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Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Review

Implication of coliforms as a major public health problem in Nigeria

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Water, the essence of life, is threatened by bacterial contamination. Coliform count is the major tool to determine the bacteriological quality of water. The determination is quite easy and informative. The different methodologies are employed depending on suitability by maximum probable number (MPN) which is the most accepted. The environment conditions like sunlight, water salinity, temperature etc. provide simple concepts to justify the coliform counts at various places. Faecal coliforms are discussed here with special emphasis as these which are very significant indicators of faecal contamination. Though uncomplicated, coliform counts also determine framing policies for safe and healthy living. However, caution has to be taken while interpreting the coliform data. This paper aims to present the best for understanding the coliform data and interpreting them in a justifiable way.

Key words: Enterobacteriaceae, total coliforms, faecal coliform, *Escherichia coli*, *Enterococci*, *Streptococcus*, maximum probable number.

INTRODUCTION

Water has endless uses namely drinking, industrial, livestock, irrigation, aesthetics, boating, swimming, and fishing and so on. However, this elixir of life is being threatened by various pollutions but mainly the bacteriological pollution of water is a serious problem. Considering the bacteriological problems of water, what comes to our mind is the word 'coliform'. Since public and environmental health protection demands safe drinking water (free from pathogenic bacteria) therefore coliforms are major concern. Coliforms are single celled bacteria, classified as total and faecal coliform, where faecal coliforms are supposed to be more severe indicator of water pollution. Coliform bacteria form a part of the Enterobacteriaceae family (Kilb et al., 2003) which can also be naturally found in soil. However, faecal coliforms strictly live the gastrointestinal tract of warm-blooded animals and so originate from animal and human faecal discharges. *Escherichia coli* is a member of faecal coliform group and *E. coli* is a specific indicator of faecal pollution (Rompre' et al., 2002). Detection of disease-causing bacteria and other pathogens in water is expensive and may pose potential health hazards. Further, testing for pathogens requires large volumes of water, and the pathogens may be difficult to grow in the laboratory and isolate. However, this problem can be easily solved by testing water for faecal coliforms especially *E. coli* as because they generally live longer than pathogens and are easy to culture in a laboratory than pathogens.

DEFINITIONS OF COLIFORMS

In standard method for the examination of water and wastewater (APPHA, 2005), coliform group members are described as:

1. All aerobic and facultative anaerobic, non-spore forming,

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Gram-negative, rod-shaped bacteria that ferment lactose with gas and acid formation at 35°C within 48 h or;
2. All aerobic and numerous facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that grow as red colonies with a metallic sheen at 35°C within 24 h on an endo-type medium containing lactose.

The description of the coliform group has now included other characteristic, such as b-D-galactosidase-positive reactions (APPHA, 2005). The search for b-galactoside positive and b-galactoside-permease-positive organisms also permit a confirmation step for lactose fermentation, when the multi-tube fermentation method is used. The cytochrome-oxidase test is also used as a confirmation test to eliminate some bacteria of the Aeromonas or Pseudomonas genera that would ferment lactose.

The definition of coliform bacteria varies country wise slightly or on the organization in charge of the microbiological monitoring regulations. In Canada, the definition is the same as in the US, in some countries in Europe, the definition varies. For example, the French Standardization Association (1990) defines total coliforms (TC) as: “rod-shaped, non-spore-forming, Gram-negative, oxidase-negative, aerobic or facultative anaerobic bacteria that are able to grow in the presence of bile salts or other replacement surface active agents having an analogous growth inhibitory effect and that ferment lactose with gas and acid or aldehyde production within 48 h at 37 = 1°C. AFNOR (1990) defines other coliform groups, together with the thermo tolerant coliforms (also called faecal coliform, FC) and, more specifically, *E. coli* as thermo tolerant coliforms which have the same fermentation properties as total coliforms (TC) but at a temperature of 44 = 0.5°C. *E. coli* produces in-dole from tryptophan at a temperature of 44 = 0.5°C, gives a positive result methyl red test, is incapable to produce acetyl methyl cabinol and does not use citrate as its sole carbon source”.

The faecal coliform group includes all of the rod-shaped non-spore forming bacteria, gram-negative, lactose-fermenting in 24 h at 44.5°C, and which can grow with or without oxygen. Another type of faecal bacteria is faecal streptococcus which is normally present in large numbers in the intestinal tracts of warm-blooded animals other than humans.

ENVIRONMENTAL SIGNIFICANCE

Total coliform is abundant in the soil. Coliform are found in natural environments, of earthy origin, but drinking water is not a natural environment for them. Their presence does not necessarily imply contamination from wastewater nor the presence of other sanitation based health risks but does indicate the need for an analysis of all water system facilities and their operations to decide the route of organisms entering the water system. Public notice to water system users is required since properly constructed and maintained water should not have total coliform. Monitoring for organisms other than coliforms is also recommended by various estuarine waters (sometimes in legislation) for example, enterococci, faecal streptococci, salmonella, entero-viruses, etc. However, these recommendations and legal requirements usually apply only to bathing, recreational area or to shellfish zones.

The coliform include the following genus: *Escherichia, Klebsiella, Enterobacter, Citrobacter, Yersinia, Serratia, Hafinia, Pantoea, Kluyvera, Cedecea, Ewingella, Moelleralla, Lectercia, Rahnella, Yokenella* (Topley, 1997; Ballows, 1992). Coliforms such as *Citrobacter*, *Enterobacter* and *Klebsiella* species can also be found in natural environments such as soil, vegetation, or surface waters, where their presence is not necessarily related to faecal contamination (Leclerc et al., 2001). Faecal coliform is a subgroup of the total coliform group (American Public Health Association (APHA), 2005). Faecal coliform bacteria normally originate in the intestines of mammals, as discussed. They have a comparatively short life span compared to other coliform bacteria. Their occurrence could be related to improper disposal of sanitary waste. Immediate public notice and a boil order to the users (within 24 h) are required due to the higher likelihood of disease organisms also being present in water. Dominant in the area are *Escherichia and Enterococci* (Stevens et al., 2003).

E. coli is the main bacterium within the thermo tolerant coliform group, present in large numbers in feces at concentrations of about 10^9 bacteria per gram of faecal matter (Brenner et al., 1982). It does not multiply appreciably in the environment (Edberg et al., 2000), whereas other members of these bacteria are found naturally in water, soil and vegetation (Parch and Malheur, 2012). Also, these are universally present in large numbers in sewage but do not grow in natural waters (Environment Agency, 2002). Town (2001) reported a strong positive correlation between faecal coliform and *E. coli* bacteria. When concentrations of faecal coliform bacteria are elevated, concentrations of *E. coli* bacteria are elevated too. Compared to other faecal coliform, they have a relatively short life span. Their presence indicates a strong probability that human or animal wastes are entering the water system.

E. coli is considered to be most sensitive to environmental stresses. Its survival time in the environment is dependent on many factors, such as temperature, exposure to sunlight (UV rays), presence and types of other micro flora, and the physic-chemical characteristics of water involved (for example, groundwater, surface water, or treated distribution water). In general terms, *E. coli* survives for about 4 to 12 weeks in water containing a moderate amount of micro flora at a temperature of 15 to 18°C (Edberg et al., 2000). Regrowth of *E. coli* in water distribution systems is not a concern, since *E. coli* rarely grows outside the human or animal gut (Geld, 1996).

So far, the Guidelines for Canadian Recreational Water Quality (Health and Welfare, Canada, 1996) have suggested *E. coli* as the best indicator of faecal contamination from warm-blooded animals in freshwaters whereas the enterococci group is for marine waters (Nail, 2004). Generally, for water examination purposes, enterococci can be regarded as indicators of faecal pollution, although some can rarely originate from other environment.

Enterococci have a number of advantages as indicators over total coliforms and even *E. coli,* as they have been known to survive longer (Meters et al., 1974). Despite being less numerous than faecal coliforms and *E. coli* in human feces (Fleche et al., 1983), they are still abundant enough to be detected after significant dilution. There is a concern that enterococci are a diverse group of bacteria, and that the group contains species that are environmental and their presence in water is not necessarily indicative of faecal pollution. This concern is driven by the problems associated with the use of total coliforms as an indicator of faecal pollution. An early research report by Geld (1970) indicated that *Enterococcus facials* vary liquefactions was common in good quality water and its importance was not clearly considered if recovered in waters in concentrations of less than 100 organisms/100 mile however, more recent research on the relevance of faecal streptococci as indicators of pollution showed that the majority of enterococci (84%) isolated from a variety of polluted water sources were “true faecal species” (Pinto et al., 1999).

SETTING WATER QUALITY GOALS

As per Central Pollution Control Board (CPCB), an apex body in the field of water quality management, India, the term quality must be considered relative to the anticipated use of water. From the user’s point of view, the term “water quality” is defined as “those physical, chemical or biological characteristics of water by which the user evaluates the acceptability of water” (CPCB, 2008). The water supply must be pure, wholesome, and potable. Therefore, for setting water quality objectives of a water body, it is essential to identify the uses of water in that water body. CPCB has developed a concept of “designated best use”. According to which, out of several uses a particular water body is put to, the use which demands highest quality of water is called its “designated best use”, and consequently the water body is designated. For each of these five “designated best uses”, the CPCB has identified water quality requirements in terms of few chemical characteristics, known as primary water quality criteria. The “designated best uses” along with respective water quality criteria is given in Table 1. For aquaculture and cooling, the coliforms are not considered as there is no direct damage found till now. The CPCB, in collaboration with the concerned state Pollution Control Boards, has classified all the water bodies including coastal water in country according to their “designated best use”.

RISK TO HUMAN HEALTH

Most people are concerned about the health risk that coliform may pose. People exposed to coliform contaminated water may exhibit fever, diarrhea and abdominal cramps, chest pain, or hepatitis. During bathing, exposure to coliforms may cause urinary tract infection. While *E. coli* by itself is not generally dangerous, other pathogens of faecal origin that are health threats include *Salmonella, shield,* and *Pseudomonas aeruginosa.* Non-bacterial pathogens that may be present with faecal material include protozoans, such as *Cryptosporidium, Giardia* and viruses. Vero cytotoxic *E. coli* (Parch and Malheur, 2012). The vero-cytotoxin/shiga toxin producing *E. coli* (VTEC/STEC) group has over 200 different serotypes, including the highly pathogenic entrerohaemorrhagic *E. coli* (EHEC) with *E. coli* O157:H7 the most significant serotype that causes hemorrhagic colitis with bloody diarrhea and haemolytic uraemic syndrome better known as HUS (Bolton et al., 2009; WHO, 2004). There are also other pathogens, such as: Enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC), whose spread occurs mostly through the human faecal-oral route (Bolton et al., 2009). Several authors have reported waterborne disease outbreaks in water meeting the coliform regulations (Gofti et al., 1999).

LABORATORY METHODS FOR TOTAL COLIFORM DETECTION

All method of total coliform identification requires culturing of the sample in the presence of a special media. The culturing process requires approximately one to three days for the coliform to grow before interpreting the bacterial data. There are mainly three laboratory procedures that are majorly used to detect coliform in a water sample. However, there are many other sophisticated methods which have come up in the recent years.

Multiple tubes

This method was developed in the early 1900s. It uses some test tubes and measures the amount of gas production in another small tube called Durham’s tube during 48 h of incubation. Results are reported in terms of most probable number of organisms (MPN) per 100 milliliters of sample. Lactose and lauryl tryptose broths are used as presumptive media, but Seidler et al. (1981) and Evans et al. (1981) have observed interference of

Table 1. Use based classification of surface water in Nigeria (NAFDAC, 2008).

Designated-best-use	Class of water	Criteria
Drinking Water Source without conventional treatment but after disinfection	A	-Total coliforms organism MPN/100 ml shall be 50 or less -pH between 6.5 and 8.5 -Dissolved oxygen 6 mg/1 or more -Biochemical oxygen demand 5 days 20°C 2 mg/1 or less
Outdoor bathing (organised)	B	-Total coliforms organism MPN/100 ml shall be 500 or less -pH between 6.5 and 8.5 -Dissolved oxygen 5 mg/1 or more -Biochemical oxygen demand 5 days 20°C 3 mg/1 or less
Drinking water source after conventional treatment and disinfection	C	-Total coliforms organism MPN/100 ml shall be 50 or less -pH between 6 to 9 -Dissolved Oxygen 4mg/1 or more -Biochemical oxygen demand 5 days 20°C 3mg/1 or less
Propagation of wild life and Fisheries	D	-pH between 6.5 and 8.5 -Dissolved oxygen 4mg/1 or more -Free ammonia (as N) 1.2 mg/1 or less
Irrigation, industrial cooling, controlled waste disposal	E	-pH between 6.0 and 8.5 -Electrical Conductivity at 25°C micro mhos/cm Max.2250 -Sodium absorption ratio Max. 26 -BoronMax. 2 mg/1

non-coliform bacteria, using lactose broth. A1 broth is use to detect faecal coliforms. The tubes with a positive presumptive reaction are then subjected to a confirmatory test. This number is a statistical estimate of the mean number of coliforms in the sample. As a result, this technique is a semi-quantitative enumeration of coliforms. This is reliable, easy to implement and requires only basic microbiological training apart from being relatively economical. This method suffers from lower precision in the estimation and depends on the number of tubes used for the analysis. The method is very tiresome, time-consuming and labor intensive since many dilutions have to be processed for each water sample. Significant numbers of glassware are used and laboratory cleanup is required.

Membrane filter (MF) method

This method came up in early 1950s. It filters organisms from the water through a sterile filter with a 0.45 mm pore size which retains bacteria and then incubates the initial parent organisms on the filter paper to produce visible colonies. A minimum of 22 h incubation time is required. Results are recognized as “count” of colony forming units (CFUs) per 100 milliliters. Many media and incubation conditions for the MF method have been tested for optimal recovery of coliforms from water samples (Rice et al., 1987). Among these, the most extensively used method for drinking water analysis are the m-Endo-type media in North American (American Public Health Association (APHA), 2005) and the Tergitol-TTC medium in Europe (Association Francaise de Normalisation (AFNOR), 1990). Coliform bacteria form red colonies with a metallic gloss on an endo-type medium (incubation 24 h at 35°C for TC) or yellow-orange colonies on Tergital-TTC media (incubation 24 and 48 h at 37 and 44°C for TC and FC, respectively). Other media, like MacConkey agar and the Teepol, have been used in South Africa and Britain. However, comparisons have shown that m-Endo agar yields higher counts than MacConkey or Teepol agar (Grabow and du Preez, 1979). The chromo cult agar has been found to be an alternative to MacConkey agars.

To enumerate FC, the APHA (2005) proposed that filters be incubated on an enriched lactose medium (m-FC) at a temperature of 44.5°C for 24 h. Due to the elevated incubation temperature and the addition of rosolic acid salt reagent, few non-faecal coliform colonies may develop on the m-FC medium (APHA, 2005). Dark blue colonies confirm faecal coliform’s presence. Additionally, typical colonies with shinining may be produced occasionally by non-coliform bacteria and dark red or nucleated colonies without sheen may occasionally be coliforms. Coliform verification is therefore recommended

recommended for both types of colonies (APHA, 2005). Some improvements in the method have increased detection of injured coliform bacteria, including the development of m-T7 medium formulated specifically for the recovery of stressed coliforms in drinking water (LeChevalier et al., 1983).

Evaluation on routine drinking (Meters et al., 1986) and surface (Freier and Hartman, 1987) water samples showed higher coliform recovery on the m-T7 medium as compared with that on the m-endo medium. However, m-T7 may not be as efficient when stressing agents other than chlorine are involved. Rice et al. (1987) achieved no significant difference in coliform recovery on m-T7 compared with m-Endo LES from mono-chlorinated samples. Adams et al. (1989) found that the m-T7 medium performed no better than the medium in enumerating *E. coli and C. freundii* cells exposed to ozone. This method is much simpler than MPN, less labor intensive and requires less clean up of glassware. However, it cannot be used on muddy water. The presence of high numbers of background heterotrophic bacteria has been reported to decrease coliform recovery by MF (Clark, 1980; Burlinggame et al., 1984).

Too much crowding of colonies on m-Endo media has been linked with a reduction in coliform colonies producing the metallic shine (Hsu and Williams, 1982). The principal concern about MF is its incapability to recover stressed or injured coliforms. A number of chemical and physical factors involved in drinking water treatment, like disinfection, can cause sub lethal injury to coliform bacteria, resulting in injured cells which fail to develop colonies on a selective medium. Exposure of bacteria to chlorines like products may also result in enhanced sensitivity bile salts or replacement of surface-active agents (sodium deoxycholate or Tergitol 7) contained in some selective media (Rompre et al., 2001).

MMO chromogenic fluorogenic method

This method was developed in the late 1980s. It comprises of culturing the coliforms in the sample bottle. An incubation time of 18 to 28 h is required. The yellow color indicates the presence of total coliform and florescent condition under black light indicates *E. coli.* Results are stated as the presence/absence of coliform organisms per 100 milliliters. Non-coliform organisms are not produced, this being an advantage. The enzyme substrate, for example o-nitrophenyl-b-D-galactopyranoside (ONPG), β-Galactosidase assay (CPRG), and 4-methylumbelliferyl-b-D-glucuronide (MUG) are organism specific and where they are not, the target organism is selected for by suppressing the competing. The target population is characterized by enzyme systems that metabolize the substrate to release the chromogen/ fluorogen. This results in a colour change in the medium and/or fluorescence detected under long wave UV radiation. The most important food pathogens can be screened using chromogenic/fluorogenic media in a wide variety of food samples like *Salmonella, Camphylobacter, Listeria, Listeria monocytogenes, S. aureus,* Coliforms, *E. coli* as well as specific target organisms such as *E. coli* O157.

ANOMALY IN COLIFORM DATA

Sometimes the estimation of coliform does not lead to proper understanding of the situation. This may be due to the following reasons. When the non-coliforms are present in high numbers, (more than 200 colony forming units (CFUs) in a 100 milliliter sample), it inhibits the growth of coliforms. Coliform counts for total and faecal can vary greatly throughout the stretch of an estuary-mainly due to the dilution of freshwater with seawater which continuously changes as a result of tidal fluctuations. In an inadequately filtered well, bacteria are expected to be present. Organisms that enter a well can be there one day and die off before a second sample is taken a few days or a week later. Therefore, one may fall sick but the cause may not be detected. Variation in methods of analysis can lead to variable counts. Some bacterial tests use a filtration step while others do not. Each test uses a different media to incubate the organisms. Sometimes the bacteria themselves are counted while in other cases enzyme byproducts are measured. Some methods better detect stressed coliform species while others do not. Fully representative samples are hard to obtain since bacteria often combine together in clumps in pipes and in the sample container. Thus, in cases where there are few organisms, they may not be evenly distributed in the water. Due to high salinity the coliform count may be much below the permissible limits. However, this condition does not allow the water quality to be drinkable

COLIFORMS' ENTRY TO WATER SYSTEM

1. Open defection in the catchments area release the human waste to the water body which then meets the water through surface runoff. Animal feces also contribute in the similar way. Dellile (1987) found a strong positive correlation between penguin population and bacterial numbers in the sea water adjacent to the rockeries and also a decline in bacteria numbers with distance from store. This finding supports the correlation between cattle feces and coliforms. Thus, runoff from cattle feedlots, hog farms, dairies, and barnyards that have poor animal keeping practices where waste is not properly disposed contribute a lot.
2. Domestic sewage can be the dominant source of faecal microorganisms in the marine environment and have a significant environmental impact (Lenihan et al.,

1990).
3. Discharges from illegal or leaky sanitary sewer connections, poorly functioning septic systems, wastewater treatment plant effluent are potent contributors. Bacteria are much more abundant in soils than in water.
4. Storms flows containing high amounts of sediment are often related to high concentrations of pathogenic bacteria (Marino and Gannon, 1991). The bacteria can attach to sediment particles to escape invertebrate predators (Murdoch and Cheo, 1996). Fast-running water can carry more sediment, so higher levels of bacteria can occur during high runoff. During storm flow, a strong positive correlation has been established between faecal coliform and *E. coli* bacteria (Town, 2001).
5. Bacteria washed into the ground by rainfall or snowmelt are usually filtered out as water seeps through the soil, so properly constructed water wells do not typically harbor coliform bacteria. However, fractured bedrock aquifers close to the surface are the exceptions, nevertheless, coliform bacteria can persist within slime formed by naturally occurring ground water microorganisms.
6. The slime (or biofilm) clings to the well screen, casing, drop pipe, and pump. Bacteria can enter into a new well during construction and can remain if the water system is not thoroughly disinfected and flushed. Well construction defects such as insufficient well casing depth, improper sealing of the space between the well casing and the borehole, corroded or cracked well casing, and poor well seals or caps can allow sewage, surface water, or insects to carry coliform bacteria into the well. Unplugged abandoned wells can also carry coliform bacteria into deeper aquifers. Opening at the top of the well; rusty or damage well casing; unprotected suction line; buried wellhead; and, nearness of a well to septic tanks, drain fields, sewers, kitchen sinks, drains, animal feedlots, abandoned wells, and surface water enhance the problem. Cross-connections with wastewater plumbing can also introduce coliform bacteria into the water supply. Sometimes water sources are contaminated by coliforms existing on biofilms predominantly *Citrobacter species (*kilb et al., 2003) harbored on rubber-coated valves in the water treatment units.
7. The increase in the number of industrial farms, without soil nearby, represents an opportunity to reuse their residues for agricultural purposes, as a source of nutrients and organic matter (Rufete et al., 2006) which often contributes faecal coliforms to soil and then ultimately to water.

FAVOURABLE FACTORS FOR GROWTH

1. Water depth can influence the effectiveness of solar radiation in faecal coliform inactivation (Sinton et al., 1994). Action spectra for *E. coli* show that UVB radiation has the greatest bactericidal effect (Webb and Brown, 1976), but UVA may be more vital in the marine background, as it penetrates the water column to a greater depth (Davis-Colley et al., 1994).
2. The radiation further produces heat which again has a significant effect on coliforms. Bacteria grow faster at higher temperatures. The growth rate slows drastically at very low temperatures (Smith et al., 1994).
3. Research suggests that particles as small as 11 mm naturally occurring in surface water are able to harbor indigenous coliform bacteria and *E. coli,* subsequently offering protection from UV light at a wavelength of 254 nm and up to a dose of 40 mJ/cm^2 (Cantwell and Hofmann, 2008). This phenomenon has been observed in water with turbidities as low as 0.8 NTU.
4. High concentration of dissolved oxygen boost microbial inactivation as seen in the Antarctic (Hughes, 2003). Further, temperature and salinity play important roles in regulating the concentration of oxygen found in seawater, when oxygen is present, photochemical damage to *E. coli* enhances, particularly in the presence of UVA (Sinton et al., 1994). The combination of UV and oxygen allows the formation of highly reactive free radicals (including singlet oxygen, hydroperoxyl, and hydroxyl groups), which cause cellular damage to the coliforms (Vincent and Neale, 2000). A weak negative correlation was found between dissolved oxygen and concentration of faecal coliform bacteria and *E. coli* (Hughes, 2003).
5. Stream flow often causes dilution of sewage and other wastes. It also dilutes freshwater, further reducing the coliform count (Hughes, 2003).
6. Algal blooms act as shields and reduce the penetration of solar radiation into the water column (Hader et al., 1998).
7. Sea ice thickness and physical properties, together with the snow that collects on its surface, can result in the reduction of solar radiation input into the water column (Belzile et al., 2000).
8. Salinity can affect faecal bacterial viability with high or rapidly changing salt concentrations increasing the cell inactivation (Anderson et al., 1979). The input of freshwater from iceberg melt, snowmelt from the shore, and sewage waste contributed to the low salinity in colder areas (Hughes, 2003). Seasonal factors can affect seawater salinity such as glacial melt and can reduce salinity. In summer, salinity around a piece of melting glacier ice can vary between almost freshwater and > 30% salinity (Hudier and ingram, 1994), while in winter, salt released during sea ice formation can increase sea water salinity (Golden, 2001). Coliform mortality may be greater than before by quick and sudden charges in osmotic stress caused by passing through seawater with spatially variable salinity.

RECOMMENDATIONS

If coliform bacteria are present, the source of the problem should be identified. Re-sampling from several locations within the water system is helpful. The entire water system

may need to be thoroughly flushed and disinfected before a negative bacteria sample can be withdrawn. Sometimes it is necessary to repeat the disinfection process. Proper changes or repairs should be made in the well. After the defects are corrected, the whole water system should be disinfected and the water re-examined before drinking. Many removal and disinfection procedures have been developed to control coliforms. Fluidized sand bio filters have been effectively used to remove total coliform bacteria (Davidson et al., 2008). An overall reduction of total and faecal coliforms in activated sludge system has also been found to be significant (Kazmi et al., 2008). Further, an interrelationship of biological oxygen demand (BOD) and suspended solids (SS) has been found with coliforms which suggest that improvement of the microbiological quality of wastewater could be linked with the removal of SS. Therefore, SS can serve as a regulatory tool in lieu of a clear coliforms standard.

Photo catalysis (TiO_2) has recently emerged as an alternative technology for bacteria inactivation (McLoughlin et al., 2004). Some simple approaches may be boiling the water. Chlorine (as gas or hypochlorites), chlorine dioxide, ozone and UV radiation are common tools for disinfection of drinking water (Rizzo, 2009). A very important remedy is to use bacteriophage to remove the coliforms. This is the most natural way. Ultimately, personal hygiene has no alternative. Washing thoroughly with soap after contact with contamination can prove to be effectively safe. The information on coliforms helps the water quality managers and planners to set water quality targets and identify needs and priority for water quality restoration programs for various water bodies in the country. The famous Ganga Action Plan and subsequently the National River Action Plan are results of such exercise (Central Pollution Control Board (CPCB), 2008).

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Full Length Research Paper

Correlation of obesity indicies and blood pressure among non obese adults in Zaria, Northern Nigeria

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Epidemiological studies from different populations have reported significant association between different anthropometric indicators and hypertension in obese adults but few data are available for the non obese adults of this study population. This study assessed the association between adiposity measures and hypertension risk and analyzed various anthropometric indices (body mass index (BMI), waist-height ratio, waist-hip ratio, waist and hip circumferences) as predictors of hypertension among non obese adults of Samaru, a suburb of Zaria in Kaduna state, Nigeria. The study protocol was duly approved by the Ethical committee of Ahmadu Bello University Teaching Hospital, Shika, Zaria. This cross sectional study examined a total of 174 non obese adults, 35 to 70 years of age (male, 91; female, 83) who were randomly selected for the study. All subjects were normotensive, non obese and refrained from taking any medications known to influence energy regulation. Normal-weight BMI was defined as a range of 18.5 to 24.9 kg/m^2 and overweight BMI ≤ 27 kg/m^2 was considered in the study. Descriptive statistics, partial correlation and multiple regression analysis were used to determine the relationship between anthropometric measurements and blood pressure parameters, after controlling for age. Results showed significant ($p \leq 0.05$) relationships between the systolic blood pressure (BP), diastolic blood pressure (BP) as well as mean arterial blood pressure (BP) and the indices of adiposity in male (waist circumference, BMI and waist-height ratio) than in female (waist-height ratio) group. Waist-height ratio was the most important and consistent index of adiposity that associated with the hypertensive risk in both male and female non obese adult groups, particularly with systolic BP. It then means that a decrease in intra-abdominal fat could reduce blood pressure and should be a target in the management of hypertension.

Key words: Non obese adults, blood pressure, waist-height ratio, Zaria.

INTRODUCTION

Hypertension commonly remains undiagnosed until relatively late in its course, leading to a variety of other life-threatening conditions like kidney damage and heart failure. It is also a very prominent feature of the metabolic syndrome (MetS). Insulin resistance and central obesity, recognized as the main factors involved in the pathophysiology of the MetS, contribute to elevated blood pressure, which further promotes vascular damage in cardiac, renal and brain tissue (Sowers et al., 2004; Wang and Hoy, 2004).

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Several epidemiological studies from different populations have reported significant association between different anthropometric indicators (such as waist circumference, body mass index (BMI) and waist-height ratio) and blood pressure levels (Williams et al., 2000; Duvnjak et al., 2008; Kjeldsen et al., 2008). These associations between body fatness using different indices have been consistently observed but remain poorly understood and the mechanistic explanations for the phenomenon are still being debated and no biological model of the process has been established (Rufus et al., 2008). Studies have also revealed the existence of some populations with high %fat and central adiposity at a low BMI and they tend to develop chronic diseases compared to others (Wang et al., 1994; Tuan et al., 2009).

Obesity has been particularly recognized as a major independent risk factor for cardiovascular diseases (Despres, 2001). This is because increased body fat is accompanied by profound changes in the physiological and metabolic functions of the body, which are directly dependent on the degree of excess weight and on its distribution around the body (Sanya et al., 2009). The concept of the metabolically obese normal weight individual is based on the observation that these same characteristics may be found in normal weight individuals with disorders often associated with obesity (St-Onge et al., 2004; Tsai, 2009).

Although, waist circumference (Janssen et al., 2004), BMI (Sanya et al., 2009) and weight-height ratio (Cai et al., 2013) have been shown to be associated with hypertension in some age groups, to the best of our knowledge, no data is available for the non obese adults of Zaria, Northern Nigeria. In the present study we assessed the associations of adiposity measures to hypertension risk and analyzed various anthropometric indices as predictors of hypertension, among non obese adults of Zaria in Kaduna state, Northern Nigeria.

MATERIALS AND METHODS

A cross-sectional survey was conducted on non obese adults, aged 47.13 ± 8.10 years (male) and 44.96 ± 9.58 years (female) in March, 2013. Study participants were randomly selected from different wards in Samaru, a suburb of Zaria in Kaduna state, Northern Nigeria. The Samaru town is the fourth and the most recent addition to the Zaria suburban area. It evolves from a small colonial farming settlement to become a large community, a melting-pot, often referred to as 'the University village'. It is cosmopolitan in nature, drawing and fusing people of diverse national and international backgrounds. Data from 174 non obese adults (male, 91; female, 83) were collected. The reasons that participants dropped out were: obese, untreated cases of hypertension or diabetes, taking medicine on the day when measurements were taken or non compliance to anthropometric measurements. All candidates enrolled in this study underwent a physical examination to obtain anthropometric measures. Body weight was measured with light clothes and without shoes and was approximated to the nearest 0.1 kg on a mobile lever scale (SECA; Vogel and Halke; Germany) and height was measured to the nearest 0.1 cm using a stadiometer (SECA; Vogel and Halke; Germany). Waist circumference (WC) was measured by a non-elastic flexible tape in the standing position. The tape was applied horizontally midway between the lowest rib margin and the iliac crest. Hip circumference (HC) was measured at maximal protrusion of the buttocks. The mean of two measurements to the nearest 0.1 cm were documented.

Blood pressure (BP) was measured on the same occasion as the anthropometric measurements, while subjects were sitting and with the cubital fossa supported at heart level, after at least 5 min of rest. BP was measured using a mercury sphygmomanometer, with the appropriate cuff for the adults upper arm size. The cuffs used hand bladders long enough to circle at least half of the upper arm without overlapping, and widths that covered at least two-thirds of the upper arm. Systolic BP was defined by the onset of the first Korotkoff sound, and diastolic BP was indicated by the fifth Korotkoff sound (disappearance of Korotkoff sound). The mean arterial blood pressure (MABP) is the average arterial pressure throughout the cardiac cycle (Sabri, 2003) and is calculated as follows:

MABP = DBP + 1/3 Pulse Pressure (mm/Hg)

Pulse Pressure = Difference between systolic and diastolic Blood Pressure

Normal-weight BMI was defined as a range of 18.5 to 24.9 kg/m^2 according to National Institutes of Health/National Heart, Lung, and Blood Institute (NIH/NHLBI) (1998) criteria and overweight BMI ≤ 27 kg/m^2 was considered because BMI ≥ 28 kg/m^2 has been shown to be a significant prognostic factor for all-cause and cardiovascular mortality among adults (Asefeh et al., 2001; Ofei, 2005). Weight-height ratio was calculated as waist circumference divided by height. Waist-hip ratio was calculated as waist circumference divided by hip circumference. Study purpose was explained to all volunteers before seeking their written consent. The study protocol was duly approved by the Ethical committee of Ahmadu Bello University Teaching Hospital, Shika, Zaria.

Statistical analysis

Data was analyzed using statistical package for social sciences (SPSS Inc, version 16. 0; Chicago). Descriptive statistics of mean and standard deviation was computed by sex for age, weight, height, BMI, WC, WHtR, WHpR, systolic and diastolic BP for the purpose of data interpretation. Partial correlation analysis was used to examine the relationship between WC, WHtr, WHpr and BP after controlling for age. Correlations were considered significant at P ≤ 0.05 with critical values located at 0.2050 (male), 0.2172 (female) (Thomas and Nelson, 1996). A multiple regression analysis, adjusted for age was used to examine the influence of WC, WHtr, WHpr and BMI on the risk of hypertension in both sexes. Differences were considered significant at P ≤ 0.05.

RESULTS

Descriptive characteristics of the study population as presented in Table 1 consists of (91) male and (83) female with mean BMI of 23.13 kg/m^2 ± 2.73 (male) and 23.78 kg/m^2 ± 2.42 (female) which falls within the classified normal weight (World Health Organization (WHO), 2003). Statistical equality of means at P ≤ 0.05 showed that male participants were significantly taller and heavier with smaller HC than the female. No significant differences existed in SBP, DBP, mean arterial (MA)BP

Table 1. Descriptive characteristics of the study population

Measurements	Mean ±SD	
	Male (n=91)	Female (n=83)
Age (years)	47.13±8.10	44.96±9.58
Weight (kg)*	69.55±9.60	62.90±7.96
Height (cm)*	173.32±6.42	162.58±6.09
Waist circumference (cm)	87.90±8.17	86.87±7.70
Hip circumference (cm)*	93.37±6.12	99.27±6.48
Body mass index (kg/m^2)	23.13±2.73	23.78±2.42
Waist-hip ratio*	0.941±0.050	0.876±0.06
Waist-height ratio*	0.508±0.048	0.535±0.05
SBP (mmhg)	127.03±20.14	132.77±26.05
DBP (mmgh)	79.01±10.76	82.71±14.74
MABP (mmgh)	95.02±12.76	99.398±17.94

*Statistical significant difference of equality of means at (CI 95%, P ≤ .05; Critical value: 1.960; Df: 172).

Table 2. Correlation between measures of adiposity and blood pressure.

Parameter	Male			Female		
	SBP	DBP	MABP	SBP	DBP	MABP
WC	0.232 (0.03)*	0.266 (0.01)*	0.271 (0.01)*	0.127 (0.254)	0.114 (0.309)	0.123 (0.269)
BMI	0.271 (0.01)*	0.255 (0.02)*	0.285 (0.006)**	0.192 (0.084)	0.203 (0.067)	0.204 (0.066)
WHpR	0.229 (0.03)*	0.121 (0.255)	0.188 (0.077)	0.010 (0.927)	-0.087 (0.437)	0.045 (0.690)
WHtR	0.284 (0.007)**	0.211 (0.046)*	0.267 (0.011)*	0.223 (0.044)*	0.202 (0.069)	0.218 (0.049)*
HC	0.142 (0.180)	0.273 (0.009)**	0.229 (0.030)*	0.153 (0.171)	0.236 (0.033)*	0.205 (0.065)

**Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed) Critical value: 0.2050. P: ≤ 0.05.

Table 3. Regression analysis examining the independent contribution of obesity measures to blood pressure.

Sex	Dependent variables	Model	β	R^2	p-value
Male	SBP	Waist-Height ratio	0.312	0.098	0.003*
	DBP	Waist Circumference	0.271	0.073	0.009*
	MABP	Waist Circumference	0. 288	0.083	0.006*
Female	SBP	Waist-Height ratio	0.819	0.071	0.002*
	DBP	Waist-Height ratio	0.442	0.055	0.002*
	MABP	Waist-Height ratio	0.789	0.066	0.003*

*Significant (CI 95%, P ≤ 0.05)

and age between male and female participants (P ≤ 0.05). The mean values of WHtR (male: 0.508 ± 0.048; female: 0.535 ± 0.05) and WHpR (male: 0.941 ± 0.05; female: 0.876 ± 0.06) were slightly above the classified risk values (Kuba et al., 2013). Table 2 shows significant positive relationships between the indices of adiposity and blood pressure in both sexes. WHtR significantly correlated with SBP (Male: 0.007**, Female: 0.044*), DBP (Male: 0.046*, Female: 0.069) and MABP (Male: 0.011*, Female: 0.049*). Also, in male only, WC significantly correlated with SBP (0.03*), DBP (0.01*) and MABP (0.01*). BMI also showed significant correlation with SBP (0.01*), DBP (0.02*) and MABP (0.006**) in male not female. Regression analysis in Table 3 shows

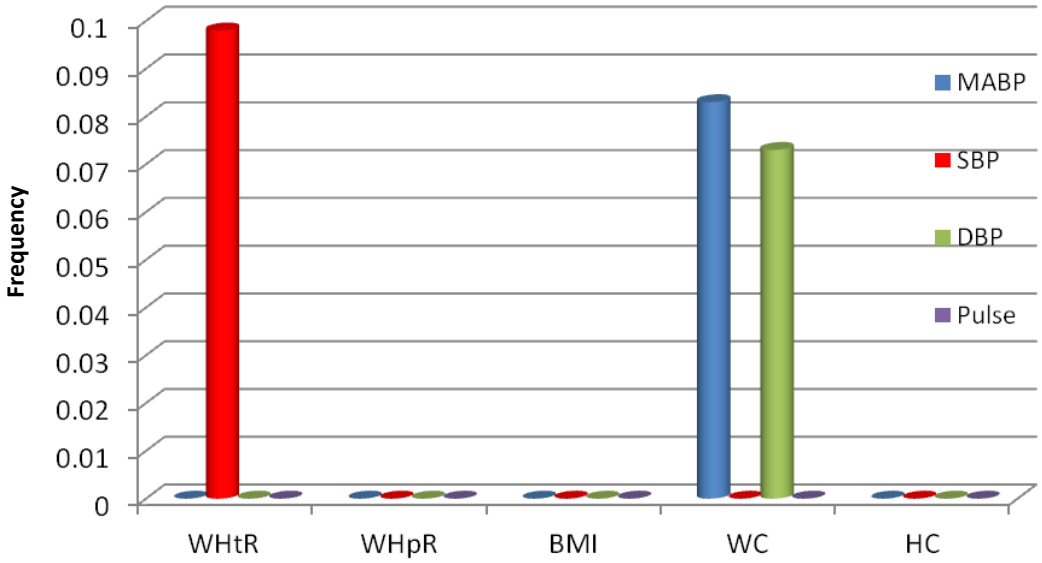

Figure 1. Predictability of measures of adiposity for blood pressure (male).

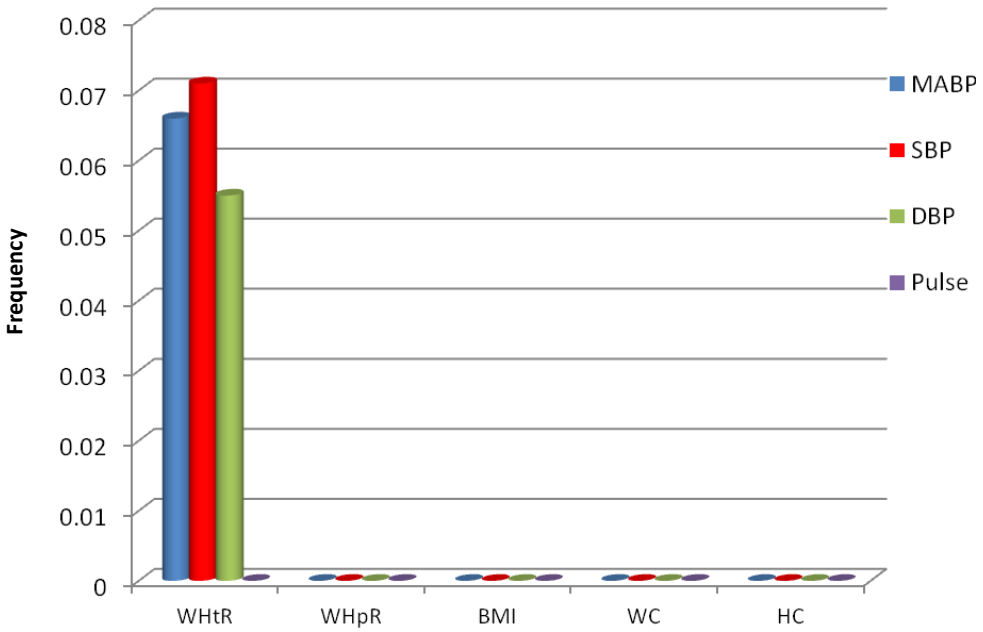

Figure 2. Predictability of measures of adiposity for blood pressure (female).

the clinical implication of large WC in male as it predicts high diastolic BP (0.009*) and mean arterial BP (0.006*), but WHtR was the most important and consistent index of adiposity that associated with the hypertensive risk in both sexes, particularly with SBP (male: r = 0.003*; female: r = 0.002*) as these were also shown in Figures 1 and 2. Using the R^2 values, Figure 3 shows the strength of the linear relationships between systolic blood pressure and waist-height ratio in male and female groups.

DISCUSSION

More suitable indices of body fat distribution have been suggested and waist-height ratio and waist-hip ratio have

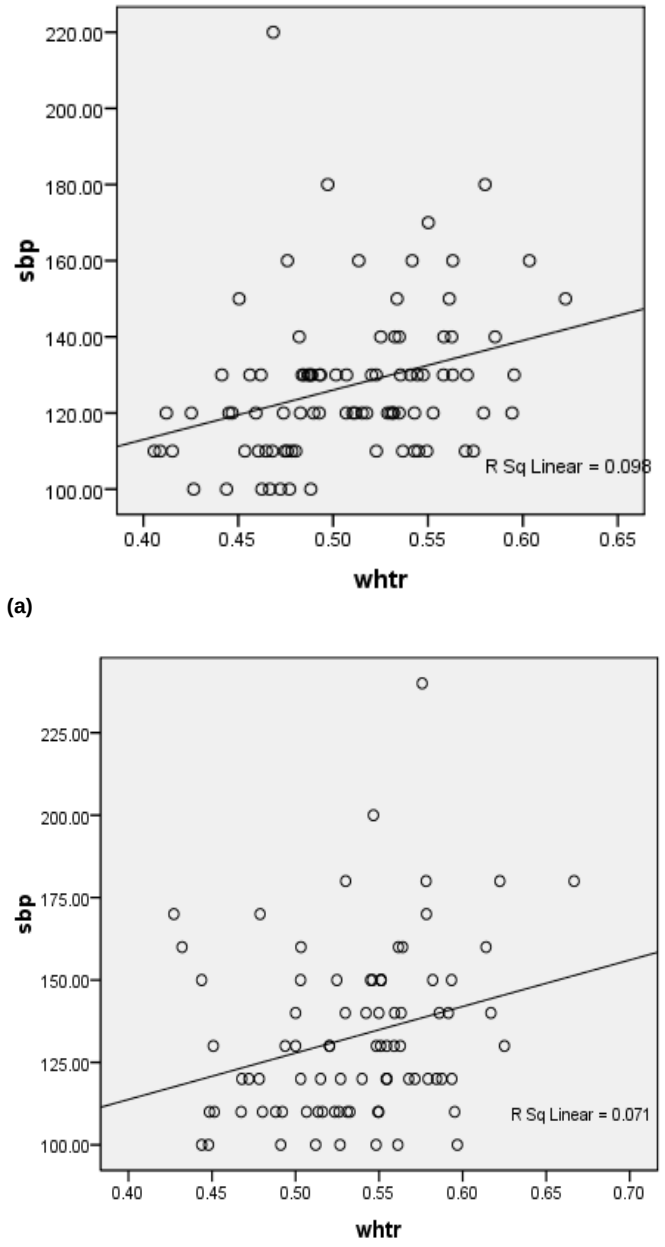

Figure 3. Relationship between Systolic blood pressure and Waist-Height ratio (Male) (Female)

been recommended. Studies have shown strong association between these indices and metabolic complications of obesity and cardiovascular risks in adults, children and different ethnic groups (Sanya et al., 2009; Falaschetti et al., 2010; Palacio et al., 2011). Most of the studied groups were obese adults; this study demonstrated that waist-height ratio was statistically superior to BMI, waist circumference and waist-hip ratio for indentifying hypertension among non obese adults. The discriminating power of waist-height ratio was lager in men than women and this is consistent with previous descriptions of non obese adults (Knowels et al., 2011; Vasan et al., 2011). This study also showed that waist circumference, BMI and waist-hip ratio correlated with blood pressure, although using regression analysis, significant influence of waist circumference, BMI and waist-hip ratio was not found, particularly in female.

Waist-height ratio takes into account the distribution of body fat in the abdominal region which has been shown to be more associated with cardiovascular risks than body weight, It adds significantly to cardiometabolic risk prediction over BMI and waist circumference in men, and it is an important index of central obesity, which is free from any bias due to hip width changes along with waist circumference of short and tall subjects (Dhall et al., 2011). The significant correlation exhibited by waist-height ratio in this study could then mean that it carries the burden of hypertension risks among non obese adults; so much so that the recommended optimal cut-off point of 0.5 for men and women (Browning et al., 2010) is higher in this study (0.508 in men and 0.535 in women). This is in agreement with the findings of Park et al. (2009) and Nambiar et al. (2010) who showed waist-height ratio to be a better predictor of cardiovascular diseases than other anthropometric measurements, including BMI, waist circumference, waist-hip ratio and skinfold thickness. These observations was also reflected in recent studies on non obese adults by Knowels et al. (2011) and Vasan et al. (2011) who proved that BMI was the least accurate predictor of hypertension.

Conclusion

In this study, waist-height ratio showed strong and consistent correlation with systolic BP > diastolic BP > mean arterial BP in both male and female groups, followed by waist circumference. This fact could then mean that waist-height ratio and waist circumference carry same information of visceral obesity. This also suggests that a decrease in intra-abdominal fat could reduce blood pressure and shows consistence with previous studies. Waist-height ratio can be calculated by individuals themselves and bias toward underestimation is minimal.

ABBREVIATIONS

WHtR, Waist-height ratio; **WHpR,** waist-hip ratio; **WC,** waist circumference; **HC,** hip circumference; **BMI,** body mass index; **SBP,** systolic blood pressure; **DBP,** diastolic blood pressure; **BP,** blood pressure; **MABP,** mean arterial blood pressure.

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Full Length Research Paper

Abattoir operations, waste generation and management in the Tamale metropolis: Case study of the Tamale slaughterhouse

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The study was conducted to assess the rate of effluent generation and management at the Tamale abattoir. It also investigated the methods adopted in processing animal carcasses, including handling/transportation to retail centres. The investigative approach to data collection was adopted in combination with desk research and other strategies. Waste material generated was estimated based on calculations by Aniebo et al. (2009). The results show that on average, about 55 cattle, 50 sheep and 20 goats were slaughtered daily at the abattoir, leading to an annual production of 2,475 tons of beef, 270 tons of mutton and 94 tons of chevon. These represent 12, 1.6 and 0.5% of the 2010 national output of meat in Ghana. The number of livestock (125) slaughtered daily results in 0.7 ton of blood, 0.5 ton of gut contents, 0.4 ton of waste tissues and 0.7 ton of bone. These translate into a total of 1,159.7 tons of blood, 822.9 tons of intestinal contents and 636.5 tons of waste tissues discharged into the environment annually. Handling and transporting carcasses to the various points of sale is generally done under unhygienic conditions, exposing the meat to all sorts of contaminants. With only one old wretched meat van serving the abattoir, majority of butchers (93%) resort to the use of other deficient means including bicycles and taxis (booth) to transport meat to the market, posing a serious threat to the health of consumers.

Key words: Abattoir, environment, intestinal content, tissue waste, biogas.

INTRODUCTION

The Food and Drug Laws/guidelines of Ghana require that imported livestock products and those produced locally meet the requirements specified under the relevant Ghana Standard for Meat and Meat Products (First Databank (FDB), 2004). Although the laws provide butchers and importers of meat with guidelines that ensure high safety and quality standards as well as a comprehensive procedure for bringing their activities into compliance with the law, enforcement appears to be a problem. Currently, activities at the Tamale abattoir apparently meet (partially) only 30% of the requirements specified under the relevant standard for meat production and none of the storage requirements (FDB, 2004).

Abattoir operations are meant to recover the edible portions of slaughtered animals for human consumption. In the process, significant quantities of waste materials including organic and inorganic solids are generated (Red Meat Abattoir Association (RMAA), 2010; Steffen & Kirsten Inc, 1989). The solid waste consists mainly of bones, undigested ingest and occasionally aborted foetuses while the liquids comprise of blood, urine, water, dissolved solids and gut contents. Some researchers

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Table 1. Data for estimating abattoir effluent.

Waste category	Cow	Goat
Blood/head (kg)	12.6	0.72
Intestinal content/head (kg)	8.0	1.25
Waste tissue (kg)	6.4	0.80
Bone/head (kg)	11.8	2.06

Source: Aniebo et al. (2011).

point out that abattoir activities are responsible for the pollution of surface and underground waters as well as air quality which indirectly affect the health of residents living within the vicinity of abattoirs (Odoemelan and Ajunwa, 2008; Patra et al., 2007; Raymond, 1977). In addition, primary producers in affected water bodies may be destroyed by such pollutants, which may directly affect fish yield, with serious consequences on diet (Aina and Adedipe, 1991).

Wrongful discharge of blood and animal faeces into streams may cause oxygen-depletion as well as nutrient-over enrichment of the receiving system which could cause increased rate of toxin accumulation (Nwachukwu et al., 2011). Humans may also be affected through outbreak of water borne diseases and other respiratory and chest diseases (Mohammed and Musa, 2012).

Abattoir waste disposal in many developing countries including Ghana has been a major challenge for years. In most cases, waste materials are disposed of without regard to sound environmental management practices, thus making them harmful to humans and other terrestrial and aquatic life. Studies from Nigeria and Ghana show that many abattoirs in the respective countries either deposit waste materials in the immediate environs or dispose of them directly into water bodies, some of which serve as sources of water for the abattoirs (Adelegan, 2002; Osibanjo and Adie, 2007; Weobong, 2001). Some people argue that the practice is mainly due to lack of or inadequate waste recovery and treatment facilities (Adeyemo et al., 2009).

In Ghana, increasing demand for animal products especially meat has led to increase in the volume of abattoir waste generation and there are growing concerns about the current situation. An enquiry into activities of the main abattoir in the Tamale metropolis showed that effluent water from the facility was highly polluted (Weobong and Adinyira, 2011), with all the measured parameters exceeding acceptable standards set by the Environmental Protection Agency (EPA) of Ghana. The study further revealed that residents within the community where the slaughterhouse is located complained of stench from the effluent, pollution of their water sources and frequent disease outbreaks among others. Concerns have also been raised about the manner in which carcasses are prepared and subsequently handled en route to the markets.

This study therefore sought to estimate the volume of meat produced at the abattoir and the corresponding effluent generated (solid and liquid) given that nothing seems to have changed by way of proper waste disposal since the last study revealed a rather worrying situation. The study as well investigated the process of meat processing and handling at the abattoir and during transportation to the markets. The outcome of the study is expected to first raise consciousness about the level of waste generation at the abattoir and the potential health and environmental implications if nothing is done to halt the situation. It is secondly expected that documentation of the findings will help to raise awareness about the unconventional methods employed in handling/ transporting meat from the abattoir to the various market centres in the metropolis and the potential for contamination.

METHODOLOGY

The investigative approach to data collection was adopted in combination with desk research (secondary data collection) and other strategies. The main abattoir in Tamale was selected for this study. Data on the number of ruminants (cattle, goats and sheep) slaughtered daily was collected (through participant observation) between April and June, 2013. This was backed with data obtained from records on abattoir operations. Additional information was collected through questionnaire administered to butchers and interviews with key informants (veterinary experts and meat inspectors) using interview schedule. Waste materials generated from abattoir operations was estimated based on calculations by Aniebo et al. (2009) (Table 1). The computations were done using average data on body weight for the respective ruminants and carcass weight per 1,000 kg.

This study also assumed that volume of waste generated from the slaughter of sheep is equal to that for goat. The estimated figures from Aniebo et al. (2009) were therefore applied to sheep. Quantity of meat produced was also estimated using data (average) from the Ministry of Food and Agriculture (MOFA) (Table 2), computed from carcass weight and number of livestock slaughtered.

Meat production (Mt) = (Off take rate (%) × Estimated population × Carcass weight of animal (kg)/1000. Carcass weight = Average livestock weight (kg) × (carcass wt%) / 100 (MOFA, 2011). For purposes of this study however, off take rate × estimated population stated in the formula was substituted with the observed number of livestock slaughtered daily at the abattoir.

RESULTS AND DISCUSSION

Entry requirements

The butcher industry is clan-related and dominated by members of the Nakohigu clan (Dagbani) meaning butchers' clan. The setup is a form of traditional/political system controlled by a Chief with various officers who play different roles as pertains in the traditional clan system. Butchers who do not belong to the regular clan but have been initiated and accepted into the business are in the minority, referred to as Bajobihi. Membership of the Tamale abattoir is currently made of 13% Bajobihi

Table 2. Data for estimating meat production.

Livestock	Av. Livestock wt. (kg)	Carcass (% of live animal wt.)	Carcass wt. of animal (kg)
Cattle	250	50	125
Sheep	25	60	15
Goats	22	60	13

Source: MOFA (2011).

Table 3. Meat production at the Tamale abattoir (Mt).

Livestock	Livestock numbers	Av. production/ day	Av. production/ year	% of 2010 production
Cattle	55	6.88	2,475	12.38
Sheep	50	0.75	270	1.60
Goats	20	0.26	93.6	0.49

Estimated using field data, 2013 and Table 2

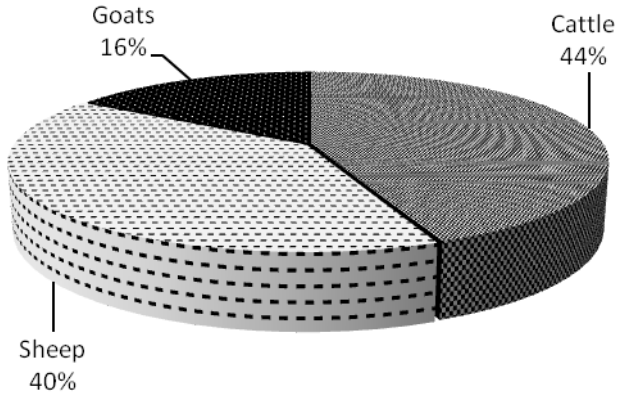

Figure 1. Composition of ruminants slaughtered daily at the Sheshegu abattoir.

and 87% Nakohigu. The current composition was described as a marked improvement of the situation 15 to 20 years earlier. According to one key informant, it was virtually impossible for individuals who are not members of the clan to operate as butchers. Traditionally, non-clan members are made to perform certain initiation rites. They present two (2) knives and an unspecified amount of money to the Chief who performs the necessary rituals. It was popularly believed that any individual (Nakohigu) who is not interested in becoming a butcher still have to go through the ritual and occasionally feed the knives with blood by participating in slaughter operations. Refusal to comply, according to some key informants, could result in madness or failure in any attempted business undertaking.

Meat production and handling at the abattoir

On the average, 125 ruminants were slaughtered daily at the abattoir. Composition of the respective ruminants slaughtered daily at the abattoir is represented in Figure 1. Table 3 shows the average (daily and yearly) estimates (Mt) of the quantity of meat produced. The results show that annually, 2,475 tons of beef, representing over 12% of the 2010 national output for Ghana (MOFA, 2011) came from the abattoir. In addition, about 1.6 and 0.5% of the 2010 national output of mutton and chevon, respectively was produced. These results show that activities at the abattoir contribute significantly to the total national meat output, providing employment for a number of people in the metropolis. There are however, serious concerns regarding the methods adopted in processing and handling of the meat as well as management of waste materials.

Waste generation and management

The 55 cattle, 50 sheep and 20 goats slaughtered daily lead to the generation of about 0.7 ton of blood, 0.5 ton of gut contents, 0.4 ton of waste tissues and 0.7 tons of bone. These translate into annual total of 1,159.7 tons of blood, 822.9 tons of gut contents and 636.5 tons of waste tissues discharged directly into the environment (Table 4). A total of 1,237.4 tons of bone that would otherwise have been part of the annual waste generation was excluded because they are often sold together with the meat. In other words, between 2005 when the abattoir was commissioned and 2013, an estimated 8,117,928 tons of blood, 5,760,300 tons of intestinal contents and 4,455,360 tons of waste tissue have been discharged into the environment. Blood and liquid intestinal fluids are washed into a drain that empties right at the premises of the abattoir (Figure 2). The drain only serves as means of carrying effluent out of the main building. These are washed by rains into nearby streams and dugouts that serve as sources of water for other communities. Solid

Table 4. Waste generation at Sheshegu abattoir.

Waste category	Cattle/day	Goat/day	Sheep/day	Total/day	Total/yr
Blood/Head(kg)	693	14.4	36.0	743.4	1,159,704
Intestinal content/Head(kg)	440	25.0	62.5	527.5	822,900
Waste tissue/Head(kg)	352	16.0	40.0	408.0	636,480
Bone/Head(kg)	649	41.2	103.0	793.2	12,37,392

Source: Field data (2013).

Figure 2. Drain carrying a mixture of blood and intestinal fluid.

Figure 3. Abattoir assistant carting intestinal waste (A) to dumping site (B) close to abattoir.

intestinal contents are collected in wheelbarrows and deposited at designated points (Figure 3A and B).

The abattoir waste materials are entirely organic that can either be composted or recycled and used for various activities, yet they are left to degrade, producing bad stench. Degrading heaps of gut contents at the site serve as breeding grounds and sanctuary for pests that become a nuisance for abattoir workers, visitors as well as residents around the facility. Bone waste is currently not a problem because they are often sold together with the meat.

The abattoir currently lacks basic facilities including cold storage facility despite the vast potential of the industry. It appears that the limited facilities provided have been run down over the years. Although officials of the veterinary services were seen inspecting meat, many other health and sanitation concerns have been ignored by the authorities. Although abattoir waste carries high levels of microorganisms that may be harmful to humans, they are an excellent substrate for generating biogas (Rabah et al., 2010). The study revealed that about 1,159.7 tons of blood, 822.9 tons of intestinal contents

Figure 4. Open burning of fur with tyres and firewood (A). Carcass processing on the floor close to a pile of gut contents (B).

and 636.5 tons of waste tissues are discharged annually. This volume of waste when properly managed (composted or digested) will in addition to reducing the sanitation and health challenges round the facility, produce other benefits (for example, manure) for farmers and biogas for home and other uses. It has been estimated that 1 kg of fresh animal waste produce about 0.03 m^3 of gas (methane) per day (FAO, 1996).

Theoretically, about 25,000 m^3 of biogas can be produced annually from the 822.9 tons of gut contents alone. It is popularly believed that the potential to generate biogas from abattoir waste is a good opportunity to enhance their activities (HDR, 2010). In other words, it could lead to improvements in efficiency and general approach to meat processing. For instance, if the abattoir is able to produce biogas for use, there will be reduced demand for firewood and lorry tyres, consequently saving some forest resources and the environment.

Meat handling and transportation

Some butchers were cited preparing carcasses on the wet, dirty floor outside the abattoir and very close to the heap of waste materials (Figure 4B). Before this stage, and immediately after animals are slaughtered, the fur is burnt off in the open using firewood and lorry tyres (Figure 4). At least five fire points were counted daily over the period, each producing smoke continuously between 7.30am and 11:00am. It was however observed that firewood constituted the greater part of the fuel. In other words, use of lorry tyres was relatively minimal compared to other places in the country where they constitute the main energy base (Nyinah, 2002). Apart from the danger it poses to the health of consumers, the practice also produce lots of smoke that pollute the area (Figure 3B). The facility is generally operating under unhygienic conditions due to lack of certain basic amenities. Since it was commissioned somewhere in 2005, it has not been rehabilitated hence most of the facilities have been run down.

Handling and transportation

There are major problems with the manner in which animal carcasses are handled during slaughter, loading and transportation from the abattoir to various points of sale in the metropolis. There is only one old, wretched meat van used to transport meat to the markets. Thus, majority (93%) of the butchers convey their meat on bicycles, motorbikes (Figure 5A) and motorised tricycles, popularly known as motor king (Figure 5B), as well as in booth of taxis under very unhygienic conditions. In most cases, meat is simply packed and transported without regard to safety measures. These practices affect the quality of meat sold on the market, with serious consequences to the health of consumers. For instance, Adzitey et al. (2010) in a study to ascertain the quality of chevon and mutton sold in three major meat shops in the Tamale metropolis concluded that samples from all the shops were contaminated with microbes (*Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Salmonella* spp. and *Escherichia* coli). They indicated that although the bacterial count for the samples were below 107 where spoilage occurs (Warriss, 2001), the presence of certain strains of these bacteria cause food-borne infections.

Conclusion

Operations at the Tamale abattoir contribute significantly to meat supply in the municipality. However, there are serious problems with the conditions under which car-casses are processed. Carcass handling in the course of

Figure 5. Images showing chevon (A) and Beef (B) ready to be transported to the market.

transportation to various retail centres within the municipality is also poor. But for the wrong approach to waste management, the volume of effluent generated at the abattoir is a potential resource that can be utilised to enhance operations as well as serve other sectors of the economy. For instance DeCo is a registered Ghanaian NGO that produce organic fertilizer for small-scale farmers (DeCo, 2011). It operates decentralized composting plants in the Northern region of Ghana using various kinds of biodegradable waste materials. Collaboration between the existing statutory regulatory bodies, municipal assemblies and major stakeholders (including DeCo) will help to address some of the pressing challenges of waste management at the abattoir. There is also the need for more robust monitoring and sanction regime (FDB, 2004) by the Veterinary Services as well as Food and Drugs Authority to ensure that meat processing and handling conform to the basic health and environmental standards.

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Full Length Research Paper

First mass screening of the human population to estimate the bio-load of *Mycobacterium avium* subspecies *paratuberculosis* in North India

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Bio-load of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) was estimated in the first mass screening of human population in Mathura region of South Uttar Pradesh. 48,919 samples were collected between December, 2010 and March, 2013 from Pathology laboratories, 26,390 were screened by indigenous ELISA kit, IS*900* blood and stool PCR, IS*1311* PCR_RE and stool microscopy. From 23,196 serum samples screened by indigenous ELISA, 34.0% were positive for MAP infection (Mathura - 35.4% and Agra 14.2%). Percent prevalence of MAP infection was 28.3, 41.8, 37.4, 29.5, 41.1, 40.7, 42.5, 36.5 and 51.2 in patients suspected for diabetes, liver disorders, anaemia, thyroid, tuberculosis, typhoid, abdominal disorders, inflammatory illness and ion imbalance, respectively. 3093 blood samples screened by IS900 PCR, 8.4% were positive (Mathura - 9.2% and Agra -7.9%). Percent prevalence of MAP was 4.8, 7.0, 20.0, 4.9, 17.8, 7.6 and 12.7 in patients suspected for diabetic, liver disorder, skin disorders, anaemia, Malaria, typhoid and apparently normal individuals, respectively. 101 stool samples screened by microscopy, 5.9% were positive and of these 2.9% were confirmed by IS*900* PCR. IS*1311* PCR_RE bio-typing showed 'Indian Bison Type' was the most prevalent biotype. The study indicated large scale exposure of human population to MAP in the Mathura region of South Uttar Pradesh and 'Indian Bison Type' biotype was most prevalent.

Key words: Blood PCR, bio-load, Crohn's disease, Indian bison type, indigenous ELISA, *Mycobacterium avium* subspecies *paratuberculosis*.

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) cause chronic inflammation of intestines in animals and human beings. Chronic inflammatory bowel disease (IBD) or Crohn's disease (CD) share certain clinical and histo-pathological similarities with Johne's disease (JD) and is fast emerging as major disease of public health significance and a potential human infection (Singh et al., 2011a; Singh et al., 2012b). MAP is slow-growing, obligate intracellular fastidious pathogen difficult to grow in culture therefore, it is challenging to detect bacilli in suspected human patients. MAP survives wide range of environmental conditions (pasteurization

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temperature, low pH, and high salt concentration, etc). This superior survival efficiency and dormancy allows pathogen to be more insidious in humans (Whittington et al., 2005). MAP colonizes in animals for years without developing clinical disease. Sub-clinically infected animals shed MAP in their milk; (Shankar et al., 2010) and feces thereby contaminating pastures (Singh et al., 2012c). MAP has been recovered from pasteurized milk (Grant et al., 2002; Millar et al., 1996; Ellingson et al., 2005), infant formula made from pasteurized milk (Hruska et al., 2005), surface water, soil (Hruska et al., 2005; Whan et al., 2005), cow manure ‘‘lagoons’’ that leach into surface water and municipal tap water (Collins et al., 2003), providing multiple routes of transmission to human population. Cow manure in solid and liquid forms is applied as fertilizer in agricultural land (Grewal et al., 2006; Gill et al., 2011).

Studies in India in last two decades showed high prevalence of MAP in domestic (goats, sheep, cattle and buffaloes) and wild (hog deer, blue-bulls, bison, etc) ruminants, other animals (camels, rabbits, etc), primates and human beings (Singh et al., 2012a). Herd prevalence of subclinical MAP in Europe and North America has been reported to range from 21.0 to 70.0%, evidence supporting MAP may be a cause of CD (Rosenfeld and Bressler, 2010). MAP has been detected in the tissues and blood of CD patients with a greater frequency than those without CD (Naser et al., 2004; Sanderson et al., 1992), human breast milk of a patient with CD, positive antibodies to MAP antigens in blood samples of CD patients as compared to controls (Naser et al., 2000). *NOD2/CARD15* gene has previously been shown to be a gene of susceptibility leading to the development of CD (Ogura et al., 2001; Goyette et al., 2007). *NOD2/CARD1* mutations result in a defective innate response to bacterial infection and, possibly, ineffective clearance of intracellular MAP. Earlier studies in India showed sero-prevalence of MAP in suspected human population was estimated as 23.4% from different geographical regions of North India (Singh et al., 2011b). Study reported moderately higher presence of MAP antibodies in human population, which necessitates programs for reducing the bio-load of MAP in the environment and in the animal population (Singh et al., 2011a).

Information on presence and levels of MAP infection in animals and human population outside developed countries is extremely limited (Rajya and Singh, 1961). MAP has also been associated with Type-1 Diabetes (Sechi et al., 2008), autoimmune thyroiditis (D’Amore et al., 2010), multiple sclerosis (Cosu et al., 2013a), autism (Dow, 2011), sarcoidosis (EL-Zaatari et al., 1996), rheumatoid arthritis (Moudgil et al., 1997), autoimmune hepatitis (Miyata et al., 1995), primary biliary cirrhosis (Vilagut et al., 1997), scleroderma (Danieli et al., 1992), Kawasaki disease (Yokota et al., 1993), Behcet’s disease (Direskeneli and Saruhan-Direskeneli, 2003) and Takayasu’s arteritis (Aggarwal et al., 1996). Information on the association of MAP with different human health problems is yet to be recognized and taken seriously by the medical doctors and scientists in India.

Data on genetics and genomics of MAP offered promise that molecular diagnostic strategies may overcome limitations of conventional microbiologic tests used for this fastidious organism (Semret et al., 2005). Insertion element IS*900* is found in 14 to 18 copies per genome of MAP and has been widely used as target sequence for PCR (Moss et al., 1991; Autschbach et al., 2005). The present first large scale screening of human population of Mathura and Agra districts aimed to estimate serological and molecular prevalence of MAP in the human population suspected with different types of health disorders.

In the first large scale screening of human population of the Mathura and Agra regions, the study aimed to determine serological and molecular prevalence of MAP in the human population suspected with different clinical disorders using microscopy, indigenous ELISA test and IS*900* blood and fecal PCR.

MATERIALS AND METHODS

Collection of samples

The work has been approved by the Institute Ethical Committee (IEC) and patients were informed in detail about the sampling and work to be done on their samples (CPCSEA Registration no., 207). A total of 48,919 human samples (Table 1) were collected from 14 different Pathology laboratories located in Mathura and Agra cities from 1st December, 2010 to 31st March, 2013 on daily basis. Of these 48,919 samples, 26,390 (23,196 serum, 3093 blood and 101 stool samples) were randomly screened for the presence of MAP using indigenous ELISA, PCR and microscopy (Table 2).

Indigenous ELISA

Serum samples were screened by ‘Indigenous ELISA kit’ standardized for the screening of human samples using soluble protoplasmic antigen (PPA) prepared from the novel native ‘Indian Bison type’ biotype of MAP strain ‘S 5’ isolated from a terminal case of JD in a Jamunapari goat at CIRG (Sevilla et al., 2005). Serum samples from earlier studies and collected from Crohn’s disease patients confirmed for MAP infection in IS*900* PCR and healthy MAP negative person were used as positive and negative controls, respectively in ELISA. Optical densities (OD) were read at 450 nm. Results were considered accepted if the ratio between mean OD value of the positive and that of negative control was ≥ 4 times. OD values were transformed and expressed as sample to positive (S/P) ratio (Collins, 2002) to determine the status of MAP infection as in the equation below. Serum samples in the S/P ratio range (≥ 0.40) was categories as cut-off and were considered positive for MAP infection.

IS*900* PCR

DNA from human blood samples was isolated and subjected to specific IS*900* PCR. MAP specific primers unique to MAP (IS*900* P90/91) (Millar et al., 1996) were procured. Primers sequences used were:

Table 1. Profile of human clinical samples collected from different pathology laboratories from Mathura and Agra region of south Uttar Pradesh in India between 1 December, 2010 to 31 March, 2013.

Region	Pathology laboratories	Human beings (n)	Samples collected		Paired samples	Stool
			Blood	Serum		
Agra	Arpana pathology	978	893	89	4	4
	Pavan pathology	97	65	65	33	23
	Jivan Jyoti pathology	229	142	149	62	12
	Pankaj pathology	417	109	311	3	3
	Sandhya pathology	979	726	341	88	33
	Dr. Lahiri pathology	80	75	58	53	9
Subtotal A		**2780**	**2010**	**1013**	**243**	**84**
Mathura	New Rangeshwar Pathology Centre	28791	7832	22522	1563	10
	Brij centre pathology	989	424	709	144	2
	Rama path.	804	335	592	123	2
	Sushila Hospital	61	36	36	11	-
	Varsha pathology	408	162	275	29	-
	Pathak pathology	19	19	5	5	-
	Swarna Jayanti Hospital	1053	463	749	159	3
	Mathura laboratory	7795	4901	5009	2115	-
Subtotal B		**39920**	**14172**	**29897**	**4149**	**17**
Total		**42400**	**15882**	**32936**	**4392**	**101**

Table 2. Region-wise distribution of blood and serum samples processed from different pathology laboratories from Agra and Mathura region.

Places	Samples (n)			Total
	Serum	Blood	Stool	
Mathura	21,649	1,130	17	22796
Agra	1,547	1,963	84	3594
Sub-total	23,196	3093	101	26390
Total	46,392	6186	202	-

$$\text{S/P ratio} = \frac{\text{O.D 450 nm of the sample} - \text{O.D 450 nm of the negative control}}{\text{O.D 450 nm of the positive control} - \text{O.D 450 nm of the negative control}}$$

1. Forward primer- P90 5'- GAA GGG TGT TCG GGGCCGTCGCTTAGG -3'
2. Reverse primer- P91 5'- GGC GTT GAG GTC GATCGC CCA CGT GAC -3'

Briefly, PCR was set up in volume of 50 µl, using 1 to 5 ng template DNA, 5 µl of 10×PCR buffer, 2.5 mM $MgCl_2$, 0.2 mMdNTPs, 10 pmol of each primer and 5 U *Taq* polymerase. Thermal cycling conditions were set as initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 10 s, annealing at 63°C for 10s, extension at 72°C for 10 s and final extension at 72°C for 3 min. Product size of 413 bp was considered positive, after separation on 2.0% agarose gel stained with ethidium bromide.

IS*1311* PCR

IS*900* PCR positives were subjected to IS*1311* PCR using M*56* and M119 primers (Sevilla et al., 2005) with some modifications. Briefly, PCR was set up in volume of 25 µl, using 0.5 to 1.0 ng template DNA, 2.5 ml of 10×PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 1 U Taq (Promega, Madison, WI). Thermal cycling was as follows: initial denaturation at 94°C for 3 min, followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. An amplicon size of 608 bp was considered positive in IS*1311* PCR, after separation on a 2.0% agarose gel stained with ethidium bromide.

IS*1311* PCR-restriction endonuclease analysis (REA)

IS*1311* PCR-REA was carried out according to Sevilla et al., (2005). Briefly, the reaction was carried out in a volume of 30 µl, containing 20 µl positive IS*1311* PCR product, 3 µl 10×buffer and 2 U of each endonuclease *HinfI* and *MseI* (Fermentas, USA). The reaction mixture was incubated at 37°C for 1.5 h. Band patterns were visualized after electrophoresis on 4.0% agarose gel and staining with ethidium bromide. Genotype profiles were interpreted according to Whittington et al., (2001).

Microscopic Examination and isolation of Bacterial DNA from stool samples

Two grams of stool sample was grounded in sterilized distilled water (10 to 12 ml) in sterilized pestle and mortar. Grounded

Table 3. Status of MAP infection in the human population of Mathura district using Indigenous ELISA test based on S/P ratio method of Collins (2002).

Samples screened *(n)*	S/P Ratio	Status of MAP infection	Sero-status *n* (%)	Cummulative total
23,196	0.00-0.09	Negative	6147 (26.5)	Total Negative 15303 (66.0%)
	0.10-0.24	Suspected	4582 (19.7)	
	0.25-0.39	Low positive	4574 (19.7)	
	0.40-0.99	Positive	6482 (27.9)	Total Positive 7893 (34.0%)
	1.0-10.0	Strong Positive	1401 (6.0)	

Table 4A. Sero-status of MAP infection in the human population of Mathura district suspected with non-infectious clinical conditions.

S/N	Clinical profile of samples	Samples *(n)*	Strong positives *n* (%)	Positives *n* (%)	Total positives *n* (%)
1	Diabetes	9816	420 (4.2)	2360 (24.0)	2780 (28.3)
2	Liver disorder	2219	147 (6.6)	781 (35.1)	928 (41.8)
3	Anemia	2416	209 (8.6)	696 (28.8)	905 (37.4)
4	Thyroid Disorder	3024	149 (4.9)	746 (24.6)	895 (29.5)
5	Ion Imbalance	995	133 (13.3)	377 (37.8)	510 (51.2)
6	Abdominal Disorder	54	03 (5.5)	20 (37.0)	23 (42.5)
7	Lipid Profile	265	03 (1.1)	121 (45.6)	124 (46.8)
8	Others (Urea,UA,LH,PRL)	467	24 (5.1)	115 (24.6)	139 (29.7)
	Sub Total	19,256	1088 (5.6)	5216 (27.0)	6304 (32.7)

Table 4B. Sero-status of MAP infection in the human population of Mathura district suspected with infectious clinical conditions.

S/N	Clinical Profile of samples	Samples *n*	Strong Positives *n* (%)	Positives *n* (%)	Total Positives *n* (%)
1	Typhoid	2824	200 (7.0)	950 (33.6)	1150 (40.7)
2	Tuberculosis	316	35 (11.0)	95 (30.0)	130 (41.1)
3	Inflammatory illness	230	25 (10.8)	59 (25.6)	84 (36.5)
4	Others (VDRL,TORCH)	570	53 (9.2)	172 (30.1)	225 (39.4)
	Sub Total	3940	313 (7.9)	1266 (32.1)	1589 (40.3)

material was centrifuged at 1557 × g for 1 h at room temperature; smears prepared from middle layer, stained with Ziehl-Neelsen (ZN)staining and were observed under oil immersion for presence of pink staining acid-fast short rods indistinguishable to MAP. Middle layer was also decontaminated using 0.9% hexa decyl pyridinium chloride (HPC) (Singh et al., 1996). After decontamination, DNA was isolated from the sediment pellet according to method of Whipple et al., (1991) with some modifications. DNA from positive samples by microscopy, were also screened using IS*900* PCR .

RESULTS

Indigenous ELISA kit

23,196 serum samples were screened, 7893 (34%) were positive (Table 3) for the presence of anti-MAP antibodies/MAP infection. From Mathura (*n*=21,649) and Agra (*n*=1,547) districts, 35.4 and 14.2% were positive, respectively. Of the various suspected non-infectious and infectious pathological conditions for which the serum samples were submitted, 28.3, 41.8, 37.4, 29.5, 41.1, 40.7, 42.5, 36.5 and 51.2% were positive for MAP infection from the cases of diabetes, liver disorder, anemia, thyroid disorders, tuberculosis, typhoid, abdominal disorders, inflammatory illness, ion imbalance, respectively (Table 4A and 4B). Age-wise persons in the age group of >40 yr showed higher prevalence of MAP, however sex-wise, there was no significant difference between male and female population with respect to MAP infection (Table 5).

IS*900* Blood PCR

Screening of 3093 blood samples by IS*900* blood PCR, 262 (8.4%) were positive for MAP infection. From Mathura (*n*=1130) and Agra (*n*=1963) regions, 9.2 and 7.9% blood samples were positive, respectively. Of the various suspected infectious and non-infectious pathological conditions for which the blood samples were submitted to the pathologies laboratories, 4.8, 7.0, 20.0,

Table 5. Sex-wise sero-status of MAP infection in the human population of Mathura and Agra regions.

Regions	Males		Females		Total *n* (%)
	Samples	Positives *n* (%)	Sample *(n)*	Positive *n* (%)	
Mathura	11289	4054 (35.9)	10,360	3618 (34.9)	7672/21649 (35.4%)
Agra	757	102 (13.4)	790	118 (14.9)	220/1547 (14.2%)
Subtotal	12046	4158 (34.5%)	11150	3736 (33.5%)	7894/23,196 (34.0%)

Table 6. Detection of MAP infection by IS*900* blood PCR in the human population of Mathura district suspected for non-infectious and infectious clinical conditions

S/N	Sampling parameter	Samples processed (*n*)	Positives *n* (%)
Non-infectious health problems			
1	Lipid Profile	121	5 (4.1)
2	Diabetes	451	22 (4.8)
3	Liver disorder	71	5 (7.0)
4	Kidney Dysfunction	70	0 (0)
5	Thyroid Disorder	63	0 (0)
6	Anemia	749	37 (4.9)
	Sub-Total	1525	69 (4.5)
Infectious diseases			
7	Typhoid	39	3 (7.6)
8	Tuberculosis	10	0 (0)
9	Others (VDRL, TORCH)	16	0 (0)
10	Skin disorder	5	1 (20.0)
11	Malaria	56	10 (17.8)
	Sub-Total	126	14 (11.1)
Others			
12	Normal Healthy Individuals	1246	159 (12.7)
13	Blood grouping	196	20 (10.2)
	Sub Total	1442	179 (12.4)
Total		**3093**	**262 (8.4)**

4.9, 17.8, and 7.6% positive blood samples belonged to cases of diabetes, liver disorders, skin disorders, anemia, malaria and typhoid, respectively (Table 6). Screening of blood samples of 1442 apparently normal individuals, 12.4% were positive in IS*900* blood PCR (Table 6). Sex-wise there was no significant difference between male and female population with respect to MAP infection (Table 7).

Microscopic examination and isolation of DNA for IS*900* PCR on stool samples

Of the 101 blood samples screened, 5.9% (6) and 2.9% (3) were positive in microscopy and IS*900* PCR, respectively. Of 17 stool samples from Mathura region none was positive by microscopy. However, of the 84 stool samples from Agra region, 7.1% (6/84) were positive for acid-fast bacilli (AFB) indistinguishable to MAP (Table 8). Of these AFB positive samples; 3.5% (3/84) were positive by IS*900* PCR (Table 7, Figure 1). Geno-typing of representative IS*900* PCR positive DNA showed presence of 'Indian Bison Type' biotype in the two regions.

DISCUSSION

Present study was first attempt to estimate bio-load of MAP infection in the human population of Mathura and Agra regions by large scale screening of human samples submitted to different pathological laboratories in the

Table 7. Sex-wise presence of MAP infection by 'IS*900* PCR in blood sample'.

Region	Males		Females		Total *n* (%)
	Samples *(n)*	Positives (%)	Samples *n)*	Positives *n* (%)	
Mathura	503	58 (11.5)	627	47 (7.4)	105/1130 (9.2)
Agra	936	85 (9.0)	1027	72(7.0)	157/1963 (7.9)
Subtotal	1439	143 (9.9)	1654	119 (7.1)	262/3093 (8.4)

Table 8. Screening of stool sample by microscopy and IS*900* PCR.

Region	Stool samples *(n)*	Positive *n* (%)	
		Microscopy	IS*900* PCR
Agra	84	6 (7.1)	3 (3.5)
Mathura	17	0 (0)	0 (0)
Total	101	6 (5.9%)	3 (2.9%)

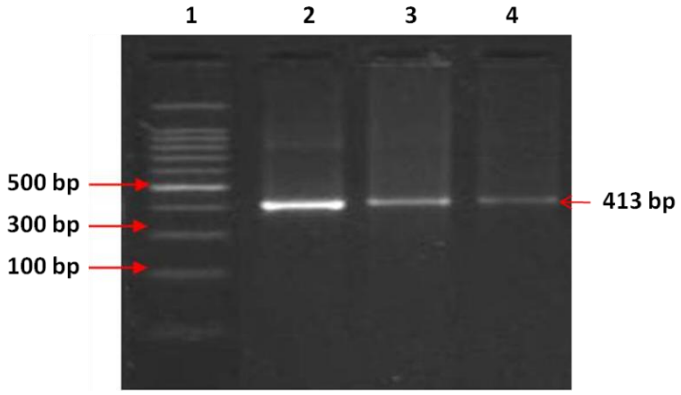

Figure 1. MAP specific amplicons (413bp) using IS*900* specific primers. Lane 1: 100bp ladder, lane 2: Positive control, lane 3-4: DNA samples.

region. Our earlier pilot studies has confirmed the presence of MAP infection in the confirmed and suspected cases of Inflammatory Bowel Diseases (Crohn's disease), animal workers suffering with chronic colitis (suspected for IBD), animal and non-animal keepers living on the periphery of big cities and apparently normal human beings (Singh et al., 2011a; Shisodiya et al., 2009). Present Indian medical science is more inclined towards clinical management of IBD & CD and totally disregards the role of MAP in these clinical conditions. In general, little attention is paid towards research especially against infections of animal origin, e.g., MAP. In absence of interest in MAP it was difficult to get samples (biopsies, blood, serum etc.) from cases of IBD/CD. Therefore in the present study, the strategy adopted was to estimate presence and level of MAP infection by first large scale screening of human population, irrespective of any particular disease condition from Mathura and Agra region. For this purpose serum and blood samples were collected from different pathology laboratories, where blood, serum and stool samples were submitted for various infectious and non-infectious health disorders. Previously developed 'indigenous ELISA test' for other livestock species (Collin et al., 2005, Pruvot et al., 2013) has been standardized for the screening of human serum samples (Singh et al., 2011c). Using 'indigenous ELISA kit' in the present investigation indicated high (34%) bio-load of MAP in the human population indicating heavy exposure to MAP infection. Studies by other workers also reported presence of MAP in human population particularly in patients of CD and diabetes (Greenstein et al., 2003; Bitti et al., 2012). A population based study reported 35% sero-positivity rates however; there was no difference in rates between CD patient, UC patients and healthy controls (Collins et al., 2000). Similarly in present findings also there was no significant difference in the rates of MAP infection between patients suspected for suffering with infectious (32.7%) and non-infectious (40.3%) clinical conditions. Earlier studies by Singh et al., (2008) reported high sero-positivity in CD patients (100%), animal attendants (75%) and apparently normal human beings (38%). In a sero-survey of animal keepers suspected and not-suspected for CD showed that 12.9 and 4.2% were positive by the indigenous ELISA test (Shisodiya et al., 2009; Singh et al., 2011a) while screening of animal attendants who worked with goatherds endemic for MAP infection had higher prevalence of MAP infection as compared with person with no history of contact with animals. In another study by Singh et al. (2011c), Sero-prevalence of MAP was higher in CD patients (80%), as compared to ulcerative colitis patients (4.5%) and apparently normal persons (15.3%) using 'indigenous adsorbed ELISA test. The further reported sero-prevalence of MAP in another group of apparently normal human beings as 23.4%. Geographical region-wise, 34.0, 33.3, 32.8, 25.0, 23.0, 17.7% and 12.5% serum

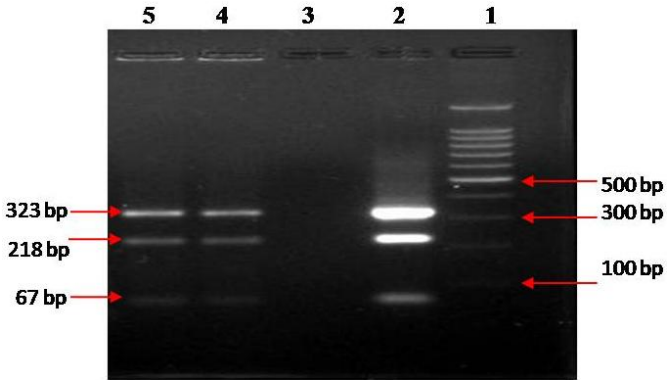

Figure 2. IS*1311* PCR-REA analysis. Lane 1: 100bp DNA ladder, lane 2: Positive control DNA, lane 3: Negative control, lane 4 and 5: Digested DNA sample ('Indian Bison Type')

samples were positive from the states of Punjab, Uttarakhand, New Delhi, Himanchal Pradesh, Haryana, Uttar Pradesh and Jammu and Kashmir, respectively. Presence of higher bio-load of MAP in animals (domestic and wild ruminants) (Kumar et al., 2010) and animal products (milk and milk products) (Shankar et al., 2010) indicated animals are source of MAP infection to human population directly (by contact and consumption of animal products) and indirectly (through contact). Animal keepers and attendants stand at higher risk and falling easy prey to exposure of MAP through heavy to very heavy load of MAP bacilli in animals and environment.

The study also analysed 3091 blood samples of human beings from Mathura region using IS*900* blood PCR, wherein 8.4% (262) were found positive for MAP infection. Of these 262 positive human beings, 4.5, 11.1 and 12.4%% positive samples were from non-infectious, infectious clinical conditions and apparently normal individuals, respectively. Skin disorders, malaria, typhoid, liver disorder, anaemia, diabetes and lipid profiles were the major suspected clinical conditions for which the blood samples were submitted to pathology laboratories and found positive for MAP infection in IS*900* blood PCR. Whereas in ELISA, serum samples were positive for all the suspected clinical conditions for which the samples were submitted (Table 4a and Table 4b).

Screening of stool samples by microscopy and IS*900* PCR, 5.9 and 2.9% were positive for MAP respectively (Table 8). Presence of acid fast bacilli indistinguishable to MAP in stool samples using microscopy was an interesting findings, which we have also reported in our previous studies (Singh et al., 2008). However, studies in other parts of the world reported cell wall deficient (CWD) forms of MAP in human beings (Greenstein, 2003). Presence of heavy load of MAP in human stools should be considered alarming. Recently, it has been reported that MAP antigens have the capacity to induce colitis in mice (Momotani et al., 2012). Further investigations are required if these AFB have any role in developing the disease.

Naser et al. (2004) also detected MAP bacilli in blood of 50% patient with CD and 22% of patients with UC. Presence of MAP in blood, suggests that it may be distributed to different organs and may play pathological role in at different sites of infection. Presence of MAP in blood has been directly related with etiological role in CD (Naser et al., 2009). However, presence of MAP in blood of healthy individuals in present study has been reported earlier also (Singh et al., 2011c) has given rise to controversies and food for especially those people who contradicts the role of MAP in CD.

However, recent advancements in MAP research indicated the presence and role of MAP in patients with various diseases such as Type-1 Diabetes (Sechi et al., 2008), autoimmune thyroiditis (D'Amore et al., 2010), multiple sclerosis (Cossu et al., 2013a), autism (Dow, 2011), sarcoidosis (EL-Zaatari et al., 1996) and autoimmune arthritis (Moudgil et al., 1997). Our study correlates with the finding of some authors that have claimed the association of MAP infection with autoimmune disorder viz. Type-1 Diabetes and thyroid disorder etc.

Earlier, it has been reported that mycobacterial heat shock protein (HSP65), shares sequential and conformational elements with several human proteins and it can be predicted that by molecular mimicry mecha-nisms, MAP can stimulate auto-antibodies resulting auto-immune disorders like CD, type 1 diabetes, autoimmune (Hashimoto's) thyroiditis, and multiple sclerosis etc. Mycobacterial HSP65 has also been implicated in the pathogenesis of rheumatoid arthritis, autoimmune hepatitis, primary biliary cirrhosis, scleroderma and Kawasaki disease (Dow, 2012). It has also been pre-dicted that MAP HSP60/65 triggers anti-GAD (pancreatic glutamic acid decarboxylase) antibodies that destroy the pancreas (Jones et al., 1993; Naser et al., 2013). Further it has been reported that sera from children with type I diabetes shows strong sero-reactivity to MAP specific protein MAP3738c (Cossu et al., 2013b). Further, it has been shown that sera from diabetes patients react against MAP Hsp65 protein (Naser et al., 2013). In our present study also, 28.3 (2780/9816) and 4.8% (22/451) patients suspected for diabetes were found positive for MAP infection in 'indigenous ELISA and IS*900* blood PCR, respectively.

IS*1311* PCR_RE method specifically designed for the bio-typing of MAP by Whittington et al. (2001) was used to characterize native strains and in India, a new biotype of MAP 'Indian Bison type' has been reported as principal biotype infecting different animal species, animal products and human beings (Shankar et al., 2010; Singh et al., 2013). This assay (Figure 2) helped us to further give an insight that this particular biotype of MAP has accumulated genetic differences compared to MAP 'K 10' and other international MAP isolates (Fiocchi, 1998).

Unlike JD in animals, MAP has been found as cell wall deficient form in CD patients (Greenstein, 2003); however, in India, cell wall containing MAP has been recovered from animal healthcare workers (suspected for CD). In later studies, these cell walls containing MAP were genotyped as 'Indian bison type' genotype of MAP (Singh et al., 2009a). These findings indicated that 'Indian bison type' strain may be capable of initiating disease that manifests similarly to JD in animals. 'Indigenous ELISA' using PPA from 'Indian bison type' biotype MAP of goat origin was superior when compared with imported commercial ELISA kits for screening of animals was also useful in screening of human samples for MAP infection (Singh et al., 2011c). Presence of MAP in patients suspected for infectious and non-infectious clinical conditions showed that besides association with Inflammatory bowel disease (Crohn's disease), MAP may also play an important role in other health disorders and cases of colitis and other abdominal ailment in India. Due to the presence of MAP in the milk of animals (Shankar et al., 2010) and human (Naser et al., 2000) it is highly likely that MAP will be passed on to the next generation through milk, thereby creating endemicity of infection in a population or colony. In India people may get infected by other means as MAP has also been reported from environment soil and river water (Singh et al., 2012c) and abdominal disorders very common in India. Presence of MAP in human population with various suspected pathological conditions and from apparently normal individuals indicated large scale exposure of human population to MAP in Mathura region of South Uttar Pradesh in India. Both serological and molecular tests helped establishing presence of MAP organism in clinical samples and possible association with various pathological and physiological conditions.

Conclusion

The superior survivability of MAP allows the pathogen to be more insidious for human health. Despite the full genome sequencing, little information is available about the prevalence of this pathogen under the Indian condition. The present work has been carried out by combining both serological and molecular tools viz., faecal culturing, Indigenous ELISA kit, blood PCR targeting IS*900* and IS*1311* sequences as well as PCR_RE, in order to detect in better way the organism in clinical samples of human patients and find out its association with various pathological and physiological conditions, so that the researchers and diagnosticians can have a better understanding of the epidemiological status of the disease, CD. Present study reports high burden of MAP in human population and further studies are required to address the heavy load of MAP in different disease conditions in North Indian population.

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Full Length Research Paper

The use of chart review to elucidate the epidemiology of leprosy in the Mbingo leprosarium of Cameroon

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The WHO introduced MDT for the treatment of leprosy in 1982 because dapsone monotherapy was lifelong and resistant. "(he objective of this study was to determine the impact of MDT on leprosy control and its epidemiology in Mbingo leprosarium'. Patients who attended the Mbingo leprosarium from 1961 to 1998 were identified through a thorough manual review of hospital records in 2002. A structured data collection form containing information on sex, age, type of disease, province of origin, date of admission and whether it was a new case, transferred, relapsed, readmission, discharged, absconded or defaulted. Patients with incomplete data Were dropped from the investigation. The review was carried out before and after the introduction of MDT in 1982. 1045 case files comprised of 271 for the period 1961 to 1967 and 774 for 1982 to 1998 were reviewed. The epidemiological trend of leprosy showed peak values in 1964,1984,1986 and 1991 and a decrease in 1967, 1982 and 1998. In the pre-MDT period, admissions increased from 4(0.4%) in 1961 to 70 (10.9%) in 1964and decreased to zero in 1982. Since MDT implementation, admissions increased to 39 (6.1 %) in 1986 and a continuous gradual drop till 1998. Immediately after I\II)T implementation many patients \\ere cleared *from* the registers with peak values in 1984, 1986, 1991 and 1993. 283 (27.1%) new admissions, 60 (5.7%) transfers, 10 (1.0%) readmissions, 20 (1.9%) relapses, 15 (1.4%) defaulters, 14 (1.3%) deaths and 373 (35.7%) discharges were reported from 1992 to 1998.

Key words: Leprosy, multi-drug therapy, Mbingo, leprosarium, epidemiology, Cameroon

INTRODUCTION

The drug of choice for leprosy treatment was dapsone but because of widespread resistance and life-long

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treatment, patients became discouraged (WHO, 1982). The WHO introduced multi-drug therapy (MDT), a combination of three drugs (dapsone, rifampicin and clofazimine) for multi-bacillary leprosy and two drugs (dapsone and rifampicin) for pauci-bacillary cases (WHO, 1982; Anonymous, 1992). MDT enjoys a high degree of patient acceptability, absence of treatment failure, very low relapse rates following completion of treatment; compliance is high because of the fixed and relatively short duration of treatment, low frequencies of side-effects, cost effective and it cures the patient (Noordeen, 1995; The Star, 1997; WHO, 1995; ILEP, 1998). Based on the MDT strategy, the WHO targeted to eliminate leprosy as a public health problem from endemic countries by 2000 (WHO, 1991).

The leprosy prevalence in Cameroon in 2002 was 1.35/10000 and pauci-bacillary defaulter rate was 22.2% and detection of new cases was decreasing (Diallo et al., 2002) but 574 new cases were detected with 79 child cases giving a prevalence of 0.45/10000 and detection rate of 3.71% in 2004 (AFRO, 2004). Leprosy has been integrated into the primary health care system in Cameroon (Daumerie et al., 1991). In the enclave Essimbiland in Menchum division of northwestern Cameroon, leprosy is still a public health problem of primary importance after the year 2000 with case-detection among children on the increase (Nsagha, 2002; Provincial Delegation of Public Health, 2008). Work by Nsagha and colleagues in Cameroon indicated that social stigma (Nsagha et al, 2011a) and rehabilitation (Nsagha et al, 2011b) are major epidemiological determinants of leprosy elimination because of high prevalence in Boyo and Menchum divisions (3.4/10,000 and 4.5/10,000 respectively) (Diallo et al, 2002; AFRO, 2004; Provincial Delegation of Public Health, 2008). These divisions still have high leprosy prevalence (1.7/10,000 for Menchum and 2/10,000 for Boyo) in the North West Region of Cameroon (Provincial Delegation of Public Health, 2008). All leprosy cases in this locality are treated at the Mbingo leprosarium, hence, this study was undertaken to determine the impact of MDT on leprosy control and the epidemiology of the disease.

METHODS

Study area

The Mbingo leprosarium was established in 1954 by missionaries of the Cameroon Baptist Convention church. The leprosarium has a general health care service headed by a doctor and two nurses, a technical section for the manufacture of prosthesis and many vocational rehabilitation activities. The leprosarium is surrounded by many smaller villages but since its creation, more than 1500 leprosy patients have been discharged but because of the high social stigma, about 400 of these discharged patients could not return to their villages of origin; they live in different villages around the leprosarium such as Mbingo II, Mejang and Baingo. Within the premises of the leprosarium, there are discharged patients living in Dr. Jones' quarter and NewHope village. This study was conducted from June to August 2000.

Review method

Patients who attended the Mbingo Baptist leprosarium from 1961 to 1998 were identified after a thorough manual review of hospital records. A structured data collection form containing relevant items of information was used for this purpose. Information was extracted from different medical records and arranged in a logical sequence. The search was carried out manually. This exercise took place over a period of three months and covered the number of patients admitted and discharged during 1961 to 1998. The information collected was in two parts. The first section focused on the characteristics of patients on admissions and data collected included the sex, age, type of disease, province of origin and date of admission and whether the admission was a new case, transferred case from another clinic, a relapsed case or a readmission. The second section was based on discharged cases. The same parameters mentioned above were studied but it was investigated whether the cases were officially discharged, or they absconded or defaulted and if they were transferred out or dead. Patients who had incomplete data in this survey were dropped from the investigation. The registers were reviewed year by year.

The classification (WHO, 1982; International Leprosy Congress, 1948; Ridley and Jopling, 1966) of the patients was based on provisional clinical diagnosis by the leprosy control supervisor or the medical officer and upon laboratory results. Readmissions were cases that were treated in the leprosy colony of Mbingo or elsewhere but who came back for either reversal (type I) or erythema nodosurn leprosum (type 2) leprosy reactions. Patients who were discharged but who constantly consulted the hospital for other illness or the sequelae of leprosy such as ulcer care were not considered as readmissions. The chart review in the Mbingo leprosarium was carried out before and after the introduction of MDT in 1982. The records were not kept in the pre-MDT periods (1968 to 1981), hence only records from 1961 to 1967 and 1982 to 1998 were reviewed. Patients with incomplete data were excluded from the study. The authorization to carry out the work was obtained from the Cameroonian Ministry of Public Health (Nº D76/A/MSP/SESP/SG/DRH/SDGP/SFS).

Data management and analysis

The structured data collection forms were checked for incomplete forms and edited for the use of correct codes, including range and consistency errors. The data were analyzed using Epi-Info after a double entry by two data clerks. Data summaries such as proportions and percentages and testing of the working hypothesis (MDT had no impact on leprosy control) were also carried out using the chi-square and Fisher exact tests.

RESULTS

A total of 1045 files comprised of 271 for the period 1961 to 1967 and 774 for 1982 to 1998 were reviewed. Record keeping on leprosy in the study area was generally poor as important demographic variables on religion, occupation, educational, marital status, laboratory diagnosis, type of rehabilitation activities, presence or absence of deformities, disabilities and types were lacking.

Yearly pattern of leprosy admissions and discharges (1961 to 1998)

For the period 1961 to 1998, 1045 leprosy patients

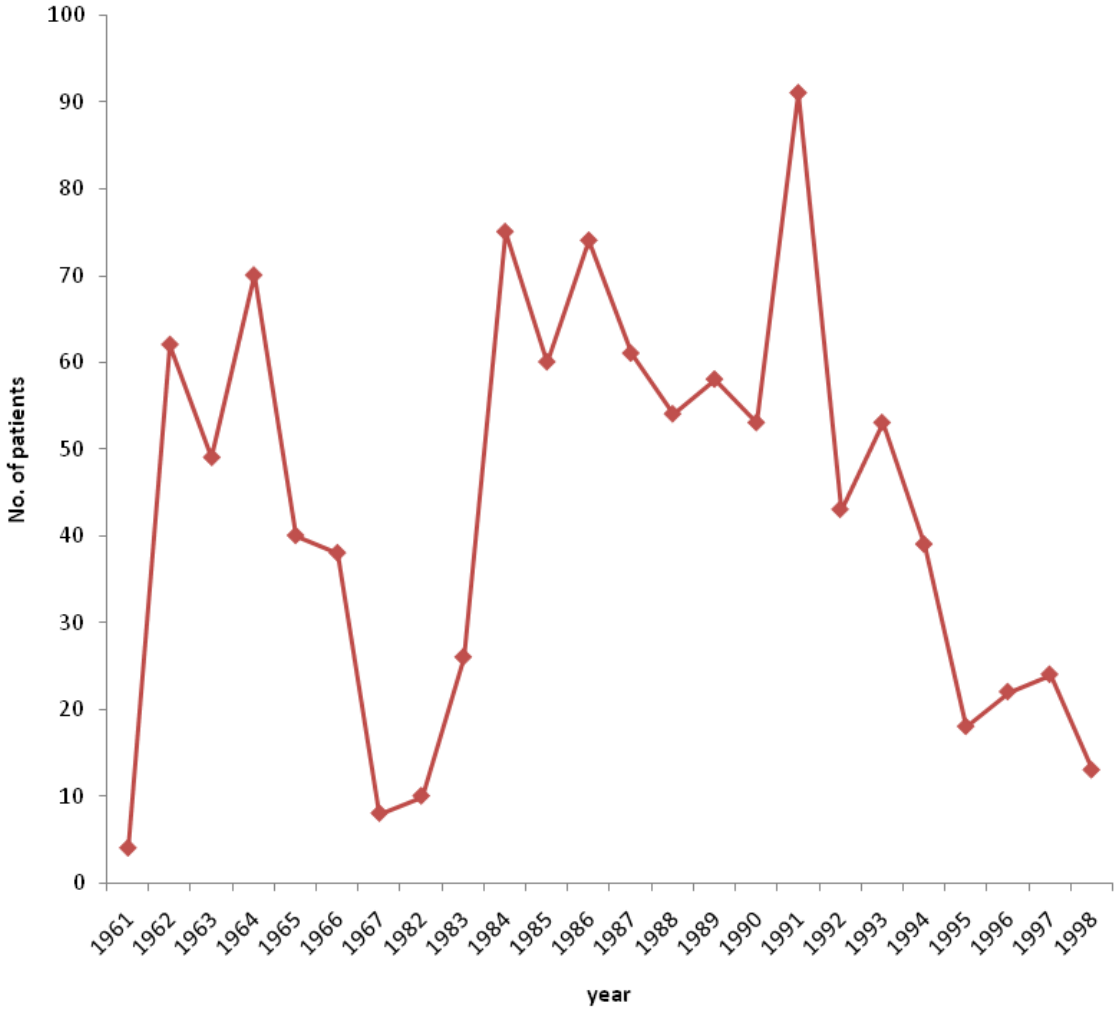

Figure 1. Yearly distribution of all leprosy patients between 1961-1998 in the Mbingo leprosarium.

consisting of 644 (61.6%) admissions and 401 (38.4%) discharges were registered in the leprosarium. The highest number of admissions was 70 (6.7%) in 1964 in the pre-MDT era and 39 (3.7%) in 1986 in the post-MDT period. The mean age of the 1045 leprosy patients was 26.48±10.68 years. The epidemiological trend of leprosy over the years showed peak values in 1964, 1984, 1986 and 1991 (Figure 1). There were marked decrease in the number of leprosy patients in 1967, 1982 and 1998. But the deepest trough was observed in 1967. In the pre-MDT period, the number of leprosy admissions increased from 4 (0.4%) in 1961 to a peak of 70 (10.9%) in 1964 and decreased to zero in 1982 immediately after the introduction of MDT. Since MDT implementation in 1982 in the Mbingo leprosarium, admissions increased to 39 (6.l%) in 1986 and maintained a continuous gradual drop till 1998 (Figure 2). There were no leprosy discharges from 1961 to 1967 during the pre-MDT era for which data was available. Immediately after MDT implementation many leprosy patients were cleared from the registers with peak values of discharges occurring in 1984, 1986, 1991 and 1993 (Figure 2).

Distribution of leprosy from 1961-1998 according to gender, type of leprosy, new admissions, transfers, readmissions, relapses, deaths and defaulters

Women were slightly in the majority with a sex ratio of 92 males to every 100 females. In the seven-year period of the pre-MDT era for which data was available, there was a male preponderance constituting 51.3% of the total admissions. The situation was reversed post-MDT with males accounting for only 46.6% of the total admissions and discharges during this period. The distribution of patients by sex appears uniform in each year of study.

During the pre-MDT period (1961 to 1967), the most common type of leprosy was tuberculoid (TT) with 175 (16.7%) cases. The highest registered number of leprosy was 70 (6.7%) in 1964 and TT constituted the most

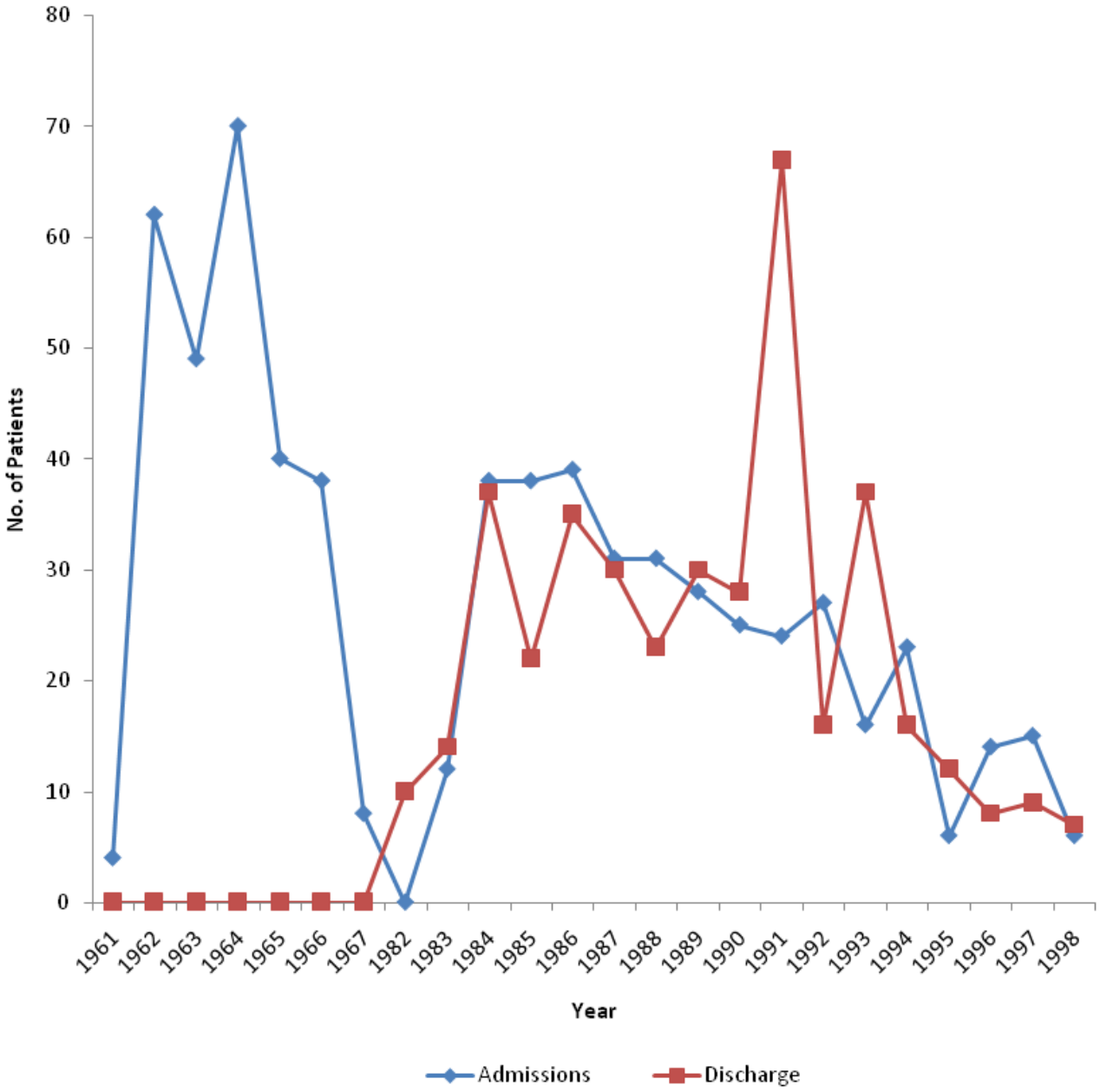

Figure 2. Yearly pattern of leprosy admissions and discharges in the Mbingo leprosarium from 1961 to 1998.

frequent type with 52 (5.0%) cases. The commonest types of leprosy in the post-MDT period (1982-1998) were borderline tuberculoid (BT) 264 (25.2%), borderline borderline (BB) 158 (5.0%) and lepromatous (LL) 193 (18.5%) cases.

For the period 1961 to 1967, 180 (17.2%) new admissions, 61 (15.7%) transfers, 20 (2.2%) readmissions and 8 (0.8%) relapsed cases of leprosy were registered in the leprosarium. No default, death and discharged cases were recorded during this period. 283 (27.1%) new admissions, 60 (5.7%) transfers, 10 (1.0%) readmissions, 20 (1.9%) relapses. 15 (1.4%) defaulters, 14 (1.3%) deaths and 373 (35.7%) discharged cases were reported from 1992 to 1998. There was significant variation over the years in the different types of admissions and discharges ($p<0.05$). There were no records of absconders, discharges and deaths in the pre-MDT period.

Distribution of leprosy patients according to nationality and province (1961 to 1998)

The highest number of leprosy patients was from

Table 1. Age and sex distribution of leprosy patients by division and period in the Mbingo leprosarium (1961 to 1998)

Period	Age group (Years)	Division										
		Mezam		Menchum		Boyo		Bamboutous		Others		Total
		M	F	M	F	M	F	M	F	M	F	
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Pre-MDT (1961 to 1967)	<20	15 (1.4)	8 (0.8)	7 (0.7)	6 (0.6)	0	0	0	0	42 (4.0)	14 (1.4)	92 (8.8)
	20+	19 (1.8)	32 (3.0)	25 (2.4)	33 (3.2)	1 (0.09)	0	0	0	30 (2.9)	39 (3.7)	179 (17.1)
	Sub Total	34 (3.3)	40 (3.8)	32 (3.0)	39 (3.7)	1 (0.09)	0	0	0	72 (6.9)	53 (5.0)	271 (25.9)
Post-MDT (1982 to 1998)	<20	5 (0.5)	4 (0.4)	24 (2.3)	16 (1.5)	0	0	2 (2.0)	20 (0.2)	10 (1.0)	5 (5.0)	68 (6.5)
	20+	43 (4.1)	79 (7.6)	97 (9.3)	116 (11.1)	4 (0.4)	4 (0.4)	66 (6.3)	115 (11.0)	110 (10.5)	72 (6.9)	706 (67.6)
	Sub Total	48 (4.6)	83 (7.9)	121 (11.6)	132 (17.6)	4 (0.4)	4 (0.4)	68 (6.5)	117 (11.2)	120 (11.5)	77 (7.4)	774(74.1)
Pre-and Post-MDT (1961 to 1998)	<20	20 (1.9)	12 (1.2)	31 (3.0)	22 (2.1)	0	0	2 (0.2)	2 (2.0)	52 (5.0)	19 (1.9)	160 (15.3)
	20+	62 (5.9)	111 (10.6)	122 (11.7)	149 (14.3)	5 (0.5)	4 (0.4)	66 (6.3)	115 (11.0)	140 (13.4)	111 (10.6)	885 (84.7)
	Total	82 (7.8)	123 (11.8)	153 (14.7)	171 (16.4)	5 (0.5)	4 (0.4)	68 (6.5)	117 (11.2)	192 (18.4)	130 (12.5)	1045 (100)

M = Male; F = Female; MDT = Multi-drug theory

the North West Province was 785 (75.1%) followed by the West Province 209 (20.0%) of Cameroon. The proportion of cases from the North West Province during the post MDT period (69.5%) was lower than that in the pre-MDT period (96.1%). There were only 5 (0.5%) foreigners including 4 (0.4%) Nigerians and 1 (0.1%) American. Results showed that 80% of these foreigners were admitted in the post-MDT period.

Distribution of leprosy patients by division, age and sex from 1961-1998

The records revealed that the highest number of leprosy patients were from Menchurn division with 324 (31.0%) cases in the post-MDT period with only 21.9% in the pre-MDT period followed by Mezam 205 (19.6%) cases and Boyo 9 (0.9%) cases. During the pre-MDT era, Menchum, Mezam and Boyo divisions had 71 (6.8%), 74 (7.1 %) and 1 (0.09%) cases respectively but from 1982 to 1998, Menchum, Mezam and Boyo divisions had 253 (24.2%), 131 (12.5%) and 8 (0.08%) cases respectively. Bamboutous division registered 185 (17.70/0) cases from 1982 to 1998. Details of age and sex distribution of the leprosy patients shown in Table 1 indicate that there was a statistically significant difference between the divisions ($p<0.05$). For both the pre-MDT and post-MDT periods, more females than males had leprosy. But from Table 1, it was found that there was male preponderance in each of the divisions for patients less than 20 years of' age. The age and sex distribution of leprosy patients was statistically significant in Mezam ($p = 0.00$) and Bamboutous ($p = 0.01$) divisions.

Classification of leprosy patients according to age and type of disease

The Havana classification (indeterminate (I), tuberculoid (TT), lepromatous (LL)) of leprosy (1948) was used for cases reported between 1961 to 1967. The Ridley/Jopling classification (1966) (TT, BT (borderline tuberculoid), borderline borderline (BB), borderline lepromatous (BL)), LL was used from 1982 to 1998. The commonest type of leprosy in the study area before the implementation of MDT was TT with 175 (16.7%) cases. During the post-MDT era, the commonest types of leprosy were BT 264 (25.2%) and LL 193(18.5%) cases. For the period of 1961 to 1998, leprosy was more common among those above 20 years than the young ones; 179 (17.1%) from 1961 to 1967 and 706(67.5%) from1982 to 1998 (Table 2).

Epidemiological trend of incident leprosy in the leprosarium (1961 to 1998)

The secular trend of incident leprosy shows three humps: 1962, 1964 and 1985 as demonstrated in. After the sudden peak, the incidence of

Table 2. Distribution of types of leprosy by age in the study area (1961-1998).

Period	Age Group (Years)	Types of Leprosy						
		I	TT	BT	BB	BL	LL	Total
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Pre-MDT (1961 to 1967)	<20	5 (0.5)	65 (6.2)	0	0	0	22 (2.1)	92 (8.8)
	20+	21(2.0)	110 (10.5)	0	0	0	48 (4.6)	179 (17.1)
	Sub Total	26 (2.5)	175 (16.7)	0	0	0	70 (6.7)	271 (25.9)
Post-MDT (1982 to 1998)	<20	0	9 (0.9)	23 (2.2)	18 (1.7)	8 (0.8)	10 (1.0)	68 (6.5)
	20+	0	81 (7.8)	241 (23.0)	140 (13.4)	61 (5.8)	183 (17.5)	706 (67.6)
	Sub Total	0	90 (8.6)	264 (25.2)	158 (15.1)	69 (6.6)	193 (18.5)	774 (74.1)
Pre and Post-MDT (1961 to 1998)	<20	5 (0.5)	74 (7.1)	23 (2.2)	18 (1.7)	8 (0.8)	32 (3.1)	160 (15.3)
	20+	21 (2.0)	181 (18.3)	241 (23.0)	140 (13.4)	61 (5.8)	231 (22.1)	885 (84.7)
	Total	26 (2.5)	255 (25.4)	264 (25.2)	158 (15.1)	69 (6.6)	263 (25.2)	1045 (100)

I=Indeterminate leprosy, TT=tuberculoid leprosy, BT=borderline tuberculoid leprosy, BB=borderline borderline leprosy, BL=borderline lepromatous leprosy, LL= lepromatous leprosy

leprosy cases recorded increased in 1962 with 50 (10.8 %) cases and 1964 with 48 (10.4%) cases. It decreased to zero level in 1982. However, these numbers began to increase in 1983 sharply reaching a peak in 1985. Thereafter, admissions gradually slowed down Figure 3.

Distribution of incident leprosy in the leprosarium by division (1961 to 1998)

Among the 180 (38.9%) incident cases in the leprosarium before the implementation of MDT (1961 to 1967) in 1982, 50 (10.6%) cases were from Menchum, 48 (10.4%) from Mezam and 1 (0.2%) from Boyo (p=0.45). From 1982 to 1998, the number of incident cases increased from zero to 283(61.1%) with 95(20.5%) cases from Menchum, 37 (8.0%) from Mezam and 2 (0.4%) from Boyo ($p < 0.05$). For the period 1961 to 1998, therefore, the highest number of incident cases admitted was from Menchum division with 145 (31.3%) cases followed by Bamboutous division with 76 (16.4%) cases during the post-MDT period.

Age, sex and type of incident leprosy cases in the study area (1961 to 1968)

Of the 180 (38.9%) incident cases from 1961 to 1967, 135 (75.0%) were above 20 years and 45 (25%) were below 20 years compared to 247 (87.3%) and 36 (12.7%) respectively for 1982 to 1998. The differences in the distribution of incident leprosy with age during the pre and post-MDT periods were statistically significant ($p<0.05$). From 1961 to 1998, there were more females with new leprosy 257 (55.5%) versus 201 (44.5%) respectively. The sex difference and types of new leprosy cases was not statistically significant (p=0.17). A distribution of types of new cases of leprosy with age showed that people older than 20 years were more than the younger ones; 380 (82.1%) versus 83 (17.9%) respectively but this was not statistically significant (p=0.11). The commonest types of new leprosy cases were TT: 126 (27.2%) cases from 1961 to 1967 and BT: 86(18.6%) cases and BB: 80(17.3%) cases from 1982 to 1998. A statistically significant difference between age, sex and type of leprosy was observed for BT among new admissions ($p<0.05$).

DISCUSSION

Impact of MDT on leprosy transmission

MDT has been very effective in the treatment of leprosy coupled with its cost effectiveness, low side effects, low relapse rates and high compliance (WHO, 1995). According to Meima (2002) the reasons for declining trends in the transmission and incidence of leprosy may be related to several factors: the period during which *Mycobacterium leprae* is transmitted, which can

Figure 3. Secular trend of new admissions of leprosy in the study area from 1961 to 1998.

be reduced by early case detection and chemotherapy, BCG vaccination, which is widely administered as a preventive measure against tuberculosis but appears to afford more protection against leprosy than tuberculosis (Fine and Smith, 1996) and socioeconomic conditions, which are thought to play an important role in leprosy (International Leprosy Association Technical Forum, 2002). Economic improvement may result in a decline in incidence such as housing conditions, number of persons per household per room, family size and nutritional factors. Possible protection of tuberculosis against leprosy (Fine, 1982), either by immunization or by competing risk can also reduce the incidence of leprosy.

A further problem is the delay between onset of disease and detection. For instance, in the ALERT control programme in Ethiopia, the average detection delay exceeded two years. The ease of transmission of leprosy is not known. The group at risk of developing leprosy might be small, possibly due to genetic factors (leprosy infection is suggested to be more common than

leprosy disease (Fine, 1982; Noordeen, 1985) or because close contact is important. Close contact household and family, neighbours, social and business contact has been suggested to play a key role in transmission (van Beers et al., 1999). It is well possible that close contacts of a leprosy patient become infected rapidly. If close contact is indeed important, this may lead to a rapid decrease in the patient's opportunities to transmit *M. leprae*. Thus, "early" detection may still be too late to prevent much of transmission by subsequent treatment. Other factors which could limit the impact of leprosy control have also been suggested, including carriage of *M. leprae* in the nose, persistence of *M. leprae* in the soil, and animal reservoirs (Blake et al., 1987; Reich, 1987; Kazda et al., 1990; Klatser et al., 1993). Studies by Nsagha et al., (2009) have highlighted a number of operational barriers to MDT usage ranging from insufficient coverage, lack of comprehensive and continuous health care, MDT shortage, ignorance, insufficient use of resources and lack of confidence in leprosy treatment because of the occurrence of deformities among treated cases.

Epidemiological trend of leprosy in the Mbingo leprosarium

The increase in the number of discharged cases over the years may be due to the curative effect of MDT which encouraged self-reporting for treatment. The new admissions of leprosy in MBH followed a secular trend from 1985 to 1993 and periodic trends occurred between 1994 and 1997. The number of new cases of leprosy has been on the decrease in the leprosarium since the implementation of MDT in 1982. This secular trend could be partly due to the implementation of MDT in the leprosarium in 1982 (WHO, 1982; Provincial Delegation of Public Health, 1996). MDT is known to have reduced the number of leprosy cases in the world from 10 to 12 million in 1990 to 1.6 million in 1998 (WHO, 1998). The decrease in the secular trend could also be due to the integration of leprosy into the primary health care system in Cameroon in 1991 (Daumerie, 1991; Nsom, 1999) which enabled many health centers at the level of the districts to handle leprosy cases. The records revealed that leprosy was more common in the age group of people above 20 years. This agrees with the findings of Noordeen, (1985) and Berthe et al., (1990) who discovered that in endemic areas there is a clear peak of leprosy in the older age group.

Geographical distribution of leprosy cases in the leprosarium from 1961 to 1998 in the Mbingo leprosarium

The results clearly indicate that in the study area, Menchum division contributed to the bulk of leprosy in the leprosarium. The uneven distribution of leprosy in Boyo, Menchum and Mezam divisions is a common characteristic of leprosy because this uneven pattern has been reported by many leprosy workers (Noordeen, 1985; Brightmer, 1990). The factors contributing to the geographical variations are not quite clear except that they probably involve several factors such as opportunities for exposure (dressing habits, occupational hazards such as in farming) and genetic factors (Noordeen, 1985). Other factors such as the nutrition and social class rating of the subjects may also be considered. The Essimbi people of Menchum division live in small over-crowded one-room houses that could facilitate the transmission of leprosy if one household member is suffering from the disease (Nsagha, 2002). The proximity of Menchum division to the leprosarium may enhance the capacity of inhabitants to have a better knowledge of the disease, which encouraged self-reporting. The remote and enc1aved nature, bad roads and limited health facilities in Menchum division may also be contributing factors (Nsagha, 2002). Menchum division is one of the least populated divisions of the North West Province with a population of 119,921 (Provincial delegation of Public health, 1996) but the chart review showed that the bulk of leprosy patients in the province came from there even though the population is very scanty. This pattern corresponds with that of other researchers who have remarked on the apparent association in tropical Africa of areas of highest leprosy rates coinciding with areas of sparse population density (Brightmer, 1990; Hunter and Thomas, 1984).

An association noted in Ethiopia (Berthe et al., 1990) of high leprosy prevalence corresponding to the highland regions of the country does not occur in this study area of Cameroon because Menchum Division is not a plateau. The chart review has revealed that leprosy is generally on the decrease in the study area. The decline of leprosy may be due to the natural course of the disease and to changes in the socio-hygieno-economic conditions in these areas (Nsagha, 2002). As has occurred in other parts of Nigeria (Waaldijk, 1989) the decline in the prevalence of leprosy may be related to the increased activity of the leprosy supervision whereby many health centers in the districts take care of leprosy nowadays contrary to the years before 1991 when leprosy was handled only by vertical programmes of the leprosarium (Nsagha, 2002).

From the chart review, new cases of leprosy from Boyo division have always been the least. Taking into consideration that many of the patients in the leprosarium act as reservoirs of infection, genetic factors and environmental sanitation of inhabitants from this division need to be taken into account when interpreting these results. Inhabitants of Boyo division may have developed immunity to clinical leprosy.

The records from the Provincial Delegation of Public

Health in the North West Province (Provincial Delegation of Public Health, 1996) showed that Boyo and Menchum divisions had prevalence above the WHO standard of elimination of leprosy as a public health problem. But chart review results in the Mbingo leprosarium from 1982 to 1998 showed very few cases from Boyo division. The high number reported by the provincial delegation may be due to the fact that Boyo division has a better health infrastructure for the treatment of leprosy (the leprosarium) and some cases in the surrounding divisions and regions prefer to seek medical care in the leprosarium, sometimes to escape the social stigma attached to the disease in their communities (Nsagha, 2002). This situation has been observed from Yemen (Al-Qubatic and Al-Dobai, 1999).

Bamboutous division is the most easily accessible division from the French speaking Cameroon with respect to the leprosarium (about 45 km). Inhabitants from this division could have a better awareness of the disease because of its proximity to the leprosarium and tend to self-reporting more than the other regions. The leprosarium, being a Baptist leprosy hospital, inhabitants from Bamboutous division could prefer to come there instead of going to government clinics because of the respect they will receive from the mission hospitals considering the high social stigma attached to leprosy. The high number of incident leprosy patients from Bamboutous division could also mean there are many cases of leprosy there. The backlog of patients from Menchum and Bamboutous will continue to provide the pool of leprosy infection in the study area, which can hinder the effective control of the disease. The Ministry of Public Health should initiate special action projects for the elimination of leprosy (SAPEL) and leprosy elimination campaigns (LEC) in these areas in collaboration with the WHO.

The commonest ethnic groups in the study area are Bekom, Essimbi and Mankon but 31.0% leprosy cases from 1961 to 1998 were from Essimbi land. The Ethnic variation could be geographical than ethnic (Noordeen, 1985). Leprosy is said to cluster in specific geographical locations (Ong, 1999). Danielsein and Boek (1848) made the astute epidemiological observation that 'leprosy tends to cling to specific families' and this has supported and propagated the idea that leprosy is hereditary and therefore genetic.

Sex distribution of leprosy in the Mbingo leprosarium

Among the 1045 leprosy patients from 1961 to 1998, there were more females (545) than males (500). Among 463 new cases of leprosy from 1961 to 1998, there were 257 females and 206 males. For the period, 1961 to 1967, results showed that more new cases of leprosy were among females than males, 97 (53.9%) versus 83 (46.1%) (P>0.05). For the period of 1982 to 1998, there were still more incident leprosy cases among females than males, 160(50.5%) versus 123(43.5%) (p= 0.00). In 1985, Noordeen reported that the male:female ratio of leprosy in Cameroon was 2:1. A similar ratio was reported from Yemen (Al-Qubatic and Al-Dobai, 1999).

The sex distribution of leprosy in Cameroon has changed with more females having leprosy than males. The occurrence of more cases among females than males from chart review in the leprosarium may be due to environmental factors. In the study area, farming is carried out mostly by women and during the hot seasons, they work partially bare body. The women therefore have increased exposure opportunities to infection because leprosy bacilli have been reported to be present in the soil in Norway (Kazda, 1990). Among the environmental factors, differing clothing habits are sometimes mentioned (Cochcrane, 1947). Even though women and men in many parts of Africa dress alike and thus cover their bodies to about the same extent (Noordeen, 1985), women in the study area, culturally don't wear trousers that could cover their legs. This may be a predisposing factor for leprosy infection. In the leprosarium, all the leprosy workers observed were men. It could mean that the examination of women in the area by these male workers is less' complete and satisfactory since culturally women do not undress in front of men and also women are always shy when examined by male health workers (Nsagha, 2002). This may explain why more infectious forms were discovered among males than females. Males in general are exposed to greater risks of infection as a result of their more exposed life style (Noordeen, 1985; Al-Qubatic and Al-Dobai, 1991) and may tend to have leprosy more than females.

Proportion of multi-bacillary and pauci-bacillary cases of leprosy in the Mbingo leprosarium

It was observed that among the admissions in the leprosarium from 1961 to 1967, there were 70 (23.8%) lepromatous cases of leprosy compared to 201 (74.2%) pauci-bacillary cases and from 1982 to 1998, there were 420 (54.3%) lepromatous cases compared with 354(45%) tuberculoid cases. In Africa, a low proportion of lepromatous cases has been reported (Cap, 1981). In this study, a significantly high prevalence of multi-bacillary forms of leprosy was observed, sufficient to make the distribution different from most parts of Africa (Nsagha, 2002). This discrepancy was also observed in Ethopia (Berthe et al., 1990). Noordeen (1985) noted that in areas where leprosy is dying out, the few cases that occur do have a predominance of lepromatous leprosy. This could be due to the fact that multi-bacillary leprosy takes a much longer time to be treated (two years) as against 6 months for the tuberculoid type using the current WHO MDT since the bacillary load is much.

Relapsed leprosy in the Mbingo leprosarium

From 1961 to 1967, there were 8 relapsed cases of leprosy with 3 (37.5%) occurring in 1962 and 2 (25:0%) in 1963 and 1966. Twenty relapsed cases of leprosy were recorded from 1982 to 1998. More females suffered relapsed leprosy from 1982 to 1998 than males (54.54% versus 45.46%). Even though MDT was introduced in the center in 1982, cases of relapses were documented after MDT implementation. In the leprosarium milieu, the increased bacillary load could lead to further disease progression or relapse (Kyriakis et al, 1994) because of the presence of many infectious forms. Relapses could have also been due to discharged cases from dapsone monotherapy or wrong classification. Relapsed leprosy can hinder the control of the disease since these cases can transmit the infection to other community members.

Leprosy readmissions in the Mbingo leprosarium

Readmissions for leprosy reactions were observed entirely with multi-bacillary leprosy (BB, BL, LL) even though the registers did not indicate whether readmissions were for type 1 (reversal reaction) or type 2 (erythema nodosum leprosum) leprosy reactions. The bacterial load is higher in multi-bacillary than pauci-bacillary leprosy, hence, readmissions were recorded only among patients with the former type of the disease. The decrease in number of readmissions from 1961 to 1998 could be due to the effectiveness of MDT in the treatment of leprosy.

Defaulters of leprosy treatment in the Mbingo leprosarium

Out of 173 discharged patients between 1982 to 1998 in the Mbingo leprosarium, 14 (1.3%) were defaulters but there were no defaulters from 1961 to 1967. Studies in Tanzania showed that 3% of patients indicated ignorance as a reason for default while 27% defaulted for unknown reasons (Hertroijs, 1974). Illiteracy and ignorance have also been reported as reasons for default from India (Bhgagoliwal et al, 1979). Deficient knowledge of leprosy determinants such as free treatment as well as the social stigma are some factors that need to be considered when interpreting why the patients defaulted.

Mortality of leprosy patients in the leprosarium

No deaths were reported from 1961 to 1967 compared to 14 deaths registered among the patients from 1982 to 1998 but the registers could not indicate whether the deaths were due to complications of leprosy or some other causes. It is note worthy that leprosy can disfigure and mutilate but it is not fatal. Leprosy is rarely an immediately cause of death but its psychosocial implications are enormous. In some communities in the world, because of the social stigma of leprosy, patients are killed or some decide to take away their life because of the frustration from the social stigma (Bryeceson and Pfaltzgraff, 1990).

CONCLUSION

The majority of the leprosy cases were from Menchum Division where Essimbiland is found. Since 1982, MDT has reduced the burden of leprosy in the leprosarium as many patients have been discharged. Record keeping on leprosy was generally poor as medical records from 1967 to 1982 were not available. Also, important demographic variables such as religion, occupation, educational, marital status, laboratory diagnosis, type of rehabilitation offered, presence or absence of deformities, disabilities and types were lacking.

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Full Length Research Paper

The understanding and perception of service providers about community-based cervical screening in Nigeria

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Community-based cervical screening based on the single visit approach using the visual inspection approach (VIA) and treatment with cryotherapy is the most appropriate method in limited resource settings in the short- to medium-term for the control of cervical cancer. The study is aimed at assessing the service providers understanding and perception of community-based cervical screening. A cross-sectional study of 31 health workers providing cervical screening services in Ogun State, Nigeria, using closed and open ended questions was carried out. There was a huge turnover of health workers who had been involved in cervical screening. Over 90% of the health workers opined that screening should commence before the age of 25 years. Their opinion of the recommended screening interval for cervical cancer varied; with 54.8, 16.1 and 29.0%, giving 3, 2 years and 1 year, respectively. The VIA is the most recommended screening test by the health workers (74.2%). The majority of health workers (87.1%) felt that the logistic and technical support provided for the cervical screening program was not adequate. Cervical cancer screening is thought to be of low priority within the health system by 45.2% of the respondents while 32.3% think that it is of moderate priority. The majority of the health workers (90.3%) said that the health authority in their local government do not budget funds for cervical cancer prevention. The service providers perceive the need for an urgent improvement in the community-based cervical screening through awareness creation, reduction in health worker turnover, and support and integration of cervical screening services.

Key words: Cervical screening, community, health workers, perception, understanding.

INTRODUCTION

Globally, more than 520,000 new cases of cervical cancer are diagnosed annually with about 85% of these cases in developing countries (WHO/ICO Information Centre on human papillomavirus (HPV) and Cervical Cancer (HPV Information Centre), 2010). In developed countries, the incidence of cervical cancer has declined by over 70% in the last 50 years due to accessible organized cervical cancer screening using the Papanicolaou smear and treatment of pre-cancers (Curado et al., 2007). The cervical cancer mortality in the United States has also decreased by 70% over the past five decades following the introduction of pap smear (Saslow et al., 2002).

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Similarly, in Finland, the incidence of cervical cancer reduced by more than 80% following the introduction of an organized cytology-based screening program (Strengthening cervical cancer prevention in Europe, 2007).

The successes reported in developed countries have not been replicated in sub-Saharan African, where cervical cancer remains the commonest cause of cancer deaths and accounts for over a fifth of all cancers in women (Parkin et al., 2003). It is estimated that between 60 and 75% of women in sub-Saharan Africa who develop cervical cancer live in rural areas and have no access to cervical screening (Parkin et al., 2002). It is possible that the circumstances in these low resource settings with widespread poverty, weak health infrastructure, and other basic challenges make it impossible for organized cervical cancer screening based on Pap smear to be successful. Other possible reasons include high level of competing health needs, wide spread poverty, and inappropriate health seeking behavior (Ezeanochie et al., 2010). There was therefore a need to reevaluate the use of cytology as the primary tool for cervical cancer screening in limited resource settings.

Nigeria has a population of 40.43 million women aged 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year, 14,550 women are diagnosed with cervical cancer and 9,659 die from the disease. Cervical cancer ranks as the second most frequent cancer among women in Nigeria, and the second most frequent cancer among women between 15 and 44 years of age. About 23.7% of women in the general population are estimated to harbor cervical HPV infection at a given time, and over 90% of invasive cervical cancers are attributed to HPVs 16 or 18. It is projected that in 2025, there will be 22,915 new cervical cancer cases and 15,251 cervical cancer deaths in Nigeria (WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre), 2010).

Cervical cancer prevention worldwide is based on screening women using conventional cytology (Pap smear). Pap smear screening was developed in 1928 and named after the inventor, Dr. George Papanicolaou (Ferlay et al., 2004). Pap smear programs, also known as cytological screening programs, have achieved impressive results in reducing cervical cancer incidence and mortality in some developed countries. Indeed, it has been estimated that cervical cancer incidence can be reduced by as much as 90% where screening quality and coverage are high (Eddy, 1986). For example, in Finland, a national cervical cancer screening program that was launched in 1963 decreased the cervical cancer rate to 5.5 cases per 100,000 women, a rate that is among the lowest in the world (Hakama et al., 1975). In contrast to developing countries, where about 80% of all new cases exist, it has been estimated that only 5% of women have had a Pap smear in the last 5 years (Herdman and Sherris, 2000).

Pap smear is a multistage process that involves sampling cells from the transitional zone of the cervix using either a wooden spatula or a brush. The cells are smeared on a glass slide and appropriately fixed, while the slide is sent to a trained cytopathologist for review and detection of abnormality. This multistage process can take several weeks before the results are available to the client, although in well-organized programs results can be available sooner. A promising cytology-based method is the liquid-based cytology (LBC) which is more sensitive (94.4%) than pap smears (De Vuyst et al., 2005) and has a potential to reduce the number of visits by clients for unsatisfactory smears and in addition provides specimen for HPV DNA testing. LBC is however, more expensive than the Pap smear and requires technical laboratory support for successful implementation. This drawback is an important challenge to its widespread use in limited resource settings.

Various studies have shown different degrees of sensitivity and specificity for Pap smear when conducted at different settings. In a study conducted at Kenya, Pap smear had a sensitivity and specificity of 83 and 94.6%, respectively (De Vuyst et al., 2005). In another study in India, pap smear had a sensitivity and specificity of 53.7 and 50%, respectively (Sherwani et al., 2007), while in another study carried out in rural Peru, Pap smear had a sensitivity and specificity of 26 and 99%, respectively (Almonte et al., 2007). Hence, clients often require more than one smear for a reliable diagnosis to be made before planning for treatment of premalignant lesions detected. This affects client compliance to screening programs especially because premalignant lesions are asymptomatic.

Even where screening coverage is high, the non-detection and treatment of premalignant lesions defeat the overall objective of reducing new cases of cervical cancer. Conventional Pap smear screening is therefore not the ideal technology of choice for a successful population-based screening program in Nigeria, a developing country.

In recent times, the visual inspection approach (VIA) using either 3 to 5% acetic acid or Lugol's iodine solution to stain the cervix has been promoted and used for cervical cancer screening programs in developing countries. Visual inspection with acetic acid is a simple, painless screening procedure that takes about 5 min to perform. The client is counseled on the procedure and an informed consent is obtained. With the client placed in alithotomy position, a pelvic examination is done, and a 3 to 5% acetic acid or vinegar solution is applied with a swab on stick within the transitional zone of her uterine cervix and result read after about a minute. The result could be normal if there is no color change or positive if a dense white color is noted within the area of application

of the 3 to 5% acetic acid. The VIA have a specificity and sensitivity of 73.3 and 80.0%, respectively (De Vuyst et al., 2005; Sankaranarayanan et al., 2003). This is comparable to rates found for conventional pap smears. The benefit of the visual approach over Pap smear is that the results are immediate and as a result treatment could be offered on the spot for precancerous lesions.

The single visit approach (SVA) involves offering cervical screening with the visual inspection method using either 3 to 5% acetic acid or Lugol's iodine and treatment offered to clients with noted precancerous lesions. The treatment offered an abnormal VIA screening as either an excision treatment or an ablative method using cryotherapy. Cryotherapy is a painless procedure which takes about 15 to 20 min to perform; it is relatively inexpensive compared to other treatment modalities and is effective for premalignant lesions. The VIA can be performed by lower cadres of health personnel, does not require high tech expensive technology and infrastructure to perform, is very affordable, and provides almost the same result as the Pap smear used in developed nations. Besides, the health care providers can be trained within 1 to 2 weeks (Sankaranarayanan et al., 2003). In addition, it has been observed in some studies that the VIA reduces the percentage of women lost to follow-up and the need for multiple visits to the health facility (Jeronimo et al., 2005).

However, the VIA is best suited forwomen whose transformation zone is still visible in the ecto-cervix during speculum examination. Hence, its use may be limited in screening post menopausal women (Sankaranarayanan et al., 2003).

The HPV DNA test is more sensitive than Pap smear in detecting high grade dysplasia in older women (Duarte-Franco et al., 2007; Gravitt et al., 2010). The direct detection of HPV in cervical specimen may therefore offer an alternative or complement to population-based cytological screening. The advantages of HPV DNA testing over cytology are that it does not require the same level of technical expertise as cervical cytology; it is amenable to large-scale population-based screening and identifies women with current disease and those at risk of developing the disease over the next 2 to 3 years (Gravitt et al., 2010). However, HPV DNA testing is more expensive and may not be readily available or accessible in limited resource settings. The cost of HPV DNA testing should reduce in future and the need for its use in limited resource settings in cervical cancer screening programmes may become more compelling.

In view of the challenges associated with ensuring high-quality cytology-based services in limited resource setting, and the high cost of conducting HPV DNA screening, an organized VIA approach is a useful alternative to the conventional cytology and HPV DNA testing in screening for cervical cancer. It offers a comparatively cheaper screening platform with minimal manpower requirements. The problem of "loss to follow up" associated with cytology-based screening is avoided with a single visit "screen and treat" model, thereby offering treatment for screen-positive persons. However, there is a role for cytology-based screening in focal communities where there is availability of the required man power and a motivated client base. This SVA using VIA and treatment with cryotherapy is the most appropriate in limited resource settings in the short- to medium-term pending the increased accessibility and affordability of newer techniques such as HPV DNA testing (Royal Thai College of Obstetricians and Gynecologists (RTCOG)/JHPIEGO Cervical Cancer Prevention Group, 2003).

Between 2005 and 2009, the World Health Organization supported a study of the effectiveness and acceptability of VIA and Cryotherapy in six African countries namely Madagascar, Malawi, Nigeria, Tanzania, Uganda, and Zambia. The project was structured and implemented in two phases; an initial local demonstration which was followed by a national scale-up programme. The Nigerian project site for the WHO multi center study was based in the Ogun State, one of 36 States in the Nigerian federation. A total of 100 healthcare workers in 49 health facilities in Ogun State were trained and equipped to undertake the VIA in their community settings. Women requiring cryotherapy were referred to the project headquarters (Centre for Research in Reproductive Health, Sagamu) where the resources for cryotherapy were only available (Dada, 2011).

Years into the project, there is a need to assess the community-based cervical screening in Nigeria. The focus heart of this study is to assess the project from the perspective of the health workers who are involved.

MATERIALS AND METHODS

Study area

Ogun State is entirely in the tropics. Located in the Southwest zone of Nigeria with a total land area of 16,409.26 square kilometers, it is bounded on the West by the Benin Republic, on the South by Lagos State and the Atlantic Ocean, on the East by Ondo State, and on the North by Oyo and Osun States. It is situated between latitude 6.2°N and 7.8°N and longitude 3.0°E and 5.0°E. There are 20 local government areas and 236 political wards in the Ogun State, Nigeria. The WHO equipped 49 VIA centers in the state.

Study population

The projected population of Ogun State, Nigeria, is about 4,280,090. The population of women between the ages of 20 and 64 years was put at 921,712 with an annual growth rate of 2.8%. The projected population of women between the ages of 20 and 64 (ages with the most significant risk of cervical changes) in the Ogun State is about 1,058,184. A total of 100 health workers were trained by the WHO to give VIA services in the state.

Table 1. Health workers' understanding about cervical screening.

Knowledge about cervical screening	Frequency	Percentage
Target age group for screening		
>35years	3	9.7
18–60years	10	32.3
25–60years	2	6.5
Child-bearing age	4	12.9
Middle age	2	6.5
Sexually active women	6	19.4
Youth	4	12.9
Total	31	100.0
Screening interval (years)		
1	9	29.0
2	5	16.1
3	17	54.8
Total	31	100.0
Screening tests to use		
Do not remember	7	22.6
Pap smear	1	3.2
VIA	23	74.2
Total	31	100.0
Management of positive screening test results		
Chemotherapy	2	6.5
Counselling, health education	1	3.2
Cryotherapy	15	48.4
Do not remember	7	22.6
Referral	6	19.4
Total	31	100.0

Study design

The study design is cross-sectional.

Sampling

Total sampling was adopted to reach all the 100 health care workers that were trained under the WHO project.

Data collection

Self-administered questionnaires were provided to the health workers who were trained on the VIA. The questionnaire which contains both open- and close-ended questions was adapted from "Planning and Implementing a cervical cancer prevention and control programme" (a publication of the program for Appropriate Technology in Health-PATH) and standardized to suite the Research needs.

Data analysis

The data obtained were double-entered into using Microsoft Excel package and screened. Analysis was done using the statistical package for social sciences (SPSS) Software version 16. Data are presented in the form of frequencies and percentages. The open-ended questions were analyzed by content analysis.

RESULTS

Out of the 100 intended questionnaires, 40 were successfully delivered, while 31 were returned. There was a huge staff turnover making it impossible to reach the majority of trained health workers. Many had resigned and moved to other employments, some had retired while others had been transferred out of sites offering the VIA services. A total of 5,346 women were screened for cervical cancer in the period 2007 to 2010 at these health facilities. Large variations were observed in the implementation of screening programmes in the various facilities. Indeed, at more than half of the health facilities, less than 2 women were screened per month on average.

The staff turnover rate was quite on the high side. A lot of the health workers who had the VIA training had either retired, resigned to seek other appointments, or have been posted to departments that has nothing to do with the subject under evaluation. Out of the 31 health workers interviewed, 15 (48.4%) of them were no longer involved with cervical cancer screening. Table 1 shows the knowledge and understanding of the health workers about cervical screening. The responses given by health workers about the age to initiate cervical screening was quite diverse, but generally within the risk age group for cervical cancer. Over 90% of them opined that screening should have commenced by the age of 25 years. The responses given by health workers about the target group for cervical screening were diverse. The ages between 18 and 60 years was given by 32.3% of respondents; 19.4% said sexually active women while 12.9% each said women of child-bearing age and youth but generally within the risk age group for cervical cancer.

There was a varied response to the recommended screening interval for cervical cancer with 54.8, 16.1 and 29.0%, giving 3, 2 years and 1 year, respectively. The VIA is the most recommended screening test by the health workers (74.2%), while 7 of the 31 health workers (22.6%) could not remember the appropriate screening test. A total of 67.8% of the health workers would offer either a referral or cryotherapy to a woman with positive result while 22% would not know what to do. Table 2 shows the perception of the health workers concerning the support they receive for the programme. The majority of health workers (87.1%) felt that the logistic and technical support provided for the cervical screening program was not adequate.

Table 2. Health workers' perception of the support for community-based cervical screening.

Perception of support	Frequency	Percentage
Adequacy of logistic and technical support		
No	27	87.1
Yes	4	12.9
Total	31	100.0
Prioritization of cervical cancer prevention		
High	7	22.6
Moderate	10	32.3
Low	14	45.2
Total	31	100.0
Budgetary allocation for cervical cancer prevention		
Yes	3	9.7
No	28	90.3
Total	31	100.0
Adequacy of resources allocated		
Yes	2	66.7
No	1	33.3
Total	3	100.0

Cervical cancer screening is thought to be of low priority within the health system by 45.2% of the respondents while 32.3% think that it is of moderate priority. Only 22.6% place it as a high priority issue within the health system. The competing health priorities identified by the health workers in order of popularity include HIV/AIDS, malaria, tuberculosis, immunization and polio eradication, reduction of maternal and child mortality rate, family planning, and diabetes. Others are prevention of sexually transmitted infections and teenage pregnancy and sickle cell anaemia. The majority of the health workers (90.3%) said that the health authority in their local government do not budget funds for cervical cancer prevention. However, two of the three health workers who said they had budgetary allocations found the resources to be appropriate.

The health workers identified certain weaknesses of the policies and guidelines for cervical cancer prevention. There is a general lack of political will and backing by governments at all tiers. Cervical screening is accorded a low priority within the health system. There is inadequate funding, lack of manpower, insufficient equipment, and other consumables for the VIA screening. Only a few centers have the necessary resources for cervical screening. The non-integration of cervical cancer programme into routine prevention programmes is a major weakness of the current setting. In the absence of a nationally accepted and clear-cut policy of cervical cancer prevention, the present practice may not be sustainable.

They suggested some of the following measures to address the weaknesses in policies and guidelines for cervical cancer prevention. There should be advocacy to gain political will and support of the government, policy makers, and law makers. Awareness creation on the morbidity and mortality of cervical cancer with the inclusion of cervical cancer education in school curriculum is regarded as the most important step to promoting cervical screening. There is a need for the enactment of a national policy and the creation of national prevention programme and its integration with other routine prevention programmes. There also must be a deliberate manpower development and adequate resource allocation for cervical cancer screening. Provision of free cervical screening and treatment for those who are positive that is accessible to all who require it is very important. Prioritizing cervical screening within the health sector was also recommended. There is a need for the development of a multisectoral, local, and international partnership for the prevention of cervical cancer.

There were also weaknesses in the provision of cervical cancer screening services.There is a low patient turnout due to a generally low level of awareness of cervical cancer and screening among the populace. This coupled with the fact that patients are sometimes required to pay and a lack of commitment by health personnel due to poor motivation ensure that services are not accessible to women who should have them. Others include lack of sustainability due to staff turnover, inadequacy of consumables, absence of budgetary allocation for cervical screening, shortage of skilled personnel, and follow-up problem. There is lack of hospital management and government support and shortage of fund coupled with incessant industrial action by hospital staff.

Various measures were proposed to address the weaknesses of the cervical cancer screening services. The recommendations include advocacy to gain political will and support of the local government authorities; awareness creation on the morbidity and mortality of cervical cancer and a deliberate manpower development and adequate monthly resource allocation for cervical cancer screening. Monitoring and evaluation with a view to provide ways to improve the programme is also crucial.

DISCUSSION

The health workers demonstrated a good understanding of cervical screening. They understood that cervical screening should be done routinely beginning from early adulthood till later in life. However, there was an apparent diversity in their opinion concerning the specifics. The

apparent lack of a widely circulated guideline on cervical screening in Nigeria would be responsible for this. Clinical guidelines provide recommendations to assist practitioners in providing appropriate health care based on scientifically valid research. Studies show that these have significant potential to enable provider organizations to improve quality without increasing costs (Wagner, 1999; Grimshaw and Russell, 1993; Marquez, 2001). It could have been due to the fact that most of the health workers did not have continued training on cervical screening. It is widely accepted that continuous medical training tends to reinforce knowledge and improve health-care delivery and health outcomes (Brennan et al., 2006; Davis et al., 1999; Umble and Cervero, 1996; Robertson et al., 2003).

There was inadequate logistic and technical support for the programme apparently and no local funding support for the program in most cases. This can be attributed to the low priority attached to cervical screening in the Nigeria health sector despite the high morbidity and mortality associated to cervical cancer in the country. This is similar to what is seen in Southern Africa where despite the fact that cervical cancer is the leading cause of cancer death among women, new research reveals that governments' attempts to address the disease have been inadequate. There is still a lack of clear and comprehensive national cervical cancer management guidelines and policies in the region. Neither Namibia nor Zambia has comprehensive guidelines on the management of the illness. Where guidance is available, it tends to be inadequate, focusing on screening, with limited guidance about other forms of prevention or treatments (Southern Africa Litigation Centre Report, 2012). Currently, many low- and middle-income countries have health systems that do not meet the requirements for chronic care. In recent years, many of them have invested in vertical national programmes to address HIV/AIDS, tuberculosis, and malaria to the detriment of other diseases such as cervical cancer (Borisch, 2010).

The weaknesses in the program are those that pertain to policy and guidelines which are not well disseminated and those related to service delivery. There is a low level of awareness of cervical cancer and screening among the populace. The non-integration of cervical cancer programme into routine prevention programmes is a major weakness of the current setting. In the absence of a nationally accepted and clear-cut policy of cervical cancer prevention, the present practice may not be sustainable. Many studies in the sub-Saharan Africa and indeed developing countries have shown similar findings (Lyimo and Beran, 2012; Louie et al., 2009; Mutyaba et al., 2007; Denny et al., 2006).

It is therefore important to develop a robust integrated programme with well-disseminated guidelines and continuous training for health providers that plays up the importance of cervical cancer control. A deliberate measure must be put in place to address the high turn-over of health personnel. It is hereby emphasized that at the heart of every cervical cancer control programme both in Nigeria and indeed the developing nations is the creation of awareness about cervical cancer and screening.

Conclusion

The service providers perceive the need for an urgent improvement in the community-based cervical screening. Awareness creation, funding, logistic, and technical support for the programme and integration of services are some of the issues needing attention to boost cervical screening. There must be concerted efforts to reduce the turnover of staff that have had training in and are thus involved with cervical screening.

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Full Length Research Paper

Knowledge, attitude to hypertension and lifestyle habits of rural dwellers in Owerre-Nkwoji, Imo State Nigeria

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Hypertension accounts for 13% of global mortality, with 25% prevalence in Nigeria. Current trends in management is lifestyle modification. Information on lifestyle habits is needed for effective intervention programe. A study was done among rural dwellers between ages 20 and 75 in Owerre- Nkwoji, Imo State, to determine their knowledge, attitude to hypertension and lifestyle habits. Information gathered would be used to plan an intervention programe to increase awareness and promote healthy lifestyles. The study design is descriptive and simple random sampling method was used in selection of village for the study. An instrument used was a questionnaire with sections on background, knowledge, attitude and lifestyle habits. Descriptive and inferential analysis was done. Findings revealed awareness on hypertension as 116 (96.7%). 88 (73%) were aware of sudden death and stroke in the community. Participants with high knowledge were 32 (26.7%), average 40 (33.3%) and low 48 (40.0%). There was positive attitude to hypertension prevention. Sixty-four (53.3%) believed that hypertension can be sent by one's enemy. Unhealthy lifestyles elicited include use of alcohol, table salt and stimulants. There is a need to correct misconceptions. Readiness for improved health was elicited. As people recognize that lifestyles and behavior significantly affect health, they may assume responsibility for avoiding high risk behaviors.

Key words: Attitude, hypertension, knowledge, lifestyle habits, rural-dwellers.

INTRODUCTION

Hypertension is recorded as a major type of cardiovascular disease which affects one billion people worldwide and claims the life of seven million (American Heart Association, 2006). It accounts for 13% of global mortality (Katib, 2004) with 25% prevalence in Nigeria (Danbauchi, 2007). A current trend in management is lifestyle modification (Simeon and Zieve, 2008). This study serves as a pilot study for a larger study in the same environment. Information on lifestyle habits is needed for effective intervention for health promotion programes. The participants' knowledge, attitude to hypertension and its preventive measure, lifestyle habits in relation to hypertension as well as blood pressure screening were assessed for this purpose.

MATERIALS AND METHODS

Study was descriptive, done among rural dwellers between ages 20 and 75 in Owerre-Nkwoji, Imo State. The study population was 120 rural dwellers (44 males and 76 females aged between 20 and 75 years). A village was randomly selected out of the six that made up the town through balloting. Informed consent was obtained from the town union leader as well as from each participant. The first household was identified with tossing of the coin at the center of the village square. Elements of the study were drawn from alternate households until the required number was reached. Instrument for data collection were: a structured questionnaire which has four sections that is, demography, knowledge, attitude and lifestyle habits. Measuring tools for blood pressure, weight and height were standardized. Face and content validity was ascertained by experts. Back to back translation was done using 10 respondents from the community to ensure congruence. The measuring tools were tested

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Table 1. Frequencies and percentage of responses on knowledge of hypertension.

Awareness and knowledge variables	Statement	Responses		
		Yes (%)	No (%)	Do not know (%)
Awareness	Is your BP reading known to you?	24 (20)	72 (60)	24 (20)
Cause	Much thinking (worries) is the main cause of HBP	104 (86.7)	4 (3.3)	12 (10)
Diagnosis of HBP	Diagnosis of HBP is made by any health worker	20 (16.7)	100 (83.3)	0 (0.0)
	HBP is detected on sight	28 (23.3)	20 (16.7)	52 (43.0)
	HBP is detected by blood pressure measurement	92 (76.7)	28 (23.7)	0 (0.0)
Symptoms	Severe headache with heat sensation is a symptom of HBP	56 (46.7)	12 (10)	52 (43.3)
	Symptom of HBP include pain side of neck	36 (30.0)	28 (23.3)	56 (46.7)
Risk factors	Alcohol can bring about HBP	44 ((36.6)	36 (30.0)	40 (33.3)
	Use of table salt can bring about HBP	8 (6.7)	84 (70.0)	28 (23.3)
	Obesity can bring about HBP	36 (30.0)	32 (26.7)	52 (43.3)
Management of HBP	HBP is best managed in Prayer Houses	36 (20.0)	48 (40.0)	36 (30.0)
Prevention	Regular exercise can prevent HBP	76 (63.3)	8 (6.7)	36 (30.0)

for validity and reliability before putting them into use. Blood pressure,weight and height measurements were recorded with appropriate tools. All questionnaires were retrieved as it was interview administered, through training research assistants. Data when validated was found to be congruent. Analysis of Crobach's alpha for English 1, Vernacular (Igbo) and English 2 was 0.63. Reliability of tool using Crobach's coefficient was computed and value was 0.76. Data was entered using statistical package for social sciences 17.0. Descriptive analysis was done which displayed the frequencies of various variables; the mean values as well as the standard deviation (SD). Body mass index (BMI) and blood pressure classification was done. Inferential analysis of association between dependent and predisposing variables were computed using Chi square.

RESULTS

Age ranged from 20 to 75 (mean 36, SD ± 12.83). Sixteen (13%) had no formal education; 20 (17%) primary; 48 (40%) secondary and 36 (30%) had tertiary education. Seventy six (63%) were jobless; 24 (20%) civil servants; 12 (10%) farmers; 8 (7%) are petty traders. Household income; lower class was 68 (57%), lower middle class was 48 (40%), and middle class was only 4 (3%). None were upper class. The overall awareness level was 44%. Findings revealed that 116 (96.7%) were aware that the term "hypertension" refers to high blood pressure. Twenty four (20%) were aware of their blood pressure readings while 96 (80%) are unaware. Responses on awareness of incidents of stroke and sudden deaths in the community was 88 (73 .3%) each. Overall knowledge was 60%. Knowledge were 32 (26.7%), average 40 (33.3%) and low 48 (40.0%). Knowledge of stroke as a complication of hypertension: Yes; 36 (30.0%); No 8 (6.7%) while 'do not know' was 76 (63.3%).

There was a positive attitude to hypertension prevention. Sixty eight (56.7%) strongly agreed and 52 (43.3%) agreed that hypertension is of serious health concern. Twenty (16.7%) strongly agreed and 52 (43.3%) agreed that it is important to check one's blood pressure regularly even though 16 (13.3%) strongly agreed and 36 (30.0%) agreed that hypertension is not for young people. There were myths about hypertension (Tables 1 and 2).

Unhealthy lifestyles elicited include use of alcohol, table salt and stimulants (Figure 1). The relationship between age and knowledge was not significant; Pearson X^2 = 0.563, P-value = 1.151,

Table 2. Myths expressed by respondents about hypertension.

Statement	Responses		
	Yes (%)	No (%)	Do not know (%)
HBP is sent through juju/remote enemy attack	80 (66.7)	36 (30.3)	4 (3.3)
HBP can be from food poisoning	56 (46.7)	36 (30.0)	28 (23.3)
Stroke is due to knock on the head by spirits	52 (44)	40 (33)	28 (23)

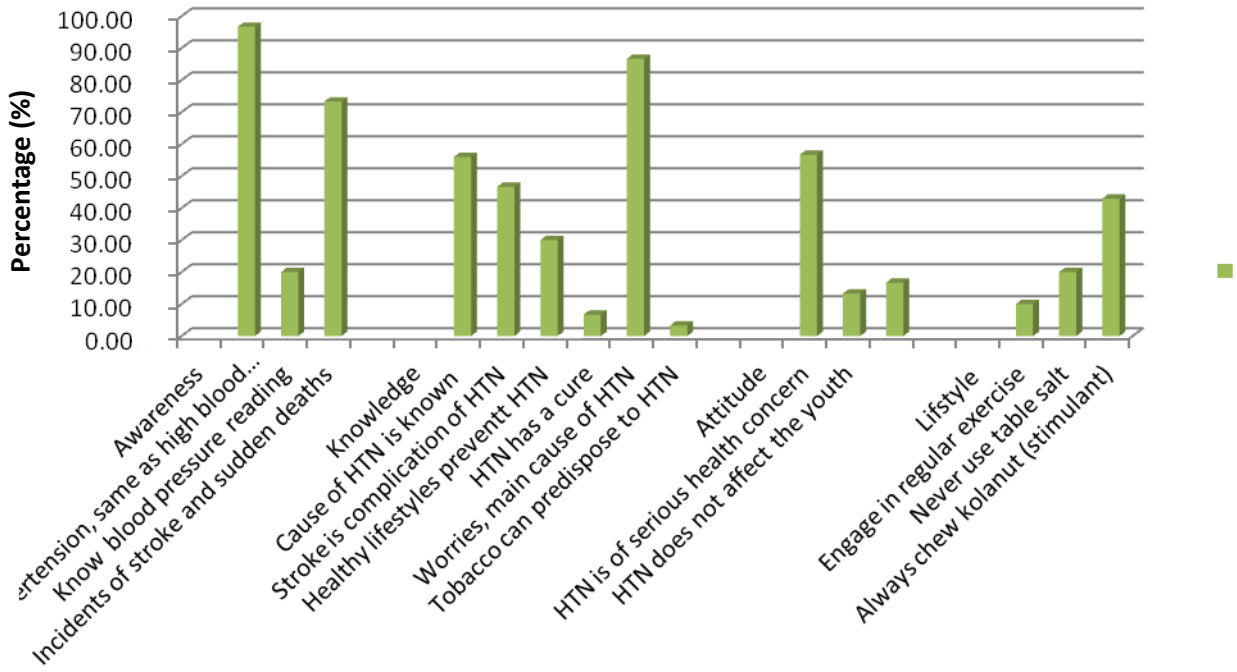

Figure 1. Responses on awareness, knowledge, attitude and lifestyle habits. Note: HTN is hypertension.

df = 2, even though high knowledge for ages 40 and above were 37.5% while less than 40 was 22.7%.

The relationship between sex and knowledge was not significant; Pearson X^2 = 0.237, P-value 2.880, df = 2 though there was high knowledge for males - 68.0% and females 32.0%. The relationship between age and attitude was not significant; Pearson X^2 = 0.697, P-value = 0.151, df = 1, even though result showed negative attitude for ages 40 and above as 37.5% and less than 40 as 45.5%. Relationship between sex and attitude, Pearson X^2 = 0.249, P-value = 1.330, df = 1 was also not significant, but negative attitude for males was 20.0% and females 48.0%. The relationship between age and lifestyles was not significant; Pearson X^2 = 0.657, P-value = 0.197, df = 1 though negative lifestyle for ages 40 and above as 50.0% and less than 40 was 40.0%. The relationship between sex and lifestyles was also insignificant; Pearson X^2 = 0.410, P-value = 0.679, df = 1 though result of negative lifestyle for males was 60.0% while females were 40.0%. Result of blood pressure revealed that fifty six (47%) had a normal blood pressure reading. BMI showed participants were not obese (Figure 2).

DISCUSSION

Awareness of participants on hypertension issues was 44% as against the 50% in the study by Ike et al. (2010). Misconception was also apparent in this study as in theirs. For example, stroke and hypertension can be caused by gods or enemy, respectively. Attitude to hypertension was negative especially among participants, as majority strongly disagreed with having hypertension in their lifetime and does not affect young people. Apparently these participants will not go for a routine blood pressure check based on their belief. It was also noted that majority of the participants could not differentiate between daily activities and regular exercise as it was strongly agreed that daily activities was same as regular exercises unlike in the study by Ohata et al. (2005) where all participants appreciated a 12 week

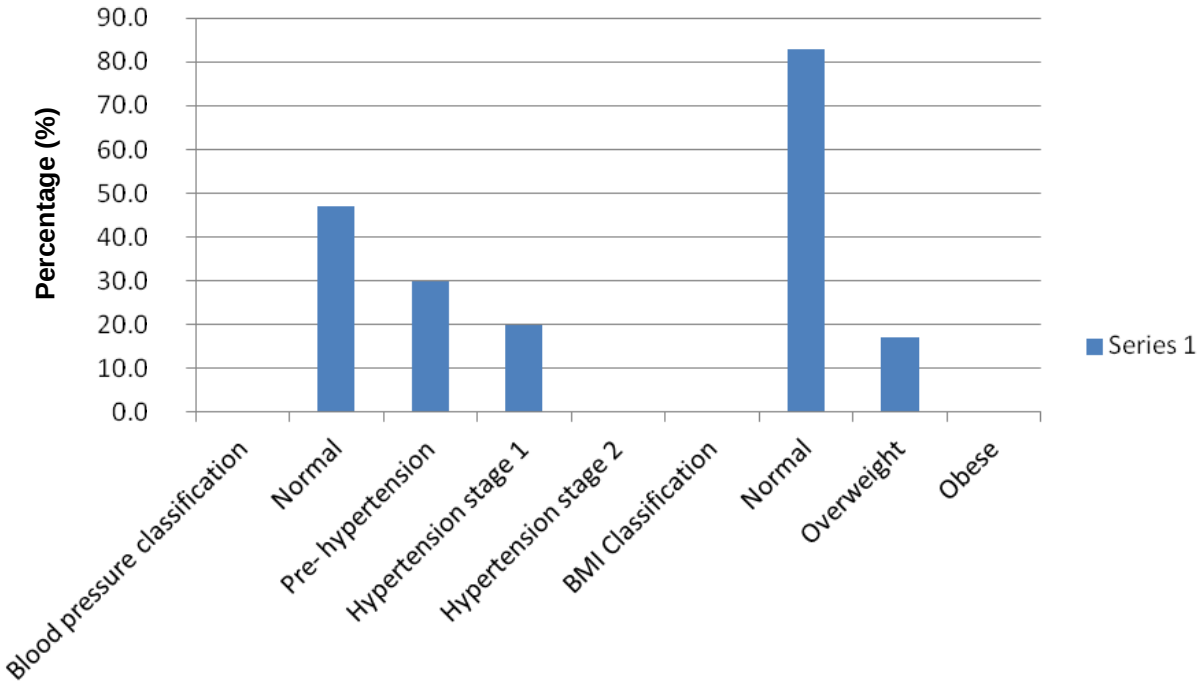

Figure 2. Classification of respondents' blood pressure and BMI status.

regular exercise as an intervention. Identified risk factors were in line with Wang et al. (2006) and Omorogiwa et al. (2009) in their studies. Smoking was not identified as a risk factor as in the report by Ricks (2004) and Aghaji (2008) but snuff was enlisted. The unhealthy lifestyles in relation to nutrition include the chewing of kolanut, use of table salt, and alcohol as in the studies by the aforementioned researchers.

Conclusion

Even though majority was aware that the term 'hypertension' refers to high blood pressure, the overall level of awareness was poor. Majority agreed that hypertension is a serious ailment and that adoption of healthy lifestyles will curb the menace, which implies that there would be health seeking behavior among the population. Myths about hypertension need urgent intervention. The findings of this study cannot be generalized because of the population size. There is therefore need for population focus study. Information gathered would be used to plan an intervention programme to increase awareness and promote healthy lifestyles.

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Full Length Research Paper

Prevalence of japanese encephalitis and its modulation by weather variables

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Japanese encephalitis (JE) is a major public health problem in India. To study the influence of climatic factors on JE, cases and the transmitting mosquito species were analysed during 2001 to 2006. To know the status of Japanese encephalitis virus activity in human population, sero-epidemiological studies were undertaken in villages of Kurnool district, Andhra Pradesh, India. Similarly, mosquitoes were sampled from study areas at bimonthly intervals during 2001 to 2006 and identified to species level. The collected mosquitoes were screened for JE virus by using an antigen-capture enzyme linked immunosorbent assay. Out of 2051 samples collected from the study areas, 156 (8%) sera samples were found to be positive for JE virus. The highest number of JE positive cases was observed in 2005 (14%), followed by 2003 (10.6%) and 2001 (9.1%). The seasonal pattern on occurrence of JE cases clustered among different seasons (that is, monsoon, winter and summer) showed that JE cases occurred in all seasons of the year. The vector, *Culex tritaeniorhynchus* per man hour density was correlated with (minimum and maximum temperature, $p < 0.035$ and $p < 0.013$), whereas *Culex gelidus* was positively correlated with rainfall ($p < 0.05$). JE transmission in temperate areas is dependent on climatic factors; however this study suggests that effects of weather variables such as rainfall, temperature and relative humidity might be responsible for increase of vector populations and also the JEV infection. Apart from these, the other factors like agricultural practices, virus amplifying hosts such as pigs and its density and virus reservoirs might also play a major role in the disease transmission in the study areas.

Key words: Epidemiological survey, vector surveillance, Japanese encephalitis virus, climatic variables.

INTRODUCTION

Japanese encephalitis (JE) is one of the leading forms of viral encephalitis and is more prevalent in South Asia, Southeast Asia, East Asia and the Pacific (Solomon, 2006; Fischer et al., 2008). Mostly children and young adults are afflicted with JE in rural and suburban areas where rice cultivation and pig farming coexist (Fischer et al., 2010). In India, Japanese encephalitis virus (JEV) is an important pediatric public health problem (Kanojia et al., 2003; Srivastava et al., 2003) and was first reported in 1955 (Saxena and Dhole, 2008). Subsequently, JE epidemic have been reported in as many as 25 states/union territories of India (Kabilan et al., 2004). Recently in Uttar Pradesh, Bihar, and Andhra Pradesh, JE has emerged as a perennial public health problem (Arunachalam et al., 2009). An epidemic of JEV was reported in 2005 in Gorakhpur, Uttar Pradesh, India. It was the most severe epidemic in 3 decades; 5,737 persons were affected in 7 districts of Uttar Pradesh

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and 1,344 persons died (World Health Organization (WHO), 2006).

JE virus has been recovered from 19 mosquito species in different parts of India, and the prominent vectors are *Culex tritaeniorhynchus* Giles and *Culex vishnui* Theobald (Murty et al., 2010). The JEV is mainly transmitted by the mosquito *Cx. tritaeniorhynchus* in India, which prefers to breed in irrigated paddy fields (Upadhyayula et al., 2012). Wading ardeid water birds serve as reservoirs for this virus, but virus regularly spills over into pigs, members of the family of equidae and humans are generally thought to be dead-end hosts. The epidemiology of JE virus is not well understood and only little research has been done. This menace has now grown to be a serious infectious disease in developing countries like India. Hence, the present study is undertaken to investigate the influence of various climatic factors on JEV transmission in Kurnool district, Andhra Pradesh.

MATERIALS AND METHODS

Study area

Kurnool district of Andhra Pradesh, India was selected for the present study as highest number of JE cases were reported from this region since 1996. The area is located between 15.83°N, 78.05°E and its total population is 1,724,795. Most of the population is affianced in agricultural practices and are of low socioeconomic status. Patchy paddy fields and water loggings are commonly seen in and around Kurnool district that promotes the vector breeding. Domestic animals such as cattle, pigs and poultry commonly share the habitat with human population. This district is generally warm and humid during most parts of a year. The agricultural activities are at their peak during the Southwest monsoon which persists between June and October of the year. During the study period (2001 to 2006), the maximum temperature ranged from 30.3°C (December, 2005) to 42.8°C (May, 2003). Out of the 69 Primary Health Centers (PHCs) of Kurnool district, six areas namely Peddathumbalam, Nandanapalli, Nandikotkur, Gudur, Cherukulapadu and Kurnool have been selected for this study which showed highest number of JE cases since 1996. Data was collected on epidemiology, entomological and environmental parameters in index areas which are mentioned in the manuscript.

Epidemiological survey

In order to know the status of JE virus activity in human population, sero-epidemiological studies were undertaken in the study areas of Kurnool district. The blood samples were collected from the school children (6 to 10 years old) through finger prick method. The sera of school children were examined by haemagglutination inhibition (HI) test following protocol of Clarke and Casals (Arunachalam et al., 2009) for identifying the flavivirus antibodies (JE/West Nile/Dengue).

Mosquito collection

Mosquitoes were sampled from identified villages at bimonthly intervals during 2001 to 2006 and identified to species level (Arunachalam et al., 2009). Both blood engorged and unfed adult mosquitoes, resting on bushes and thatched roofs of cattle sheds and human dwellings were trapped from different parts of the village. Fully fed mosquitoes were held for 24 to 48 h for digestion of blood meals. Later the captured mosquitoes were segregated into different pools (50 mosquitoes per pool) and were screened for JEV infectivity by using an antigen-capture enzyme linked immuno-sorbent assay (ELISA) for the initial screening of flavivirus and inoculation of *Toxorhynchites splendens* (Wiedemann) combined with an indirect immunofluorescence assay (Toxo-IFA) which was performed to confirm infection with JEV. Virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1000 females tested (Arunachalam et al., 2009).

MIR = No. of positive pools / Total no. of specimens tested × 1000

Meteorological data

Besides epidemiological and entomological studies, data on other parameters like maximum and minimum temperature, rainfall, relative humidity and wind speed were collected month wise from meteorological department, Hyderabad, Government of India during study periods.

Ethics statement

The study received ethical clearance from the Ethical Committee which was constituted in our institutes (Indian Institute of Chemical Technology & Centre for Research in Medical Entomology) affiliated to Ministry of Science & Technology and Ministry of Health & Family Welfare, Govt. of India. This ethical committee has approved to carry out the research work. The consent of the subjects who provided the blood sample was minors. Hence written consent was obtained from the parents/guardians before the commencement of epidemiological survey. Similarly, entomological survey was also conducted in private land/private residences after obtaining the written consent from the respondents.

Statistical analysis

Data analysis was done with the SYSTAT statistical package. Most of the JE cases occured in July to September (monsoon period). In order to understand the disease transmission dynamics, the samples were also collected during winter (November to January) and summer (March to May) seasons. The incidences of JE, mosquito density and MIR were correlated with climatic factors by using Spearman's rank correlation method.

RESULTS

Out of 2,051 samples collected from the study areas (Figure 1), only 156 (8%) sera samples were found to be positive for JE virus by HI method. The highest number of JE positive cases was observed in 2005 (14%), followed by 2003 (10.6%) and 2001 (9.1%). Between the years 2002 and 2003, an increasing trend of JE cases was observed but the year 2004 had less number of cases

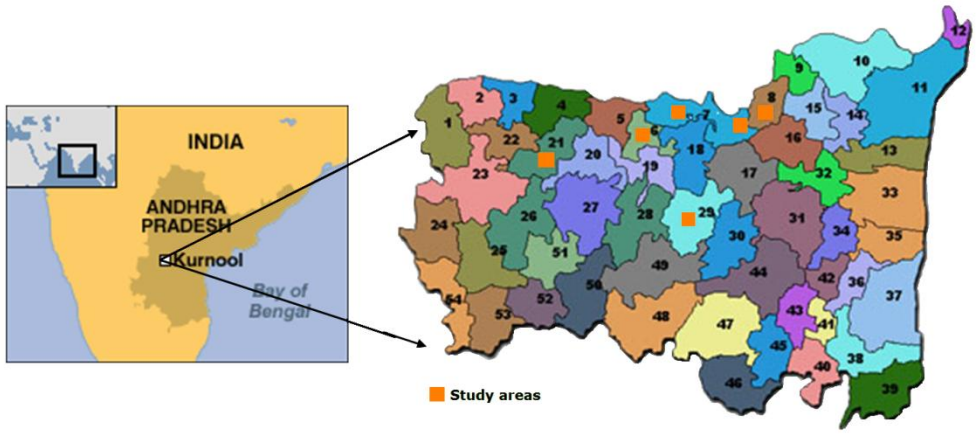

Figure 1. Map showing the locations of study areas in Kurnool district of Andhra Pradesh, India.

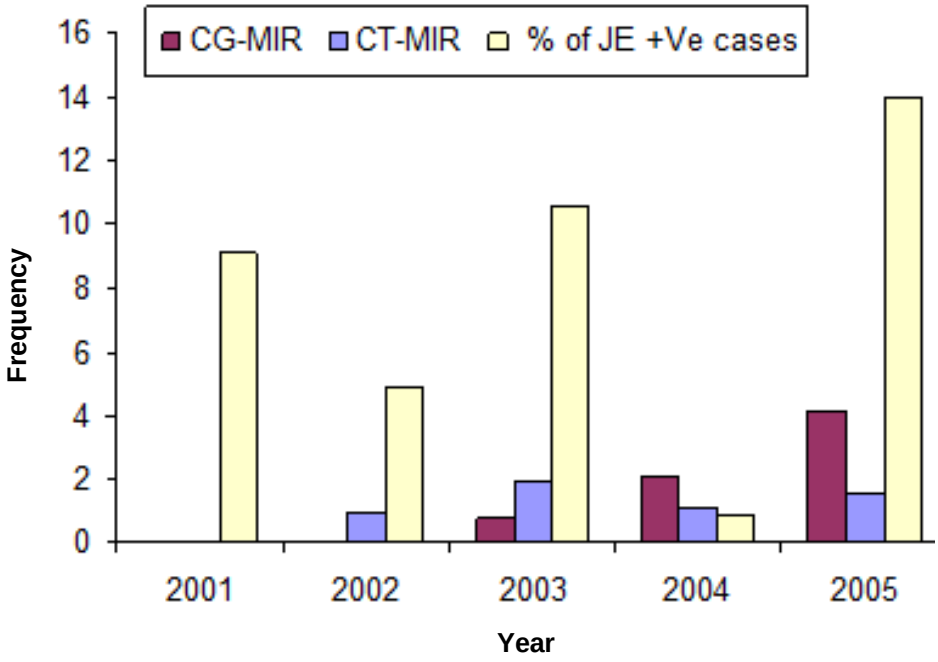

Figure 2. Year wise JE cases (%) and minimum infection rate of *C. tritaeniorhynchus* and *C. gelidus* in Kurnool district of Andhra Pradesh.

reported (Figure 2). The percentage of JE positive cases are found to be weak to moderate correlation with minimum infection rate (MIR) of *Cx. tritaeniorhynchus* ($r = 0.26$, $p < 0.6$) and *Cx. gelidus* ($r = 0.36$, $p < 0.5$).

To understand the disease transmission dynamics, sero-epidemiological survey was also carried out during different climatic seasons of the year. From the study, it is noticed that number of cases were mostly recorded during the monsoon period (from July and attains highest number in September). Among the seasonal data on JE cases, highest number of positive cases was recorded during summer season of the year 2005 (Figure 3). Weak to moderate correlation was observed in the number of JE positive cases while comparing with per man hour (PMH) density of *C. tritaeniorhynchus* ($r = -0.35$, $p < 0.4$) and *C. gelidus* ($r = -0.43$, $p < 0.3$). Similarly, correlation analysis has been done with weather variables and incidence of JE cases during the study period (Figure 4). The results in Table 1 indicate that the maximum, minimum temperature, relative humidity and rainfall were

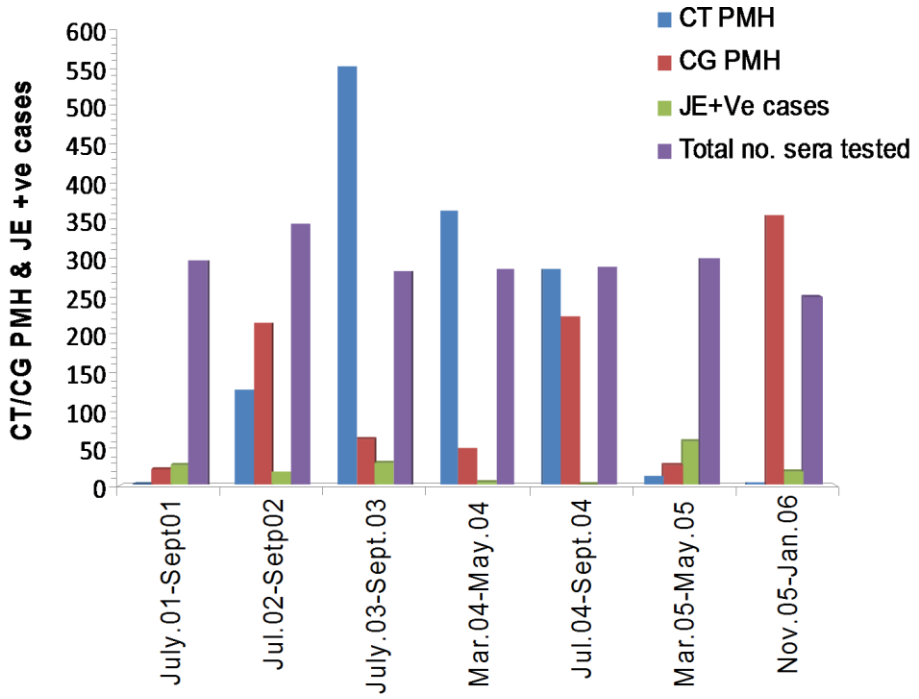

Figure 3. Seasonal distribution of JE cases and vector density in Kurnool district of Andhra Pradesh.

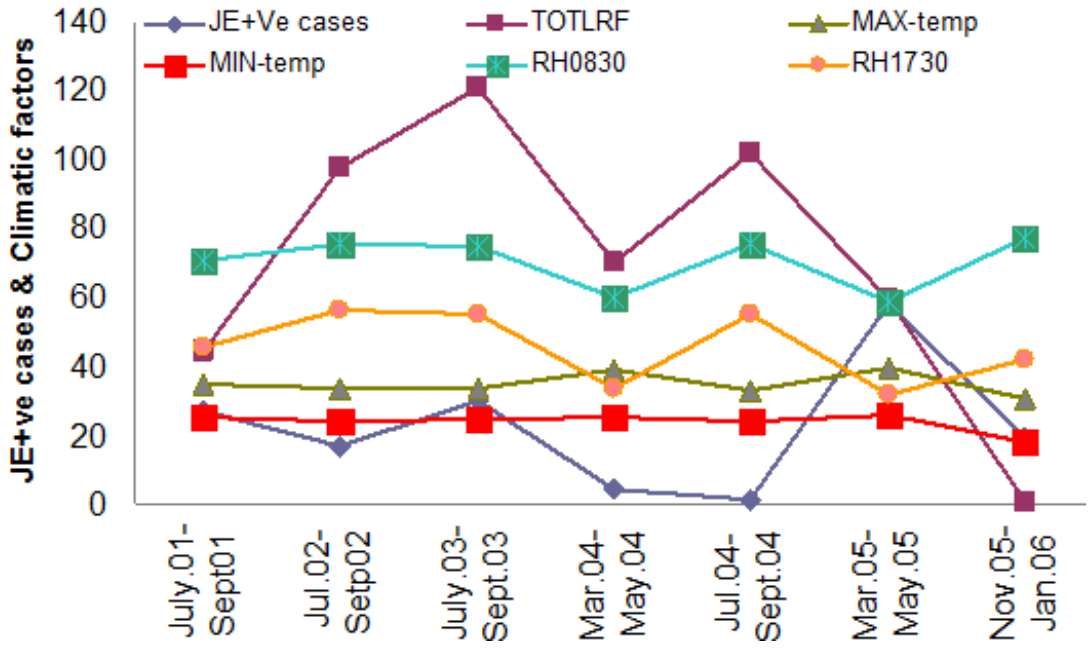

Figure 4. Incidence of JE positive cases in relation with temperature, rainfall and relative humidity in Kurnool district of Andhra Pradesh.

found weak to moderate correlation on the number of JE cases.

In Table 2, the correlation coefficient was evaluated between climatic variables and vector density of *C.*

Table 1. Correlation between climatic variables and JE cases in Kurnool district of Andhra Pradesh, India.

Climatic variables	r-Value	p-Value	95% CI
Rainfall	-0.152	0.745	-0.812-0.679
Maximum temperature	0.372	0.411	-0.529-0.879
Minimum temperature	0.225	0.627	-0.636-0.836
Relative humidity at 08.30 h	-0.381	0.399	-0.881-0.522
Relative humidity 17.30 h	-0.387	0.391	-0.883-0.517

Table 2. Correlation between climatic variables and per man hour density of *C. tritaeniorhynchus* and *C. gelidus*.

Climatic variables	*C. tritaeniorhynchus*			*C. gelidus*		
	r-Value	p-Value	95% CI	r-Value	p-Value	95% CI
Rainfall	-0.317	0.489	-0.864-0.573	0.753	0.05*	-0.001-0.961
Maximum temperature	-0.789	0.035*	-0.967 to -0.087	0.027	0.953	-0.741-0.765
Minimum temperature	-0.861	0.013*	-0.979 to -0.309	0.276	0.549	-0.602-0.852
Relative humidity at 08.30 h	0.684	0.09	-0.142-0.949	0.084	0.858	-0.714-0.787
Relative humidity at 17.30 h	0.346	0.447	-0.550-0.872	0.358	0.43	-0.541-0.875

*P<0.05.

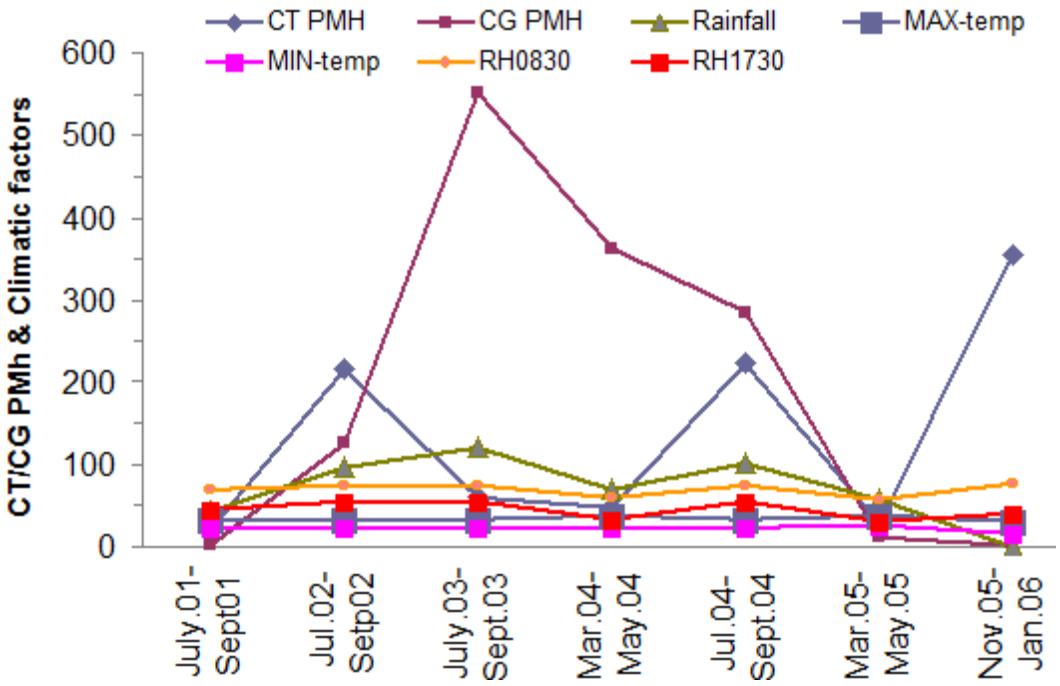

Figure 5. Seasonal prevalence of *C. tritaeniorhynchus* and *C. gelidus* with temperature, rainfall and relative humidity in Kurnool district of Andhra Pradesh.

tritaeniorhynchus and *C. gelidus*. In *C. tritaeniorhynchus* PMH density found correlation exists with temperature ($p < 0.035$ and $p < 0.013$) (Figure 5), whereas *C. gelidus* was positively correlated with rainfall ($p < 0.05$). The MIR showed moderate correlation with *C. tritaeniorhynchus* ($r = 0.338$, $p < 0.289$) and *C. gelidus* ($r = 0.373$, $p < 0.268$) PMH density. Similarly, *C. tritaeniorhynchus* MIR were correlated with weather variables and the MIR values had

Table 3. Correlation between climatic variables and minimum infection rate of *C. tritaeniorhynchus* and *C. gelidus*.

Climatic variables	*C. tritaeniorhynchus* MIR			*C. gelidus MIR*		
	r-Value	p-Value	95% CI	r-Value	p-Value	95% CI
Rainfall	0.227	0.714	-0.819-0.924	0.759	0.137	-0.373-0.983
Maximum temperature	-0.095	0.879	-0.902-0.859	-0.841	0.074	-0.989-0.160
Minimum temperature	0.327	0.591	-0.780-0.939	-0.414	0.488	-0.950-0.737
Relative humidity at 08.30 h	0.362	0.55	-0.765-0.943	0.855	0.065	-0.110-0.990
Relative humidity at 17.30 h	0.438	0.46	-0.724-0.952	0.751	0.144	-0.389-0.982

shown that weak to moderate correlation exist, whereas *C. gelidus* showed strong correlation significance with maximum temperature ($p < 0.074$) and relative humidity ($p < 0.065$) (Table 3).

DISCUSSION

JE is a major public health problem in India and mainly affects the children and young adults (Swami et al., 2008). It is estimated that 3 billion people live in Japanese encephalitis virus (JEV) endemic regions and the disease incidence are about 50,000 cases and 10,000 deaths annually (Fischer et al., 2008). From this study, out of 2051 samples collected, 156 (8%) sera samples were found positive for JEV. The year-wise JE positives cases during the study period (2001 to 2006) are presented in Figure 2 which shows that the JEV infection in this area is in the immediate past. To understand the prevalence of JE cases among the different seasons of a year, it was noticed that highest number of JE cases were mostly reported in JEV transmission period that is, monsoon period (July to September) (Figure 3). Higher number of cases during this period may be due to the moderate to high rainfall and cultivation of paddy fields (preferred breeding place of *Culex*). These suitable conditions help the mosquitoes to breed and transmit the disease at higher rate than the rest of the months of a year (Sarkar et al., 2012). However, JE cases were also reported during summer seasons of a year 2004 and 2005 (no data available for year 2001 to 2003 for summer season).

To observe the disease transmission dynamics during summer season (that is, March to May) of the years, it is noticed that higher numbers of JE positive cases were recorded (1.4% in 2004 and 19.3% in 2005). Occurrence of JE cases during summer seasons may be due to high precipitation, suitable temperature for JEV propagation in mosquitoes. In contrast, heavy rainfall (April, 64 mm and May, 113.7 mm) was recorded during summer season of 2005 and coincidentally highest numbers of JE cases were reported (19.3%) during this period. Similar kind of reports was also observed in Eastern China, where JE cases were reported in summer season (Upadhyayula et al., 2012). Higher number of cases may be due to creation of many breeding sources for *Culex* mosquito species in paddy fields and might be responsible for the increased risk of JE cases (Khan et al., 1996).

Sero-epidemiological surveys were also conducted in winter season (that is, November, 2005 to January, 2006) where (7.6%) of JE cases were reported. JE cases during winter season may be due to second phase cultivation period of paddy where there were sufficient numbers of mosquito breeding places. Similar kinds of data were also reported on JE cases during winter season (Sarkar et al., 2012; Khan et al., 1996). Based on these study reports, JE tends to be endemic, and cases occur sporadically throughout the year with a peak after the start of the rainy season. Hence, it is assumed that climate variability has a direct influence on JE cases (Githeko et al., 2000). Temperature (22 to 34°C) and relative humidity (42.7 to 69.6%) are ambient conditions to facilitate the higher mosquito density as well as JE virus replication and occurrence of JE cases (Murty et al., 2010).

In the present study, it is noticed that minimum and maximum temperature were found to be positively correlated with number of JE cases but negative correlation with rainfall and relative humidity (Table 1). It is also noticed that the average rainfall during the study period was 70.5 mm and relative humidity was between (45.6 to 70.1%). Even though, with this low rainfall and low relative humidity, occurrence of many JE cases were noticed, and this may be due to the availability of sufficient numbers of breeding places for mosquitoes and suitable temperature for development and transmission of JE virus.

C. tritaeniorhynchus has also been incriminated as a major vector of JE in India as well as in many countries of Southeast Asia (Murty et al., 2010). In the present study also we have recorded that JEV was mainly transmitted by the *C. tritaeniorhynchus* and *C. gelidus* mosquito species. Their abundance was mainly noticed during the paddy cultivation periods. In Kurnool district, generally two rice crops are grown in a year (from July to November and December to April). As a result, *C. tritaeniorhynchus* showed increase in abundance during September and January, corresponding to rice crop

(Figure 3). Likewise, the vector *C. gelidus* density was also found high in JE transmission period and this vector is normally bred in the stagnated water bodies. Most of the researchers have reported that the vector density is associated with the JE cases but in the present study, the vector density that is, PMH of *C. tritaeniorhynchus* and *C. gelidus* showed negative correlation with number of JE positive cases when compared with the various climatic variables (rainfall and temperature) (Table 2 and Figure 5). Similar type of reports was found where dengue incidence was negatively associated with rainfall (Thammapalo et al., 2005).

The JEV infection rates in female *C. tritaeniorhynchus* and *C. gelidus* mosquitoes varied largely. To understand the transmission dynamics of virus by vectors and their spatial variations within a JE endemic district of Andhra Pradesh, this study was undertaken, covering the whole year. Vector susceptibility in different years showed *C. tritaeniorhynchus* MIR 1.9% with 10.6% JE cases in 2003 and 1.5% with 14% JE cases in 2005. Similarly in 2005, higher MIR was reported in *C. gelidus* (4.2%) and correspondingly 14% of JE cases were reported in the study area. Monthwise data shows that the higher MIR values were reported in the September to November and extended up to December and was least during summer season of year. This suggests that variations in infection during these different seasons are mainly responsible for transmission of JEV to humans. Thus, temporal changes might have greatly impacted the efficiency of arboviral transmission in nature, which would have significant epidemiological importance.

In our study, lower MIR in both *C. tritaeniorhynchus* and *C. gelidus* mosquitoes have been reported, similar results of lower infection rates were also observed by earlier researchers (Samuel et al., 2010). This may be due to the low virus titer and quantity in the blood meal or due to the presence of several anatomical or physiological barriers (Bi et al., 2003). However, it is also suggested that the variations in the number of JE cases and transmission of JEV may be due to human age distribution, type of mosquito's species as well as on the influence of various climatic factors for the growth, development and propagation of both virus and vectors. The climate variables such as temperature, rainfall and relative humidity might have a significant impact on the transmission of the diseases (Upadhyayula et al., 2012). Correlation analysis in this study showed that MIR of both *C. tritaeniorhynchus* and *C. gelidus* mosquito species were positively correlated to percentage of JE positive cases. Similarly, correlation of MIR with climatic factors showed positive relation with rainfall, relative humidity and negative relation with temperature (Table 3). The MIR obtained during the study period 2001 to 2005 showed correlation with the abundance of the vectors which ranged for *C. tritaeniorhynchus* (65 to 490.16 PMH) and *C. gelidus* (6 to 157.33 PMH).

This longitudinal study reveals a steady increase of JE cases from 2001 to 2005, indicating a possible public health threat in the near future. The incidence of JE infection is relatively high during monsoon period and it strongly depends on rainfall, humidity and temperature as well as the paddy cultivation. Effects of climate change on rainfall, temperature and other climatic variables may increase the vector populations and risk of JEV infection, especially in temperate regions like India. Apart from these factors, availability of high mosquito abundance, virus reservoirs, virus infected mosquitoes, development of resistance to effective insecticides, rapid globalization, population explosion and global climate change have also influenced the endemicity of this disease (Karuanaratnae and Hemingway, 2000; Ghosh and Basu, 2009). As of now, no disease surveillance studies have been carried out in this region, and this study is to make an attempt to understand the disease scenario and vector dynamics in relation with weather variables.

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Full Length Research Paper

Women in child-bearing age who are not currently pregnant are missed opportunities for malaria control in pregnancy: Evidence from 16 Local Government Areas of Nigeria

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The health of women is not only linked to reproductive issues but also to efforts in health promotion, improved nutritional status and general well being of girls and adolescents from infancy to adulthood. The aim was to evaluate the use and non-use of long lasting insecticidal nets (LLIN) among women in child-bearing age in Nigeria. This survey employed cross-sectional survey to collect data from households on coverage and use of LLINs. The study took place in 2007, five months after the distribution of LLINs, coinciding with the second raining period of the year and a time of high malaria transmission during the wet season. In the 1,756 households sampled, the ratio of women in child-bearing age to currently pregnant women was 8.2 to 1. The probability of having a woman in reproductive age in the household is 50 times more than having a pregnant woman. Overall, there was a significant difference in the proportion of pregnant women who slept under any net (χ^2 = 23.2; p = 0.000003; odds ratio (OR) = 1.89; confidence interval (CI) = 1.46, 2.46) or under an LLIN (χ^2 = 15.17; p = 0.0001; OR = 1.73; CI = 1.31, 2.28) night before survey compared to women in child-bearing age. A significant proportion of currently pregnant women took IPT1 only compared to WCBA in their last pregnancy (χ^2 = 524.04; p = 0.000000; OR = 35.2; CI = 22.6, 54). Use of LLINs among women in child-bearing age (WCBA) in Nigeria is low. Aggressive approach is needed to increase the utilization of LLIN among WCBA to improve use among pregnant women.

Key words: Women in child-bearing age, currently pregnant women, long lasting insecticidal nets, intermittent preventive treatment, malaria, school children.

INTRODUCTION

In order to set a strong basis for good maternal and infant health, it is necessary for women in child bearing age (WCBA) to engage in or be guided along healthy living. Some of the fundamental elements for a healthy life may include the consumption of healthy diet, drinking adequate clean water, doing moderate but regular exercise and having adequate rest. In addition, WCBA should avoid obesity, use of tobacco, narcotic or hard drugs and consumption of alcohol as well as avoid abusive relationships (Utah Department of Health, 2012). Furthermore, WCBA may also be able to promote their well-being and live healthier and longer lives through regular screening for cancer and other illnesses. These healthy habits in childbearing years can improve birth

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outcomes, support life-long health, and may prevent premature death for women (Utah Department of Health, 2012).

Preventive health activities are important for reducing illness and detecting disease in early, treatable stages. However, policies and guidelines addressing health of teenagers and that of women in child-bearing age are scanty in sub-Saharan Africa (SSA). This may be responsible for the region having the highest rate of teenage pregnancy (Treffers, 2003; United Nations Children's Fund (UNICEF), 2001), mostly due to early marriage, custom and tradition, high level of sexual activity, lack of general sex education and low access to affordable contraceptive option. Specifically in malaria control programs, WCBA, a group that includes teenagers and adolescents, are often not specifically considered as "high-risk group" along with pregnant women, and they should, for obvious reasons.

Looking at literature, there are few quantitative or qualitative studies that have reported the use of malaria control commodities such as long lasting insecticidal nets (LLINs) and artemisinine-based combination therapies (ACTs), among this potential "at-risk" group. Though studies have documented access, ownership, coverage and utilization (Belay and Deressa, 2008; Thawani et al., 2009; Wagbatsoma and Aigbe, 2010), as well as cost implication (Jimoh et al., 2007) and willingness-to-pay (Onwujekwe et al., 2004) for preventive (Menendez et al., 2008) or curative (Onwujekwe et al., 2012) malaria commodities among general population and in reference to pregnant women, hardly has there been a reference to WCBA. Most Malaria Control Programs at country level either focus primarily on pregnant women and neglect WCBA or lump them together under the umbrella of "universal coverage" (Roll Back Malaria, 2012; Federal Ministry of Health, 2010). The symptoms relating to this deficiency may not be unconnected with the perceived low utilization of intermittent preventive treatment (IPT) and LLIN among pregnant women (KEMRI-Wellcome, 2012).

Though WCBA may not be currently pregnant, they could have been pregnant earlier and the pregnancy was either carried to term or aborted. Furthermore, some of them may be currently pregnant but are yet unaware of the pregnancy while some are likely to get pregnant within a month after LLIN has been distributed, thus missing out in ownership and use of the commodity. In some cases, especially in first pregnancy, tradition demands that a woman does not tell others that she is pregnant until the pregnancy shows and by this time she is already infested with malaria parasites. In Nigeria, malaria is responsible for up to 11% of maternal death (Federal Ministry of Health, 2004). The World Health Organization (WHO) Expert Committee on Malaria recommends that intermittent preventive treatment (IPTp) and long lasting insecticidal nets (LLIs) be used to mitigate the effects of malaria in pregnancy (WHO, 2004).

Since malaria in pregnancy increases the risk of maternal anaemia, maternal mortality, abortion, prematurity, intrauterine growth retardation, intrauterine death and low birth weight (Anya, 2004; van Geertruyden et al., 2004; Tako et al., 2005), there is need to protect not only those who are currently pregnant but also all those who have the greatest potential to be pregnant at any time - the WCBA. We sought to examine the pattern of ownership and use of malaria commodities among WCBA in 16 Local Government Areas of Nigeria. Our aim was to evaluate the extent of utilization of LLIN among this potentially "at-risk" group with a view to improve on this, if found inadequate, so as to achieve further reduction in the morbidity as well as mortality associated with the disease among the WCBA specifically and among pregnant women in general.

MATERIALS AND METHODS

This population-based study relating to use of LLINs among WCBA used household data on Nigerian females aged 15 to 49 years who resided in selected 16 Local Government Areas (LGAs) in the 6 zones of the country during the period 2007 through 2008. The study compares these data with those from currently pregnant women. Currently, pregnant women using LLIN were used as the primary referent population because data on them provide more precise rates with which to compare use of LLIN among WCBA.

Study population

We targeted WCBA, (12 to 49 years) who self-identified as Nigerians belonging to and resident in the area of study and not just visiting. These comprise of those in South-west zone who are predominantly of the Yoruba ethnic group whose ancestors occupy the geographic area that is now known as Owo in Ondo State, Ogo-Oluwa in Oyo State, Irepodun/Ifelodun in Ekiti State and both Badagry and Ikorodu in Lagos state. Those that reside in South-east and South-south zones of the country occupy different geographical zones and are respectively of the Ibo, Ijaw, Edo, Ibibio and other ethnic groups. The Ibos in land-locked South-east zone occupy Aninri in Enugu State and Ukwa West in Abia State; the coastal Ijaws in South-south zone are in Brass within Bayelsa State, and Ogu-Bolo in Rivers State; the Aniomas occupy Oshimili North in Delta State and the Binis are in Edo State. The other component of the study population are of Hausa Fulani stock in North-west (Bungudu in Zamfara State) and in North-east (Gulani in Yobe State). The North-central zone (Ilorin in Kwara State) consists of mixed ethnic groupings of Yorubas and Hausa/Fulani stock mainly.

The study design

The study was a cross-sectional multi-stage random cluster-sample survey designed to estimate with acceptable precision selected ITN indicators in areas where integrated LLIN-EPI campaigns were conducted in Nigeria. The populations to be covered by the survey, the universe of all, were the households in the 48 LGAs, children age 0 to 5 years, pregnant women and WCBA (15 to 49 years). In this report, emphasis is laid more on LLIN coverage among WCBA.

Sample size calculation

This has been described elsewhere (Afolabi et al., 2009). Briefly,

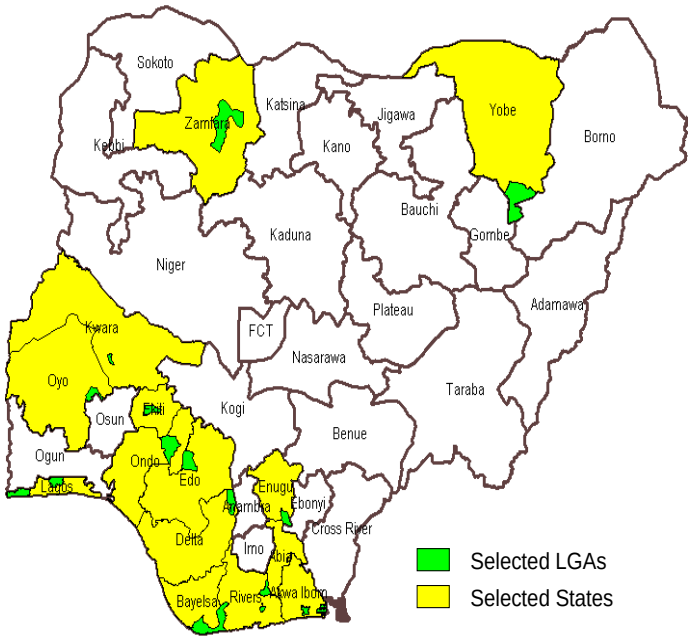

Figure 1. Map of Nigeria showing States and LGAs selected for the survey.

the 2006 population census puts the population size of the 48 LGAs in the study areas at 8,546,280 and the average household size in Nigeria is estimated to be 5 persons (National Population Commission, 2003) translating to about a total of 1,709,256 households in the 48 LGAs. Therefore, to achieve a 3% precision (level of error) with 95% confidence level, assumed proportion of 0.5 and presumed desired change of 20%, a sample of 1,712 households was required for meaningful analysis (the sample size was adjusted to none response rate of 10%). This translated to 107 households per LGA and 10.7 per cluster. Rounding up the cluster size to 11 households therefore meant drawing a minimum sample size of 1,760 (110 households per selected LGA, and 11 per cluster).

Selection of the households

A multi-stage sample design aimed at selecting 1,760 households from 16 LGAs drawn from 24 states was adopted with equal allocation to all the 16 LGAs (that is, 110 households per LGA). The first stage was the selection of 16 LGAs from all the project states. The list of all the 48 LGAs and the states in which they are located was first arranged by geo-political zone. This was to eliminate the concentration of LGAs to be selected in a particular geo-political zone. The 16 LGAs were then selected using systematic sample selection procedure. The second stage involved the selection of enumeration areas (EAs), which for the purpose of this survey were the clusters, from each LGA. To ensure that all EAs in the selected LGAs were given an equal chance of being selected, the frame of all localities and their EAs in the selected LGAs were obtained and 10 clusters systematically selected from each LGA. The third stage was the selection of households from each of the 10 selected clusters. Thrice the number of required households (that is, 33 households) was listed and then 11 households systematically selected. A sketch maps of the selected clusters and households and a brief description of how to reach them was then prepared. Since population wise, the EAs are not equal in size, the household listing continued until the required number of 33 households was obtained. Those who fell outside the age range of 15 to 49 years, those just visiting and hospitalized patients were excluded from the study. Participants whose ages were not ascertained were also excluded.

Data management including analysis

Source of data on the use of LLIN was the household survey carried out in 16 LGAs using a pre-tested questionnaire served to household heads by field-workers who were trained for three days. Where the household head was absent, a proxy was interviewed to complete the data from such household. The questionnaire was pilot-tested at Kuje, FCT, an area not involved in the study and relatively far away from Ilorin, a study site within the same zone. The main independent variables were socio-economic status which included educational status, employment and income level. Primary data were manually entered into questionnaires on the field and double-checked for an error by field supervisors. Where an error, an omission or inappropriate information was detected, the field worker was asked to go back to the household where the error was detected. The data were then collated and entered into IBM compatible desk top computers located within WHO premises, Asokoro, Abuja. EPI-INFO version 6 statistical software was used to perform the analysis. Non-parametric tests were used for determining the significance of associations of variables. The overall prevalence of missing data in this study was less than 5%. Average (Av.) number of WCBA or of PW per HH was calculated by the simple formula:

$$\text{Average} = \frac{No.\ of\ WCBA\ (or\ PW)}{No.\ of\ households\ surveyed}$$

Where WCBA = Women in child-bearing age and PW = pregnant women.

Ethical review

The protocol for this survey was well-scrutinized before being approved by the National Malaria and Vector Control review board. Written or verbal informed consent was received from all participating households.

RESULTS

This study was conducted in 16 LGAs within 14 states of the 6 geo-political zones of Nigeria (Figure 1). In all, 1,756 households were surveyed (Table 1) in which there was an average of 0.17 currently pregnant woman (CPW) per household, compared to 1.4 WCBA per household, indicating that, for every 1 CPW there was 8.2 WCBA who have the potential to be pregnant at any time, if they are not yet obviously pregnant. There were 0.18 CPW to 1.4 WCBA in South-East zone, 0.19 CPW to 1.3 WCBA in South-south, 0.13 PW to 1.3 WCBA in South-West, 0.27 PW to 1.7 WCBA in North-West, 0.08 PW to 1.5 WCBA in North-Central and 0.18 PW to 1.4 WCBA in North-East zones, respectively. The proportion of WCBA, compared to CPW, was significantly higher in South-West ($\chi^2 = 4.80$; $p = 0.03$; OR = 1.37; CI = 1.03, 1.81) and North-Central ($\chi^2 = 6.37$; $p = 0.01$; OR = 2.35; CI = 1.19, 4.64) than in other zones. The proportion of WCBA in surveyed HH was considerably higher than that of

Table 1. Distribution of currently pregnant women (CPW) and women in child-bearing age (WCBA) in households of survey (2007).

Parameter	Total HH	No. of currently PW				No. of Women in child-bearing age					
		0	1	2	Total	0	1	2	3	4	Total
State-LGA					**South-East zone**						
Abia-Ukwa West	110	92	18	0	18	2	76	21	7	4	155
Enugu-Aninri	110	89	21	0	21	8	75	11	9	7	152
Total	220	181	39	0	39*	10	151	32	16	11	307*
*χ^2 = 0.03; p = 0.87; OR = 0.97; CI = 0.68, 1.39											
					South-South zone						
Akwa Ibom-Eket	110	86	24	0	24	2	95	10	2	1	125
Akwa Ibom-Mbo	110	89	18	3	24	20	30	21	18	21	210
Bayelsa-Brass	110	94	16	0	16	20	52	25	9	4	145
Delta-Oshimili North	110	98	12	0	12	27	71	8	4	0	99
Edo-Owan West	111	90	20	1	22	6	83	15	7	0	134
Rivers-Ogu Bolo	107	81	25	1	27	10	66	21	6	4	142
Total	658	538	115	5	125*	85	397	100	46	30	855*
*χ^2 = 3.08; p = 0.08; OR = 0.80; CI = 0.63, 1.03											
					South-West zone						
Ekiti-Irepodun/Ifelodun	109	94	13	2	17	15	57	17	10	10	161
Lagos-Badagry	110	95	14	1	16	0	93	13	2	2	133
Lagos-Ikorodu	110	97	13	0	13	1	79	24	4	2	147
Ondo-Owo	110	105	5	0	5	10	82	11	5	2	127
Oyo-Ogo Oluwa	110	89	21	0	21	8	67	23	5	7	156
Total	549	480	66	3	72*	34	378	88	26	23	724*
*χ^2 = 4.80; p = 0.03; OR = 1.37; CI = 1.03, 1.81											
					North-West zone						
Zamfara-Bungudu	110	84	22	4	30*	1	49	47	9	4	186*
*χ^2 = 1.98; p = 0.16; OR = 0.75; CI = 0.50, 1.12											
					North-Central zone						
Kwara-Ilorin West	110	102	7	1	9*	8	65	19	11	7	164*
χ^2 = 6.37; p = 0.01; OR = 2.35; CI = 1.19, 4.64											
					North-East zone						
Yobe-Gulani	109	90	18	1	20*	18	45	30	14	2	155*
χ^2 = 0.03; p = 0.86; OR = 0.96; CI = 0.59, 1.55											
Total	1756	1475	267	14	295	156	1085	316	120	17	2384

In all for every PW (pregnant women) there are 8.2 WRA.

CPW and consequently, having a WCBA in a HH is about 50 times more likely than having a CPW (χ^2 = 1949.45, p = 0.0000, OR = 49.32, CI = 40.21, 60.50) (Table 2).

Use of LLINs

Eight hundred and ninety one (51%) of households surveyed had any net while 738 (42%) had insecticide treated nets in the form of LLINs (Figure 2). The highest percentage of the ownership and assumed usage of “any net” (88%) and of LLIN (84%) was in Owo LGA in Ondo state, South-west Nigeria. Ilorin West LGA in Kwara state recorded the lowest “any net” (17%) and the lowest ITN/LLIN (6%) use. In general, LLINs were found more in

Table 2. Percent distribution of currently pregnant women (CPW) and women in child-bearing age (WCBA) in households of survey (2007).

Parameter	Frequency (%)	
	Women in child-bearing age	Women in child-bearing age
Household with	1598 (90.8)	295 (16.8)
Household without	162 (9.2)	1475 (83.2)
Total	1760	1760

$\chi^2 = 1949.45$; p = 0.0000; OR = 49.32; CI = 40.21, 60.50.

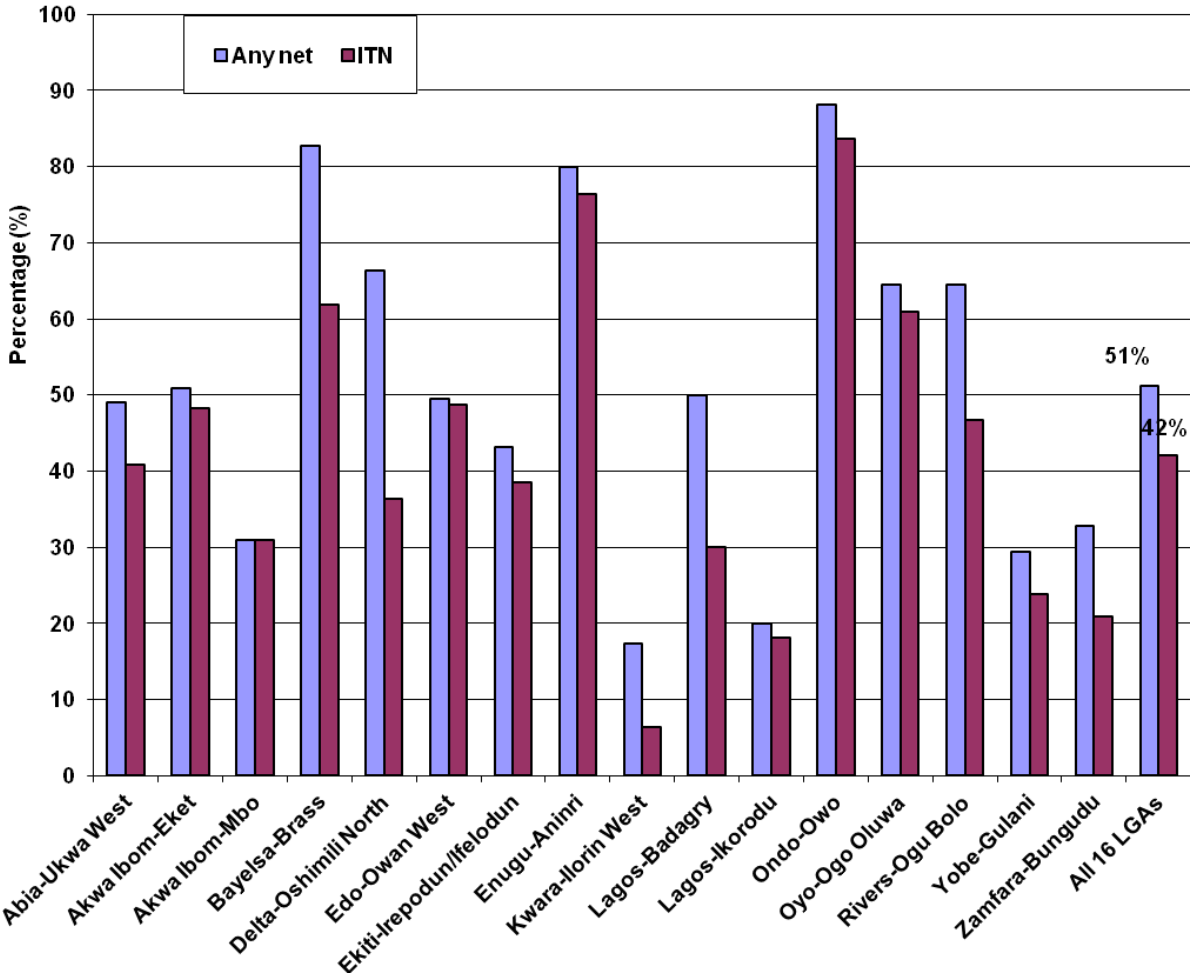

Figure 2. Percentage of households with any mosquito net and ITN by LGA.

southern states of the country than in the northern states. Figure 3 illustrates the percentage of households with at least 1 mosquito net of any type by wealth quintile. The ownership of LLIN ranged from 53% among the poorest to 56% among the least poor. Almost equal percentage of households had any net hanging (69%) and LLIN (68%) hanging during the survey. Ikorodu LGA in Lagos state had the lowest percentage of "any net" and of LLIN hanging while Owo in Ondo state and Gulani in Yobe state had the highest percentage (91%) of "any net" and of LLIN hanging (Figure 4). The percentage use of LLINs by CPW and by WCBA in different zones of the country was staggered. For example, 36% of CPW and 23% of WCBA slept under LLIN the night before survey in South-east zone, 0% CPW and 12% WCBA did so in North-Central zone. Overall, there was a significant difference in the proportion of pregnant women who slept under any net ($\chi^2 = 23.2$; p = 0.000003; OR = 1.89; CI = 1.46, 2.46) or under an LLIN ($\chi^2 = 15.17$; p = 0.0001; OR = 1.73; CI = 1.31, 2.28) night before survey compared to WCBA.

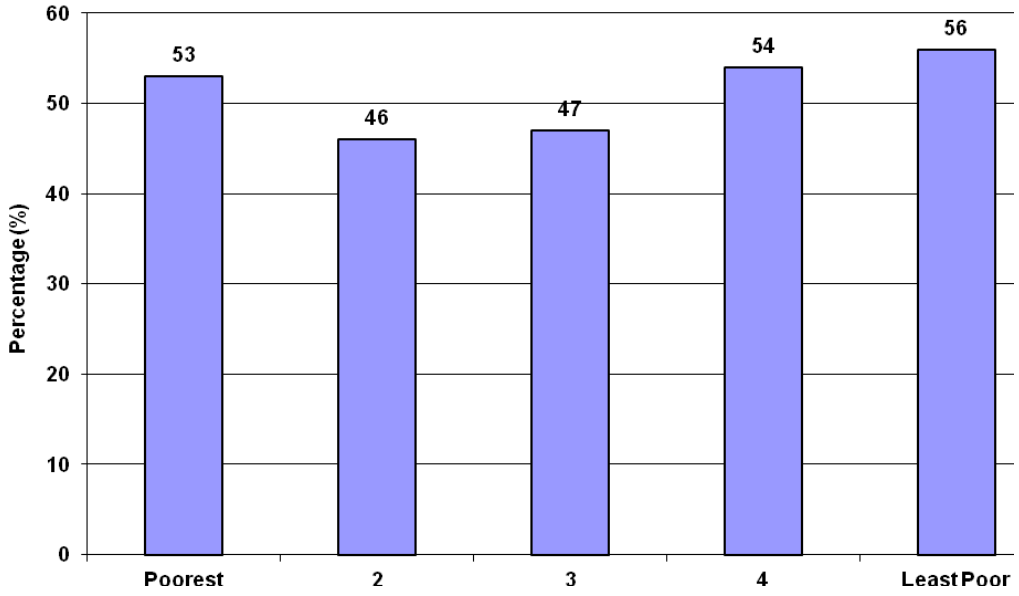

Figure 3. Percentage of households with at least 1 mosquito net of any type by wealth quintile.

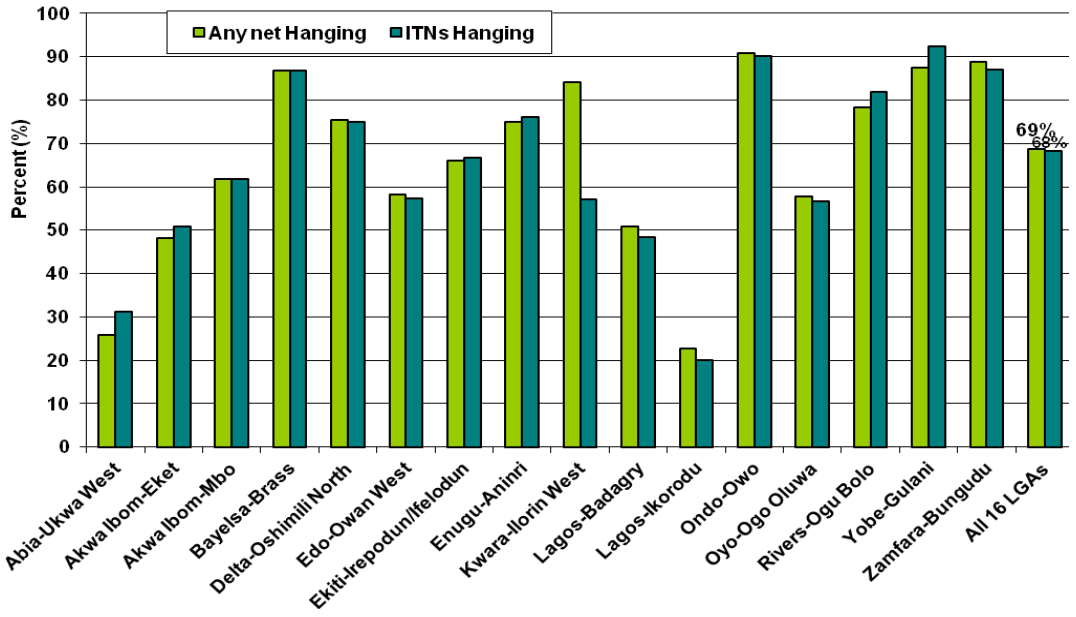

Figure 4. Percentage of households with mosquito nets of any type that have any net hanging and percentage of households with ITNs that have ITNs hanging.

Table 3 also shows that CPW are about twice as likely to sleep under any net or under an LLIN than WCBA.

Use of IPT in current and in past pregnancy

Data was collected on use of sulphadoxine-pyrimethamine (SP) as intermittent preventive treatment (IPT) of malaria during present or immediate past pregnancy. Results from this study show that, overall, a significant proportion of CPW took IPT1 only, compared to the last pregnancy of WCBA (χ^2 = 524.04; p =0.000000; OR = 35.2; CI = 22.6, 54) and that CPW were 35 times more likely to take at least IPT1 compared to WCBA. Furthermore, CPW were about thrice more likely to take IPT1 and IPT2 (χ^2 = 7.85; p = 0.005; OR = 2.56; CI = 1.29, 5.05) than WCBA in their last pregnancy (Table 4). Responses from CPW and WCBA also showed

Table 3. Proportion of currently pregnant women (CPW) and women in child-bearing age (WCBA) who slept under any net or under Long Lasting Insecticidal Nets (LLINs) in households (HH) within various Local Government Areas (LGAs) night before survey (2007).

Parameter	CPW				WCBA		
	Total HH	Total	Frequency (%)		Total	Frequency (%)	
			Slept under any net	Slept under LLIN		Slept under any net	Slept under LLIN
State-LGA				**South-East zone**			
Abia-Ukwa West	110	18	3(16.7)	3(16.7)	155	7 (4.5)	6 (3.9)
Enugu-Aninri	110	21	11(52.4)	11(52.4)	152	63(41.4)	63 (41.4)
Total	220	39	14(35.9)!	14(35.9)*	307	70 (22.8)!	69 (22.5)*
! χ^2 =3.22; p=0.07; OR=1.90; CI=0.94, 3.84					*χ^2=3.4; p=0.06; OR=1.93; CI= 0.95, 3.92		
				South-South zone			
Akwa Ibom-Eket	110	24	6 (25.0)	6 (25.0)	125	14 (11.2)	14 (11.2)
Akwa Ibom-Mbo	110	24	7 (29.2)	7 (29.2)	210	22 (10.5)	18 (8.6)
Bayelsa-Brass	110	16	9 (56.2)	6 (37.5)	145	79 (54.5)	63 (43.4)
Delta-Oshimili North	110	12	8 (66.7)	2 (16.7)	99	38 (38.4)	22 (22.2)
Edo-Owan West	111	22	6 (27.3)	6 (27.3)	134	17 (12.7)	16 (11.9)
Rivers-Ogu Bolo	107	27	15 (55.6)	12 (44.4)	142	53 (37.3)	50 (35.2)
Total	658	125	51 (40.8)!	39 (31.2)*	855	223 (26.1)!	183 (21.4)*
!χ^2 =11.7; p=0.0006; OR=1.95; CI=1.33, 2.88;					*χ^2=5.97; p=0.01; OR=1.67; C =1.10, 2.51		
				South-West zone			
Ekiti-Irepodun/Ifelodun	109	17	3 (17.6)	3 (17.6)	161	13 (8.1)	13 (8.1)
Lagos-Badagry	110	16	3 (18.8)	2 (12.5)	133	26 (19.5)	13 (9.8)
Lagos-Ikorodu	110	13	0 (0.0)	0 (0.0)	147	5 (3.4)	5 (3.4)
Ondo-Owo	110	5	5 (100.0)	5 (100.0)	127	52 (40.9)	49 (38.6)
Oyo-Ogo Oluwa	110	21	7 (33.3)	7 (33.3)	156	28 (17.9)	27 (17.3)
Total	549	72	18 (25.0)!	17 (23.6)*	724	124 (17.1)!	107 (14.8)*
!χ^2 = 2.77; p = 0.09; OR =1.61; CI = 0.91, 2.84					*χ^2 = 6.26; p = 0.01; OR =2.07; CI = 1.16, 3.70		
				North-West zone			
Zamfara-Bungudu	110	30	8 (26.7)!	4 (13.3)*	186	35 (18.8)!	23 (12.4)*
!χ^2 = 0.99; p = 0.32; OR =1.57; CI = 0.65, 3.82					*χ^2 = 0.02; p = 0.88; OR = 1.09; CI = 0.35, 3.41		
				North-Central zone			
Kwara-Ilorin West	110	9	0 (0.0)!	0 (0.0)*	164	20 (12.2)!	19 (11.6)*
χ^2 = not valid							

Table 3. Contd.

North-East zone							
Yobe-Gulani	109	20	7 (35)!	6 (30.0)*	155	26 (16.8)!	23 (14.8)*
!χ^2 = 3.82; p = 0.05; OR =2.67; CI = 0.97, 7.34					χ^2 = 2.93; p = 0.09; OR = 2.46; CI = 0.86, 7.06		
Overall	1756	295	98 (33.2)!	80 (27.1)*	2391	498 (20.8)!	424 (17.7)*
!χ^2=23.2; p=0.000003; OR =1.89; CI = 1.46, 2.46					χ^2=15.17; p=0.0001; OR = 1.73; CI = 1.31, 2.28		

Table 4. Results of χ^2 analysis of currently pregnant women who took IPT and other medications and women in child-bearing age who took IPT and other medications in their last pregnancy.

Zone	Currently pregnant women				WCBA-last pregnancy			
	n	Frequency (%)			n	Frequency (%)		
		Took IPTI only	Took IPTI+IPT2	Took other medications		Took IPTI only	Took IPTI+IPT2	Took other medications
SE	39	31 (79.5)	2 (5.1)	24 (61.5)	307	1 (0.3)	7 (2.3)	136 (44.3)
SS	125	43 (34.4)	3 (2.4)	35 (28.0)	855	13 (1.5)	89 (10.4)	271 (31.7)
SW	72	6 (8.3)	4 (5.6)	32 (44.4)	724	9 (1.2)	57 (7.9)	331 (45.7)
NW	30	7 (23.3)	0 (0.0)	8 (26.7)	186	2 (1.1)	4 (2.2)	26 (14.0)
NC	9	2 (22.2)	0 (0.0)	2 (22.2)	164	2 (1.2)	13 (7.9)	98 (59.8)
NE	20	2 (10.0)	0 (0.0)	2 (10.0)	155	2 (1.3)	8 (5.2)	22 (14.2)
Total	295	89 (30.2)*	9 (3.1)**	103 (34.9)***	2391	29 (1.2)*	178 (7.4)**	884 (37.0)***

*χ^2=524.04; p=0.000000; OR =35.2; CI = 22.6, 54.8, **χ^2=7.85; p=0.005; OR =2.56; CI = 1.29, 5.05 and ***χ^2=0.48; p=0.49; OR =1.09; CI = 0.85, 1.41

that about 35% of CPW and 37% of WCBA took other medications during their pregnancy, though the difference did not reach a level of significance.

DISCUSSION

This study, the first to provide a detailed description of use of LLIN and IPT among women in child-bearing age in Nigeria, reveals several notable characteristics and determinants of malaria control among this group of people in the population. Firstly, women in child-bearing age far out-number pregnant women in the households. This alone puts them in a condition whereby they can get pregnant at any time and therefore are potentially an “at risk” group in malaria endemic areas of the country. Secondly, though a large number of WCBA are present in households, very few of them slept under protective measures such as LLIN when compared to currently pregnant women. From all indications, African women have restricted access to fiscal and societal powers which contributes to their attaining inferior levels of education and lack of autonomy (Birn et al., 2009).

Almost one-fifth of illnesses and deaths among WCBA in developing countries are pregnancy related and at least one woman dies every minute (529,000/year) due to maternal causes such as primary hemorrhage, infection, eclampsia (seizures), obstructed labor, complications from abortion and ectopic pregnancy (WHO, 2005).

These illnesses could be prevented or at least reduced by providing needed support against infection to WCBA. Women in child-bearing age need strength before they become pregnant especially younger ones who may be experiencing their first pregnancy. The provision of LLIN is a sure means to make certain that WCBA do not enter their pregnancy already in anaemic state (Brieger, 2012).

Malaria in pregnancy is a major public health concern in Nigeria and other sub-Saharan African countries as it has many deleterious effects on both the mother and the fetus underscoring the significance of making available to this group of people adequate and "effective protection and case management" (Crawley et al., 2007). There are multiple barriers to women in child-bearing age, especially the poorest who are likely to have malaria (Somi et al., 2007), accessing formal health care such as perceived quality of care (Litvack and Bodart, 1993), lack of knowledge as well as distance from health services (Mwenesi et al., 1995) transport cost and treatment cost (Nyamongo, 2002; Onwujekwe et al., 2008).

Women in child-bearing age are not specifically targeted by information, education and communication (IEC) or behavioral change communication (BCC) of malaria control programs. Most of the previous reports on malaria morbidity and mortality have not considered non-pregnant WCBA as a distinct group but rather have included them as a heterogeneous group represented as teenagers or adolescents. This might be due to the fact that this is a diverse group whose age ranges from 12 to 49. For precisely this reason, malaria control programs should identify and target, initially, the younger WCBA, especially students. This may involve collaborating with line-ministries such as Education, Youth, Sports and Social Developments. Younger WCBA can be reached at schools, during sporting events, at youth meetings, at musicals and through specific radio and television programs.

By focusing on WCBA population, we have undertaken an initial step towards clarifying the minimal utilization of LLINs and neglect of this group of women in malaria control programs. For example, only 21% of WCBA compared with 33% of currently pregnant women slept under any net and only 18% of WCBA compared with 27% of pregnant women slept under an LLIN night before survey. To increase the percentage of pregnant women that have access to and who utilize malaria control commodities, it is imperative to "catch them young" at least at the earliest stage of becoming a woman or better still at the primary school level. Understanding the dynamics of use of LLIN by WCBA is essential for developing effective prevention and intervention strategies to reduce the burden of malaria not only in this population but also among pregnant women and in the society at large.

This study has begun the process of identifying the characteristics of WCBA who are at risk of malaria when not pregnant and are at higher risk of the disease when pregnant. Notably, not being fully protected while not pregnant might constitute an elevated risk factor for WCBA during pregnancy. This elevated risk may be associated not only with pyrexia in pregnancy but also with maternal conditions such as placenta praevia, pre- and/or post-partum haemorrhages, abortion and anaemia. Congenital malformation (Patel and Adhia, 2005), intra-uterine growth retardation (Allen et al., 1998), miscarriage (Friedrich, 2012) and still birth (Bader et al., 2012.) are possible effects on the fetus while low birth weight (Kabanywanyi et al., 2008) and death in the first year of life due to immature lungs are possible effect on the baby. Health facilities should be organized to provide Integrated Management of Women's Illnesses (IMWI), not only for screening of malaria among WCBA or during pregnancy but also for communicable and non-communicable diseases such as cervical and breast cancers, hypertension, diabetes, pregnancy-related illnesses, reproductive health, toxoplamosis, HIV and even mental health.

Malaria control programs should focus on distributing LLINs to primary school pupils who are likely to influence their parents, siblings, peers not at school and the entire community to sleep under an LLIN. Furthermore, strategies such as focused antenatal care (FANC), IEC and behavioral change communication (BCC) should also specifically target WCBA with appropriate health messages regarding sleeping under LLINs. In this way, use of LLIN in pregnancy will increase and maternal and child morbidity and mortality will decrease in accordance to the expectations of the Millennium Development Goals (MDGs).

Limitations

Several limitations of this study should be noted. One is selection bias, due to the nature of the survey. Thorough screening prior to sample selection would have minimized selection bias and ensure group homogeneity. Some of the women in child-bearing age could not produce authentic evidence of their ages. Another limitation is attrition, and this was minimized where possible. Thirdly, we did not ask the WCBA specifically of other medications used during their last pregnancy.

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