used for the PCR mixes containing 0.2µM of each primer, 1.5µM, MgCl, 0.1µM dNTPs, 1 unit Taq polymerase, 1X of buffer and 5µl of DNA template for a final volume of 50µl. The revelation was made on a GelDoc Bioanalyzer (BioRad) after electrophoresis on 1.5% agarose gel.

Study of sensitivity

It was made from a dilution series from the DNA extracted of the *Cowpoxvirus* viral strain for *Orthopoxviruses* and a positive DNA MCV sample.

Study of the specificity

It was realized by carrying out PCR from the

primers specific to OPV (EACP1, EACP2) and MCV (MCV1, MCV2) in the presence of DNA from different microorganisms mentioned in Table 1.

RESULTS

The PCR carried out using our positive Cowpox virus DNA control allowed us to validate our method of amplification. Indeed, two samples corresponding to the pure sample and the dilutions 10^{-1} of our controls were positive (FIG. 1). The dilution series of 10^{-2} to 10^{-5} of the Cowpox virus DNA control obtained from 200 µl of viral strain on Véro cell being negative by PCR thus fixing the detection threshold of our method at 10^{-1} for a volume of 200 µl. of viral strain. No amplification was detected with the DNAs from other pathogens frequently involved in cutaneous infections in Côte d'Ivoire (Figure 2).

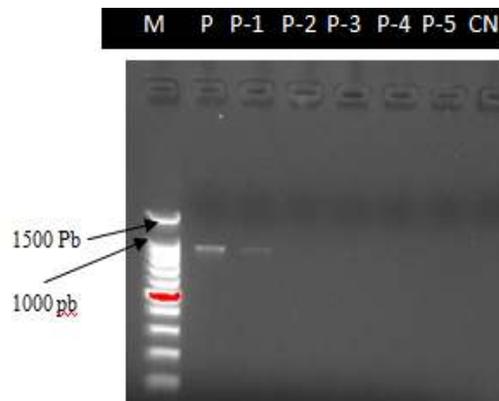


FIG 1 :DILUTION SERIES OF COXPOXVIRUS DNA

(P = undiluted Cowpoxvirus DNA, P-1 to P-5 = dilution series

CN: Negative control M: Marker (1500 bp to 100 bp)

This sensitivity could improve by performing extraction with a larger volume of viral strain.

The MCV detection tests were performed using cutaneous lesions from 21 patients diagnosed with

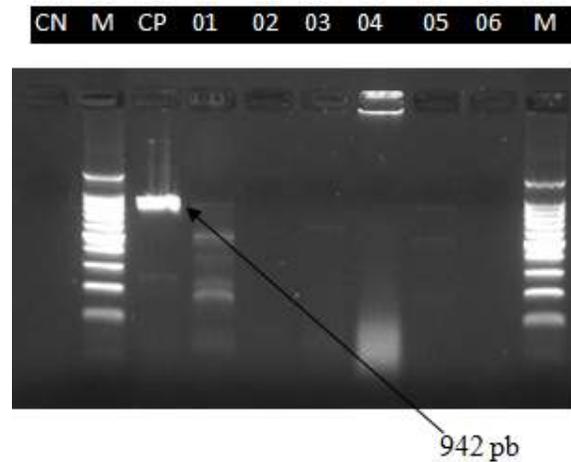


FIG 2 : SPECIFICITY TEST RESULT

CP = Cowpoxvirus (942 bp), 01 = MCV, 02 = 845 G (Measles virus, 03 = 258ub (M ulcerans), 04 = S aureus, 05 = 014 VZV (varicella

MCV infection clinically. The patients had the epidemiological and clinical characteristics presented in Table 2.

TABLE 2: CHARACTERISTICS OF THE 21 PATIENTS WHOSE SAMPLES WERE TESTED FOR THE DETECTION OF MCV

Number	Age	Sex	Clinical Lésion
1/YOP	15 Months	M	PAPULES
2/YOP	8 Years	M	PAPULES
5/YOP	24 Years	F	PAPULES
6/YOP	7 Years	F	VESICLES
7/YOP	4 Years	M	CRUSTS
8/YOP	3 Years	M	VESICLES
9/YOP	5 Years	F	MACULES
10/YOP	4 Years	F	PUSTULES
11/YOP	3 Years	M	MACULES
12/YOP	13 Years	M	VESICLES
13/YOP	7 Years	F	MACULES
14/YOP	5 Years	F	MACULES
2306	6 Years	F	VESICLES
2503	4 Years	F	PAPULES
2602	4 Years	F	VESICLES
2648	6 Years	F	PAPULES
2649	9 Years	M	PAPULES
2650	6 Years	F	PAPULES
2647	3 Years	F	PAPULES
2761	2 Years	F	Unspecified
2762	28 Years	F	Unspecified

A test for detection of MCV was carried out with the first three samples (1 / YOP, 2 / YOP, 5 / YOP). Each sample was diluted to 10^{-1} to reduce the risk of

PCR inhibition (Figure 3). The 167 bp PCR product was revealed for all three samples corresponding to positive MCV detection results.

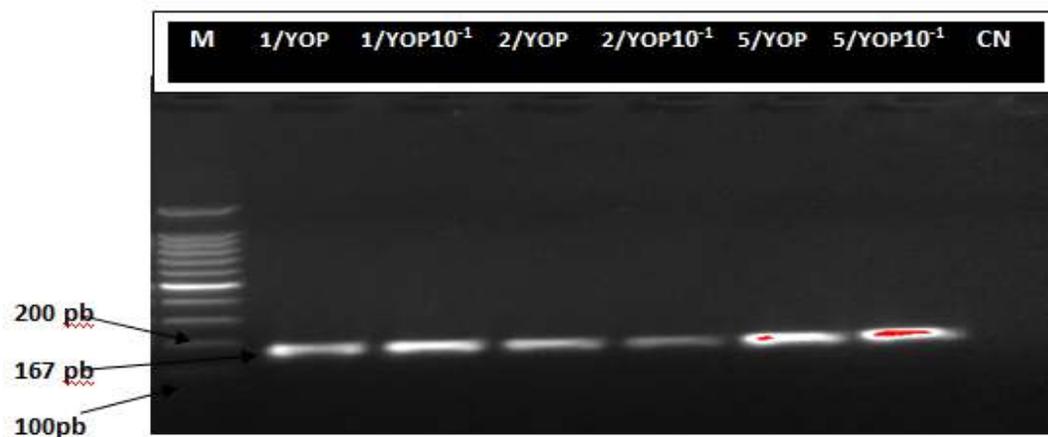


FIGURE 3: MCV PCR TEST OF SAMPLES

1/YOP, 2/YOP and 5/YOP, CN = negative control

A dilution series of 10^{-1} to 10^{-9} was carried out with the 1 / YOP sample in order to determine the sensitivity threshold of the PCR. Positive bands were observed up to 10^{-6} dilution (Figure 4). The specificity tests carried out with the DNA of other pathogens did not reveal nonspecific amplifications due to microorganisms often involved in human skin lesions. Of the 21 suspected samples of MCV

infection, 18 were confirmed on the 21, ie 85.71% agreement between the clinic and the molecular test used. Samples 2503, 2306 and 010/YOP were negative (Figure 5). Of the three negative samples, two were found to be positive for the conventional PCR detection of OPVs of sample 2503 and 010/YOP.

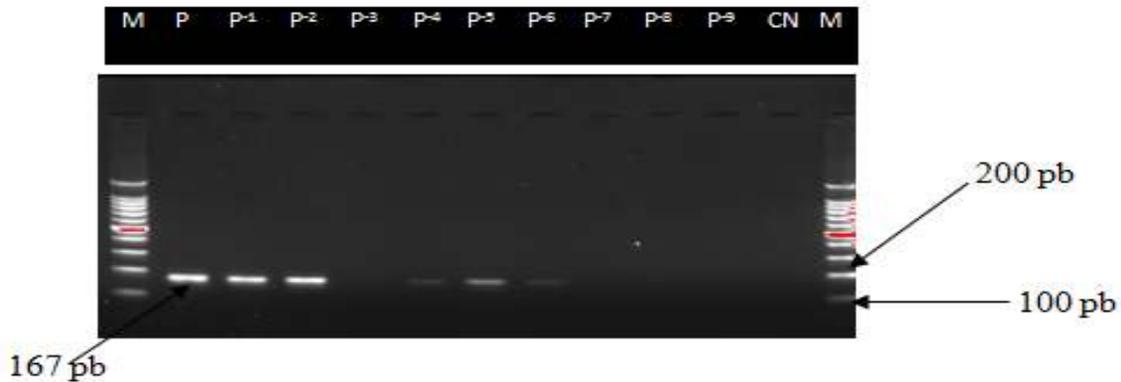


FIG 4: MCV DNA DILUTION SERIES

(P = undiluted MCV DNA, P⁻¹ to P⁻⁹ = dilution series, CN: Negative Control M: Marker (1500 bp to 100 bp)

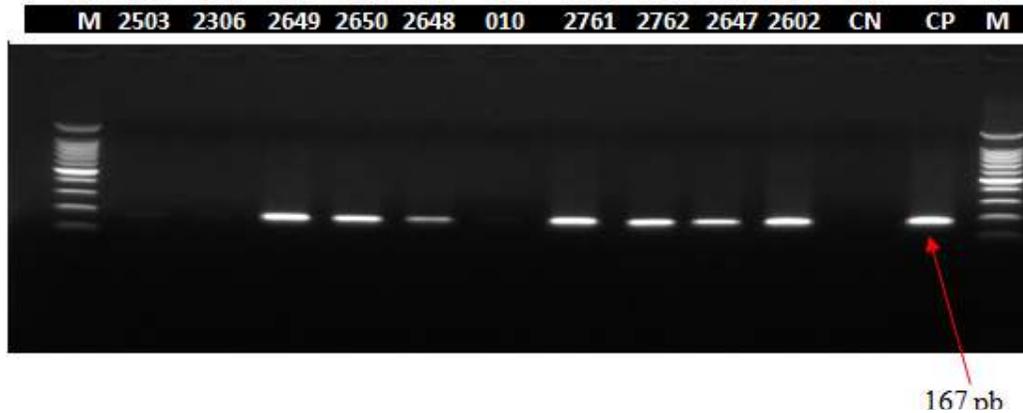


FIG 5 : RESULTS OF PATIENTS SUSPECTED OF MCV INFECTIONS

2503, 2306 and 010 = 10/YOP are negative. CN: Negative control CP: Positive control, M: Marker (1500 bp to 100 bp)

DISCUSSION

Molecular tools are increasingly being developed for the diagnosis of *Poxviruses* [13, 14, 15]. They have the advantage, on the one hand, of making a rapid and precise diagnosis and of others by avoiding the constraints of biosafety and biosurety related to the cultivation of *Poxviruses*; this development of the molecular detection for *Poxviruses* in our laboratory falls within this framework. It is one of the first studies in the establishment of the molecular diagnosis of *Poxviruses* in human medicine in Côte d'Ivoire. Despite the fact that this study may present biases related to the absence of a reference strain of

Poxvirus whose acquisition remains subject to rigorous measures in this context of global bioterrorism, this study constitutes a starting point in the implementation of the development of molecular tools for the detection of *Poxviruses* in Côte d'Ivoire. A discrepancy between the clinical diagnosis of MCV infection and the molecular outcome in three patients was observed in this study. Thus, two of the patients presented with OPV lesions. This clinical confusion is related to the similarity between the clinical lesions due to the different genera of the *Chordopoxviridae* subfamily. Thus, co-circulations have also been reported [16,

17, 18], hence the need for a biological diagnosis despite the strong clinical suspicion.

CONCLUSION

The emergence of Monkeypox virus in Central

Africa is a signal for West Africa. It is necessary to set up rapid diagnostic methods. This study is part of this approach. However, its sensitivity needs to be improved. Real-time PCR implementation is also a solution.

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RISK FACTORS ASSOCIATED WITH HIV PREVALENCE IN PREGNANT WOMEN IN BURKINA FASO, FROM 2006 TO 2014

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ABSTRACT

Purpose of the study: To determine the socio-demographic factors influencing the dynamics of HIV prevalence among pregnant women in Burkina Faso.

Material and methods: A total of 66,597 pregnant women from the 13 health regions of Burkina Faso were included in this study conducted between 2006 and 2014. Venous blood samples were collected and analyzed for the detection of HIV antibodies according to WHO / UNAIDS strategy II, using the mixed test Vironostika HIV Uniform II Plus O (Bio-Mérieux) and the test discriminating ImmunoCombII HIV-1 & 2 BiSpot (Organics). Samples with discordant results between the two tests, as well as those positive to HIV-2 or HIV-1 + 2, were retested with HIV BLOT 2.2 (MP Diagnostics). Sociodemographic data collected from the participants were correlated with their HIV status to determine key risk factors influencing HIV infection prevalence in Burkina Faso.

Results: Sociodemographic data showed that the study population consisted mainly of married women (91.2%) at their first pregnancy (27.1%) with a large majority of them being housewives (86.2%) who did not attend any form of schooling (69.4%). About 88.4% had stayed longer than a year in the health region where they initially participated in the study and 55.8% were between 20 and 29 years of age. Overall HIV prevalence significantly dropped from 2.7 % in 2006 to 1.3% in 2014. However HIV seroprevalence in this study has varied significantly according to socio-demographic characteristics including marital status, parity, occupation, education, age group and the length of stay in the women's health community ($p < 0.0001$). Factors sustaining HIV transmission included the status of being unmarried (OR=1.67 [1.42-1.97]), primigest (OR=1.64 [1.41-1.89]), having other occupations except being student (OR = 1.68 [1.20-2.33]), aged between 20-49 years (OR=3.14 [2.51-3.93]) and the duration of stay less than a year in their locality (OR=5.33 [4.61-10.16]) and these factors were identified as main risk factors associated with HIV prevalence.

Conclusion: Burkina Faso remains among the countries with concentrated epidemics despite a significant reduction in the prevalence observed in this study. The inclusion of identified risk factors in the national HIV program could improve the quality of the response to the epidemic.

Keywords: HIV-Pregnant Women-Risk Factors-Burkina Faso

FACTEURS DE RISQUE ASSOCIES A LA PREVALENCE DU VIH CHEZ LES FEMMES ENCEINTES AU BURKINA FASO, DE 2006 A 2014

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RESUME

But de l'étude: Déterminer les facteurs sociodémographiques influençant la dynamique de la prévalence du VIH chez les femmes enceintes au Burkina Faso.

Matériel et méthodes : Au total 66.597 femmes enceintes provenant des 13 régions sanitaires du Burkina Faso ont participé à cette étude, de 2006 à 2014. Leurs prélèvements sanguins ont été analysés pour la détection des anticorps anti-VIH selon la stratégie II de l'OMS/ONUSIDA, en utilisant le test mixte Vironostika VIH Uniforme II Plus O (Bio-Mérieux) et le test discriminant ImmunoCombII VIH-1&2 BiSpot (Orgenics). Les échantillons ayant donné des résultats discordants entre les deux tests, ainsi que ceux qui étaient positifs au VIH-2 ou au VIH-1+2 ont été soumis au test de confirmation HIV BLOT 2.2 (MP Diagnostics). Les données sociodémographiques recueillies chez les participantes ont été corrélées avec leur statut sérologique VIH pour déterminer les facteurs de risque.

Résultats : Les données sociodémographiques ont montré que la population d'étude était constituée principalement de femmes mariées (91,2%), primigestes (27,1%), ménagères (86,2%) et non alphabétisées (69,4%); 88,4% d'entre elles avaient séjourné 1 an ou plus dans la région sanitaire où elles ont participé à l'étude et 55,8% étaient âgées de 20 à 29 ans. La prévalence globale du VIH a baissé de 2,7% en 2006 à 1,3% en 2014. Elle variait significativement selon les caractéristiques sociodémographiques notamment la situation matrimoniale, la parité, l'occupation, la scolarité, la tranche d'âge et la durée de séjour dans la localité sanitaire des femmes ($p < 0,0001$). Les statuts de femmes non mariées (OR=1.67 [1.42-1.97]), primigestes (OR=1.64 [1.41-1.89]) ayant autres occupations que d'être élèves et étudiantes (OR=1.68 [1.20-2.33]), âgées de 20-49 ans (OR=3.14 [2.51-3.93]) et la durée de séjour <1an dans leur localité sanitaire (OR=5.33 [4.61-10.16]) constituaient des facteurs de risques associés à la prévalence du VIH.

Conclusion: Le Burkina Faso demeure parmi les pays à épidémie concentrée malgré une baisse significative de la prévalence observée dans cette étude. La prise en compte par le programme national de lutte contre le VIH des facteurs de risque qui ont été identifiés pourrait améliorer la qualité de la lutte contre cette épidémie.

Mots-clés : VIH-Femmes enceintes-Facteurs de risque-Burkina Faso

INTRODUCTION

Women represent a vulnerable fringe of the population that is more at risk to sexually transmitted infections (STI) particularly the human immunodeficiency virus (HIV) (1, 2). This is due in one hand to their genital anatomy exposing them to more infections during the sexual intercourse and in the other hand to their low socio-cultural and economic status (3, 4). Studies have shown that several factors, including age, multiple sexual partners, poverty, literacy and occupation, were associated to the occurrence of HIV infection (5, 6, 7, 8, 9, 10). Control programs that target these factors should help reduce the incidence of the infection.

In Burkina Faso, initial serosurveys reported a continuing downward trend in the seroprevalence of HIV infection over time with 7.17%, 6.15%, 4.2% and 2% in 1998, 2003, 2004 and 2005, respectively (11). In the absence of antiretroviral therapy, initial infection control programs focused on prevention of HIV transmission through screening in the general population, the etiologic diagnosis in patients, and on raising awareness through information, education and communication. Serosurveillance activities were already used to determine the national HIV prevalence and to track its dynamic over time. Certainly, the advent of highly active antiretroviral therapy (HAART) and especially its accessibility to populations of countries with limited resources have significantly impacted on the circulation of HIV in most sub-Saharan African countries. However, non-compliance to antiretroviral therapy leading to treatment failure in patients proved to be an additional risk factor associated with the spread of the virus or even death of patients.

The aim of this study was to determine the socio-demographic factors contributing to the dynamics of

HIV prevalence among pregnant women in Burkina Faso.

MATERIAL AND METHODS

Sites and period of study

The study was carried out in the 13 health regions of Burkina Faso: Boucle du Mouhoun, Cascades, Center, Center-East, Center-North, Central-West, East, South-Central, Hauts-Bassins, North, Central Plateau, Sahel and Southwest. Antibodies detection serological analyzes were carried out at the National Reference Laboratory for HIV/AIDS and Sexually Transmitted Infections (NRL-HIV/AIDS-IST) in the Department of Bacteriology-Virology of the University Teaching Hospital (UTH) Yalgado Ouédraogo in Burkina Faso. The study covered a nine-year period from 2006 to 2014.

Study population

Pregnant women between 15-49 years-old attending antenatal care visits in the selected health centers of the 13 health regions of the country were enrolled consecutively until the recommended sample size was completed: 800 in Ouagadougou in Central Region and in Bobo-Dioulasso respectively, and 400 in each of the other sites.

Collection of sociodemographic data and serum samples

A questionnaire was administered to all enrolled women to collect sociodemographic data. Ten milliliters of venous whole blood taken in sterile dry tubes from each pregnant woman were centrifuged to collect the serum, which was aliquoted in a sterile cryotube labeled and stored at -20°C before transfer to NRL-HIV/AIDS-IST.

Serological analyses

Sera were analyzed according to the WHO/UNAIDS HIV detection strategy II (12). Briefly, each serum was analyzed by a first very sensitive mixed test, Vironostika HIV Uniform II Plus O (Bio-Merieux, France). Any negative sample to this test was classified as "negative". Those found positive were re-analyzed by a second and discriminating assay, ImmunoCombII HIV-1&2 BiSpot (Orgenics) to identify the virus type (HIV-1, HIV-2 or HIV-1+2). Any discordant results between the two tests were classified as "indeterminate" temporarily. These sera, as well as those found positive to HIV-2 or both HIV-1/2 were submitted to a confirmatory assay, HIV BLOT 2.2 (MP Diagnostics): the results obtained by Western blotting were interpreted according to the WHO criteria (12). The final results were reported as negative or positive to HIV-1, HIV-2, both HIV-1/2, or indeterminate.

Ethical Considerations

All enrolled pregnant women were informed of the purpose of the study and verbally consented for their participation. The study used the WHO uncorrelated anonymous tests. The identification of each sample was correlated with the sociodemographic questionnaire of the corresponding participant.

Statistical analysis of data

Associations between patient sociodemographic characteristics and their serologic testing results were established to identify key exposing factors

influencing the prevalence of the infection. Data analyses were conducted using statistical packages EPI INFO version 7 and the MedCalc software. The statistical significance threshold was set at 0.05.

RESULTS

Sociodemographic characteristics of the study population

In total, 66,597 pregnant women were recruited over the study period with an average of 7399.66 inclusions per year. The inclusion rates ranged from 6,093 in 2013 to 7,872 in 2010 (Table I).

Married women were the most represented marital status with 91.2% compare to 8.6%, of single women, less than 1% of widows and less than 1% divorced women. Considering the number of children, women without children (27.1%) and those with single child (23.3%) were the most represented. According to their occupation, housewives (86.2%) were the most represented.

Study participants who attended school had predominantly elementary educational level (16.7%) between 2006 and 2011 and secondary educational level (16.6%) from 2012 onwards. Average number of women participating to the study that stayed in their health region over a period longer than a year was about 88.4%.

The median age of the study participants was 24 years (range from 15 to 49). Considering the study population as a whole, women in the age group 20-29 (55.8%) were the most represented follow by those in the age group 15-19 with 17.8%.

TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Parameters	2006	2007	2008	2009	2010	2011	2012	2013	2014
Marital status									
-Married	6,722 (90.8)	6,806(90.4)	6,977(88.7)	6,541(90.0)	7,208(91.5)	7,171(92.8)	6,693(91.8)	5,573(91.5)	7,046(93.2)
-Single	671(9.1)	665 (8.8)	888 (11.3)	688 (10)	658 (8.4)	546(7.1)	590 (8.1)	515 (8.5)	509(6.7)
-Widow	4 (0.1)	4(0.1)	0	3 (0.0)	5 (0.1)	5 (0.1)	4 (0.1)	2(0.0)	1(0.0)
-Divorced	0	2 (0.0)	1 (0.0)	0	1 (0.0)	0	1 (0.0)	2(0.0)	5 (0.1)
-Cohabiting	0	49 (0.7)	0	0	0	0	0	0	0
-Unspecified	0	0	0	0	0	0	0	4 (0,06)	0
Total	7,400 (100)	7,526 (100)	7,866 (100)	7,232 (100)	7,872(100)	7,724(100)	7,288(100)	6,092(100)	7,560 (100)

Parity									
0	1,981 (26.8)	1,950(26.3)	2,128(27.1)	1,915(26.5)	1,891(24.1)	1,999(26.0)	1,847(25.4)	1,519(24.9)	1,767(23.5)
1	1,586 (21.5)	1,611(21.7)	1,718(22.0)	1,512(20.9)	1,803(23.0)	1,749(22.7)	1,618(22.3)	1,338(22.0)	1,750(23.3)
2	1,234 (16.7)	1,153(15.5)	1,331(17.0)	1,226(17.0)	1,375(17.5)	1,310(17.0)	1,293(17.8)	1,145(18.8)	1,335(17.7)
3	913 (12.4)	939 (12.7)	988 (12.6)	936 (13.0)	975 (12.4)	937(12.2)	943 (12.9)	777 (12.8)	1,007(13.4)
4	627(8.5)	695 (9.4)	661(8.4)	661 (9.2)	701 (8.9)	681(8.8)	678 (9.3)	558 (9.2)	689(9.1)
5	403 (5.5)	456 (6.2)	472 (6.0)	414 (5.7)	460 (5.9)	441(5.7)	431 (6.0)	338 (5.5)	473 (6.3)
6 to 14	634 (8.6)	606 (8.2)	537 (6.9)	558 (7.7)	642(8.2)	582 (7.6)	454 (6.3)	416 (6.8)	502(6.7)
Total	7,378 (100)	7,410 (100)	7,835 (100)	7,222(100)	7,847(100)	7,699 (100)	7,264 (100)	6,091 (100)	7,523(100)
Occupation									
-Housewife	6,676 (90.2)	6,747(89.6)	7,047(89.7)	6,349(87.8)	6,888(87.5)	6,598(85.4)	6,018(82.5)	4,921(80.8)	6,153(81.3)
-Pupil/student	224 (3.0)	277 (3.7)	281 (3.6)	345 (4.8)	360 (4.6)	426 (5.5)	467 (6.4)	355 (5.8)	541(7.2)
-Trader	275(3.7)	218 (2.9)	235 (3.0)	183 (2.5)	248 (3.2)	349 (4.5)	338 (4.6)	282 (4.6)	430(5.7)
-Public servant	103(1.4)	115 (1.5)	119 (1.5)	105 (1.4)	133 (1.7)	140 (1.8)	182 (2.5)	128 (2.1)	178 (2.4)
-Artisan	33 (0.4)	105 (1.4)	24 (0.3)	56(0.8)	121 (1.5)	20 (0.3)	103 (1.4)	120 (2.0)	70 (0.9)
-Stallkeeper	20(0.3)	0	2(0.0)	1(0.0)	34(0.4)	0	22 (0.3)	19 (0.3)	2(0.0)
-Other	69(1.0)	65 (0.9)	151 (1.9)	192(2.7)	88 (1.1)	193 (2.5)	168 (2.3)	267 (4.4)	192(2.5)
Total	7,400 (100)	7,528 (100)	7,859 (100)	7,231 (100)	7,872 (100)	7,726 (100)	7,298 (100)	6,092(100)	7,566 (100)
Level of instruction									
-Primary	1,130 (15.3)	1,141(15.2)	1,311(16.7)	1,059(14.6)	1,191(15.1)	1,144(14.8)	1,001(13.8)	940 (15.4)	933 (12.3)
-Secondary	772 (10.4)	938 (12.5)	998(12.7)	1,048(14.5)	1,064(13.5)	1,080(14.1)	1,199(16.5)	1,004(16.5)	1,257(16.6)
-High	133 (1.8)	48 (0.6)	37 (0.5)	67 (0.9)	79 (1.0)	90 (1.2)	118 (1.6)	113 (1.9)	145 (1.9)
-Alphabetized	230 (3.1)	480 (6.3)	402 (5.1)	330 (4.6)	379 (4.8)	365 (4.8)	337(4.6)	243 (4.0)	383(5.1)
-Not alphabetized	5,129 (69.4)	4,919(65.4)	5,116(65.0)	4,727(65.4)	5,158(65.5)	5,002(65.1)	4,620(63.5)	3,791(62.2)	4,844(64.1)
-Unspecified	0	0	0	0	0	0	0	0	0
Total	7,394 (100)	7,526 (100)	7,864 (100)	7,231 (100)	7,871 (100)	7,681 (100)	7,275 (100)	6,091 (100)	7,562 (100)
Time spent in the health region									
<1 year	992 (13.5)	1,031(13.7)	889 (11.3)	874 (12.1)	860 (11.0)	809(10.5)	890 (12.2)	651 (10.7)	684(9.0)
≥1 year	6,373 (86.5)	6,490(86.3)	6,970(88.7)	6,356(87.9)	6,976(89.0)	6,903(89.5)	6,396(87.8)	5441(89.3)	6,879(91.0)
Unspecified	0	0	0	0	0	0	0	0	0
Total	7,368 (100)	7,521 (100)	7,859 (100)	7,230 (100)	7,836 (100)	7,712 (100)	7,286 (100)	6,092(100)	7,563 (100)
Age groups (years)									
15-19	1,460 (19.7)	1,474(19.6)	1,538(19.5)	1,277(17.7)	1,329(16.9)	1,347(17.4)	1,196(16.4)	943 (15.5)	1,328(17.6)
20-24	2,324 (31.4)	2,443(32.5)	2,495(31.7)	2,183(30.2)	2,542(32.3)	2,403(31.1)	2,284(31.3)	1,922(31.5)	2,164(28.6)
25-29	1,750 (23.6)	1,776(23.6)	1,885(23.9)	1,916(26.5)	1,907(24.2)	1,914(24.8)	1,869(25.6)	1,620(26.6)	1,953(25.8)
30-34	1,109 (15.0)	1,067(14.2)	1,177(14.9)	1,155(15.9)	1,249(15.9)	1,253(16.2)	1,205(16.5)	1,006(16.5)	1,317(17.4)
35-39	579 (7.8)	587 (7.8)	605(7.7)	564 (7.8)	658 (8.3)	647 (8.4)	585 (8.0)	481 (7.9)	659(8.7)

40-44	159 (2.1)	154 (2.0)	144(1.8)	126 (1.7)	161(2.0)	143(1.9)	142 (2.0)	106 (1.7)	124(1.6)
45-49	19(0.3)	24 (0.3)	22 (0.2)	11 (0.2)	26 (0.3)	18 (0.2)	13 (0.2)	15 (0.2)	17(0.2)
Unspecified	0	0	0	0	0	0	0	6(0.1)	0
Total	7,400 (100)	7,524 (100)	7,866 (100)	7,232 (100)	7,872 (100)	7,725 (100)	7,294 (100)	6,093 (100)	7,562 (100)

Annually prevalence of HIV

Yearly HIV seroprevalence steadily decreased from 2.7% in 2006 to 1.3% in 2014 (Table II). However, a significant increase in seroprevalence was observed between 2008 (2.0%) and 2009 (2.2%) ($p = 0.000$; $Chi2 = 18$).

The majority of sera were diagnosed with HIV 1 or HIV 2 infections (antibodies to these viruses were detected every year) contrary to HIV-1+2 co-infection (Table II).

TABLE II: ANNUALLY REPARTITION OF HIV PREVALENCE OVER THE STUDY PERIOD

Year of study	Number tested	HIV-positive					Prevalence [IC95%]
		HIV-1	HIV-2	HIV-1+2	Total		
2006	7,400	186	10	4	200	2.7 [2.4-3.1]	
2007	7,576	160	7	0	167	2.2 [2.0-2.7]	
2008	7,866	152	7	2	161	2.0 [1.8-2.4]	
2009	7,232	146	8	6	160	2.2 [1.9-2.6]	
2010	7,872	124	6	0	130	1.6 [1.4-2.0]	
2011	7,702	124	6	0	130	1.7 [1.4-2.0]	
2012	7,294	116	4	0	122	1.7 [1.4-2.0]	
2013	6,093	81	5	4	90	1.5 [1.2-1.8]	
2014	7,562	93	2	0	95	1.3 [1.0-1.5]	

HIV seroprevalence according to the socio-demographic characteristics of the participants

Marital status

HIV seroprevalence was consistently higher (2.86) in single pregnant women than in married pregnant women (1.77) (Table III). This difference was statistically significant ($p < 0.0001$; $Chi2 = 673.9$), despite the general decline in rates of HIV infections over the years.

Parity

HIV seroprevalence varied significantly according to the parity of the women (Table III) and it appears higher in nulliparous women than multiparous women ($p < 0.0001$, $Chi2 = 484.1$).

Occupation of pregnant women

Rates of HIV infections were higher in traders, public servants and artisan respectively (3.20%, 3.15%, 3.98%) (Table III), than in pupil/student (1.12%) or in housewife (1.77%). The differences observed between

HIV seroprevalence by type of occupation were statistically significant ($p < 0.0001$). HIV prevalence was significantly higher (1.80) among other occupations than among pupil/students (1.12) and the difference was statistically significant ($p < 0.0001$; $Chi2 = 1007.8$).

Level of education of pregnant women

HIV seroprevalence varied significantly between in secondary (2.71%), primary (2.68%), tertiary (1.92%), literate (1.52%) and not alphabetized (1.60%) ($p < 0.0001$; $Chi2 = 34.0$.) The difference between literate and not alphabetized was not statistically significant ($p = 0.69$, $Chi2 = 0.157$).

Residency time in health regions

The rate of HIV infection varied according to the women length of stay in their locality. Rate was higher

(3.16%) in women who stayed for a shorter duration year (1.71%) over the study time. This difference was statistically significant ($p<0.0001$, $Chi^2=465.7$).

Age of pregnant women

The results obtained during the serosurveillance years showed that HIV infections were more pronounced in women of 35 to 39 years old (3.09%) than in the other

in their locality than those who stayed longer than a age groups (Table III). The lowest seroprevalence (0.68%) was found in the 15-19 years age groups. The observed difference in prevalence between the age groups was statistically significant ($p<0.0001$; $Chi^2=872.8$).

TABLE III: GLOBAL HIV SEROPREVALENCE BY SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PREGNANT WOMEN, 2006-2014.

Parameters	Positive	Negative	Total (n)	Prevalence (%)
Marital status				
Married	1,081	59,656	60,737	1.77
Single	164	5,566	5,730	2.86
Widow	3	25	28	10.71
Divorced	0	0	0	0
Cohabiting	4	45	49	8.16
Parity				
0	221	16,776	16,997	1.30
1	304	14,381	14,685	2.07
2	267	11,135	11,402	2.34
3	194	8,221	8,415	2.30
4	120	5,170	5,290	2.26
5	80	3,808	3,888	2.05
6 to 14	72	4,859	4,931	1.46
Occupation				
Housewife	1,019	56,378	57,397	1.77
Pupil/student	37	3,239	3,276	1.12
Trader	82	2,476	2,558	3.20
Public servant	38	1,165	1,203	3.15
Artisan	26	626	652	3.98
Stallkeeper	1	99	100	1.00
Other	56	1,329	1,385	4.04
Level of instruction				
Primary	264	9,586	9,850	2.68
Secondary	227	8,135	8,362	2.71
High	16	814	830	1.92
Alphabetized	48	3,101	3,149	1.52
Not alphabetized	697	42,609	43,306	1.60

Time spent in the health region				
<1 year	243	7,437	7,680	3.16
≥1 year	1,007	57,777	58,784	1.71
Age groups (years)				
15-19	82	11,810	11,892	0.68
20-24	235	20,525	20,760	1.13
25-29	452	16,138	16,590	2.72
30-34	292	10,246	10,538	2.77
35-39	166	5,199	5,365	3.09
40-44	22	1,237	1,259	1.74
45-49	2	163	165	1.22

Risk factors associated with HIV infection

Unmarried women (single, cohabiting, widowed and divorced) were 1.67 times more likely to be infected with HIV than married women. Nulliparous women (women with no child) were 1.64 times more likely to be infected with HIV than those with one or more children.

All other occupations combined were 1.68 times more likely to be infected than pupil/students. The risk of HIV infection was 3.14 times higher among women aged 20-49 than among those aged 15-19. The pregnant women with a residence time of less than one year in a health region was 5.33 times more likely to be infected with HIV than those with duration of stay of one year or more. Odds ratio were respectively for primary, secondary and high: 0.98[0.82-1.18]; 1.40[0.84-2.33]; 1.41[0.85-2.36]. These various risk factors to HIV infection are reported in Table IV.

DISCUSSION

Sociodemographic characteristics of the study population

Study participants were predominantly married women (91.2%), and primigravidae accounted for almost 27.1%, with a majority of them being housewives (86.2%), and not alphabetized (69.4%). Almost 90% of them stayed longer than a year in the initial health region they were enrolled in the study, and about half of them were between 20-29 years of age. These rates are descriptive of the African context, as national HIV sentinel surveillance studies were generally conducted in pregnant women who are accessible during antenatal care visits (13).

Global HIV Prevalence

Overall HIV seroprevalence decreased significantly over time from 2.7% in 2006 to 1.3% in 2014. However, Burkina Faso remains one of the countries with "moderate prevalence" globally (1% -3.9%) and a

generalized epidemic among pregnant women (>1%) according to the WHO classification (14). Such a decrease was also reported in the 15 to 49 years old pregnant women in other studies, particularly in West African countries from 4.3% to 2.9%, and Eastern African countries from 3.6% to 2.9% (15), in Malawi from 15.0% to 10.6% (16), and in Uganda from 28.3% to 25.1% (17) even though most of these countries had higher national prevalence than Burkina Faso.

HIV prevalence and marital status of pregnant women

Despite significant variations in HIV prevalence in the study population over the 9-years period, results showed that infection rates were consistently higher in single women (2.86%) than in married women (1.77%). In spite of the variation in the prevalence between the various statuses, it appears in this study that being unmarried represented an additional risk factor increasing the likelihood of getting infected with HIV in comparison to the married participants (OR=1.67 [1.42-1.97]). These results were significantly high in divorced women (18.9%) in Tanzania (18) and never-married women (6.8%) in Uganda (19). Santelli *et al.*, (9) identified married status as a risk factor of HIV infection in 15-24 years old women [Adjusted IR Ratio (AIRR) = 0.55, 95%CI: 0.37-0.81]. The results were also different from those reported in Ethiopia, suggesting that married women were 3.29 times more at risk of infection with HIV (95% CI [0.43 -20.00]) than unmarried women (20). On the other hand, they are comparable to those found in Uganda (21) in 15-49 years old women that reported a lower risk of having HIV among brides compared to those never married (AIRR= 0.26, 95%CI: 0.16-0.42) and also comparable to those in Tanzania (22) where unmarried women (6.8%) were more likely to be infected with HIV than married women (5.4% 95% CI: 1.13-1.45, $p<0.05$) and divorced (5.1%).

TABLE IV: RISK FACTORS ODDS RATIO

Parameters		Number of samples		<i>p</i> value	Odds Ratio
		Positive	Negative		
Marital status	Unmarried	171	6,636	0.000	1.67 [1.42-1.97]
	Married	1,081	59,656		
Parity	1 children or more	1,037	48,611	0.000	1.64 [1.41-1.89]
	0 children	221	16,997		
Occupation	Other types of occupation	1,166	60,744	0.000	1.68 [1.20-2.33]
	Pupil and student	37	3,239		
Age group (years)	20-49	1,169	53,508	0.000	3.14 [2.51-3.93]
	15-19	82	1,810		
Length of stay	<1 year	243	7,437	0.000	5.33 [4.61-10.16]
	1 year or more	1,007	57,797		
Education level	Primary	264	9,586	0.88	0.98 [0.82-1.18]
	Secondary	227	8,135		
	Primary	264	9,586	0.19	1.40 [0.84-2.33]
	High	16	814		
	Secondary	227	8135	0.17	1.41 [0.85-2.36]
	High	16	814		

HIV Prevalence and women's occupation

The prevalence of HIV declined over the years in all occupational categories. However, it remained significantly lower in student (1.12%) when compared to other occupations considered all together (1.88%). Our results were comparable to those reported in Tanzania (18) with a higher prevalence among traders (13.1%) and the data reported by Mengistu *et al.*, (20) in Ethiopia where women traders had 2.07 times more risk (95% CI [0.46-8.85]) of being infected with HIV. In addition, the study indicated that being student was not associated with the occurrence of infection conversely to all other occupations (OR = 1.68 [1.20-2.33]). However, a study in Uganda (9) showed that student status was a factor associated with a high risk of HIV infection among women aged 15-24 years (AIRR= 0.22).

Prevalence of HIV infection and level of education

HIV prevalence was not statistically significant between non-alphabetized participants and those with some level of literacy. Furthermore, school level (primary, secondary and high) was not a risk factor associated with HIV infection (OR=0.98[0.82-1.18];

1.40[0.84-2.33]; 1.41[0.85-2.36]). Several studies conducted in other countries have reported contrasting results with high prevalence in non-alphabetized women 13.4% in Tanzania (18) and 3.9% in Uganda (19). This contrasted the results observed in Sekondi-Takoradi, Ghana (23), which reported that pregnant women at the secondary and tertiary levels were less likely to be infected with HIV than those who did not attend primary school (OR=0.53). It was different also from the data reported in India by Darak *et al.*, (24) indicating that pregnant women with less than 11 years of schooling were significantly more at risk of contracting HIV [Adjusted Odds Ratio (AOR) = 2.4].

HIV prevalence and age participants

HIV seroprevalence was significantly higher ($p<0.0001$) in the 25-29, 30-34 and 35-39 years age-group. This age range corresponds to the most active period of sexual and fertility life for women in Burkina Faso. The very low prevalence (0.68%) in adolescents (15-19 years) during the nine years period of the study would indicate a behavioral change towards compliance with HIV prevention measures.

The study also found that the 20-49 years age group was a potential risk factor for HIV infection compared to the 15-19 years age group (OR=3.14 [2.51-3.93]; $p=0.000$). These results are similar to those obtained in Tanzania which showed that women of 25-34 years old (COR=1.97, 95%CI: 1.79-2.16, $p<0.05$) and those older than 35 years (COR=1.88, 95%CI: 1.62-2.17, $p<0.05$) were more at risk of being infected compared to those in the age group of 15-24 years (22). It was also similar to results revealed in India, which reported higher risk for HIV infection in women of 25 years old onwards (AOR: 1.38; 95% CI: 1.17 to 1.61) compared to those less than 25 years old (24). However, Mengistu and *al.*, (20) found no statistically significant difference between age groups and HIV prevalence.

Prevalence and parity of women

The prevalence significantly varied with parity ($p<0.0001$). It exceeded 2% in women with 1 to 5 children and was less than 1% in nulliparous and the large multiparous (6 to 14 children) women. Primigradae were 1.64 times more likely to be infected with HIV than other parities combined (OR=1.64 [1.41-1.89]). These results were different from those reported in Uganda (19) where prevalence was higher (7.5%) in women with 3 children than women who had 1 child (4.1%) and in Ethiopia (20) with high prevalence (12.2%) in multipara than primipara (9.2%). This result could be useful to direct control efforts towards the most vulnerable groups for the prevention of mother to child transmission (MTCT) in Burkina Faso.

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Prevalence and length of stay of women in the health region

Prevalence was significantly higher (3.16%) in women with a residence time less than a year in the initial enrollment health region ($p<0.0001$). Consequently, a shorter duration appeared as a risk factor associated with HIV infection in the study population (OR=5.33 [4.61-10.16]). This result is different from that observed in Tanzania (22) where there was no significant difference between the risk of having HIV and the length of stay of women in their residency. Our results could convey the existence of high-risk sexual behavior in women with shorter duration of stay (26) and the mobility of people living with HIV (27). Migration is a phenomenon that can expose women to more risk of HIV infection (7, 8, 10, 28).

CONCLUSION

Based on the results of this study carried out in Burkina Faso over nine years period, it appears that HIV prevalence has significantly decreased in the country over time. This is an encouraging finding, and represents the will and expectations of all actors involved in the fight against HIV and also testifies for the adequacy of programs developed and implemented to control HIV transmission in the population of Burkina Faso. However, Burkina Faso still remains among the countries with generalized epidemics. Risk factors associated with HIV infection were unmarried, first pregnancy, occupation, age group 20-49, and population mobility.

Awareness raising programs for students and teenage women should be extended to other socioeconomic strata and older women to reduce risk factors independently of prevalence in Burkina Faso.

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RESIDUAL MOTHER-TO-CHILD TRANSMISSION OF HIV IN BURKINA FASO

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ABSTRACT

Background: Burkina Faso is one of the countries in West Africa most affected by the HIV/AIDS pandemic, despite the implementation of a mother-to-child HIV transmission prevention program as a strategy to reduce the risk of vertical transmission of the disease.

Objective: To assess the current risk of mother-to-child transmission of HIV in Burkina Faso.

Materials and methods: A prospective study was conducted between December 2014 and July 2016, in the 13 health regions of Burkina Faso. Women who were screened HIV-positive during a prenatal consultation were followed until delivery. Their babies received dry blood spot (DBS) at birth, at week 6 and at 1 year, to screen for HIV.

Results: Overall, 186 pregnant women were included in the study, with a mean age of 29.17±6.13 years. Of their children, 430 DBS actually received a PCR test, giving a 91.1% PCR implementation rate. After analyses, 6 (1.3%) babies were identified as carriers of HIV1. The newborn's serological status was associated with delivery pattern (p=0.000), the administration of antiretroviral drugs to the mother after delivery (p=0.0064), the administration of Nevirapine to the newborn at birth (p=0.022), the use of contraceptive methods after delivery (p=0.028) and the presence of breast affections/infections since delivery (p=0.013).

Conclusion: The results of our study are encouraging and demonstrate the effectiveness of interventions in the mother-to-child prevention program (PMTCT) for HIV-positive pregnant women can be improved through early initiation of triple therapy in early pregnancy and improved adherence to antiretroviral (ARV) therapy.

Keywords: Burkina Faso, HIV/AIDS, mother-to-child transmission, antiretroviral drugs, pregnant women

RÉSIDUS DE LA TRANSMISSION MÈRE-ENFANT DU VIH AU BURKINA FASO

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RÉSUMÉ

Contexte : Le Burkina Faso fait partie des pays les plus touchés par la pandémie du VIH/sida, malgré la mise en place d'un programme de transmission mère/enfant du VIH comme stratégie de prévention de la transmission verticale.

Objectif : l'objectif était d'évaluer le risque résiduel actuel de transmission du VIH de la mère à l'enfant au Burkina Faso.

Matériel et Méthode : Il s'agit d'une enquête prospective simple à visée descriptive et analytique réalisée entre décembre 2014 et juillet 2016, dans les 13 régions sanitaires du Burkina Faso. Les femmes reçues en consultation prénatale dépistées séropositives ayant donné leur consentement éclairé étaient suivies jusqu'à l'accouchement. Leurs enfants ont bénéficiés d'un prélèvement Dry Blood Spot (DBS) à la naissance, à la 6ème semaine de vie et à un an pour la recherche du VIH par la PCR.

Résultats : Au total 186 femmes enceintes ont été enregistrées dans l'ensemble des sites de l'étude dont l'âge moyen était de 29,17 ans avec un écart type de $\pm 6,13$. A partir des enfants nés de ces femmes, 430 DBS ont effectivement bénéficié d'un examen PCR soit 91,1% de taux de réalisation de PCR. A la suite des analyses six (06) enfants étaient porteurs de VIH1 soit 1,3% de la population d'enfants testés. Le statut sérologique du bébé était associé au mode de délivrance ($p = 0,000$), à l'administration de médicaments antirétroviraux à la mère après l'accouchement ($p = 0,0064$), à l'administration de la névirapine au nouveau-né à la naissance ($p = 0,022$), à l'utilisation de méthodes contraceptives après l'accouchement ($p = 0,028$) et à la présence des affections/infections au sein depuis l'accouchement ($p = 0,013$).

Conclusion: Les résultats de notre étude sont encourageants et démontrent que l'efficacité des interventions dans le programme de prévention de la transmission mère l'enfant (PTME) pour les femmes enceintes séropositives peut être améliorée par l'initiation précoce de la trithérapie au début de la grossesse et l'amélioration de l'observance des antirétroviraux (ARV).

Mots-clés: Burkina Faso, VIH / SIDA, transmission mère-enfant, médicaments antirétroviraux, femmes enceintes

INTRODUCTION

Burkina Faso is one of the countries in West Africa most affected by HIV/AIDS. The first estimate of HIV seroprevalence in the general population in 1997 was 7.1% (1,2). In the pregnant population, mean HIV seroprevalence in seroprevalence sites (sentinel sites in the health district where mother-to-child transmission of HIV is actively monitored) was 6.5% in 2002 (3, 4). In 2006, this figure was estimated at 2.5% and at 2% in 2010, according to a joint United Nations Program on HIV/AIDS/World Health Organization (UNAIDS/WHO). This reduction in seroprevalence required a series of measures to control the disease, initially based on education sensitization and information. Since the mid-1980s, several researchers have studied mother-to-child transmission of HIV. These studies were needed to provide estimates on mother-to-child HIV transmission, demographic forecasts, to compare rates of transmission in different epidemiological contexts and to understand the determinants of mother-to-child transmission to identify factors amenable to interventions and counseling services (individual counseling and care for mothers and children) (2).

As of 2000, a strategic plan to combat HIV/AIDS and sexually transmitted infections (STIs) was adopted by the Burkina Faso government. The strategic plan identified areas for action, including reducing the spread of HIV as a result of a national program to prevent mother-to-child transmission of HIV (PMTCT/HIV). Since 2002, Burkina Faso has implemented a program to prevent mother-to-child transmission of HIV, aimed at increasing the number of women giving birth to children free from HIV/AIDS. The first PMTCT/HIV program was implemented between 2002 and 2005 and was used as a prevention strategy against vertical transmission. The program included the provision of higher-quality and lower-risk obstetric care, the administration of Nevirapine to the mother peripartum (2 mg/kg bodyweight) and the choice between exclusive breast milk substitutes or exclusive breastfeeding for up to 4 months,

followed by early weaning (5,6). The second program, implemented between 2006 and 2010, used the same strategies, except the antiretroviral (ARV) regimen, which included the administration of three ARVs: one from the 28th week of pregnancy, three peripartum and two postpartum (5,6,7). This program reduced the risk of vertical transmission to less than 5%, when properly applied (WHO, 2004). The third PMTCT/HIV program, for the period between 2011 and 2015, proposed prophylaxis or ARV treatment protocols from others, to reduce mother-to-child transmission of HIV. Secure feeding and ARV prophylaxis were also offered to the newborn infant. This program uses two sequential protocols in options A and B, (initially 3 years for option A and 2 years for option B). Option A was introduced in 2010 and includes a single dose of ARVs for women (if their CD4 count is over 350) from the 14th week of pregnancy, as well as ARV during labor and delivery, and for 1 week after birth. Option B, introduced by WHO at the same time as option A, consists of antiretroviral combination therapy from the 14th week of pregnancy until 1 week after the end of breastfeeding, to 1 year (8). A requirement for moving from one option to the next is to bring together the human, material and financial means necessary to make this change. In the end, option A lasted for 4 years, from 2011 to 2014. Other innovations include early prophylactic care that corresponds to the 14th week of pregnancy and the concept of safe breastfeeding. The aim of this program, which is based on WHO option B+ (trithérapie as soon as the mother is notified of her HIV-positive status and treatment of the child (NVP/AZT) for 4 to 6 weeks), is to eliminate mother-to-child transmission of HIV in Burkina Faso.

A previous residual transmission cross-sectional study, conducted in 2008, highlighted a very high prevalence of 3.3% (9). It is in this context the present study was initiated; with the aim of assessing the impact of the intervention on residual vertical transmission of HIV and to identify its determinants, to reduce the risk of further transmission.

MATERIALS AND METHODS **Study design and sampling**

This prospective study was conducted between December 2014 and July 2016, in the Central and Hauts-Bassins regions of Burkina Faso. Convenience sampling at two levels (district and region) was performed and took into account all 13 health regions in the country. Health districts with the highest HIV prevalence among pregnant women were selected. In addition, PMTCT sites with the best immunization coverage (at 6 or 10 weeks) were also selected. Overall, 10 PMTCT sites in each of the three major districts of the Central and Hauts-Bassins regions and one or two PMTCT sites in other areas of the country were selected. Data collection involved 34 PMTCT sites in 14 health districts.

Target population

The target population was pregnant women identified as HIV-positive during the prenatal consultation screening and HIV-positive breastfeeding women and their babies, who gave their informed consent to participate in the study.

Sample size

The sample size was estimated at 155 pregnant women and 132 children. The number of HIV-positive women needed was estimated as follows: The total number of HIV-positive women expected in the district on the number of PMTCT sites in the district should be divided by four (the number of quarters in a year). Total number of HIV-positive women expected in the district = number of HIV-positive women expected per PMTCT site during the recruitment phase, which is 3 months. The number of children needed was estimated on the basis of the expected number of children born to HIV-positive mothers in PMTCT sites retained for 3 months.

Data collection

Enrollment of pregnant women was done during the prenatal consultation, regardless of the stage of pregnancy. A questionnaire was developed to gather data on socio-demographic characteristics and to identify risk factors for mother-to-child transmission. This included monitoring of women in the study from pregnancy to postpartum, compliance with refocused schedules for prenatal consultations, compliance with the applicable PMTCT protocol (by infected pregnant women, infected parturients, nursing mothers, exposed children), type of delivery (surgery or vaginal delivery, at home or at the health center) and invasive practices during childbirth, newborn and infant feeding patterns and the evolutionary phase of HIV infection.

Dry blood spot (DBS) samples were obtained from babies and used for PCR at birth, at 6 weeks of life when screened negative at birth, and finally at

1year, when the first two tests were negative by PCR. A child was declared negative after three negative PCR tests. The sampling was performed on the newborn's heel on the lateral or medial side of the foot. Cards (DBS) were dried at laboratory temperature, out of direct sunlight on a rack, for at least 3 hours or overnight. They were then stored in plastic bags with a desiccant at -20° C.

Laboratory analysis

In the laboratory, DNA extraction was performed using the extraction kit on the DBS. After extraction, DNA amplification was performed using the real-time PCR kit for the qualitative or quantitative detection of HIV-1 cellular DNA. The evolution of the amplification is represented by a sigmoid-like curve, which can be divided into two phases. At the beginning of the exponential amplification phase, the time when the signal leaves the background noise corresponds to a number of cycles called Ct (threshold cycle); during this exponential amplification phase, the quantity of PCR products obtained at each moment directly depends on the initial copy number. The second phase is a plateau phase, which corresponds to a slowing-down of the amplification of the reaction because of depletion of reagents.

Reading and interpretation of results

The PLC is equipped with a system enabling analysis of the results. It determines the threshold value of the reaction as well as the Ct value of each standard of the range i.e. the intersection between the threshold value and the amplification curve. The Ct values corresponding to the samples of unknown values are reported on the right of the abscissas, and then the number of copies of DNA/PCR is extrapolated. Results are qualitative.

Ethical Considerations

The study was approved by Burkina Faso Ethics Committee for Health Research through proceedings N° 2014-8-101.

RESULTS

Socio-demographic characteristics

A total of 186 pregnant women were enrolled on the study, across all the study sites. Of the participants, 70.4% were from the central region, 9.7% from Hauts-Bassins and 7.5% from the northern regions. Age ranged from 17-43 years, with a mean age of 29.17±6.13 years. Overall, 47.8% of women had not attended school, 21.5% had primary and 25.2% had secondary school level education. Less than 2% of participants had a higher education level. **Mother's medical history** More participants who shared their positive serology results with their partner gave birth to uninfected newborns, compared with those who did not share their results (p=0.009).

Information on delivery
In this study, 95.5% of women had a full-term pregnancy and 96% delivered in a health center (63.2% in the health center where they had their prenatal consultations and 32.8% in other health

centers). In total, 90.9% of women had vaginal deliveries however, 4.5% of the study population gave birth prematurely and 4.01% gave birth at home.

TABLE 1: BIVARIATE ANALYSIS OF A SAMPLE OF HIV-POSITIVE PREGNANT WOMEN IN BURKINA FASO (N=186)

Variable	Terms	Newborn's Serology			Chi-square Value	P Value Meaning
		Negative (%)	Positive (%)	Aggregate (%) (All women irrespective of the serology of the baby)		
Age group of surveyed individuals	Under 25 years	20.4	33.3	20.9	0.678	0.712 (ns)
	25-34 years	53.5	50.0	53.4		
	Above 35 years	26.1	16.7	25.8		
Literacy Level	Illiterate	48.8	33.3	48.3	2.078	0.721 (ns)
	Primary school	20.3	16.7	20.2		
	Secondary school	25.0	50.0	25.8		
	Higher education	1.7	0.0	1.7		
	Other	4.1	0.0	3.9		
Marital Status	In couple without co-spouse	73.3	50.0	72.5	8.087	0.044 (**)
	In couple with co-spouse	17.4	16.7	17.4		
	Single /divorced	5.2	33.3	6.2		
	Other	4.1	0.0	3.9		
Residence Area	Urban	73.8	83.3	74.2	0.273	0.512 (ns)
	Rural	26.2	16.7	25.8		
Occupation	Public Sector employee	4.7	16.7	5.1	11.529	0.073 (ns)
	Private Sector employee	4.1	0.0	3.9		
	Merchant	14.5	0.0	14.0		
	Farmer	1.2	0.0	1.1		
	Pupil /student	1.2	16.7	1.7		
	Housewife	67.4	66.7	67.4		
	Other	7.0	0.0	6.7		

ns = not significant, ** = significant at 5% ; Marital status (couple, or divorced/single) was significantly associated with the newborn's positive serological status (p=0.044).

TABLE 2: BIVARIATE ANALYSIS OF THE MOTHER'S MEDICAL HISTORY IN A SAMPLE OF HIV-POSITIVE WOMEN IN BURKINA FASO (N=186).

Variable	Terms	Newborn's Serology			Chi-Square Value	Meaning
		Negative	Positive	Aggregate		
Number of prenatal consultations corresponding to the start of ARV treatment.	CPN 1	62.4	40.0	61.4	3.287	0.349 (ns)
	CPN 2	25.7	40.0	26.3		
	CPN 3	7.3		7.0		
	CPN 4	4.6	20.0	5.3		
How many weeks do you estimate the length of this ARV treatment before childbirth	Less than 4 weeks	7.8		7.5	0.419	0.674 (ns)
	At least 4 weeks before delivery	92.2	100.0	92.5		
Sharing results with your partner	Yes	55.7	0	53.7	7.211	0.009 (**)
	No	44.3	100.0	46.3		

ns = not significant, ** = significant at 5%

There was a significant association between the newborn's serological status and the pattern of delivery (p=0.000); the risk is high when it comes to a guided delivery. Managing the woman without the partner's support increased the risk of HIV transmission for the newborn (p=0.009). Antiretroviral drug administration to the mother after delivery (p=0.044) and administration of Nevirapine to newborns at birth was associated with a lower rate of seropositivity in newborns

receiving Nevirapine at birth, compared with those who did not receive this treatment (p=0.022).

PCR results

Three (1.96%; 95% confidence interval [CI] 0-4.6) babies were HIV-positive at birth. Two (1.34%; 95%CI 0-3.4) babies were HIV-positive at 6 weeks of age (both of these babies were born negative). One baby (0.78%; 95%CI 0-2.3) was HIV-positive at 12 months of age. This baby was negative at birth but could not be tested at 6 weeks of age because his mother was missing.

TABLE 3: BIVARIATE DATA ANALYSIS ON DELIVERY

Variable	Terms	Newborn's Serology			Chi-Square value	Meaning
		Negative	Positive	Aggregate		
Provide information on delivery pattern	Natural	8.6	0.0	8.3	20.527	0.000 (***)
	Artificial	10.5	0.0	10.1		
	Guided	79.0	66.7	78.6		
	Don't know	1.9	33.3	3.0		
Were there any complications during the delivery?	Yes	12.9	0.0	12.4	0.883	0.445 (ns)
	No	87.1	100.0	87.6		
Mother's treatment during labor	Nvp	13.3	20.0	13.5	1.709	0.635 (ns)
	AZI/3TC	10.1	0.0	9.8		
	None	20.9	40.0	21.5		
	Other	55.7	40.0	55.2		
Did the mother receive antiretroviral drugs after delivery	Yes	93.6	66.7	92.6	10.106	0.044 (**)
	No	6.4	33.3	7.4		
Did the newborn receive nevirapine at birth	Yes	96.8	66.7	95.7	12.686	0.022 (**)
	No	3.2	33.3	4.3		
Did the newborn received other ARV than Nevirapine since his birth	Yes	9.7		9.3	0.640	0.551 (ns)
	No	90.3	100.0	90.7		
Did the newborn already make HIV test (PCR) at birth	Yes	90.7	100.0	91.1	0.610	0.565 (ns)
	No	9.3	0	8.9		

ns = not significant, ** = significant at 5%

FIGURE 1: MOTHER-TO-CHILD TRANSMISSION RATE

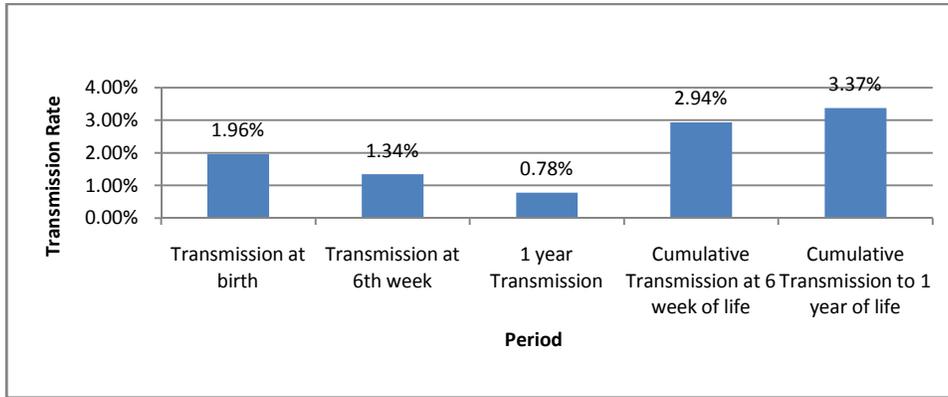


TABLE4A: BREASTFEEDING AND POSTPARTUM MONITORING.

Variable	Terms	Newborn's Serology			Chi-Square Value	Meaning
		Negative	Positive	Aggregate		
Newborn feeding pattern since his birth	Exclusive breastfeeding	90.8	66.7	89.9	4.174	0.126 (ns)
	Mixed breastfeeding	8.6	55.5	9.5		
	Artificial feeding	0.7		0.6		
Has he drunk water, fruit juice, traditional products since his birth?	Yes	36.5	50.0	37.0	0.705	0.703 (ns)
	No	57.4	50.0	57.1		
	Don't know	6.1		5.8		
Has the newborn been sick since his birth?	Yes	44.1	60.0	44.7	0.492	0.399 (ns)
	No	55.9	40.0	55.3		
If AME is practiced, please specify the length	0-3 months	16.8	33.3	17.3	3.08	0.214 (ns)
	0-5 months	13.7		13.3		
	0-6 months	69.5	66.7	69.4		
After AME, how long has the woman screened seropositive proceeded with breastfeeding?	0 months	1.2		1.1	3.664	0.160 (ns)
	6 months	20.2	66.7	21.8		
	12 months	78.6	33.3	77.0		

TABLE 4B: BREASTFEEDING AND POSTPARTUM MONITORING

Variable	Terms	Newborn's Serology			Chi-Square value	Meaning
		Negative	Positive	Aggregate		
Did the mother have postnatal consultation	Yes	85.2	80.0	85.0	0.103	0.561 (ns)
	No	14.8	20.0	15.0		
ARV treatment used by the mother screened positive	Yes	93.0	100.0	93.1	0.227	0.806 (ns)
	No	7.0	0.0	6.9		
After delivery, does she use a family planning method	Yes	52.1	0.0	50.3	5.247	0.028 (**)
	No	47.9	100.0	49.7		
Is the newborn under prophylaxis with Cotrimoxazole	Yes	86.2	75.0	85.9	0.405	0.459 (ns)
	No	13.8	25.0	14.1		
Have you be subject to breast affections/infections since delivery	Yes	3.7	50.0	5.1	17.268	0.013 (**)
	No	96.3	50.0	94.9		

Ns = not significant, ** = significant at 5%

Table 4b shows that mothers who used contraceptive methods after delivery had fewer seropositive babies than those who did not ($p=0.028$). Mothers who reported having post-partum breast affections/infections had more positive babies than those who did not($p=0.013$).

DISCUSSION

The main objective of our study was to assess the current residual risk of mother-to-child transmission of HIV in Burkina Faso. Out of a sample of 186 HIV-positive women, six (1.3%) of their children were positive for HIV-1. This national study involved 14 districts out of 13 health regions in Burkina Faso. However, it should be noted that the contribution of the Bobo Dioulasso districts to the study was quite small. Out of the three health districts, Do, Dafra and Karangasso-Vigué, which were expected to contribute a minimum of 60 samples, only Karangasso-Vigué district provided 16 samples, reducing the sample size of the study. More participants from the Hauts-Bassins region would have made it possible to obtain a larger sample enabling us to analyze data for both regions (Central and Hauts-Bassins) separately. However, all these data have helped to better understand the rate of residual HIV transmission in Burkina Faso.

In the present study, six exposed children were born HIV1-positive, three of them at birth and two were positive at 6 weeks. At 1 year, there was an additional positive child. These results are encouraging compared with previous sero-surveillance data in Burkina Faso, where the estimated percentage of children infected with HIV through vertical transmission from their HIV-positive mothers who have given birth in the last 12 months in 2013, 2014, 2015 was 5.72%, 5.30% and 4.95%, respectively (10). In other countries such as China, the vertical transmission rate was reported as 6.7% in 2013 (11) and in Ukraine in 2010 it was reported as 4.1% (12), despite the implementation of PMTCT programs. In addition, similar vertical transmission rates have been reported in other countries, particularly South Africa,

where surveys conducted in 2010 and 2011 revealed vertical transmission rates of 3.5% and 2.7%, respectively (13).

MTCT is responsible for the majority of HIV infections in children, with 10,000 new cases of infected newborns each year in Burkina Faso (UNDP, 2001). With a lack of specific action to reduce the risk of transmission, estimated rates of mother-to-child or vertical transmission range between 14% and 25% in Europe and United States and between 13% and 42% in developing countries (14). In 1997, the rate of MTCT was very high in developing countries, up to 25% and 35%, while in France and in the United States, the rate was less than 5% (15). The two main reasons for this are breastfeeding practices and access to drugs to reduce mother-to-child transmission. Furthermore, it is recognized that under a PMTCT intervention, the MTCT rate may fall below 5% (13). In developed countries, MTCT rates have declined recently, sometimes to less than 2%, because of the effectiveness of interventions to prevent this transmission (16). By 2015, some countries such as Cuba had already successfully eliminated MTCT (UNAIDS/WHO, 2015). In Burkina Faso, risk factors for mother-to-child transmission of HIV are related to pregnancy (nutritional status, sexually transmitted infections, anemia), labor/delivery (traumatic obstetric procedures) and extended breastfeeding until the age of 2 years (6; 17).

It is important to note the difficulty of comparing different studies on the rate of mother-to-child transmission of HIV, because of the multiplicity of methodological approaches. In our study, all mothers of HIV-positive children started with triple therapy, at least 4 weeks before delivery. However, during and after childbirth, nearly 21.7% and 8.4% of women, respectively, received no ARV treatment. Among those who received it during labor, about 40% were on triple therapy, 13.3% treated with Nevirapine and about 10.8% with AZT/3TC. After delivery, only 50% of women benefited from triple therapy and 9% from dual therapy (AZT 3TC). Considering the above, the

B+ option was not respected, i.e. starting triple therapy, which will be continued for the remainder of their life time as soon as the diagnosis is made in the mother and child and administration of NVP or AZT (2 mg/kg/day) single-dose for 4 to 6 weeks, irrespective of the newborn feeding method.

One of the issues this study assessed as part of the mother's history was the sharing of her serological status with her spouse. Almost half of women surveyed (48.9%) already knew their serological status before pregnancy. Of these, 89% knew they were HIV-positive. However, 47.9% of women in the study were unable to share their HIV status with their partners and none of the HIV-positive women had shared their HIV status with their partners. The reasons given include fear of being rejected by their partner, stigmatization and conflicts in the home. This shows the importance of the community in supporting HIV-positive women and the involvement of men in the TME program. This family dimension of PMTCT is a reality. Indeed, previous studies and experiences of the actors showed that women have difficulty in revealing their HIV serological status to their partners, especially during pregnancy, even if access to treatment that now enables the diagnosis makes sharing less difficult than in early 2000 (18).

In this study, two variables were significantly associated with newborn positive serology: use of contraceptive methods after childbirth and breast affections/infections since childbirth. There were also higher rates of the following variables in mothers with HIV-positive children: mixed breastfeeding, ingestion of water, fruit juice and traditional products, an episode of disease in the newborn (skin infections were reported in three children), exclusive breastfeeding practice between 0 and 3 months, breastfeeding for 6 months and non-use of family planning (FP) by the mother.

According to data from the early 1990s, the estimated risk of breast milk transmission in HIV-positive women was about 15%, if breastfeeding was continued for 2 years or more (19). The risk of transmission through breastfeeding in women with recent (postpartum) infection was nearly twice as high (20).

In our study, the two babies who were positive at 6 weeks were negative at birth. In the first case, exclusive breastfeeding was performed for 3 months and in the second case, exclusive breastfeeding was continued for 6 months. Factors that may increase the risk of MTCT during breastfeeding, according to WHO, include oral thrush and/or oral ulcers of the newborn and cracks, crevices, mastitis and mammary

abscesses in the mother. None of these factors were found in the two positive infants. There was no statistically significant correlation between breastfeeding and HIV serology in our study. This could be because of the limited number of babies found seropositive during the breastfeeding period.

In this study, triple therapy onset was delayed (after the third month of pregnancy) and the irregularity or even absence of ARV treatment during delivery and postpartum was observed in more than half of HIV-positive mothers. The first late prenatal consultation, ARV unavailability and insufficient sensitization of women on the need to take these drugs are elements that we found in this study. If the PMTCT protocol is followed appropriately, it can significantly reduce the risk of mother-to-child transmission of HIV-1 or even reduce the HIV-1 vertical transmission rate to 0.0% (21). Also, our study found that mother-to-child transmission of HIV was higher in women who had vaginal delivery; 20% compared with 14% for women who gave birth by caesarian.

CONCLUSION

The residual MTCT rate was 1.96% at birth, 1.34% at 6 weeks and 0.78% at 12 months. The cumulative rate at 6 weeks was 2.94% and from birth to 12 months was 3.37%. These results provide hope in the fight against HIV in Burkina Faso. The analysis of factors impacting MTCT has demonstrated the effectiveness of PMTCT interventions for HIV-positive pregnant women. These interventions could have been improved through the early commencement of triple therapy in early pregnancy and improved adherence to ARV therapy. No HIV-positive women in the study who gave birth to an HIV-positive baby shared her HIV status with her partner. Women's partners should be involved in the implementation of the program to help these women and ensure their proper care. Early and permanent community involvement must be effective throughout the continuum of the provision of integrated care (maternal neonatal and child health/PMTCT). The community plays an important role in HIV management within couples and therefore contributes to the success of the PMTCT program.

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ORIGINAL ARTICLE

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SCHOOL BASED MASS DE-WORMING INITIATIVE IN SOUTH-WEST NIGERIA

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ABSTRACT

Background: The public health implications of helminthic infection in developing countries were generally agreed by many researchers to include poor growth and poor school performance among others. But the role of school based mass de-worming in combating the menace of helminthiasis remains controversial. Several studies have assessed the impacts of mass de-worming with conflicting results. This study was designed to evaluate the impact of antihelminthic mass chemotherapy on changes in growth indices and school absenteeism.

Materials and methods: Albendazole tablets were administered by school teachers to pupils after data and stool sample collection. Follow up data were collected 6 months later for impact assessment. Ponderal growth retardation was defined as BMI under 5-percentile.

Results: Overall helminth infection rate was 373/1442 (39%) of the pupils before the intervention. *Ascaris lumbricoides* (n=247; 25.8%) and hookworm (n=89; 9.3%) were the most common. At enrolment 19.6% of children with and 11.8% without helminth infections had BMI below the 5-percentile. These figures were reduced to 9.2% and 8.8% after de-worming respectively. No effect of de-worming was seen on longitudinal growth. The number of helminth infected children with >25% absenteeism reduced by 12.5%, while the reduction rate was 6.8% in the uninfected group.

Discussion: The difference in response to de-worming between infected and uninfected children strongly support the beneficial effect of de-worming on growth and school absenteeism. The intervention could be administered by school teachers without formal healthcare training, thus allowing integration of the programme into existing structures.

Keywords: Helminthes, Absenteeism, Preventive Chemotherapy.

ÉCOLE EN FONCTION DE MASSE DES VERMIFUGES INITIATIVE DANS LE SUD-OUEST DU NIGÉRIA

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ABSTRAIT

Les implications de santé publique de l'infection à helminthe dans les pays en développement ont été généralement acceptées par de nombreux chercheurs d'inclure un croissancement médiocre et mauvais résultats scolaires entre autres. Mais le rôle de l'école base massive des vermifuges dans la lutte contre la menace de l'helminthiase reste controversée. Plusieurs études ont évalué les effets de masse des vermifuges avec des résultats contradictoires. Cette étude visait à évaluer l'impact des antihelminthiques chimiothérapie massive sur l'évolution des indices de croissance et de l'absentéisme scolaire.

Matériels et méthodes : Albendazole comprimés ont été administrés par les enseignants de l'école aux élèves après prélèvement de données ou de selles. Suivi de données ont été recueillies à 6 mois plus tard pour l'évaluation de l'impact. Retard de croissance pondérale a été défini comme IMC sous 5 percentile.

Résultats : Dans l'ensemble le taux d'infection de helminthes était 373/1442 (39 %) des élèves avant l'intervention. *Ascaris lumbricoides* (n = 247 ; 25,8 %) et l'ankylostome (n = 89 ; 9,3 %) étaient les plus fréquentes. Lors de son inscription, 19,6 % des enfants atteints et 11,8 % sans helminthes infections avaient IMC inférieur au 5-centile. Ces chiffres ont été réduits à 9,2 % et 8,8 % après l'administration de vermifuges respectivement. Aucun effet de l'administration de vermifuge a été observé sur la

croissance longitudinale. Le nombre d'helminthes contaminés des enfants avec > 25 % l'absentéisme réduit de 13,9 %, tandis que le taux de réduction était de 7,2 % dans le groupe non infecté.

Discussion : La différence en réponse à des vermifuges entre enfants infectés et fortement appuyer l'effet bénéfique de déparasitage sur l'absentéisme de croissance et de l'école. L'intervention pourrait être administrée par des enseignants sans formation formelle de soins de santé, permettant ainsi l'intégration du programme dans les structures existantes.

Mots clés: Helminthes, absentéisme, chimiothérapie préventive.

BACKGROUND

Despite the evidence indicating that school based mass deworming is one of the best ways to secure the future of children in developing countries, sustainability of the program as well as effective mass coverage continue to hinder the control of Soil Transmitted Helminths (STHs). (1) Chronic infections with intestinal helminths are important health factors that may influence school performance, reduce social competence and regular school attendance. Studies suggested that helminthiasis are associated with lower literacy levels by 13% and lower earnings later in life by 43%. (2) The total lost years of schooling due to worm associated absenteeism amount to over 200 million years, mostly in developing countries. (3) Also, IQ loss in poor countries associated with helminth infection is estimated as 3.75 points per worm infection. (4) The World Health Organization estimated that about 280 million children that are in need of deworming live in Sub-Saharan African Region where Nigeria is number one with 21%. (5) Despite the fact that antihelminthic drugs are not expensive, the cost of mass deworming is not sustainable. For instance data from the Partnership for Child Development (PCD) showed that the cost of school-based deworming was estimated to be around

50 US cent per child per year. But the actual cost of the antihelminthic tablet was 3 US cent per 400mg dose (albendazole) per child, (6) most of the cost is actually spent on training and other logistics. This means that if the cost of training and logistics could be eliminated, more money will be freed up to sustain the program. This was what motivated us to evaluate the effectiveness of a mass deworming program involving only school teachers without formal healthcare training.

MATERIALS AND METHODS

Study site and population: This study was carried out at Ilero town, South-West of Nigeria. A previously

published research work from this community showed that open defecation was a common practice while tap water was also not available. (7) The town has about 7 public primary schools consisting of about 1442 pupils.

Study design: The study was designed as an effectiveness study with minimal influence of the research team. The study was carried out in the mid of the school year between April and Sep 2013. The aim of the study was explained to the children and their parents/guardians who also consented freely to participate in the study. The height in centimeter and weight in kilogram of each child was measured. The school attendance registers were checked to record the number of days that each child was absent during the school term. School absenteeism was evaluated in the 100 days the school was open prior to the intervention and 100 days after the intervention. Data from the primary 6 pupils were not included in analysis because they were not available for follow up, having graduated before the revisit period. Data from pre-school children below the age of 2 years were also excluded from analysis because they had no attendance register. We were therefore left with 957 pupils to work with. Though all the pupils in the community benefited from the deworming tablets

Stool specimen collection: The children were given wide mouth, screw cap plastic bottles with clear instruction on how to transfer feces into it in the school premises. Children that were deemed too young were assisted with trained personnel in specimen collection. Peanut sized stool samples were preserved with about 5mls of 5% formalin. The specimen was thoroughly mixed with the preservatives using applicator stick to ensure good preservation.

Ethical approval and Drug administration: The study was approved by the research and ethic committee of Federal teaching hospital, Abakaliki. Teachers were asked to administer 400 mg of chewable albendazole tablets to the pupils irrespective of their age at a cost of about 5 US cents per child.

The process was explained to the teachers a week before the program and they were encouraged to use their social contacts to sensitize the community.

Laboratory analysis: Preserved stool samples were centrifuged and examined by direct microscopy for ova of parasites by 2 independent microscopists, and

any discrepancy was resolved by having a third opinion.

Data analysis: Data of weight, height, age and sex were used to calculate weight for age, Body Mass Index (BMI) and height for age using WHO growth tables and software. Children were classified as reduced ponderal growth when BMI was less than 5 percentile (% ile) according to the WHO classification scale, those with BMI between 5 to 85 % ile were regarded as normal weight while those with BMI above 85%ile were classified as overweight. (8,9) Similarly, height for age was used as indicator for linear growth. The number of days of absenteeism before and after intervention were grouped into ranges from those who were absent for less than 5% through those who were absent for more than 25%. Because it was considered unethical to randomize children to treatment or placebo we used a different approach to document the effect of deworming. Thus, we divided the children into two arms for the data analysis, one consisting of children with detectable helminths and the other without helminths prior to treatment.

RESULTS

We observed that the teachers complied reasonably with the deworming instructions given, and that the

community was effectively sensitized, judging by the consent and high acceptance rate from the pupils. We enlisted a total of 957 children (473 boys and 484 girls) with a median age of 8 years and in the range 2-16 years. Three hundred and seventy three (39%) of all the pupils in the community were infected with at least one helminth species. *Ascaris lumbricoides* (25.8%) and Hookworm (9.3%) were the commonest followed by mixed infection with the two parasites (1.8%), (table 1). The helminth positive children were almost twice as likely as the helminth negative children to be underweight prior to deworming (19.6% vs. 11.8%, Table 2, p=0.0001). Six months after deworming the prevalence of low underweighted pupils reduced to about 9% in both groups of children.

TABLE 1: HELMINTHS DETECTED IN CHILDREN PRIOR TO DEWORMING

Helminth	N (%)
Ascaris	247 (25.8)
Hookworm	89 (9.3)
Ascaris+Hookworm	17 (1.8)
Trichuristrichuria	6 (0.6)
Enterobius	5 (0.5)
Other mixed infections	9 (0.9)
Negative	584 (60.8)
Total	957

TABLE 2: PREVALENCE OF ABNORMAL BMI BEFORE AND AFTER DEWORMING BY HELMINTH CARRIER STATUS.

		BMI group, N (%)			Total
		Underweight	Normal weight	Overweight	
Before deworming ¹	Helminth Negative	69 (11.8)	479(82.2)	35(6.0)	583 (100) ²
	Helminth Positive	73(19.6)	288(77.2)	12(3.2)	373 (100)
	Total²	142	767	47	956
After deworming	Helminth Negative	51 (8.8)	475(81.8)	55(9.5)	581 (100)
	Helminth Positive	34(9.2)	296(80.4)	38(10.3)	368(100)
	Total³	85	771	93	949(100)⁴

¹Significant difference between helminth negative and helminth positive children, p=0.0001; ²Anthropometric measurements missing from 1 child; ³Helminth status assessed prior to deworming; ⁴Follow up was missing for 7 children

ABLE 3: EFFECT OF DEWORMING ON SCHOOL ATTENDANCE

	Number of pupils with more than 25% absenteeism		N, (%)
	Before deworming	After deworming	Difference
Worm negative pupils	87 of 583(14.9)	47 of 581(8.1)	40(6.8)↓
Worm positive pupils	68 of 373(18.2)	21 of 368(5.7)	47(12.5)↓

↓= decrease in number

DISCUSSION

The high prevalence of helminth infection seen in this community (39%) is a likely consequence of lack of potable water combined with the poor sewage disposal methods in this community, (report published elsewhere). (7) A survey of four primary schools in Kenya showed a similar prevalence ranging from 31% to 48.9%, (10) while 58.3% prevalence was also reported among Ethiopian children (11) in communities with similar challenges of water, sanitation and hygiene. A higher prevalence (68.2%) was once found in a similar study in Nigeria by Dada-Adegbola *et al* (12) while Kirwan *et al* reported 50% prevalence in a similar Nigerian study. (13)

Our data suggested a causal relationship between intestinal helminth infection and ponderal growth changes in the study population. Factors responsible for growth retardation of children in developing countries are complex and multifactorial. Apart from helminth infection, other factors attributable include undernutrition, low family income and large family size (14). This may explain why some studies found little or no effect of deworming intervention on weight gain.(15,16) However; other studies found deworming to significantly improve weigh gain. The study of Ethiopian children (17) also found an association between helminth infection and underweight among the school children, and the weight-for-age z-scores of the children significantly increased four weeks after treatment for helminth infection, with a single dose of albendazole. A clinical trial in India also showed that a single albendazole treatment of helminth infected school children lead to a significant weight gain.(17)

Studies evaluating the impact of deworming on school attendance have generated conflicting reports. For instance, the Kenyan study by Muguel and Kramin (18) showed that deworming improved school attendance. This was supported by another study in Jamaica which also showed that helminth infection

was associated with poor school attendance and deworming led to a significant improvement in absenteeism 6 months after treatment, a benefit that was more pronounced in infected children who were also stunted. (19) On the other hand, Davey *et al* (20) recently concluded that benefit of deworming on school attendance remains controversial. It is not

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unlikely that the initiation of our study could have sensitized the teachers to improve on school attendance record keeping which could be a confounding factor. Also as a predominantly farming community, seasonal variation in farming activities such as planting, weeding and harvesting could also interfere with school attendance, which we may not be able to account for. We therefore like to suggest that the deworming effect on absenteeism observed in our study needs cautious interpretation and should be subjected to further investigation that will take into consideration all possible variables.

By limiting our role during the deworming exercise to that of observership and data collection at few time points, the results of our study showed that school based mass deworming could be effectively carried out by the teachers without investing most of the donor funds in formal training. This in turn could free more funds for drug procurement and reduce donor fatigue which is a major obstacle to sustainability of mass deworming programs. Minimizing the interference of the research team also allows us to have a more realistic picture of the real life situation and the effectiveness of the program as opposed to efficacy studies, which require more control and follow up by the investigation team.

Studies that have employed community integrated approach in deworming programs reported that the approach is effective and feasible. (21-23) Also a study about community perception of school based deworming in Turkey showed that 87.4 % of the parents were aware of school health programs and 99% of them approved of teachers' role in providing health education and administering deworming tablets to pupils. (24) Our exercise cost about 5 US cent per 400mg dose of albendazole, indicating that about 2000 children could be effectively dewormed with just 100 USD. This suggested that deworming could be done effectively at minor expenses through interaction between public health promoters and the community. However, monitoring of large scale efforts of integrating deworming in existing facilities is important in order to detect obstacles to this approach. Our data support the usefulness of regular deworming and indicate that an effect will last at least 6 months. It seems possible to motivate schools to support the program at minimal cost. Further studies are needed to evaluate and monitor rolling out of this program.

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LATERAL GENICULATE NUCLEUS HISTOPATHOLOGY IN THE RAT EXPERIMENTAL MODEL OF AFRICAN TRYPANOSOMOSIS

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ABSTRACT

Trypanosomosis is an infectious disease of humans and animals characterized by sleep/wake disturbances and disruptions in other circadian rhythm activities. The disease is caused by protozoan parasites of the genus *Trypanosoma* and transmitted by the bite of infected tsetse flies of the *Glossina* species. Although trypanosomosis has a well known aetiology, histopathological studies on brain regions involved in the control of circadian rhythms are scanty. Lateral geniculate nucleus works in conjunction with the suprachiasmatic nucleus, the master circadian rhythm pacemaker, in regulating circadian rhythms. The purpose of this study was to investigate the effect of *T.b.brucei* infection on the histology of the lateral geniculate nucleus, a brain region that can serve as an alternative secondary circadian rhythm pacemaker when the master pacemaker fails. Twelve control and twelve experimental male albino rats were used in this study. The experimental rats were inoculated intraperitoneally with 0.2ml of infected blood containing 1×10^4 *T.b.brucei* parasites. The infected animals were allowed to go through the full course of infection and sacrificed when they were *in extremis*. Each rat was decapitated and the brain immediately extracted from the skull. The brain was fixed in 10% buffered neutral formalin for at least 48 hours. The brain was later removed from the formalin solution and a coronal section made. The coronal section was processed histologically and stained using the haematoxylin and eosin method. The stained slides were observed under a microscope and photomicrographs taken. Histological alterations, including tissue degeneration, infiltration and proliferation of cells, and perivascular cuffing were observed in the lateral geniculate nucleus of infected rats. Lateral geniculate nucleus cannot, therefore, serve as an alternative secondary circadian rhythm pacemaker during trypanosome infection.

Keywords; Trypanosomosis, Lateral geniculate nucleus, Histopathology, Circadian rhythm

HISTOPATHOLOGIE DU NOYAU GENICULAIRE LATÉRAL DANS LE MODÈLE EXPERIMENTAL DE RAT DE LA TRYPANOSOMOSE AFRICAINE

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ABSTRACT

La trypanosomose est une maladie infectieuse des humains et des animaux caractérisée par des troubles du sommeil /sillage et des perturbations dans d'autres activités de rythme circadien. La maladie est causée par des parasites protozoaires du genre *Trypanosoma* et transmis par la morsure des mouches tsé-tsé infectées de l'espèce *Glossina*. Bien que la trypanosomose ait une étiologie bien connue, les études histopathologiques sur les régions cérébrales impliquées dans le contrôle des rythmes circadiens sont rares. Le noyau géniculé latéral travaille en conjonction avec le noyau suprachiasmatique, le stimulateur cardiaque du rythme circadien, dans la régulation des rythmes circadiens. Le but de cette étude était d'étudier l'effet de l'infection par *T. b. brucei* sur l'histologie du noyau géniculaire latéral, une région du cerveau qui peut servir de stimulateur cardiaque secondaire circadien lorsque le stimulateur maître échoue. Dans cette étude, on a utilisé douze rats albinos mâles expérimentaux et douze témoins. Les rats expérimentaux ont été inoculés par voie intrapéritonéale avec 0.2 ml de sang

infecté contenant 1×10^4 parasites *T. b. brucei*. Les animaux infectés ont été autorisés à passer par le cours complet de l'infection et sacrifiés quand ils étaient in extremis. Chaque rat a été décapité et le cerveau a immédiatement été extrait du crâne. Le cerveau a été fixé dans du formol neutre tamponné à 10% pendant au moins 48 heures. Le cerveau a ensuite été retiré de la solution de formol et une section coronale a été réalisée. La coupe coronale a été traitée histologiquement et colorée selon la méthode de l'hématoxyline et de l'éosine. Les lames colorées ont été observées sous un microscope et des photomicrographies prises. Des altérations histologiques, y compris une dégénérescence tissulaire, une infiltration et une prolifération des cellules et un brassard périvasculaire ont été observées dans le noyau géniculé latéral des rats infectés. Le noyau géniculé latéral ne peut donc pas servir de stimulateur cardiaque du rythme circadien secondaire pendant l'infection trypanosomienne.

Mots clés; Trypanosomose, Noyau géniculé latéral, Histopathologie, Rythme circadien

INTRODUCTION

Trypanosomosis, also known as sleeping sickness in humans and Nagana in cattle, occurs only in 36 sub-Saharan Africa countries where there are tsetse flies that can transmit the disease. The disease continues to be a major health problem in sub-Saharan Africa where it threatens the health and productivity of humans and livestock, causes massive economic losses and severely constraints the continent's socio-economic development. The disease is caused by protozoan parasites of the genus *Trypanosoma* and is transmitted by the bite of an infected tsetse fly of the genus *Glossina*(1).

The disease progresses in two distinct stages. The first or early stage of the disease, also known as the haemolymphatic phase, is defined by the restriction of the trypanosomes to the blood and lymph system. The second or late stage of the disease, also known as the neurological phase, is characterized by the presence of the parasites in the cerebrospinal fluid(2). In the absence of treatment, trypanosomosis is invariably fatal to both humans and livestock.

The causative agents of trypanosomosis, the *Trypanosoma* parasites, show early invasion in brain areas that lack a blood-brain barrier, such as the pineal gland and median eminence(3). From here, the trypanosomes invade other brain regions including the thalamus and hypothalamus where they cause inflammatory responses that may lead to disruptions in endogenous circadian rhythms like the sleep/wake cycle.

The lateral geniculate nucleus, located in the thalamus, is the primary relay centre for visual information received from the retina of the eye. It receives input directly from the retina via the retino-hypothalamic tract (4). Besides being a major visual processing centre, the lateral geniculate nucleus also participates in the regulation of circadian rhythms through its projections, via the geniculo-hypothalamic tract, to the supra-chiasmatic nucleus, the master circadian rhythm pacemaker in the hypothalamus (5).

Thus, the lateral geniculate nucleus, through the geniculohypothalamic tract, provides a secondary, indirect photic input to the suprachiasmatic nucleus as well as an alternate input which has an important role in entrainment of circadian rhythms (6). This is further supported by other studies (7-11) that have reported that the lateral geniculate nucleus is an important component of the circadian timing system and is responsible for the integration of photic and non-photoc information to modify suprachiasmatic nucleus activity. Since the histology and functioning of the suprachiasmatic nucleus is altered during trypanosomosis (12), this study investigated the effect of *T.b. brucei* on the histology of lateral geniculate nucleus in an attempt to find out if this brain centre can serve as an alternative circadian rhythm pacemaker during trypanosome infection.

MATERIALS AND METHODS

Experimental Setup

Twenty four male albino rats, aged 3-3½ months and weighing 200-220g, were used in this study. The rats were randomly divided into two groups, control and experimental, of twelve rats each. The rats were housed at room temperature in the mini-laboratory animal house in the Department of Biological Sciences, University of Eldoret, Kenya, where the study was carried out. They were housed three per cage and were exposed to 12/12 hours of light/dark cycle throughout the study period. The rats had access to food (mice pencil, Unifeed Millers Ltd, Kisumu, Kenya) and clean water *ad libitum*.

Two weeks prior to data collection, the rats were observed and accustomed to routine handling. They were also screened for ectoparasites and each rat was injected subcutaneously with 0.01ml of Ivermectin (Ivermin®, Sinochem Ningbo Ltd., China), a broad spectrum parasiticide that effectively controls both ectoparasites and endoparasites(13). The experimental protocol got the approval of the Department of Biological Sciences ethics committee on care and use of animals for research purposes.

Infection of Experimental Rats

An isolate of the parasite *T.b. brucei*(ILTat1.4) was obtained from the International Livestock Research Institute (ILRI), Nairobi, Kenya. The parasite was originally obtained from the blood of a naturally infected cow in Uhombo, Kenya. The isolate was injected intraperitoneally into a donor rat for the purpose of expanding the stablitate for subsequent inoculation into the experimental group rats. The donor rat was put in a cage and transported to the animal house at the University of Eldoret.

The donor rat was monitored for the presence of parasites daily by direct microscope observation of trypanosomes in wet smears of blood samples obtained from tail bleeds. When parasitaemia was established five days post-infection, the donor rat was anaesthetized with ether and 2ml of blood obtained from it through cardiac puncture. One millilitre (1ml) of this blood was diluted with 2ml of phosphate buffered saline solution (pH 7.4). Then, 0.2ml of this blood, containing about 1.0×10^4 live *T.b. brucei* parasites was injected intra-peritoneally to each of the twelve rats in the experimental group. The number of parasites was determined using the Neubauer haemocytometer method(14). Rats in the control group were, concurrently, injected intra-peritoneally with 0.2ml normal saline.

Organ Harvesting and Histological Studies

All the twelve infected experimental rats were allowed to go through the full course of infection and sacrificed when they were *in extremis*. For every experimental rat sacrificed, a control rat was sacrificed too. Each rat was anaesthetized with ether and then decapitated. A firm cut along the midline of the skull (through both parietal and frontal bones) was made using a sharp knife. Both parietal and frontal bones were tilted thus exposing the brain. The brain was then gently lifted out of the skull and

immediately put in 10% buffered neutral formalin where it was fixed for at least 48 hours.

One week later, three brains were randomly selected from each group for further processing. Each of the selected brains was removed from the formalin solution and a coronal section of the brain, across the thalamus, was made. The coronal section was processed histologically using an automated tissue processor(Global Medical Instrumentation Inc., USA). Paraffin blocks were sectioned with a manual rotary microtome (Leica Biosystems, Germany) at 5 μ m thickness. The thin sections of the coronal section were mounted on glass slides and stained using the standard staining technique of haematoxylin and eosin (15). The stained slides were observed under a light microscope (Euromex, Holland) and photomicrographs taken using a camera (Canon EOS, Canon Inc., Japan) attached to the microscope.

RESULTS

Parasite Detection and Physical Observation of the Rats

Parasites were detected in the tail blood of experimental rats five to eight days post-infection. The experimental rats showed no signs of disease for the first fifteen days post-infection. Thereafter, they showed apparent fatigue, decreased activity, lack of appetite, discharge from the eyes and nose, and paralysis of limbs and tail. On the other hand, the control rats showed no signs of infection throughout the study period. They were also of normal behaviour, appetite, and general activity.

The lateral geniculate nucleus of control rats showed normal architecture composed of normal neurons and glial cells. The nuclei of the neurons were round and quite distinct. On the other hand, the lateral geniculate nucleus of experimental rats showed neurons with smaller and shrunken nuclei, and inflamed and enlarged blood vessels (Figure 1).

LATERAL GENICULATE NUCLEUS HISTOLOGY

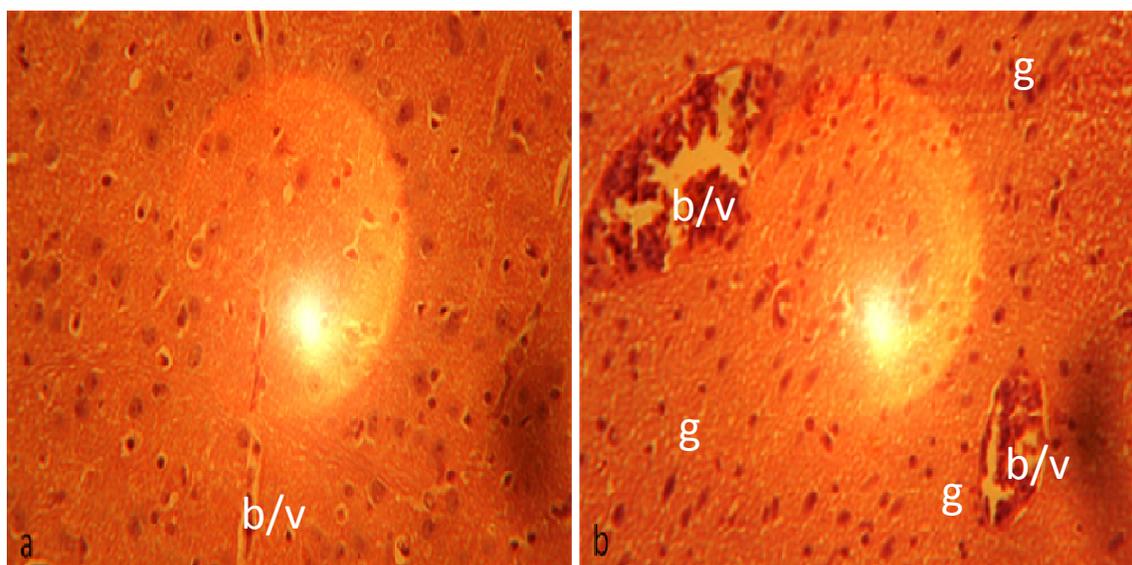


FIGURE 1. PHOTOMICROGRAPH OF CORONAL SECTION THROUGH THE LATERAL GENICULATE NUCLEUS OF A) CONTROL, AND B) *T.B. BRUCEI*-INFECTED RAT. (g = glial cell; b/v = blood vessel). Note the enlarged and inflamed blood vessels in the experimental rat. Mg x400

DISCUSSION

Although the lateral geniculate nucleus is one of the main components of the visual pathway and an important component of the circadian timing system, it seems to be one to which least attention is paid in histopathological studies. In the present study, the neuronal nuclei in the LGN of experimental rats were smaller and shrunken (pyknotic) compared to those of their matched controls. In addition, the blood vessels became inflamed and enlarged (perivascular cuffing) and there was marked infiltration and proliferation of cells notably glial cells, lymphocytes, plasma cells and macrophages. Such histological alterations could have affected the neuronal network between the lateral geniculate nucleus and the supra-chiasmatic nucleus. The integration role of the lateral geniculate nucleus in modifying supra-chiasmatic nucleus activity was, consequently, affected. The entrainment to a 12/12h light/dark cycle was disrupted in the experimental rats and this could explain the disruption of the sleep/wake cycle observed in these animals.

A study by Watts *et al.* (16) reported that lesions in the lateral geniculate nucleus result in destruction of not only the geniculo-hypothalamic tract, but also the hypothalamo-geniculate projection originating in the

supra-chiasmatic nucleus and terminating in the lateral geniculate nucleus. Similarly, damage to the lateral geniculate nucleus has been reported to impair performance on brightness discrimination tasks (7). These findings support the view that the integrity of the lateral geniculate nucleus is necessary for entrainment of circadian rhythms (8).

The findings of the present study indicate that trypanosomiasis causes histological changes in the lateral geniculate nucleus of infected rats. The integration role of the lateral geniculate nucleus in modifying the activity of the supra-chiasmatic nucleus, and in synchronization of circadian rhythms, is hence affected. Lateral geniculate nucleus cannot, therefore, act as an alternative secondary circadian rhythm pacemaker during trypanosomiasis.

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Conflict of Interest: The authors declare that there are no competing interests.

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PREVALENCE OF TRYPANOSOMIASIS IN SHEEP IN THE KACHIA GRAZING RESERVE, KACHIA, KADUNA STATE, NIGERIA

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ABSTRACT

An investigation was carried out in the Kachia grazing reserve in Kaduna, Nigeria, to determine the prevalence of trypanosomiasis among sheep. The reserve has had a history of high prevalence of the disease and farmers in the area are known to ignore the control of trypanosomiasis in sheep and goats and focus more on cattle. The sheep studied showed lacrimation, pale mucous membranes, hair loss, lameness and tick infestation. Blood samples from 110 sheep were collected and examined by using the Standard Trypanosome Detection Method i.e. Haematocrit Centrifugation Technique (HCT), Buffy Coat Method (BCM), and Giemsa stained thick and thin blood films. The packed cell volume (PCV) of each animal was also determined. An overall point prevalence rate of 40.9% (45 positive) was recorded. The average PCV of the infected sheep (19.6 ± 0.45) appeared lower but statistically not significant ($p > 0.05$) than that (18.6 ± 0.51) in those non-infected. The trypanosomes observed were *T. congolense* (40.0%), *T. Brucei* (28.8%), *T. vivax* (17.7%) and mixed infections (13.3%). The potential of small ruminants serving as reservoirs of infection for cattle, insufficiency of professional Veterinary services, absence of alternative trypanosomiasis control methods other than chemotherapy and poor land use practices which forces migration of herds and complicates the control of the disease in the area were discussed.

Keywords: Prevalence, Trypanosomiasis, Sheep, Grazing reserve, Chemotherapy, Reservoir.

PREVALENCE DE LA TRYPANOSOMIASE CHEZ LES MOUTONS DE LA RESERVE DE PATURAGE DE KACHIA, KACHIA, ETAT DE KADUNA, NIGERIA.

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RÉSUMÉ

Une étude a été menée dans la réserve de pâturage de Kachia à Kaduna au Nigeria, pour déterminer la prévalence de la trypanosomiase chez les moutons. La réserve a eu une histoire de prévalence élevée de la maladie dans les moutons et les agriculteurs dans la région sont connus pour ignorer le contrôle de la trypanosomiase dans les moutons et les chèvres et se concentrer davantage sur le bétail. Les moutons étudiés ont présenté des larmolements, des muqueuses pâles, une perte de cheveux, une boiterie et une infestation de tiques. Des échantillons de sang de 110 moutons ont été recueillis et examinés en utilisant la méthode de détection de trypanosomes standard, c'est à dire Technique d'hématocrite par centrifugation (HCT), Méthode Buffy Coat (BCM), et des films de sang Giemsa colorés, épais et minces. L'hématocrite (PVC) de chaque animal a également été déterminé. Un taux de prévalence ponctuelle globale de 40,9% (45 positifs) a été enregistré. Le PCV moyen des moutons infectés ($19,6 \pm 0,45$) est apparu plus faible mais statistiquement non significatif ($p > 0,05$) que celui ($18,6 \pm 0,51$) chez les sujets non infectés. Les trypanosomes observés étaient *T. congolense* (40,0%), *T. brucei* (28,8%), *T. vivax* (17,7%) et les infections mixtes (13,3%). Le potentiel des petits ruminants servant de réservoirs d'infection pour le bétail, l'insuffisance des services vétérinaires professionnels, l'absence de méthodes alternatives de lutte contre la trypanosomiase autres que la chimiothérapie et les mauvaises pratiques d'utilisation des terres qui obligent la migration des troupeaux et compliquent le contrôle de la maladie dans la région ont été discutés.

Mots clés : Prévalence, Trypanosomiase, Moutons, La réserve de pâturage, Chimiothérapie, Réservoirs.

1.0. INTRODUCTION

Trypanosomiasis is an important constraint to the development livestock and agriculture in sub-Saharan Africa with estimated annual losses due to direct and indirect effects of the disease running into billions of dollars (\$5 billion US dollars yearly) or 3 £billion pounds annually (1, 2). It is estimated that without the presence of tsetse in sub-Saharan Africa, 90 million additional cattle could be produced (3). Losses due to the disease include; reduction in herd sizes as a result of livestock deaths and drop in calving rate, reduced market value of animals as a result of loss in condition, drop in milk production, reduced workefficiency of draft animals and prevention of mixed farming (4).

Small ruminants play an important role in the rural economies of sub-saharan Africa. They are kept mainly to generate income, as savings and for ceremonial purposes. They also serve as valuable supplement to cattle in term of animal protein supply for the teaming population including the provision of manure for field crops (5).

In spite of the importance of these animals to the rural poor farming communities (dwellers), research into the incidence trypanosome infection in sheep and goats is limited. Current research is changing notions about the importance of the disease among small ruminants (5,6,7,8). Most worrisome also, is the potential that small ruminants have in serving as reservoirs of infection for cattle (5,1). Trypanosomosis seems to be reemerging as a very important livestock disease in Nigeria, assuming major clinical importance in small ruminants and extending to previously designated tsetse free zones (6). This prompts the need for more detailed work in the area. Previous reports in Northern Nigeria range from 1.6% in sheep and 1.0% in goats (9) to 35.2%, 7.5%, 9.14% respectively (10,11,5) in small ruminants found in Benue, Gombe & Plateau States. A survey carried out by the Nigerian Institute for Trypanosomiasis Research (NITR) in the Kachia grazing reserve (12) revealed an infection rate of 9.49% in sheep and 5.08% in goats.

This study seeks to update information on the prevalence of trypanosomiasis in small ruminants in the area, as adequate information will allow for proper planning of control activities (13,14) as well as serve as valuable scientific data.

2.0. MATERIALS & METHODS

2.1. Study Area
The Kachia grazing reserve (KGR) lies between latitudes 10° 03' and 10° 03' N and longitudes 7° 55'

and 8° 06'E. It is 780m above sea level in the south east of Kaduna state, which is about 90 kilometers from Kaduna town. It is the major part of Ladduga district of Kachia Local Government Area in Kaduna State, Nigeria and has an area of 33,411 hectares with an estimated population of 18,000 people, 50,000 cattle and 30,000 sheep & goats. The area lies within the sub humid zone, which is characterized by a dry season period from November to April, and a rainy season from May to October. The vegetation consists of the typical Northern Guinea savannah Woodland. Rainfall ranges between 1000-1200mm per annum.

The grazing reserve is divided into 6 blocks and each of the blocks consists of several settlements which are communities with separate names. The settlement patterns are mainly hamlets and farm compounds. Most families have goats & sheep. Goats usually graze near homes while sheep usually graze alongside cattle. Each block has a school, an earth dam and at least 2 boreholes. The first and central block (Nassarawa) is where the clinics, drug store, community centre and market are located. The study was carried out during the rainy season (August/September) of 2010.

2.2. Experimental Method

In each block, one village is selected in a day as a converging point: A total of 110 animals were sampled with 26, 37, 22, 15 & 10 in blocks 1, 2, 3, 4, and 5 respectively. In each block, animals that could be sampled along with cattle were randomly selected from each farmer by the Veterinarian. Three millilitre (3 ml) of blood was collected from the jugular vein into sample bottles containing ethylene diamine tetra acetate (E.D.T.A). Parasitological examination was done in the Laboratory using the Standard Trypanosome Detection Method i.e. Haematocrit centrifugation technique HCT(15), Buffy coat method, BCM(16), and Giemsa stained thick & thin films. The packed cell volume (PCV) of each animal was also determined while trypanosome species were identified based on their motility using the BCM and morphological features from Giemsa stained films.

2.3. Statistical analysis

The data obtained from this study was analyzed using the student's t-test. A p-value of <0.05 or less was considered significant.

3.0. RESULTS

A summary of the results can be seen in the table below. A total of 45 (40.9%) animals were found positive with 4, 8, 12, 15 & 6 animals in blocks 1, 2, 3, 4 & 5 respectively. The highest prevalences were in blocks 5 (60%) & 4 (100%) and the most encountered species of trypanosomes was *T. congolense* (40.0%).

PCVs (Packed Cell Volume) as low as 11% but the average PCV among infected animals was 19.6±0.45 and among uninfected 18.6±0.51 but was not statistically significant. Most samples showed very

high parasitaemia. There were also no specific clinical signs attributable to Trypanosomosis observed on the animals.

TABLE 1: SUMMARY OF RESULTS

Blocks	1	2	3	4	5	Total
Number Sampled	26	37	22	15	10	110
Number Positive	4	8	12	15	6	45
Point prevalence	15.3%	21.6%	54.5%	100%	60%	40.9%
Species: <i>T. brucei</i>	1	4	2	4	2	13 (28.8%)
<i>T. congolense</i>	2	4	7	3	2	18 (40.0%)
<i>T. vivax</i>	1	-	3	2	2	8 (17.7%)
<i>T. brucei/T. congolense</i>	-	-	-	2	-	2 (4.4%)
<i>T. b / T. c / T. v</i>	-	-	-	4	-	4 (8.8%)

4.0. DISCUSSION

The prevalence rate of 40.9%, is quite high. This is undoubtedly contributing to the poor condition & performance observed in the sheep of the reserve. Infection rates were also higher in blocks farthest from the main entrance into the reserve which also has proximity to occasional professional veterinary services as roads are less motor able in the more interior parts of the reserve (3, 4 and 5) and also farmers tend to treat less in these blocks compared to the more exterior ones (1 & 2) who are less enlightened as it were. *T. congolense* and *T. brucei* were also the most prevalent species identified which differs from previous reports in the reserve (12) and elsewhere (5,11) where *T. vivax* has been implicated as more dominant in sheep & goats. However, it is interesting to note that this result tallies with a concurrent study in cattle where *T. congolense* and *T. brucei* were also highest. This brings to fore the issue of the potential of small ruminants as reservoirs of infection. This study, however, needs to be carried out with more sensitive diagnostic tools for greater clarity.

Of note is also the absence of alternative control methods other than chemotherapy. Most herdsmen administer drugs themselves or employ the use of quacks (17) mostly in order to save costs but also due to an insufficiency of professional Veterinary services. There is no resident Veterinarian in the reserve. These practices lead to poor drug use patterns such as under dosing, use of fake & wrong drugs as well as poor handling and administration of trypanocides. This may be creating a problem of drug resistance in the area (17). Alternative control methods such as the use

of traps, odour baits & insecticide treated cattle usually help to reduce frequent treatment and help prevent or delay the development of drug resistance (18). The Nigerian Government's Grazing Reserve Act of 1964 was promulgated as a response to the problem of alienation of grazing lands. The law was also taken as one of the policy measures to address some of the constraints confronting livestock development in Nigeria. Thus, grazing reserves were established not only to protect grazing lands from crop farming and provide easier access to them by pastoralists but also to encourage the sedenterization of nomadic/transhumant pastoralists through legally secured titles to grazing land & water as well as a means of promoting livestock development (19). Other than the KGR, Kaduna has gazetted and earmarked other grazing reserves in Anchau, Kagarko and Birnin Gwari. However, the KGR is the only functional and organized among them all. And so, though the area was set aside exclusively for grazing of livestock, it has gradually become a settlement for Fulani herdsmen who have unfortunately also taken up crop farming and other commercial activities. This has gradually reduced the pasture available, forcing migration during the dry season, further complicating the control and containment of the disease in the area both in and outside the reserve.

5.0

CONCLUSION

This study has shown that trypanosomiasis is still a problem among small ruminants and may present a greater problem to cattle by acting as reservoirs of infection thereby threatening food security in the area and the region at large. It is necessary that more studies be carried out with more sensitive diagnostic

techniques for greater clarity of the problem. Other control activities also need to be embarked upon in order to reduce drug pressure and thereby prevent or delay the development of drug resistance. Better management practices also need to be carried out by

authorities over the reserve in order to provide professional health services and systems as well as proper land use practices so as to ensure the optimal use of the reserve and contain & control the spread of diseases.

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COMPARATIVE BONE MARROW RESPONSES OF ALBINO RATS EXPERIMENTALLY INFECTED WITH SINGLE AND MIXED SPECIES OF *TRYPANOSOMA CONGOLENSE* AND *TRYPANOSOMA BRUCEI* AND ABILITY TO CONTROL ANAEMIA

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RUNNING TITLE: BONE MARROW CHANGES IN AFRICAN TRYPANOSOMOSIS

ABSTRACT

Effect of *Trypanosoma congolense* and *T. brucei* mixed infection on ability of the bone marrow to respond to anemia was investigated in albino rats. This was with the view of assessing the possible impact on recovery rate from anemia following chemotherapy of African trypanosomiasis. The investigation involved descriptive evaluation of packed cell volume and corresponding bone marrow cytological changes associated with single and mixed infection of *T. congolense* and *T. brucei*. It involved laboratory based experimental infection of albino rats as research models. A total of 32 adult albino rats of mixed sexes were used for this investigation. The rats were randomly grouped into three groups, A, B, C made up of 8 rats each, and infected with *T. congolense*, *T. brucei* and mixed infection of these species. Eight other rats served as the uninfected control group. Parameters measured included weekly packed cell volume (PCV) and differential bone marrow cytology of the different groups of infected and control rats at the end of 21 days post infection (PI). At the end of 21 days PI, the anemia characterized by drop in PCV was most severe in the mixed infection group, and least in *T. brucei* group with tendency for self-recovery from anemia. The bone marrow responses in the mixed infection group was however weak and inferior to that of *T. brucei* and *T. Congolense* groups. Poor erythropoietic response in the mixed infection group despite significant fall ($P < 0.05$) in PCV level was believed to arise from severe renal and hepatic pathology resulting to subnormal erythropoietin release and severe stem cell injury. This is believed would cause longer time to be taken by mixed infection animals to recover from anemia after chemotherapy. It is concluded that *T. congolense* and *T. brucei* mixed infection result to marked incapacitation of the bone marrow and ability for recovery from anemia. This suggests that supportive administration of synthetic erythropoietin may be required in trypanosome specie mixed infection situation due to severe pathological effects on the kidney and liver resulting to impaired erythropoietin biosynthesis and slow recovery from anemia following chemotherapy in African trypanosomiasis.

Keywords: Anemia, bone marrow, mixed infection, rats, trypanosomiasis, erythropoietin.

DES REPONSES COMPARATIVES DE LA MOELLE OSSEUSE DE RATS ALBINS INFECTES EXPERIMENTALEMENT AVEC DES ESPECES UNIQUES ET MIXTES DE *TRYPANOSOMA CONGOLENSE* ET DE *TRYPANOSOMA BRUCEI* ET LA CAPACITE DE CONTROLER L'ANEMIE

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TITRE COURANT: LES CHANGEMENTS DE MOELLE OSSEUSE DANS LA TRYPANOSOMIASE AFRICAINE

RESUME

L'effet de *Trypanosoma congolense* et *T. brucei* infection mixte sur l'aptitude de la moelleosseuse à répondre à

L'anémie a été étudiée chez les rats albinos. Il s'agissait d'évaluer l'incidence possible sur le taux de récupération de l'anémie consécutive à la chimiothérapie de la trypanosomiase africaine. L'étude portait sur l'évaluation descriptive de l'hématocrite et les modifications cytologiques correspondantes de la moelle osseuse associées à l'infection unique et mixte de *T. congolense* et *T. brucei*. Il a impliqué l'infection expérimentale de rats albinos commodes de recherche. Un total de 32 rats albinos adultes de sexmixte ont été utilisés pour cette enquête. Les rats ont été groupés aléatoirement en trois groupes, A, B, C, qui composait de 8 rats par groupe, et infecté par *T. congolense*, *T. brucei* et infection mixte de ces espèces. Huit autres rats ont servi de groupe témoin non infecté. Les paramètres mesurés incluaient l'hématocrite hebdomadaire (PCV) et la cytologie de la moelle osseuse différentielle des groupes différents de rats infectés et témoins à la fin de 21 jours après l'infection (PI). À la fin de 21 jours PI, l'anémie caractérisée par la chute du PCV a été la plus sévère dans le groupe infection mixte, et moindre dans le groupe *T. brucei* avec la tendance à l'auto-récupération de l'anémie. Les réponses de moelle osseuse dans le groupe infection mixte étaient cependant faibles et inférieures à celle de *T. brucei* et *T. congolense*. Une mauvaise réponse érythropoïétique dans le groupe d'infection mixte en dépit d'une chute significative ($P < 0.05$) du taux de PCV était considérée de provenir d'une pathologie rénale et hépatique sévère résultant au déchargement de l'érythropoïétine sous-normal et des lésions graves des cellules souches. On pense que ce la causerait plus de temps à être prises par les animaux d'infection mixte pour récupérer de l'anémie après la chimiothérapie. Il est conclu que l'infection mixte *T. congolense* et *T. brucei* résulte à l'incapacité marquée de la moelle osseuse et la capacité de récupération de l'anémie. Ceci suggère qu'une administration d'un grand soutien d'érythropoïétine synthétique peu être requise dans une situation d'infection mixte de ces espèces de trypanosomes due à des effets pathologiques graves sur le rein et le foie résultant de l'altération de l'érythropoïétine - biosynthèse et de la récupération lente de l'anémie après chimiothérapie dans la trypanosomiase africaine.

Mots clés: Anémie, moelle osseuse, infection mixte, rats, trypanosomiase, érythropoïétine.

INTRODUCTION

Bone marrow responses play key roles in the pathogenesis of anemia in African trypanosomiasis as they determine ability for haemopoietic cell regeneration and control of anemia in infected hosts (1-4). Anemia constitutes a major pathological feature of trypanosomiasis in man and animals (5,6). Bone marrow factors involved in dyserythropoiesis include ineffective erythropoietic activities and erythrophagocytosis by macrophages in the bone marrow, spleen and other tissues (5,7).

We had reported that even though *Trypanosoma congolense* and *T. brucei* are pathogenic to animals, they cause severe anemia under mixed infection which arises probably from combination of differences in the pathogenic mechanisms of the different species of trypanosome (8). Although trypanosome mixed infection occurs naturally in the field (9), the effect on ability of the animal to control the development of anemia has not been reported. Similarly, in parts of Central Africa, sleeping sickness in man arising from *T. rhodesiense* and *T. gambiense* mixed infection is not unlikely.

In this study, we investigated the effects of *T. congolense* and *T. brucei* mixed infection on ability to respond to and recover from anemia using rats as model. Such findings are likely to find relevance in the proper chemotherapy of trypanosomiasis due to mixed trypanosome species infections and prevention of the post treatment lingering effects of anemia, a major cause of death in African trypanosomiasis.

MATERIALS AND METHODS

A total of 32 adult albino rats were used for the investigation. The rats were bred at our Research Station in Vom, Plateau State, Nigeria and brought to Kaduna for the study. Commercially prepared rat cubes and water were fed to the rats *ad libitum* throughout the course of investigation. At the end of one week acclimatization period, the rats were randomly grouped into three groups; A, B, C and control groups made up of 8 rats each. Trypanosome species used were *T. congolense* (Bassa) and *T. brucei* (Lafia). Both parasites were obtained from cattle during field survey and cryopreserved in liquid nitrogen from where they were first sub-passaged into donor rats and then into the experimental rats.

The rats in group A were inoculated with *Trypanosoma congolense*, 1×10^3 parasites while group B was inoculated with *T. brucei* with the same number of parasites. Group C was inoculated with 0.5×10^3 each of *T. congolense* and *T. brucei*. All inoculations were intraperitoneal (IP). Parameters measured included packed cell volume (PCV) as described by Dacie and Lewis (10), and estimation of mean differential bone marrow counts of both control and trypanosome infected rats at the end of 21 days post infection. Bone marrow smears were obtained from the right femur of 5 rats randomly selected from each group, and three rats that remained in group C by day 21 post infection. The smears were air dried, fixed for 20 minutes in absolute alcohol, stained with Giemsa's stain and examined by light microscopy as described by Anosa et al (7). A minimum of 500 marrow cells were counted per rat and differentiated.

RESULT

At the end of 21 days PI, the anemia characterized by drop in the mean PCV was most severe in group C with *T. congolense* and *T. brucei* mixed infection ($P < 0.05$). The percentage overall drop in PCV at the end of 21 days PI was 8.5%, 6.3% and 17.0% for groups A, B, and C respectively (Table 1). However, the bone marrow in Group B, infected with *T. brucei* was most hyperplastic due to erythroid hyperplasia (Table II) followed by Group C and Group A. This led to the fall in myeloid: erythroid (M:E) ratio to the value of 1.02 ± 0.31 : 1, 0.50 ± 0.04 : 1 and 0.82 ± 0.1 in groups A, B and C respectively as against the value of 1.27 ± 0.05 : 1 for control rats. Similarly granulocyte maturation rate dropped from the value of 3.81 ± 1.10 in control rates to 3.09 ± 0.82 , 2.62 ± 0.77 and 1.37 ± 0.01 for rats in groups A, B, and C respectively.

Lymphocytes in the bone marrow of infected groups were less in number compared to those of control rats. Similar changes were observed in the marrow plasma cell counts. There was however increased cellularity of the monocyte cell lineages which was most ($1.6 \pm 0.45\%$, $P < 0.05$) in group C with mixed infection followed by group B and least in group A. Macrophage hyperplasia followed the same pattern with that of monocytes in the infected rats with macrophages being most numerous ($3.40 \pm 0.97\%$, $P < 0.05$) in the marrow of rats in group C with mixed infection. However more significant numbers of mitotic cells were encountered in the bone marrow of rats in group B infected with *T. brucei* following by group C and least in group A.

DISCUSSION

The overall changes in the mean PCV of infected rats resulting to severer anemia in the mixed infection group support our earlier findings in *T. congolense* and *T. brucei* mixed infection of rats (8). This is believed to arise from the combination of different mechanisms of pathology associated with the trypanosome species which have to do with differences in the preferential sites of localization in the tissues of infected hosts (11,12). The bone marrow of *T. brucei* infected rats was relatively most hyperplastic and responsive. This was characterized by marked erythroid hyperplasia and increase in numbers of mitotic figures which were dominantly of erythroid origin and severe fall in the M:E ratio. This supports earlier observations in *T. brucei* infected deer mice (13) and horses (14) in which marked erythroid hyperplasia also resulted in very high reticulocyte responses in the infected animals. Similar responses were observed in vervet

monkeys infected with the human infective *T. brucei gambiense* (15). Anosa et al (1,7) observed erythroid hyperplasia in cattle infected with *T. vivax* and *T. congolense* respectively. However, *T. congolense* resulted to myeloid hyperplasia in infected cattle (16) while mild reticulocyte responses occurred in *T. congolense* and *T. vivax* infected sheep (17). This study confirms that the superior reticulocyte responses in *T. brucei* infections arise from high erythropoietin activities resulting to selective stimulation of erythropoiesis above granulopoiesis. This seemed to have been responsible for the recovery from low PCV by day 21 PI in the *T. brucei* - infected group B while such improvement in PCV did not occur in the other groups, especially in the mixed infection group.

Even though anemia characterized by drop in PCV level was most severe in the mixed infection group, erythroid responses were relatively weak compared to the more superior responses in the *T. brucei* group. This is believed to have been responsible for the persistent and most severe anemia in the mixed infection group.

The inability of the bone marrow of the mixed infected group to respond well in the face of severe drop in the PCV value of infected rats suggests that, there were subnormal erythropoietin activities which probably arose from marked pathology of the liver and kidneys, organs involved in the biosynthesis of erythropoietin which controls erythropoiesis; and severe stem cell injury. Hepatic and renal pathology occur commonly in trypanosomiasis of man and animals (18, 19) and is believed to play roles in the pathogenesis of anemia.

Relatively marked increases in macrophage numbers in the mixed infection group suggest that there was also marked erythrophagocytosis by macrophages in the marrow of the rats which contributed to the severe anemia observed in this group. Increase in the monocyte numbers may have been also due to their increased demand as macrophages in the mixed infected group. This is supported by the identification of numerous macrophages with engulfed red and white blood cell lineages in the bone marrow of mixed infected group. The roles of macrophages in the pathogenesis of anemia have already been described (3, 4).

Further analysis of cytological changes in the bone marrow of infected rats also suggested that there was depression of the granulocytic precursors at all levels but particularly the more mature stages such as metamyelocytes, bands

and segmenters which constitute marrow storage pool or reserves. This resulted in the lower granulocyte maturation rate, which is the ratio of

non-mitotic to mitotic granulocytes. Similar observations were reported by Anosa et al (7) in *T. vivax* - infected calves.

TABLE I: SUMMARY OF THE PCV VALUES OF CONTROL AND INFECTED RATS

Experimental Group	Pre-infection	Day 21 PI (% Drop)
Control	51.7 ± 1.3	49.7 ± 3.8 (3.9)
Group A	47.0 ± 3.7	43.0 ± 2.8 (8.5)
Group B	48.0 ± 2.3	45.0 ± 2.9 (6.3)
Group C	47.0 ± 1.7	39.1 ± 2.3 (17.0)*

*= P<0.05

TABLE II: DIFFERENTIAL MARROW CELL COUNTS (%) OF CONTROL AND TRYPANOSOME INFECTED RATS AT 21 DAYS PI

Cell Types	Control Rats	<i>T.c.</i> Infected Rats	<i>T.c.</i> Infected Rats	Mixed Infected Rats
Erythroid Cells	35.2 ± 3.57	43.66 ± 6.59*	54.23 ± 2.71*	45.80 ± 1.12*
Myeloid Cells	44.5 ± 0.21	46.34 ± 0.27	27.17 ± 3.44*	37.40 ± 1.34
Myeloid:Erythroid Ratio	1.27 ± 0.05:1	1.02 ± 0.31:1	0.50 ± 0.04:1*	0.82 ± 0.1:1*
Granulocyte Maturation Rate	3.81 ± 1.10	3.09 ± 0.82	2.62 ± 0.77	1.37 ± 0.01*
Other Cells:				
Lymphocytes	15.6 ± 2.11	6.70 ± 3.82*	10.61 ± 2.89*	9.01 ± 0.09*
Plasma Cells	0.70 ± 0.35	0.16 ± 0.23	0.10 ± 0.14	0.4 ± 0.10*
Monoblasts/Promonocytes/ Monocytes	0.9 ± 0.87	0.53 ± 0.19	1.20 ± 1.42*	1.6 ± 0.45*
Macrophages	1.40 1.74	1.20 ± 0.09	2.00 ± 1.24	3.40 ± 0.97*
Unclassified	0.30 ± 0.29	0.35 ± 0.21	0.50 ± 0.71	0.40 ± 0.31
Damaged Cells	1.01 0.14	0.55 ± 0.49*	1.31 ± 0.16	0.80 ± 0.10
Mitotic Cell	0.21 ± 1.31	0.51 ± 0.45	2.88 ± 0.44*	1.20 ± 0.81*

T.c.= *Trypanosoma congolense*; *T.b.* = *Trypanosoma brucei*; *= P< 0.05

This drop was most severe in the mixed-infected group which may have resulted from marked granulophagocytosis by macrophages in the bone marrow of the mixed infected rats. Although similar marrow cytological changes occurred in the *T. congolense* - infected rats, they were inferior to those of *T. brucei* and mixed infection groups. This supports earlier reports of mild reticulocytosis associated with *T. congolense* infection in sheep (17). This confirms the beneficial effect of synthetic erythropoietin administration in the management of anemia in trypanosomiasis due to mixed infections (20). It was concluded that *T. brucei* precipitated most superior marrow erythropoietic response in

infected rats resulting in apparent recovery from anemia in the *T. brucei* - infected group. Even though anemia was most marked in the mixed infection group, bone marrow responses were weak and inferior to those of *T. brucei* group as the marrow was relatively less hyperplastic. This arose probably from subnormal erythropoietin release due to severe pathology of the kidney and liver (21, 22) as a result of combined effects of the peculiar pathogenic mechanisms of the parasites which has to do in part, with the differences in sites of localization in infected hosts. Such peculiar differences may have also caused severe stem cell injury and marked antigenic activation of macrophages in the mixed infection group

leading to macrophage hyperplasia and massive erythrophagocytosis. These acting together may incapacitate the bone marrow's ability to respond to anemia and recovery following chemotherapy. Although further studies were needed to confirm such findings in more natural hosts such as cattle, sheep and goats, and humans with mixed *T. rhodesiense* and *T. gambiense* infections, these observations suggest that supportive administration of synthetic erythropoietin may be required to enhance

recovery from anemia arising from infections due to mixed species of African trypanosomes.

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BRIEF COMMUNICATION

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TRYPANOSOMIASIS IN A MIGRATING HERD OF CATTLE IN KADUNA STATE NIGERIA

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ABSTRACT

The aim of this study is to evaluate the prevalence and impact of Trypanosomiasis on a herd of migrating/pastoral cattle. A herd of 50 white Fulani cattle migrating from a suburban area of Abuja to Afaka in Igabi Local Government Area of Kaduna State, Northern Nigeria, were examined and screened for Trypanosomiasis on request. The animals showed clinical symptoms of lacrimation, emaciation, depression, lethargy and enlarged superficial lymphnodes which were reportedly not present before the trek. 40 of the animals were screened by parasitological means (hematocrit, buffy coat methods and thin and thick blood smears). 15 out of the 40 animals sampled were positive for trypanosomes (37.5% prevalence). *Trypanosoma congolense* was the only *Trypanosoma* species identified. Trypanosomiasis was observed in the herd examined and laboratory examination corroborated the observed clinical signs. The results, as well as the role of migration and transhumance pastoralism in disease occurrence are discussed.

Key words: Trypanosomiasis, trypanosomes, cattle, Fulani, migration, Nigeria.

LA TRYPANOSOMIASE DANS LA MIGRATION D'UN TROUPEAU DE BOVINS DANS L'ÉTAT DE KADUNA NIGERIA

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RÉSUMÉ

Le but de cette étude est d'évaluer la prévalence et l'impact de la trypanosomose sur la migration d'un troupeau de bétail pastoral. Un troupeau de 50 bovins Peuls blanc migration à partir de la banlieue d'Abuja à Afaka dans Igabi du gouvernement local de l'État de Kaduna, au nord du Nigeria, ont été examinés et un dépistage de la trypanosomose sur demande. Les animaux ont présenté des symptômes cliniques de l'emaciation, larmoiement, la dépression, la léthargie et superficielle lymphnodes élargie qui auraient été pas présent avant le trek. 40 de ces animaux ont été éliminés par des moyens (hématocrite parasitologique, méthodes d'extraction de la couche leucoplaquettaire et frottis de sang fine et épaisse). 15 des 40 animaux de l'échantillon étaient positifs pour des trypanosomes (37,5 % de prévalence). *Trypanosoma congolense* était le seul *Trypanosoma* espèces identifiées. La trypanosomose a été observée dans le troupeau examiné et examen en laboratoire corroborent les signes cliniques observés. Les résultats, ainsi que le rôle de la migration et le pastoralisme transhumant dans les cas de maladie sont discutées.

Mots clés: la trypanosomiase, trypanosomes, bovins, Peul, la migration, le Nigeria.

INTRODUCTION

Trypanosomiasis still remains the major disease preventing optimum livestock production in Africa. With over 48 million cattle at risk, 3 million livestock deaths annually and up to US\$5billion lost yearly in livestock production and mixed agriculture, it is ranked among the top ten cattle diseases in the world (1, 2, 3). Nigeria, which has the highest number of cattle in Africa, stands to increase her cattle

production over three folds if trypanosomiasis and tsetse are controlled (4).

The bulk of Nigeria's cattle are reared extensively through transhumance pastoralism by the native Fulani. This system involves seasonal migration to secure food and water for cattle herds and also to avoid disease outbreaks (5). The unique ability of Fulani pastoralists in carrying herds across different

terrains to places of their choice has led to their being used to herd cattle for cattle owners who cannot do it themselves or who cannot afford to transport them by road when they are in need of a new location for their herds. The herd in this study were herded by Fulani men from a farm in Orozo (a suburban area around Abuja, Nigeria's capital) to Afaka (in the outskirts of Kaduna city, Northern Nigeria) due to poor feeding conditions (absence of pasture) in the former area and also security concerns.

MATERIALS AND METHODS

In the month of April 2011, a team of research staff from the Nigerian Institute for Trypanosomiasis Research (N.I.T.R) were invited by the herd owner to examine the herd located in Afaka village in the outskirts of Kaduna city, Nigeria. There were 50 animals in all. The herdsman had reported that the animals were in poor condition having rough hair coat, lacrimation and loss in weight despite a good appetite. These signs were reportedly not present before the trek from the Abuja farm. The trek was in the month of February and lasted 10 days. This information was corroborated by the visiting clinician at the farm. Clinical examination revealed lacrimation, emaciation, depression, lethargy and enlarged superficial lymphnodes.

Four milliliters of jugular blood was collected from 40 of the animals using syringes and needles and the contents transferred into commercially obtained sample bottles containing ethylene diamine tetra acetic acid (EDTA). The blood samples were kept cool by placing them in cold boxes containing ice packs after collection. Parasitological examination was carried out in the N.I.T.R. Laboratory using the haematocrit centrifugation technique, HCT (6), buffy coat method (BCM) (7) and Giemsa stained thin films made after BCM examination. The packed cell volume (PCV) of each animal was also determined while trypanosome species were identified based on their motility using the BCM and morphological features from Giemsa stained films.

RESULTS

The prevalence rate obtained after examination of the samples was 37.5% (15 out of the 40 animals sampled). *Trypanosoma congolense* was the only *Trypanosoma* species identified.

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DISCUSSION AND CONCLUSION

The occurrence of trypanosomiasis observed in this herd is not an uncommon feature in migrating herds. Grazing land and stock-routes top the list of Fulani's demands from the government. This is because the expansion of the grazing reserves and maintenance of stock routes will boost livestock population, lessen the difficulty of herding, reduce seasonal migration, and enhance the interaction among farmers, pastoralists, and rural dwellers (5, 8).

11 of the 23 known species of tsetse are found in Nigeria (4). These fly belts exist in different parts of the country including the areas through which these animals migrated. This allows for adequate exposure to the vector and the disease even if they weren't before the trek. However, the trek alone is enough to elicit enough stress in the animals to weaken their immunity and allow the progress of a disease even with very little exposure to the disease or its vector. The promotion and maintenance of stock routes would allow for proper resting, treatment and quarantine of migrating animals. Also the use of rail systems is far cheaper but has failed and is not an option for cattle owners today.

This accounts for the poor production of livestock in the region as well as the low profitability due the large amounts of money spent on treatment of diseases (over US\$35 million annually in Africa on trypanocides alone according to 9).

Trypanosoma congolense has consistently been observed to be most prevalent and important in cattle populations (10, 11).

The occurrence of trypanosomiasis in migrating herds is most likely to reduce if the proper structures for herding and transportation of the regions cattle are put in place. Before then, herders have the option of prophylactic treatment of their animals before treks and also the use of insecticide pour-ons or spot-ons to reduce or prevent attack by biting flies. Points for adequate rest feeding and drinking should also be fitted into the travel schedule before embarking on the journey.

In conclusion, trypanosomiasis was observed in the herd examined and laboratory examination corroborated observed clinical signs.

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