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MOLECULAR STUDY OF NUCLEPROTEIN GENE OF RABIES VIRUS ISOLATES FROM DOG BRAINS.

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Rabies is an endemic disease in Nigeria and it produces disease in warm-blooded animal species. In this report, molecular techniques have been used to study seven rabies isolates from the brain of Dogs in Lagos. Analysis of the 1400-b.p RT-PCR products of the N gene and the views by sequencing and restriction endonuclease analysis enabled division of isolates into 3 types. The conclusion from the study is that RT-PCR and restriction endonuclease analysis of the amplified products of the N gene would allow identification and differentiation of rabies virus strains in a location and in different parts of Nigeria.

INTRODUCTION.

Rabies is an endemic disease in Nigeria (1). There had been an under reporting of the disease in the country despite the awareness of its danger and its toll on human lives (2). In the absence of protective measures, infection with rabies virus causes severe neurological disease and death (3). The fluorescent antibody test (FAT) forms the basis for routine rabies diagnosis and together with monoclonal antibody techniques they are used for the characterization of rabies viruses. In addition, viral isolation either by newborn mouse intra cerebral inoculation test or by the rabies tissue culture infection test is also performed to allow further analysis of the etiological agent.

The recent application of molecular techniques to explore the rabies genome has permitted substantial progress in the understanding of the molecular structure and mechanism of virus (4,5). The rabies virus is known to consist of a negative strand RNA genome contained in a tight neucleocapsid composed of the nucleoprotein (N), which is enclosed by a bullet shaped outer capsid made up of a glycoprotein (G) and a membrane protein (M2) (6). The virus genome encodes a further two proteins L (the polymerase) and M1 (the phosphoprotein), both of which occur in small quantities in the virus particle where they are associated with the N protein (7,8). Detailed study of the N protein, G, L, and M proteins of rabies virus genome have been used to elucidate the nature, characteristic, phylogenetic, analysis and the epidemiology of rabies (9,10,11,12,13,).

The N gene, the major component of the internal nucleocapsid, is involved In the regulation and replication of the virus (14). It has been used to study antigenic diversity amongst rabies viruses (4) and to study the molecular basis of the antigenicity and cross protection among serotypes (15,16,17,18).

In this paper, molecular techniques such as RT-PCR, direct sequencing and restriction endonuclease analysis have been used to study the N gene of rabies Virus in isolates from brain sample of nine dogs in Lagos Nigeria. These studies also made N gene a potentially useful gene for diagnosis and epidemiological studies (12).

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MATERIALS AND METHODS

Sources of viral brain sample:

Seven brain samples collected from dogs diagnosed to be positive for rabies at Nigerian institute of medical research Lagos, Nigeria were stored at 80 C until used. The brain of two dogs diagnosed as positive for rabies were also collected from government veterinary centre in Lagos. The sample has been stored for two years before amplification of the virus.

Oligonucleotide primers:

The oligonucleotide primer rabN1 and rabN5 (10) (Table2) were used successfully to amplify the nucleoprotein (N) gene for all the nine specimens. The primers used for nucleotide sequencing were shown I table 2.

RNA extraction:

Total RNA was extracted directly from rabid brain tissue using TRIzol method which is an acid phenol / guanidium method described by Kamollvarin and his group (19) thus: in a microfuge tube, o.1gm of brain tissue dissolved in 1ml of TRIzol reagent by vigorous vortexing after which 0.2ml chloroform was added. After further vortexing for 2 to 3 minutes, tubes were spun in a microfuge centrifuge (1 x 12000g) at 4 C for 15 minutes. The upper aqueous phase was removed to a fresh tube and RNA was precipitated by the addition of 0.6ml isopropyl alcohol at room temperature for 10 minutes. RNA was pelleted by centrifugation as above for 15 minutes. The pellet was washed in 75% ethanol and dried.

Subsequently the dried RNA pellet was dissolved in diethylpyrocarionate (DEPC) treated sterile water.

RT-PCR:

FOR N gene PCR, primers RabN1 and RabN5 were used. Synthesis of cDNA was primed from 2ug of RNA using 25pmol of the positive sense primer RabN1 in a 20ul reaction, containing 50mM Tris HCL pH 8.3,75mM KCL, 3mM MgCL2 10mM DTT, dNTPs at 1mM each and 200 units Moloney murine leukaemia virus reverse transcriptases (life technologies). After incubation at 37 C for 2 hours, the reaction was inactivated by heating to 90 C for 5mins, cooled on ice and then centrifuged to collect the condensate. This cDNA product was later used as the template for PCR.

In a 200ul microfuge tube containing 14.25ul of water, the following were added: 5ul of 10 x PCR buffer (22.5mM MgCL2, 500mM Tris- HCL pH 9.2, 160mM (NH4) SO4), 5ul of cDNA sample and 0.75ul of expand enzyme (Bohringer Manheim). Also added to the above mixture were the following: 1.25ul of 10mM dNTPs, 1.25ul of 10um 5' end primer (negative sense) and 22.5ul of water. The DNA amplification was carried out in led lid Perkin Elmer thermocycler. For the N gene PCR, the temperature profile was 93 C for 10 secs, 48°C for 30 secs and 68°C for mins, then 68°C for 5 mins. PCR products were electrophoresed through 1% agarose gels ethidium bromide and visualised with UV illumination.

Direct DNA sequencing:

Direct DNA sequencing of PCR products was performed by the dideoxynucleotide chain termination method using an fmol DNA sequencing kit and 32P-labelled N gene directed internal primers according to the manufacturer's specifications (Promega). DNA sequencing comparison was performed using IBI-Pustell software.

Restriction Endonucleases of PCR Products.

PCR products were purified using the Wizard PCR clean up method (Promega) prior to analysis by restriction endonuclease digestion, performed by standard procedures (20).

Aliquots of 3ul of each amplification mixture were analysed after digestion by suitable restriction enzymes on 1% agarose gel containing ethidium bromide. The following enzymes were used for the experiment: BstbN1, Hind 3, Accl, Sca1, Eco1091, EcoR1, Dde1.

RESULTS

RNA Extraction

In the RNA extraction the concentration obtained ranged from 0.4ug/ml to 2.4ug/ml. The isolates obtained for the RT-PCR amplification were found to vary in the level purity. This was from 90% of sample 3 to 98% of sample 9 (Table 1). The RNA obtained from the rabies isolates was subjected to RT-PCR process and the DNA generated was amplified.

Analysis of Rabies Isolates PCR Products:

Of the 9 dog brain samples that were analysed, seven samples 3,4,5,6,7,8,9, that were positive for rabies by fluorescent antibody test (FAT) were positive by PCR technique. They all showed a single band at the expected site of 1,400 base pairs, when analysed in agarose gel electrophoresis. (Fig 1).

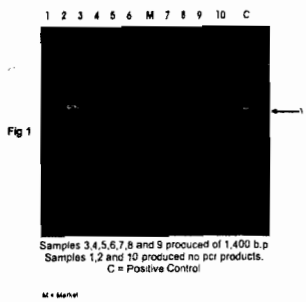

Fig 1

Samples 3,4,5,6,7,8 and 9 procuced of 1,400 b.p
Samples 1,2 and 10 produced no pcr products.
C = Positive Control

M = Marker

Genome Sequencing Determination and Analysis of the PCR Products:

Initially, partial sequencing of the PCR products of the brain samples 3,4,5,7,8 and 9 was performed using sequencing primers Nseq. 0 to Nseq. 5 and using Nseq.1 reverse primer. Both ends of the DNA were sequenced and the Nseq. 1 reverse primer was found common to the PCR products sequenced. The nucleotide sequence obtained showed that there were three distinct types of rabies virus among the samples. They were classified according to their similarities in nucleotide sequences as follows: samples 3,6,7 and 9 formed type 1; samples 4 and 5 formed type 2 and sample 8 formed type 3, though it appeared to be related to sample 4 and 5, but it was sufficiently distinct to be classified separately. One sample from each group was selected for N gene open reading frame sequencing using the additional primers detailed in Table 2. The samples selected were sample 3 for

type 1, sample 5 for type 2 and sample 8 for type 3.

In comparative analysis of sample 3 using IBI-Pustell software, samples 5 and 8 exhibited 86.3% and 85% sequence similarly respectively. However, sequence of samples 5 and 8 were 94.1% similar to each other.

The nucleotide sequences for these isolates are compared in Fig 2; positions of useful restriction endonuclease sites are also indicated.

Restricted Endonuclease Analysis:

From the nucleotide sequence analysis of each isolate using the IBI-Pustell software, a detailed restriction endonuclease site maps were determined for each sequence (Fig 2). The PCR products of the isolates were digested and the result of the enzyme digest is shown in Table 3. Samples 3,6,7 and 9 were sensitive to the following enzymes Hind 3, and Bstb1 by which they were cleaved into 2 bands of the same size. This indicated the samples to be of the same type. Samples 3,7, and 9 were grouped as type 1 by nucleotide sequencing.

Sample 4,5, and 8 were sensitive to the following enzymes Acc1, Sal 1, Sca1, and Eco01091 by which they were cleaved into 2 fragments of the same size, indicating this group of samples to be of type 2. However, samples 4 and 5 were sensitive to EcoR1 also, thereby distinguishing the two samples from sample 8 and this made sample 8 a unique type. Sample 8 is therefore designated as type 3.

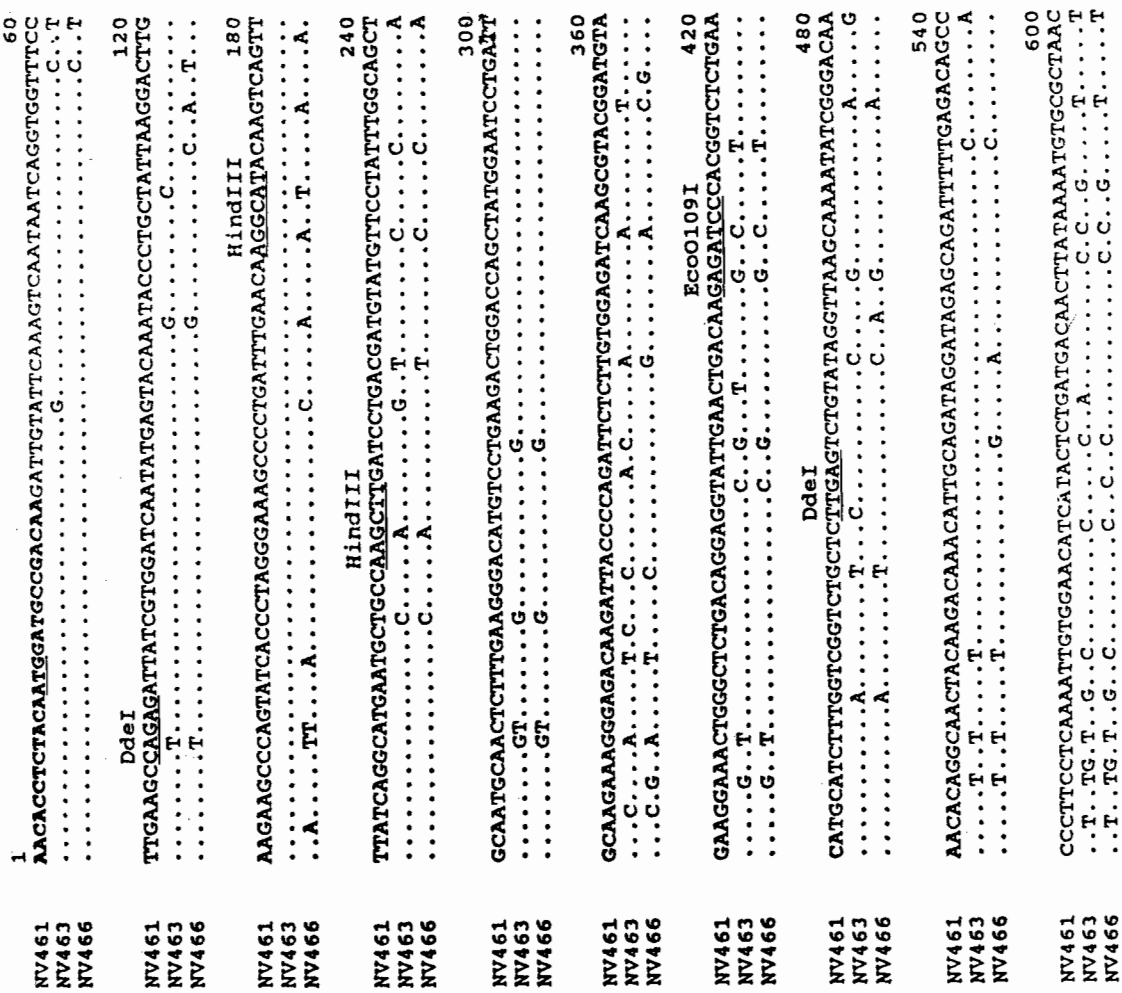

Figure 2. Comparison of the N gene nucleotide sequence of three representative rabies strains from Nigeria.

SAMPLE	OD 260	OD2 60/ 280	CONC. Ug/ml 1:500 dil.	STOCK CONC. Ug/ul	% PURITY	VOLUME OF RNA USED.u
1	0.024	1.9	1.1	0.55	94	4
2	0.011	1.89	0.5	0.25	94	6
3	0.019	1.82	0.8	0.4	90	5
4	0.059	1.83	2.4	1.2	91	1.8
5	0.038	1.92	1.6	0.8	95	2.5
6	0.036	1.95	1.5	0.75	97	2.7
7	0.044	1.95	2.0	1.0	97	2.0
8	0.042	1.88	1.8	0.9	93	2.3
9	0.019	1.96	0.8	0.4	98	5.0
10	0.010	1.90	0.4	0.2	94	6.0

Table 1
CONCENTRATION OF THE RNA OBTAINED FROM BRAIN TISSUES OF NIGERIAN ISOLATES AND THE VOLUME USED FOR PCR AMPLIFICATION

TABLE 2

PRIMERS USED FOR RT-PCR AND NUCLEOTIDE SEQUENCING

PRIMERS	NUCLEOTIDE SEQUENCE	POSITION/SENSE	
[illegible] RT Primer	[illegible]TACAATGGATG[illegible] 3'	1-36	-
[illegible] cDNA PRIMER	[illegible]GATTGA[illegible]AAGATCTTGCTCAT 3'	[illegible]14-[illegible]36	-
SEQUENCING PRIMERS			
Nseq	[illegible]TACAA[illegible]TACCT 3'	1-24	+
Nseq 1 REVERSE.	5'GACTTGTATGCCTTGTTCAAATTCGG 3'	152-176	-
Nseq 1	[illegible]TTGAACAAGGCATACAAGTC 3'	151-176	+
Nseq 2	[illegible]AAAGGAGATAA[illegible]ATCACCC 3'	301-325	+
Nseq 3	[illegible]ATATCTGGCAAAACACCGG 3'	464-488	+
Nseq 4	[illegible]AACT[illegible]GA[illegible]ACTATACC 3'	590-614	+
Nseq 5	[illegible]AAATCTCACTGCAAGGGAAGC 3'	754-779	+
Nseq 6	[illegible]TTGAGTGGGAAGT[illegible]CTT 3'	886-909	+
Nseq 7	[illegible]CAGTTTTTT[illegible]GGAAAGGGAA 3'	1048-1072	+
Nseq 8	5'CTACTTCTCCGGGGAAACCAGAAG 3'	1111-1214	+
Nseq 9	[illegible]AAGAGTTATTGCTACATGTG 3'	989-1012	+
Nseq 10	5'(AG)ATAGAGCAGATTTT([illegible])GAGA[illegible] 3'	[illegible]	+
Nseq 11	5'AAG[illegible]GA[illegible]GTGG[illegible]CAGA[illegible] 3'	[illegible]	+

The base position of each Primer refers to equivalent sequence of the PV strain rabies genome (Tordo et. al. 1986). Primers RabN 1 and RabN 5 were used for reverse transcription and amplification respectively, while Primers Nseq 0 to Nseq 11 were used for sequencing.

TYPE	EB	Hd	Ac	Sl	Sc	Ec	ER	Dd*
=3	-	-	-	-	-	-	-	- * P4 & P6
=4	-	-	-	-	-	-	-	+ * P1, P2, P3 & P5
=5	-	-	-	-	-	-	-	- * P1, P2, P3 & P5
=6	-	-	-	-	-	-	-	- * P4 & P6
=7	-	-	-	-	-	-	-	- * P4 & P6
=8	-	-	+	-	-	-	-	+ * P1, P3 & P5
=9	-	-	-	-	-	-	-	- * P4 & P6

The restriction endonucleases used in this study are represented as follows: EB,BstEl; Hd,Hind3; Ac,Accl; Sl,Sall; Sc,Scal; Ec,Eco0109I; ER,EcoRI; Dd,DdeI.
The positive sign + indicates the cleavage of the sample.
*DdeI enzyme has 6 cutting sites in the PCR products of these isolates with the positions designated as P1, P2, P3, P4, P5 & P6. For each isolate the position of cutting is shown in brackets.

TABLE 3.

SUMMARY OF RESTRICTION ENZYME ANALYSIS PROFILES OBSERVED FOR NIGERIAN RABIES ISOLATES IN THIS STUDY.

```
                 ScaI                                                  660
NV461    TGGAGCACCATACCGAACTTCCGATTCTTGGCCGGAACATACGACATGTATTTCTCCGG
NV463    .....T..T...........A....T..........C.........T......A...
NV466    .....T..T...........A....T..........C..T.......T......A...

                                                                       720
NV461    ATTGAACATCTATATTCAGCCATCAGAGTGGGCACAGTAGTCACTGCTTATGAAGATTGC
NV463    .....G.....G.....G..A..............G......GTCA.....T.T..C...
NV466    ..C..G.....G.....G..A..................T.....C...........C...

         DdeI                                                          780
NV461    TCTGGGCTGGTGTCGTTCACAGGGTTCATAAAACAGCAAAATCTCACTGCAAGGGAAGCA
NV463    ..A..A.....A.....T.............G...ATC........C......A.....
NV466    ..A..A.....A.....T.............G...ATC........C.............

                                                                       840
NV461    ATACTATATTTCTTCCAGAACAACTTCGAGGAAGAGATAAGAAGAATGTCCGAGCCAGGG
NV463    ................C..G.....G.....................T..........
NV466    ................C..AG.G..T.....................TT.........

                                                              DdeI  900
NV461    CAGGAGACTGCGGTTCCTCACTCCTATTTCATTCACTTCCGCTCGTTGGGTCTGAGCGGG
NV463    ..A.....A..T..........T........C........T..AC.A..CT....T...
NV466    ..A.....A..T..........T..................T..AC.A..CT....T...

                                                                       960
NV461    AAATCCCCGTATTCCTCAAATGCAGTAGGTCATGTATTCAATCTCATTCACTTTGTTGGA
NV463    ..G..T..T.....A..G.....T..C........G.........................
NV466    ..G..T..T.....A........C..C........G.........................

                                                                      1020
NV461    TGTTATATGGGTCAGGTCAGGTCTTTAAATGCGACGGTCATTGCTGCATGTGCTCCTCAT
NV463    ..C..C........A.....A...C.............T..............C......
NV466    ..C...........A.....A...C.......A.....T..............C......

                                                  EcoRI          BstBI 1080
NV461    GAGATGTCTGTCCTAGGGGGTTATCTAGGGGAAGAGTTTTTTGGAAAAGGCACATTCGAA
NV463    ..........T........C...T.G..A..G..A..C..C........G.....T...
NV466    ..........T........C...T.G..A..G.....C..C........G.....T.G.

                                                                      1140
NV461    AGAAGATTCTTTAGAGACGAAAAAGAACTCCAAGAATATGAGGCAGCTGAATTGACGAAG
NV463    .....G.....C........G........T..............G......C.A..A...
NV466    .AG.AG.....C........G........T...........A..G......C....A...

                                                  DdeI                 1200
NV461    ACTGATGTGGCACTGGCAGATGACGGAACCGTCAACTCTGATGATGAGGATTACTTTTCC
NV463    T.C..C.................................A.....C.....C..T..C..T
NV466    ..C..C.....................T........A.....C.....C.....C...

                                                          SalI/AccI 1260
NV461    GGTGAAACCAGGAGTCCTGAAGCCGTTTATACTCGGATCATGATGAACGGAGGCCGACTA
NV463    .TG........A...T.A.....T..C........A...........T.....T.....G
NV466    ..........A...........T..C........A...........T.....T.....G

                                                                      1320
NV461    AAGAGATCTCACATAAGGAGATATGTTTCAGTCAGTTCCAATCATCAAGCCCGCCCAAAC
NV463    ..........T...C.....................................T......
NV466    ........A..T...C..........G.........................T......

                 DdeI                                                 1380
NV461    TCATTTGCTGAGTTCCTAAACAAAACATATTCTAGTGATTCATAGAGAGTTGAATCACAG
NV463    .....C..C..A..TT.......G..G.....A.T...C.....AG.......T.G....
NV466    ......T.G..A..TT.......CGGG.....G.....C.....AG.........G....

                                                                      1440
NV461    GATTCTAGGAAACGATAACTTGTGTACATCCTTCATGAAAAAAACTAACACCTCTCCTTG
NV463    .G.G.C..A..T.T...GA....A..T....A...................TC......T
NV466    .G.G.C..A..T.C...GA.......T....A...................TC......T

                                                          1478
NV461    CGAACCATCCTAAATATGAGCAAGATCTTTGTCAATCC
NV463    .A........C.....................T.....
NV466    ..........C..G..................T.....
```

The nucleotide sequence of sample V461 (#3) is presented; bases are numbered from the start of the N gene PCR product. Only differences from this sequence are indicated for samples V463 (#5) and V466 (#8). Positions of ATG open reading frame start codons and TAG/TAA stop codons are underlined as are the recognition sequences of the discriminatory restriction endonucleases described in the text. The sequences of the amplification primers RabN1 and RabN5 are bolded.

DISCUSSION

Molecular techniques including DNA amplification have been used as invaluable tools for virus diagnosis and typing (21). These techniques have been applied to many laboratories and street isolates of rabies virus to understand the nature and character of viral genome (22). The technology has helped to improve the knowledge of relationship between rabies strains within the same geographical areas and different geographical areas.

In this study, we have applied the use of molecular techniques for the diagnosis and typing of rabies isolates collected from the metropolitan city of Lagos in Nigeria. With the RT-PCR technique, the size of the DNA of the isolates was determined to be 1,400p. The technique is also found to be useful as a diagnostic tool for confirming the presence of the virus in a specimen, by the amplification of the converted DNA.

The agreement of the results with that obtained by conventional diagnostic procedure such as FAT showed that the detection of rabies genome by PCR amplification is both specific and sensitive, more so with the highly conserved area of N gene which is invariant (9). Therefore PCR appears as a possible future technique for routine post mortem diagnosis.

The direct nucleotide sequencing of the N gene of the rabies isolate showed that the isolates could be grouped into 3 types with isolates 3,6,7, and 9 grouped into type 1, isolates 5 and 5 grouped into type 2 and isolate 8 into type 3.

Restriction enzyme analysis of amplified DNA of the N gene using a panel of eight enzymes (Bstb1, Hind 3, Acc1, Sal 1, Sca1, Eco01091, EcoR1, Dde1), divided the isolates into the same 3 types identified by the sequencing method. Though there is no record of typing of wild rabies isolates in Nigeria using molecular techniques, the study is similar to the work of Nadin-Davies et al (11), where typing of detected strains of rabies virus was carried out in an outbreak of rabies in Central Ontario. Five strains of rabies virus were identified with respect to geographical locations studied. In Lagos where Nigerian isolates were collected, three types of strains were identified and they were from neighbouring states and country (Republic of Benin). The different strains identified showed some variations in their nucleotide differences and this could be attributed to differences in wild life population densities and movement.

The molecular technique used for this study is realised to be a very simple typing method that produces result within 24 hours. It is a useful method for comparing known viral strains in a geographical area with any emerging strain in the same or a different geographical area.

CONCLUSION

Molecular biology techniques have been used to amplify and type the rabies virus isolates and 3 types were identified by both direct sequencing and restriction enzyme techniques. These showed that the techniques are very useful especially the PCR, which apart from its usage for routine diagnosis, forms powerful tool for typing and molecular epidemiological studies. Using these techniques, it would be possible to identify and differentiate similar or other different rabies virus strains that exist in the same environment and other parts of the country Nigeria and else where.

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PHAGE AMPLIFICATION TECHNOLOGY IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS: APPLICABILITY IN NIGERIA LABORATORIES.

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Though of global importance, the developing world bears the highest burden of tuberculosis (TB) worldwide and Nigeria has been rated amongst 222 countries where TB prevalence is highest worldwide. In Nigeria, diagnosis is largely by direct smear microscopy using the Ziehl-Neelson method. Studies have shown that the sensitivity of smear microscopy varies between 30 70% depending on whether direct or connected smears are examined. It is thus likely that up to half or more of TB sufferers in Nigeria are not diagnosed, automated culture and molecular methods exist but the requirement for especially dedicated, very expensive instrumentation and reagents prohibit their use in developing countries including Nigeria. The World Health Organisation recognises the need for new, affordable, rapid and highly sensitive diagnostics for use in developing countries. Phage amplification technology employs a specific mycobacteriophage which infects a live TB bacillus if represent in a sample. These replicate and lyse the cells to release progeny phage. The presence of progeny phage is detected visually as plagues on a lawn of a raid-growing, non-pathogenic Mycobacterium. Phage Amplification Technology has been evaluated and found to detect most cases missed by smear microscopy and to give results with good correlation with culture (which though highly sensitive requires 6 8 weeks incubation to give results), within 24 hours of sample preparation. It is thus faster than culture and cheaper that the new rapid automated methods, as it requires no especially dedicated instrumentation.

INTRODUCTION

Since the emergence of HIV/AIDS in the early 1980s, there has been a dramatic resurgence of tuberculosis (TB) worldwide (1,2). HIV/AIDS and tuberculosis are in a synergistic lethal alliance with one telescoping the onset of the other (3). Besides HIV/AIDS, the resurgence of TB several years after its apparent decline and its high toll on the developing world can be traced to several factors. These include economic crisis, food shortage, homelessness resulting from wars and natural disasters and overcrowding in refugee camps (4). Though a global problem, the greatest burden TB, like every other disease related to the standard of living of the people, lies with the world's poor nations. These harbour 95% of TB sufferers (5) Between 1980 and 1991, 20-67% levels of increase in the incidence rate of TB were reported for developing countries compared to 3 5% levels of increase for developed countries (6,7). Africa, South of the Sahara faces a double jeopardy. In addition to harbouring most TB sufferers, it harbours 25.3million of the 36.1 million people affected globally with HIV, a potent facilitator of the reactivation of dormant TB infection to overt TB. In 16 countries in Africa, more than 10% of adults aged 15 49 are HIV infected. Nigeria bears 10% of the African burden of HIV/AIDS and has recorded a consistent increase in HIV prevalence from 1.8% in 1993 to 3.8 in 1994, 4.5% in 1996 and 5.4% in 199. Thus of her 120 million population, 2.6 million adults are HIV-infected (8,9) HIV/AIDS does not only facilitates the reactivation of dormant TB infection to overt TB but also interferes with correct diagnosis of TB (especially with serological methods and smear microscopy) as it alters immune response to TB (10).

In Nigeria, there are a quarter of a million registered cases of TB with 25,000 new cases registered annually, (11) in the year 2000 alone, 26,641 cases were reported (120. In an epidemiology study of TB in Lagos, Nigeria, a significant increas from an incidence rate of 21% in 1982 to 42% was reported (13). This was a hospital-based study. Also, a study by Wokoma in 1990 showed a 7.74% increase in the incidence rate of TB in Port Harcourt between 1993 and 1999(14) it is important to note that disease surveillance is too incomplete to provide exact numbers of new cases and deaths occurring from TB in a year. Also, owing to the stigma attached to TB in the Nigerian society, several cases are unreported. Of the cases reported, up to a half or more are never diagnosed and are therefore not treated. This is a dangerous trend, which, amongst other things, would increase morbidity from TB, increase the spread of TB within the community, increase self-meditation, especially the use of anti-TB drugs in sub-inhibitory doses since these drugs are available over the shelf (15) and thus increase the incidence of multi-drug resistant TB. In Nigeria, TB control was incorporated into the Primary Health Care programme in 1991 and she has reported to the World health organisation (WHO) under the Directly Observed Treatment, Short Course (DOTS) programme since 1995. Available data shows that as at 1997 cohort study, treatment success was 73% amongst the cases evaluated. Case detection rate was however in the range 9-15%. Meanwhile, Nigeria has been rated among the 22 countries with the highest burden of TB worldwide (16). With increase budgetary allocation to the National TB control programme and Donor support

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there is an urgent need to beef up case finding to meet the WHO target of 70% case detention.

CONVENTIONAL DIAGNOSIS OF TUBERCULOSIS IN NIGERIA

Owing to the infectious nature of the disease, there is need for prompt initiation of rational treatment. This demands a diagnosis (17). The need for the diagnosis of TB before treatment is even stronger because Mycobacteria, like several other bacteria, have a remarkable ability to develop resistance to Antimicrobial agents even on a single exposure. For a country like Nigeria, a first requirement for rational diagnosis is that the method be cheap and easy to adapt to local laboratories under already available infrastructure while maintaining a high specificity and sensitivity. In addition, any diagnostic method that would be applicable in Nigeria must be one whose performance is not altered by the presence of HIV/AIDS because of its increasing prevalence in TB patients in Nigeria (9,13). There exist today, a multiplicity of test kits and methodologies for the diagnosis of TB. Each has its unique features and advantages. It is however not possible to say categorically that a single method meets all the demands for full diagnosis. There is often the need for a combination of two or more methods depending on the information sought, the availability of test materials and trained personnel as well as the ability of the patient to pay.

TB diagnosis in most developing countries is hinged on acid-fast microscopy (s) because it is relatively cheap and rapid. Acid-fast microscopy is a major component of the WHO's TB control strategy the Directly Observed Treatment, Short Course (DOTS) (18,19. In Nigeria, TB diagnosis under the DOTS programme of the NTLCP is by the Ziehl-Neelson (ZN) technique (20) Typical, smear microscopy is useful in identifying TB patients that expectorate acid-fast bacilli and therefore pose a public health hazard. It is also employed in identifying acid-fast bacilli from culture. Beside its use in diagnosis, it is also employed in the management of TB as it provides the opportunity to monitor smear-conversion during treatment (23).

Microscopy has several advantages. It is fast and highly specific. It is relatively cheap and easily overcomes the problem of power outages, a common occurrence in Nigeria, as slides can be examined with a light microscope in a well-illuminated laboratory. The WHO has recommended that 3 sputum samples (With at least two submitted on 2 consecutive days) should be examined and that in order to get reliable results, the whole slide must be viewed and examination done for about 15 minutes (21) There are peculiarities on ground that reduce the value of smear microscopy as a diagnosis tool in Nigeria. Generally, there is a shortage of peripheral laboratories located close to TB patients, many of who are poor, rural dwellers. Patients often have to travel several kilometres to submit samples. The possibility of complying with the WHO recommendation for the examination of three sputum samples is very bleak since it goes with the added cost of transportation and inconvenience. With the exception of the newly identified National TB Reference Laboratory in Lagos, several peripheral laboratories suffer form a shortage of adequately trained and experienced personnel. Since the identification of AFBs on a slide requires expertise, there is the risk of human error. In other words, what party A identifies as acid fast bacilli (AFBs) may be identified by party B as artefacts especially when direct smears are examined (22) as is invariably the case in peripheral laboratories in Nigeria (20).

Though considered cheap, the availability of functional microscopes and fresh slides could be a problem in many laboratories. Where slides are wasted and re-cycled as is often the case in Nigeria, there is an increase risk of misdiagnosis since acid-fast materials may be retained in scratches on recycled slides (20,23) Added to this is the fact that when the oil-immersion objective is used to examine slides, the bacilli sometime float into the oil and are carried to subsequent slides thereby increasing the risk of false-positive results (24) practically, adherence to the WHO recommendations that each slide be examined for 15 minutes is labour intensive in a busy laboratory considering the fact that three samples will be examined for each patient. Meanwhile, non-; compliance with the procedure would reduce its sensitivity.

The major drawback of smear microscopy is the variability off its sensitivity. For direct, smears, it could bee as low as 30% while for concentrated smears, it varies between 30% and 70% requiring the presence of up to 1x10-1x10 cells/ml of sputum to be

able to detect AFBs. 25. This implies that its optimal value as a diagnostic method can only be harnessed when the sample is processed (concentrated). This requires the availability of a centrifuge. Since most peripheral laboratories in Nigeria do examine direct sputum smears (20) it can be expected that more than of TB patients requiring treatment be not diagnosed and therefore, not treated. (16) The requirement for the presence of up to $1x10^4$ cells/ml of sputum to get a positive result means that smear negatively does not exclude TB. It has been reported that some of the missed cases will become infectious. In fact, active smear negative TB cases do occur and are infectious.26)

In a country like Nigeria where the prevalence of HIV/AIDS in TB patients (HIV/AIDS/TB coninfection) is on the increase (9,13) the value of smear microscopy as a diagnostic tool is further reduced because often, there is reduced number of AFBs in sputum samples (10). Under the DOTS programme in Nigeria, symptomatic patients whose sputum samples are consistently negative are diagnosed by radiographic methods (2). Though rapid X-ray results could be misleading. They lack specificity and have a low positive predicts value. Suspected TB patients may have one or more of a multiplicity of respiratory tract infections capable of causing abnormalities similar to those seen in TB. This is precisely the case with HIV/AIDS patients. Also, correct interpretation of X-ray results depends very much on the experience of the radiographer (27). Sputum culture improves the diagnosis of smear negative TB tremendously because of its high sensitivity in providing a definite diagnosis of Mycobacterium Tuberculosis. (23) Solid and liquid culture require the use of processed (concentrated/decontaminated) sputum samples because of the high levels of sputum contamination by other bacteria. Thus a centrifuge is also required. Conveniently, the TB reference Laboratory in Lagos, Nigeria uses the egg-based Lowerstein Jensen (LJ) medium. It is relatively cheap to prepare and yields good results. It however requires 6-8 weeks (or more) incubation period (as MTB is a slow0grower) before cultures are regarded as negative (3). This is therefore a very slow method. In a busy Laboratory, incubator space could be a problem. Since it involves the propagation of a highly infectious pathogen there is an increased risk of transmitting the bacillus.

Other Methods of TB Diagnosis

Besides the manual culture methods which include the use of LJ, Middlebrooks 7H11, 7H10, 7H9 and in some countries, Ogawa medium, there are automated culture methods which can detect growth within one or two weeks but usually require up to 4 weeks incubation. These include the Bactec 460 TB automated Mycobacterial Detection and Susceptibility Testing System (28,29) MB Bac/T system (Organon Teknika) and the Mycobacterial Growth Indicator Tube (MGIT™) (Becton Dickinson (30). These systems employ liquid media for the detection of MTB. It is therefore not possible be directly study the colonial morphology of the organism. In the Bactec 460 system, growth and metabolism. The use of radioactive materials is restricted in many countries. This is a major disadvantage of the Bactec 460 system. Also the culture medium employed has to be purchased specifically from the manufacture of the machine. Since it involves the incubation of the specimen into a sealed container by syringe and needle, there is the added risk of needle stick injuries. Generally, these newer (automated) methods have the advantages of high sensitivity and specificity and are comparatively faster than the conventional manual culture methods. They however require specially dedicated and expensive instrumentation. The high cost of these machine, the reagents and servicing of the machines make them unsuitable for use in most Nigerian laboratories. The conventional manual methods will therefore enjoy patronage for a very long time (31).

Most peripheral laboratories in Nigeria do not culture sputum. This is a problem, which is bound to hamper effective TB control because so far there has not been a good liaison between peripheral laboratories and the reference laboratory.

Molecular biology has been adapted in several ways to improve the diagnosis of TB. Several tests employ nucleic acid probes and gene amplification. These include the Gen-probe Amplified Mycobacterium Tuberculosis Direct test (AMTD), Strand Displacement Assay (SDA), Polymerase Chain reaction (PCR), and Lipase Chain Reaction (LCR) amongst others. These offer a rapid approach to

diagnose because of their abilities to detect a few cells a specimen within a few hours as opposed to the 6-8 weeks required for culture (32). They are however, difficult to adapt for routine use in an average laboratory (33). Under currently available infrastructure in Nigerian laboratories, it is not feasible to use of these methods because just like the automated culture methods, they require especially dedicated and very expensive instrumentation. Also, there is the need to perform every step of the procedure in a separate room to avoid cross contamination of the genetic material. Just like smear microscopy, molecular tests do not distinguish between live and dead bacilli. Therefore, treated TB can be confused with active TB. Besides, many laboratories in Nigeria lack skilled personal capable of performing these tests.

Chromatography has emerged as a useful diagnostic tool for TB in recent times. It is based on finding that each Mycobacterium specie synthesizes a unique set of mycolic acids and that a specie-specific pattern can be produced. High performance liquid chromatography (HPLC) has been reported to produce good identification results within 4 hours after culture is available. This is a rapid test that can replace an entire battery of biochemical tests. Results can be as good as 100% specificity and sensitivity for MTB complex (34). However, besides the requirement for culture, there is the added requirement for special, very expensive instrumentation and expertise for sample preparation and assay. It therefore has very limited application in Nigerian Laboratories.

Phage-based Test in the Diagnosis of Tuberculosis

The use mycobacteriophage in the diagnosis of TB is fast gaining ground today. Research into the use of phages in diagnosis dates back to 1947, when Gardner and Weiser identified phages that are specific for the Mycobacteria (35). In 1960, Redmond and cater isolated phages that specifically infect MTB and M. bovis. (36). The fact that phages are specific in their infection of hosts has been employed in developing two diagnostic methods today. These are the Luciferase Reporter phage (LRP) technology and phage amplification technology (5,37). In both methods, a specific phage that infects members of the MTB complex is used to target viable MTB in a specimen. The presence of viable cells is then determined in one of two ways. One is by the incorporation of the bacterial luciferase enzyme (Lux: AB) or the firefly enzyme (Fflux) into the phage genome that will be expressed when the phage infects viable Mycobacteria. This is the case with the Luciferase Reporter phage technology where the presence of viable cells is detected by the emission of light (38). The other detects the presence of viable MTB when progeny phages are released from the infected bacilli (5). This is method is simpler than the Luciferase phage Reporter system because results are read visually as opposed the LRP in which a luminometer or photographic film is required to detect positive results.

FASTPLAQUE TB (FPTB) ASSAY

FASTplaque TB is a commercially available diagnostic kit that detects the presence of the TB bacilli in sputum as well as other samples from TB patients. For the diagnosis of pulmonary TB, the sputum sample is processed (decontaminated and concentrated) using the NaOH-NALC (N-acety-L-cysteine) method which has been reported to concentrated more viable cells in sputum than the modified Petroff's method commonly used in Nigeria (39). The retention of as many viable cells as possible in the specimen is important because the FASTplaque assay dose not detect the presence of killed bacilli as phage replication can only occur in live cells. Processing is thus a very important step in the procedure. This is followed by the addition of the mycobacteriophage (Actiphage) to the sample in a reagent bottle. The mixture is incubated at 37oc for 1 hour to allow the phage sufficient time to infect viable MTB if present in the sample. A potent virucide (virusol) is then added. This destroys any phage that has not infected MTB cells but does not affect phage within a cell. This is incubated for 5 minutes on the bench after which the virucide is neutralised. A non-pathogenic, rapid-growing suspension (sensor cells), which is susceptible to the phage, is added. This is then plated in an agar medium, using the pour plate method and incubated overnight at 37oc. These sensor cells grow overnight to form a lawn. Plaques are seen on a positive plate (5,41,42). The underlying principle is that of phage replication. When MTB cells in the sputum sample become infected by the phage, replication occur within the

bacilli. After maturation, infected cells lyse to release the progeny phages. These become available to infect the sensor cells. Thus plaques are a confirmation that infected sensor cells are undergoing lysis. This is not possible if there were no viable MTB cells within which the phages were initially propagated. A negative plate will thus have a confluent growth with plaques.

FASTPLAQUETB VERSUS CONVENTIONAL DIAGNOSTIC METHODS

FASTplaque assay has several advantages. Assay results are available within 24 hours of sample preparation, it does not involve the propagation of the pathogen, and there is reduced risk, if any, of misdiagnosis because it does not detect dead bacilli as is the case with smear microscopy as it has the ability to detect as few as 100-300 cells/ml of sputum (43).

In one study in Cape Town, the performance of FASTplaque TB assay in the correct diagnosis of auramine **smear negative** sputum sample was compared with that of chest X-ray. FPTB detected 55% of cases, negative by auramine smear, which is even more sensitive than ZN smears. Over 70% of the chest x-rays that were suggestive of TB were from patients who did not have TB. This demonstrates the fact that chest x-ray have a negative predictive valve. These cases were confirmed by culture and/or clinical diagnosis (44). In another study at the Aga khan Hospital in Nairobi, Kenya, the FASTplaque TB was compared with AFB smear microscopy and the Bactec 460 TB culture using samples form patients, 70% of whom were co-infected with HIV. There was 100% sensitivity and 98.2% specificity. Also FASTplaque TB detected all cases missed by AFB smear microscopy (45). This demonstrates that the performance of FPTB is not affected by the presence HIV/AIDS. In yet another trial at the South African Institute of Medical Research (SAIMR), Cape Town, results from FASTplaque assay were compared with those obtained from concentrated auramine smear microscopy, solid culture (LJ) and clinical symptoms. The sensitivity of FPTB as compared with culture was 73.1% with 99.0% specificity. Also, FPTB was able to detect the presence of the TB bacilli in 55.3% of the samples that were negative by concentrated auramine smear microscopy. Overall, FPTB had positive and negative predictive values of 0.96% respectively. 46 There is therefore little or no risk of false positive and false negative results.

Similar results were obtained in an evaluation conducted at the Sindh Institute of Urolgy and Transplantation (SIUT), Karachi, Pakistan in which results from FPTB assay was compared with those from concentrated auramine smear using the Ziehl-Neelson method and LJ culture. Positive results were confirmed by PNB testing and a PCR assay specific for MTB complex. Of mall samples tested, FPTB showed 68% and 98% sensitivity and specificity in the diagnosis of smear negative TB (25). The fact that the FASTplaque assay requires no specially dedicated centrifuge and an incubator. Any laboratory, which intends to use smear microscopy maximally in diagnosing TB, would need a centrifuge. The technique can thus be applied either as a compliment to smear microscopy with better results (increased sensitivity, specificity, and positive predictive value (46,25). The essay is easy to run as it only involves simple pipetting, mixing of reagents and the pour plate culture method. It is convenient as the reagents-virucide, sensor cell, agar; growth supplement and reagent bottles all come in the same pack. It does not require extra-ordinary skills or instruments to read the results. Plaques are visible to the naked eyes. FAST plaque TB assay cannot replace culture that is more sensitive. It can however reduce the need for culture due to its ability to detect most cases that are missed by smear microscopy. It completes favourably with culture, greatly reducing the time required to obtain results from 6-8 weeks or more to 24 hours after sample preparation. While culture requires further confirmation and differentiation, FASTplaque results are conclusive of the presence of MTB complex. The WHO does not emphasize the use of culture in diagnosis except in retretment cases. Culture requires a lot of incubator space in a busy laboratory.

Under the NTLCP in Nigeria, direct smear microscopy, which is far less sensitive than concentrated smear microscopy is the method, used. (20). In contrast with smear microscopy, chest x-rays and serological methods, the presence of HIV/AIDS does not alter the results of the test (45). It is thus suitable for Nigeria where over 2.6 million adults were said to be living with HIV/AIDS as at 1999.

Coast as a Determinant of the Applicability of New Diagnosis in Nigeria.

It should be noted that despite the availability of sophisticated rapid methods for the diagnosis of TB over the years, none of these are being employed in developing countries. Therefore one of the important factors that would determine the applicability of the FASTplaque TB Tm in the Nigerian TB Control Programme is its cost as compared with those of conventional methods. TB is a disease symptomatic of poverty and so ability to pay is a major determinant of what tests are likely to be recommended for individual patients. The cost of AFB x 3 varies between six hundred and eight hundred naira (N500 N600) (about $4 - $5) in non-profit set ups. If 3 or 2 sputa samples are negative by smear microscopy, chest x-ray is conducted. This gives an additional cost of between N400- N6000 and more depending on where it is done. Culture goes for about N6000. These rates are highly variable. This could be higher in private clinics which are profit-oriented and may be lower it is subsidized. The FASTplaqueTBTM cost $200 per kit of 50 tests plus freight if one buys in small quantities from Biotec Laboratories, Ipswich, UK (47). It is difficult to guess what the actual cost per test will come to in Nigeria after freight is added, if bulk purchase attracts a discount or if the kits are bought from a local distributor. It is unlikely that the final cost for end users will be more than the WHO stipulated $5.00 for diagnostic. It has been speculated that it may be possible to reduce the cost of phage amplification technology by propagating the phages and sensor cells (M. Smegmatis, a non Mycobacterium) in a local laboratory. It has however been reported that a gene has been discovered in M. smegmatis which, when over-expressed, was found to induce resistance to infection by certain phages (40). Coupled with the problem of standardization, it may be better for now to use the commercially available kit produced by Biotec laboratories, Ipswich, UK which is tested to ensure high performance standards before plunged into the market.

Cost is unlikely to prohibit the use of FPTB in Nigeria especially if one recalls that active cases of smear negative TB do occur and are infectious. Having to wait for 6 8 weeks or more to get results of culture from the reference laboratory in Lagos before treatment is commenced increases the morbidity of TB on the patient and gives him/her adequate time to infect his/her contacts. This would in turn increase the number of people that would be requiring tests as well as treatment. Since FPTB detects most smear negative samples, treatment is commenced promptly. Besides, the use of one AFB and one FPTB increases case detection tenebrously. (46) Saving from the second and third AFBs, transportation to and from clinics to submit 3 samples and future testing and treatment of contacts could be used to pa for the diagnostic. With increased budgetary allocation to the NTLCP and donor support, the cost of diagnosis and treatment on individual patients could be significantly low.

LIMITATIONS OF THE FASTPLAQUE TBTM

The use of FASTplaque TB™ assay does have limitations. One of these is the fact that a positive result does not distinguish between all four members of the Mycobacterium tuberculosis (MTB) complex, M. tuberculosis, M. bovis, and M. africanum. This is not a problem if FPTB is used for diagnosis since all four members are aetiologic agents of TB invariably requires treatment. For purposes of research, there may be need to differentiate between members of the Mycobacterium tuberculosis complex by other means. In a sputum sample containing more than 10% of blood, the performance of the test is reduced. Also, treatment with anti-TB drugs reduces the sensitivity of the test hence results are interpreted in relation to clinical information as well as other laboratory test. Most importantly, the efficacy of the test depends on the quality and quantity of the sputum, its storage and the number of viable organisms present. To ensure a good number of live organisms, it has been recommended that the NALC/NaOH (39) method be used to process the specimen as opposed to the modified Petroff's method currently in use at the TB reference laboratory in Lagos.

CONCLUSION AND RECOMMENDATION

It is pertinent to note that for now, no diagnostic can stand on its own as the singular method for the diagnosis of TB. There may be need for a combination of laboratory methods and clinical analysis for rational diagnosis to be made. Due to the

debilitating nature of the disease, its ability to spread fast and the inherent ability of the MTB bacilli to rapidly develop resistance to anti-TB drugs, there is need for prompt diagnosis to ensure prompt treatment. Molecular, chromatographic and other automated methods require special skills to perform and specially dedicated and expensive instrumentation. It is therefore for feasible to apply these in the Nigerian TB control. In contrast, phage amplification technology is a manual method that does not require special instrumentation. It is conclusive that smear microscopy performs best with processed sputum. So also does culture. Phage amplification technology places very little demand on the scarce resources of Nigerian Laboratories because it relies on the basic instruments that ought to be available in an average laboratory. There are a centrifuge, an autoclave and an incubator.

In the diagnostic algorithm in Nigeria, FASTplaqueTB assay could be used either in the place of smear microscopy where it can afforded or in combination with one smear microscopy as opposed to AFB x 3. This combination has been reported to improve case-detection to about 91%. Most cases missed by smear microscopy will be picked up by the FASTplaqueTB assay. This would drastically reduce the number of samples to be culture.

There is an urgent need to establish a strong a liaison peripheral laboratories and the National Reference Laboratory in Lagos so that when necessary, sputum from symptomatic cases that are smear and FASTplaque negative as well as those from re-treatment cases can be submitted to the reference laboratory for culturing. This will involve the provision of more incubator space at the Reference Laboratory and the provision of vehicles for the transportation of samples.

Before FASTplaque TB™ assay can be introduced into the diagnostic algorithm in Nigeria, there is need for the evaluation of this kit at the National Reference Laboratory to ascertain excellent performance under peculiar circumstances in Nigeria. There will be need for the National TB Control Programme to embark on laboratories inspection and identification of suitable centers with a view to providing basic equipment functional microscopes, centrifuges incubator, glass and plastic wares and reagents.

There is the need for staff training and sanitization on the advantages and disadvantages of available tests as well as the new kits and how and when to apply what method or a combination of methods. Prior to introduction of this kit into the Nigerian TB control, there is the need to set performance standards as well as set up monitoring groups who will over see work in peripheral laboratory from time to time. The kits can be used in a specified region for a trial period of six months. An impact assessment must be done after this period to ascertain whether their use has significantly improved TB Control in Nigeria or not. This should precede its full incorporation into the National TB Control Programme.

There is a dire need to patronize TB control especially as it has been reported to complicate HIV/AIDS as well as predispose to infection. Beyond the expression of the political will to do so, all tiers of government must allocate adequate towards the control of TB in Nigeria, as it is difficult for only the Federal government assisted by foreign donors to shoulder the responsibility for this task. This would de-emphasize cost as a major determinant of the use of diagnostics and create an opportunity to use only the best.

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PATHOGENIC POTENTIALS OF ESCHERICHIA COLI ISOLATED FROM RURAL WATER SUPPLIES

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Electrolyte and haematological parameters in rabbits infected with pathogenic isolates of Escherichia coli from rural water supplies in Rivers State, Nigeria, where monitored. Rabbits were orally infected with suspension containing $3x10^7$ cfu /ml of Escherichia coli to induce diarrhoea, and the electrolyte (sodium, potassium and chloride ions) levels as well as the haemoglobin (hb) packed cells volume (PVC), and total white blood cell count (WBC) were determined after 48, 72, 96 hours and post infection following standard procedures. Subsequently blood samples were collected every week for 6 weeks for further estimation of WBC and HB and PVC. Results of the electrolyte (sodium, potassium and chloride ions) levels obtained revealed that significant amount of electrolytes were lost after 96 hours post-infection. Potassium ions concentration decreased by 57.2%, sodium ions by 64.6% and chloride ion concentration decreased by 59.9% as compared to the normal control rabbits not infected with E. coli.

Haematological changes were observed with an increase in haemoglobin concentration, total white blood cells count and packed cell volume from those of normal control up to day four and then decreased steadily during the monitoring period.

INTRODUCTION

Presence of *Escherichia* coli in water supply indicates faecal contamination. *Escherichia coli* has been reported in a variety of infections including gastroenteritis, urinary tract infection (Baker and Breach, 1980. Chessbrough, 1984), haemolytic colitis and haemolytic uremic syndrome (Karmali et al., 1983). The organism is also a leading cause of diarrhoea in developing countries (Alabi *et al.*, 1991) and responsible for a considerable degree of morbidity and mortality among children and adults in poor communities (WHO).

E.coli that cause diarrhoea belong to 4 major groups namely enteropathogenic E.coli (EPEC), enterotoxigenic E.coli (ETEC), enteroinvasive E.coli (EIEC) and enterohaemorrhagic E. coli (EHEC) (WHO, 1980). A number large of outbreaks of diarrhoea illness due to pathogenic *E.coli* have been related to contamination of water and death rates of 5-10 percent may be experienced among untreated infants or children (*Feachem et al*, 1983).

The pathogenicity of bacteria subsequently leading to clinical manifestation at various sites depends on certain properties of bacteria. The virulence of pathogenic organism depends on serotype and the strain specificity, infective dose and on host status (*Jawetz et al.*,1989). The incubation period is generally short and clinical symptoms occur within 5 days (usually 1-3 days) of ingesting of bacteria. Some bacteria toxins stimulate intestinal secretion, which results in the loss of electrolyte and fluid leading to dehydration. Several diarrhoea have been reported to lead to a daily loss of 5-10 litres of fluid together with large amounts of sodium, potassium and chloride ions which are responsible for a considerable degree of mortality (Weinberg, 1985). This therefore, underscores the need to embark on a study to fully elucidate the effects of pathogenic E.coli on the electrolyte and haematological parameters of experimental animals in vivo.

In this we report on levels of sodium, potassium, chloride ions, haemoglobin, packed cell volume and total white blood cell counts in rabbits orally infected with pathogenic E.coli isolated from rural drinking water sources.

MATERIALS AND METHODS

Sample collection

Using sterile 250ml glass bottles, water samples were collected from boreholes, wells, streams and rivers in the rural communities of Rivers State, Nigeria. The method for waste collection was as described by standard methods for the examination of water and wastewater (APHA, 1992).

Isolation and identification of isolates

Membrane filter technique (APHA, 1992) was employed. Water sample (200ml) was filtered under vacuum through a sterile 0.45pm pore size filter membrane (HAWG, Millipore Corp, Bed ford, U.S.A). Upon completion of filtration process, the membrane filter with retained bacteria was immediately removed with sterile forceps and placed on to eosin methylene blue (EMB) agar and incubated at 37 for 48 hours.

Identification of isolates was done following previously reported scheme (Chessbrough 1984).

Estimation of bacteria suspension.

The McFarland's standard method of estimation of bacteria suspension as described by Campbell et al. (1970) was adopted. The

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concentration of the bacterial suspension was determined by comparing it with McFarland's standard tube containing barium sulphate (varying amount of 1% barium chloride solution and 1% sulphuric acid) of desired concentration. McFarland's table for standardization of bacterial suspension was used to calculate the bacterial concentration.

Enterotoxicity Test

The isolates were screened in order to test the ability to induce diarrhoea. The sucking mouse enterotoxin activates assay as described in the manual for laboratory investigation of acute enteric infection (WHO, 1987) was adopted. The isolates were tested for their ability to produce diarrhoea in 5 days old mice. Each mouse was administered orally with bacterial suspensions ($3x10^7$ cfu/ml) and placed on a white filter paper in a cage. The infected mice and controls (not infected) were left at room temperature for 3 days and during which the faeces were observed for diarrhoea. E.coli controls strain ATCC 25922 was incubated in the study.

Inoculation of Experimental Animals.

Twelve healthy rabbits weighing 2.5 to3.0kg and without any history of clinical diarrhoea or illness were obtained from animal house of Michael Okpara University of Agriculture, Unudike. The inoculation of rabbit was carried out as described by Nielsen et al. (1995) and Pina et al (1993). Each of the six healthy rabbits was inoculated orally with 0.2ml ($6x10^7$ cfu/ml) suspension of pathogenic E.coli using plastic tube placed above the tongue and close to the throat to allow the rabbit swallow the suspension slowly. Six control rabbits were inoculation rabbits were housed differently in the laboratory to eliminate the risk of cross-infection.

Preparation Of Blood For Analysis.

After 48, 72 and 96 hours post inoculation, blood samples was collected from the inoculated and control rabbits through the heart by cardiac puncture (Campbell et al., 1970) using sterile needle and syringes. The blood samples were put in appropriate specimen containers for the estimation of electrolyte (sodium, potassium, chloride ions), haemoglobin (Hb), packed cell volume (PVC) and white blood cell count (WBC).

Subsequently blood samples were collected every week for 6 weeks for further estimation of WBC, Hb and PVC.

ESTIMATION OF SERUM ELECTROLYTE.

The blood serum samples were used to estimate chloride, sodium and potassium ions. The mercuric nitrate titration method of Schales and Schales as described by Raphael et al . (1976) was adopted for the estimation of the serum chloride. With a glass pipette, 0.2ml of blood serum was added to 1.8ml of distilled water in a clean test tube.

Therefore, 0.6ml of biphenyl carbazone indicator was added and titrated with mercuric nitrate solution from a microburette. An end point was obtained when the original salmon red colour turned to faint purple colour. The nitration of the standard chloride solution was also carried out as the test sample was determined in duplicates and the mean recorded.

For the estimation of sodium and potassium ions, flame photometer (GallenKamp Co. Ltd, England) was used and operated according to the manufacturers instruction. Measured volume (9.9ml) of deionised water was introduced into a universal bottle followed by 0.1ml of test blood serum. The bottle was capped and the content mixed by inversion. Sodium light filter (590nm) or potassium light filter (770nm) was inserted in the flame photometer and the test samples were read and recorded.

Estimation of haemoglobin concentration.

The spectrophotometric method as described by Dacie and Lewis (1994) was adopted. With a pipette, 10ml of haemoglobin diluting fluid (0.2g of potassium cyanide in 1000ml of distilled water) was dispensed into a test tube and 0.05ml of blood sample added. A spectrophotometer (spectronic 20D,Milton Roy Ltd. USA) was used to the diluted blood sample at wavelength of 625nm, using the diluted as a blank and value recorded.

Estimation of white blood cell count.

The method described by Dacie and Lewis (1994) was employed. Twenty microlitre of the rabbit blood sample was added to 0.38ml of white blood cell diluent (2% acetic acid with few drops of gentian violet). The content was mixed and a portion transferred with the aid of clean Pasteur pipette into Neubauer counting chamber (Weber Scientific International Ltd., Sussex, England) covered with coverslip. The chamber was placed on a microscope stage and the white blood cells estimated using the x10 objective lenses.

Animals	Time (hrs) following infection	Concentration of ion (mMol/L)	% decrease against control (%)
Control	0	5.6	---------
Infected	48	4.5	19.5
Infected	72	3.3	41.1
Infected	96	3.3	57.2

Table 1 :

CONCENTRATIONS OF POTASSIUM IONS OBTAINED IN THE BLOOD SERUM OF RABBITS ORALLY INFECTED WITH PATHOGENIC ISOLATES OF E.COLI WHEN COMPARED WITH NORMAL CONTROLS.

Animal	Time (hrs) following infection	Concentration of ions (mMol/)	Percentage decrease against control (%)
Control	0	129.6	----------
Infected	48	89.2	31.2
Infected	72	66.4	48.8
Infected	96	45.9	64.6

Table 2.

CONCENTRATIONS OF SODIUM IONS OBTAINED IN THE BLOOD SERUM OF RABBITS ORALLY INFECTED WITH PATHOGENIC ISOLATE OF E.COLI WHEN COMPARE WITH NORMAL CONTROLS.

Animals	Time (hrs) following infection	Concentration of ions (Mmol/l)	% decrease against control (%)
Control	0	99.5	------
Infected	48	65.8	33.9
Infected	72	50.5	49.3
Infected	96	39.6	59.9

Table 3.

CONCENTRATIONS OF CHLORIDE IONS OBTAINED IN THE BLOOD SERUM OF RABBITS ORALLY INFECTED WITH PATHOGENIC ISOLATES OF E.COLI WHEN COMPARED WITH NORMAL CONTROLS.

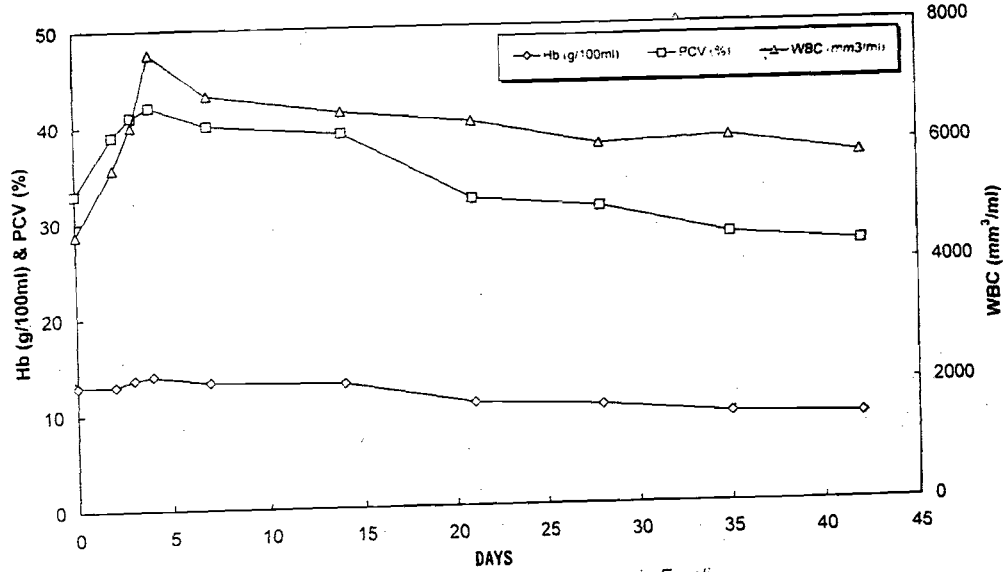

Fig. Haematological changes in rabbits orally infected with pathogenic *E. coli*

Estimation of packed cell volume

Microhaematocrit centrifugation technique as described by Dacie and Lewis (1994) was used. Capillary tube was fitted with well-mixed anticoagulated blood sample and one end of the tube sealed with plasticine. The capillary tubes containing the blood were spun at 1500rpm for minutes in microhaematocrit centrifuge (Hawksky and sons, London) and the packed cell volume read off with the haematocrit scale reader. Two independent test were carried out and the mean calculated.

Statistical analysis

Data were subjected to analysis of variance. The standard errors of difference (S.E.D) of means were multiplied by the appropriate t-statistic to test least significant difference (LSD) between means of treatment at p=0.05.

RESULTS

Electrolyte levels in the blood serum of experimental animals

Concentrations of potassium, sodium and chloride ions obtained from the analysis of blood serum collected from the infected and control rabbits are presented in tables 1-3. Potassium ions Concentration decreased from 5,6mMol/lfor the control animals to 4.5mMol/L, 3.3mMol/L and 2.4mMol/L at 96 hours respectively following inoculation (table 1). Sodium ion concentration decreased from a control value of 129.6mMol/Lto 89.3mMol/L after 48 hours following exposure to E.coli. (Table 2).

Similarly, chloride ion Concentration decreased from a control level of 99.5mMol/L to 50.5mMol/L at 72 hours and 39.6mMol/L at 96 hours of oral infection with the pathogen. Significant differences were observed in potassium, sodium and chloride ions loss at $p<0.05$.

Haematological changes in experimental animals.

The haematological responses in controls and rabbits orally infected with pathogenic E.coli shown in the figure. Haemoglobin (Hb) levels, packed cell volume (PCV) and white blood cells count showed a steady increase from control values up to the 4th day following infection. The Hb increased from 13.0g/100ml to 14.0g/100ml on the 4th day, PCV increased from 33% to 42% while WBC increased from 4600mm³/ml to 7600mm³/ml respectively on the 4th day following infection. Thereafter decrease was observed in these parameters. There is no significant difference ($p<0.05$) observed in the PCV,Hb and WBC values during the monitoring periods.

DISCUSSION

The role of E.coli in diarrhoea has been established and reported to be responsible for substantial degree of morbidity and mortality among children and adults (WHO, 1980). Diarrhoea on its own is characterised by loss of fluids and electrolytes (Tilkian et al.,1979). Results obtained in the study have revealed that experimental infection of rabbits with pathogenic isolates of Escherichia coli from drinking water source resulted in a decrease in a concentration of chloride, potassium and sodium.

Sodium ion concentration was found to decrease by 31.2,48.8 and 64.6 percentages after 48,72 and 96 hours respectively following infections with the pathogen. Potassium and chloride ions values decreased at the same time intervals when compared with the normal controls. This suggests that the infection of the rabbits with the pathogen resulted in the release of enterotoxin, which induces an outpouring of electrolyte and fluids (Robbins and Kumar, 1987). Electrolyte levels in the blood serum confirmed this.

It has been reported that loss of sodium ions from the body results in a decrease of extracellular and nervous system function (Morris, 1981), while the loss of body fluids containing large amounts of potassium ion as in diarrhoea fluids has been reported to cause hypokalaemia. Loss of chloride is characterised by acidosis.

This study has revealed tremendous loss of electrolyte that may occur in host infected with isolate of pathogenic Escherichia coli and therefore highlights the need to immediately provide portable water supplies in rural communities to prevent health hazards which could be fatal. Fluids and electrolyte depletion is one of the most grievous medical emergencies in the tropical environments in human patients (WHO, 1996). This becomes aggravated in the rural areas where there is no portable water and standard of hygiene is very poor and where medical facilities are very scarce.

As evident from the results of this study increase in haemoglobin concentration, total white blood cell count, and packed cell volume was noticed from those of control (day 0) up to the 4th day for rabbits infected with Escherichia coli and thereafter a decrease in parameters. The high values in haemoglobin concentration and packed cell volume could be due to water loss resulting from diarrhoea, which leads to haemo-concentration. The initial increase in white blood cell count values observed within the 4th day may be due to the bacteria infection. Mild to moderate elevation of the white blood cell count usually indicates disease, mainly of bacteria aetiology (Atlas, 1995). This is so because when bacteria invade the body the bone marrow is stimulated to produce and release large number of white blood cell and the number in the blood rise dramatically (Dacie and Lewis, 1994). The subsequent decrease observed in haemoglobin concentration, packed cell volume and white blood cell count after the 4th days of monitoring may be due to reduction on the severity of infection. The second possibility is that since therapy was not instituted, the rabbits generally were very ill and unable to feed normal probably due to many clinical complications (Nielsen et al., 1995). The decrease in value of these parameters observed especially in haemoglobin concentration and white blood cell count result in anaemia, immune deficiency or other haematological disorder.

The study has revealed significant loss of sodium potassium and chloride ion concentrations as well as haematological disorder in experiment animals infected with pathogenic isolates. This confirms the fact that most of the water sources in the rural communities are polluted with pathogenic bacteria. There is, therefore, the need to conduct health education continuously in the rural areas to bring about changes in individual attitudes, believes perception and values concerning water use and general environmental sanitation activities. Furthermore drilling boreholes in rural areas by government will significantly reduce the rates of water-related diseases.

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CHLAMYDIAL NEONATAL CONJUNTIVITIS (CNNC) IN ILORIN, MIDDLE BELT OF NIGERIA.

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An increasing number of babies with conjunctivitis in our center what require an urgent evaluation was observed. To evaluate Chlamydial aetiology of Neonatal conjunctivitis in our environment all babies born in the University of Ilorin Teaching Hospital over a six months period were prospectively screened for Neonatal conjunctivitis using the Center for Disease Control/World Health Organization case definition for Neonatal conjunctivitis. All patients diagnosed to have Neonatal conjunctivitis had laboratory evaluation done to identify the causes. However only those from whom the inclusion bodies of chlamydia were seen got included in this study. An empirical treatment with 10% sodium sulphacetamide eye drops was given to all patients while other additional illnesses identified were managed in a standard way. Clinical re-evaluation was done by 72 hours and 7 days of treatment for clinical cure.

A total number of 112 babies developed neonatal conjunctivitis within the study period among the 852 babies screened and chlamydia was seen in 36 (32%). The hospital based incidence for Chlamydial neonatal conjunctivitis was 42 per thousand live births. There was a male preponderance. Gestational Age ranged between 27 weeks and 44 weeks though there were more term babies. Majority were delivered by spontaneous vertex. The Mean Age of on-set of disease was 5 days with a standard deviation of 3.8 days. Premature rupture of fetal membrane occurred in 1 (3%) case. Fifteen (42%) of 36 mothers had antenatal vaginal discharge. All mothers were married. Purulent eye discharge was the commonest clinical presentation and was sometimes unilateral. All babies responded well to treatment. No complication was observed in any baby. It was concluded that chlamydia trachomatis was the leading cause of neonatal conjunctivitis in our environment and the disease is of a remarkable magnitude requiring attention in our sub region. It has similar outlook with those reported from other regions of the World.

INTRODUCTION

Chlamydia is currently claimed to be the world leading cause of both neonatal and childhood conjunctivitis (1,2,3). This organism became significant after mid 20th century in the developed countries of the world and it gradually became the leading cause of conjunctivitis. However, no significant effort is being made in this sub region of Africa either to evaluate its incidence, mode of acquisition, pathogenesis or its epidemiology. WHO reported 7-29% prevalence of chlamydia vaginal infection among pregnant women and it is estimated that 30-40% of infants born mothers infected with chlamydia will develop conjunctivitis due to the organism (4,5,6). The prevalence of maternal genital infection in Africa ranges between 3-22% while the rate of maternal child transmission was 8.1% in most of the very few studies on neonatal conjunctivitis. Reports providing requisite data on chlamydia neonatal conjunctivitis are not common in Nigeria and the region of Africa. In this report, we present the result of an investigation of chlamydia neonatal conjunctivitis in Ilorin, middle belt zone of Nigeria as requisite information for future research.

MATERIAL AND METHODS

This prospective study was carried out in the University of Ilorin Teaching Hospital. The opportunity for this study was provided by a major and broader study on neonatal conjunctivitis when al babies born in the maternity wing of the hospital were screened. The appropriate hospital committee gave an ethical clearance and consent was obtained from all mothers at onset of the study. This paper is therefore based on the patients in whom chlamydia was isolated from their conjunctiva scrapings.

Inclusion Criteria

Inclusion criterion was development of conjunctivitis within the neonatal period defined at first 30 days of life. Conjunctivitis was diagnosed based on WHO case definition (7): (a) presence of purulent eye discharge, (b) conjunctiva hyperaemia and (c) eyelid oedema. Among those who satisfy the selection criteria, laboratory evaluation for etiological diagnosis was carried out and those from whom chlamydia was isolated were studied.

Sample Collection

Palpebral conjunctiva scrapings were taken from all patents with conjunctivitis. Smears were then made on glass slides, fixed and transferred to the paediatric and child health research laboratory for giesma staining following the procedure described by Schacter (8). The staining procedure and identification of chlamydial inclusion bodies were pre-tested and perfected over a 3-month period pilot before the study.

Laboratory Method (8)

The smears were air-dried, fixed with absolute methanol for about 5 minutes, dried again under mild heat and then transferred to the paediatric research laboratory. It is then covered with a freshly prepared giesma stain for 1 hour, rinsed rapidly in 95% ethyl alcohol to remove dye, and to enhance differentiation. It is then dried and examined under the microscope for the presence of intra-cytoplasmic inclusion bodies: Elementary bodies (eb) stained reddish purple while initial bodies (ib) were more

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basophilic, staining bluish. No facilities for chlamydia immunological tests.

Treatment of patients

All patients received as empirical treatment, 10% solution of sodium sulphacetamide eye drops pending review of clinical status and laboratory results by 72 hours of therapy. Those who made clinical improvement were left on the same treatment for 7 days while those who showed no clinical response had their antibiotics changed to erythromycin.

Data handling and analysis

Data were checked for errors and omissions and subsequently entered into IBM- compatible microcomputer and then analyzed. Mean and standard deviation was done using Microsoft Excel software.

RESULTS

A total number of 112 babies developed neonatal conjunctivitis based on the case definition within the study period. However, chlamydia trachomatis was isolated from 36 infants which were included in the analysis. The rate at which each organism was identified as causing conjunctivitis in infants was as follow; Chlamydia trachomatis in 36 (32.1%), Staphylococcus aureus 15 (13.5%), Staphylococcus epidermidis 15 (13.5%), Coliform 12 (10.7%), Escherichia coli 12 (10.7%), Klebsiella spp 10 (8.9%), Pseudomonas aeruginosa 7 (8.9%), Streptococcus pneumoniae 2 (1.7%) and Streptococcus feacalis, Heamophilus influenzae and Neisseria gonorrhoeae were isolated in 1 (0.9%) case each. A total of 852 babies were born in the hospital within the study period, hence the incidence for chlamydia neonatal conjunctivitis (CNNC) was 42.3 per thousand live births.

For patients with CNNC, there were 21 (58.3%) males and 15 (41.7%) females. The term babies were 26 (72.2% while 10 (27.8%) were preterm babies. Gestational Age ranged between 27 weeks and 44 weeks. Thirty-four (94.4%) babies were delivered by spontaneous vertex while 2 (5.6%) were delivered by Caesarian section. The Mean Age of on-set of disease was 5 days with a standard deviation of 3.8 days. The earliest was 3 days and the longest 28 days. Premature rupture of fetal membrane was present in 1 (2.8%) case and absent in the rest 35 (97.2%) babies. 15 (41.7%) of mothers had antenatal vaginal discharge while 21 (58.3%) did not. All mothers were married. Five 5 (13.9%) were Primiparous 26 (72.2%), Multiparous and 5 (13.9%) were grand multiparous women.

Clinical presentation included purulent eye discharge, which may be mild, or moderate in severity in 36 (100%) of the babies and 32 (88.9%) had conjunctiva hyperemia. All babies responded well to treatment with 10% sulphacetamide eye drops. There was no complication observed in any baby.

DISCUSSIONS

Chlamydia Trachomatis accounted for about a third of all cases. This observation is in agreement with report from Kenya (9), the Gambia (10) and the United Kingdom (11), but at variance with report from Sweden where Staphylococcus species topped the list. Maternal genital infections rate during pregnancy may be responsible for this difference. CNNC cases appeared between 3 26 days with a mean of 5 days. There was male preponderance among those babies with conjunctivitis, which is in agreement with other reports (12,13). This could be due to the fact that more males were born during the study period (male: female ratio was 1.7: 1.0) and therefore it was more likely to affect male than females. Also, most of the preterm babies (> 70%) in this study were males. Preterms may need more aggressive resuscitation at birth than term babies thereby getting their eyes colonized earlier. Ages of onset was 5 days after birth (SD = 3.7) for chlamydial conjunctivitis. This was consistent with previous reports (14,15,16). Eye discharge was the most constant and most reliable clinical presentation. It was seen in all the patients diagnosed. Occasionally, it was the only criterion present for making a clinical diagnosis. This agrees with reported findings from Sweden (11) and USA (17). However, in this study no severe form was seen, C. trachomatis is known for its little or no eye discharge unlike N. gonorrhoea, which tends to present with severe purulent discharge.

Vaginal discharge was found in 15 (41.7%) of the mothers. The presence of discharge in mother may be regarded as evidence of maternal genital infection (though the specific type of infection still require evaluation). This study shows that most of the babies with CNNC were from mothers with antenatal vaginal discharge. Another report from our center showed that babies from mothers with antenatal vaginal

discharges were eight times ore likely to develop conjunctivitis (18). This was consistent with other previous reports (12). The presence of discharge increases the prosperity of getting infecting organism into the infant's conjunctiva especially during passage through the birth canal, where the number of organism can multiply rapidly. The proportion of the disease caused by C. trachomatis identified by this study strongly suggests that maternal genital tract infection with the organism is probably also very high. Hence, such National or Regional policy on prevention should include not only ocular prophylaxis for neonates, but also appropriate screening and treatment for mothers who may be infected with Chlamydia during the ante-natal period. It is established that such mothers run the risk of developing complications like perihepatitis or pelvic inflammatory disease and consequently infertility (18).

This study showed that Neonatal conjunctivitis caused by Chlamydia trachomatis responded very well to 10% sulphacetamide ophthalmic drops. This is a significant finding because the drug is much cheaper than others and readily available in our environment. The outcome of this work carried out among a cohort of neonates delivered at the UITH shows clearly that Neonatal Conjunctivitis is still a serious problem in our zone of the country. We presume that our finding is probably an important (national) index of the extent of the problem, which calls for critical attention. Although the exact incidence of visual defects or impairments in the Nigerian Child is not known, the fact is that blind children and adults begging for alms on Nigeria streets is neither rare nor common. The role of Chlamydia neonatal conjunctivitis in addition to muscles, trachomatis, and vitamin A deficiency in causing visual disability among our children calls for attention. Because of the University proven efficacy of prophylaxis in preventing neonatal conjunctivitis and possible social and economic sequalae there is an urgent need to initiate and implement a National policy on ocular prophylaxis enforceable at the Regional and grass root level to stem down the disease (18).

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PREVALENCE OF TRICHOMONAS VAGINALIS AMONGST COMMERCIAL SEX WORKERS (CSWs) IN IBADAN, NIGERIA.

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Our main objectives was to determine the prevalence of Trichomonas vaginalis among commercial sex workers (CSW's) in Ibadan, Nigeria. One hundred and sixty nine CSW's randomly selected from 18 brothels and 136 female patients without symptoms were investigated for Trichomonas vaginalis using both direct microscopy and culture methods. Thirty-seven (21.9%) of the 169 CSW's investigated had Trichomonas whilst 26(19.1%) of the 136 control subjects were positive for Trichomonas vaginalis. There was no significant difference between the CSW's with Trichomonas and the control group (p>0.001). We found no association between T. vaginalis and HIV diagnosed in the CSW's investigated. The age range of peak incidence among the CSWs and the control subjects investigated was 20-29 years. While only 6(3.6%) of the CSWs investigated were married, 88(52.1%) were single, 37 (21.8%) separated, 28 (16.6%) divorced and 10(5.9%) widowed. There was no significant difference between the CSW's with vaginal Candidiasis, Gonorrhoea and the control group but genital ulcers and HIV positively were significantly higher (p=0.000) in CSW's than the control subjects. These findings suggest that women who exchange sexual services for money can no longer be ignored. They should be involved in the control and prevention of STDs.

INTRODUCTION

Trichomonas is widely distributed all over the world and remains a common infection among female patients attending sexually transmitted diseases clinics.

In developing tropical Africa, a prevalence rate among the female patients ranges between 5-37 percent. The World Health Organization (WHO) estimate 180 million cases of Trichomonal infection are acquired annually world wide (1) and it has been found to increase the risk of transmission of HIV and predispose pregnant women to premature rupture of membranes and early labour (2,3) Some estimates suggests that one out of five sexually active women contracts Trichomoniasis during her lifetime. Majority of female patients harbouring Trichomonas vaginalis present with vaginal discharge, which is usually frothy, greenish-yellow and offensive. Unlike pre-pubertal gonococcal vulvo-vaginitis cases occasionally encountered in Ibadan, Trichomoniasis among this age group is very rare(4). Dunlop and Wisdom found no T. vaginalis even in post pubertal virgins from the study done in 1965, which confirms sexual contact as the main mode of transmission. the control of Trichomoniasis seems to health care, whereas it has remained endemic in many developing countries where control may only be possible by regular screening and treatment.(2)

The prevalence of Trichomoniasis in specific groups correlates with the general level of sexual activity. Trichomoniasis has been diagnosed in 22.3 percent of women attending gynaecological clinic in Ibadan (16), 19.6 percent of women attending special treatment clinic, Ibadan (7) and 5 percent among female patients attending out patient department in Lagos (8). Commercial sex workers (CSWs) are an important group for the transmission of a number of sexually transmitted diseases (STDs) including Trichomoniasis all over the world (9). They have held a time-honored position in STDs control, both as major reservoirs of the disease and a convenient, untraced source of infections (10). Evidence that CSWs may play a significant role in the spread of STDs such as Gonorrhoea and Trichomoniasis is accumulating (11-14). There are at present no comprehensive or reliable data on the prevalence of STDs especially Trichomoniasis among CSWs in Nigeria, however, evidence from studies in other countries show that it is quite high (15,9). In a study done in Osoba in 1972, 3(15.8 percent)of the 19 CSWs screened had Trichomoniasis.similar study done by Tanyuksel et al in Turkey on CSWs showed that 25 percent of them were positive for Trichomonas vaginalis. The aim of the present study was to determine the prevalence of Trichomonas vaginalis among CSWs in Ibadan.

MATERIAL AND METHODS

STUDY POPULATION.

Commercial sex workers (CSWs) working in 18 brothels that were randomly selected in Ibadan municipality were included in this prospective study. The study was done between February 1998 and March 2000, in cooperation with the health-nursing officers attached to the special treatment clinic, University College Hospital, the Directors of the brothels and the "presidents" of the inmates.

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Name, age, place of work, other occupation , parity ,and marital status were noted. Symptoms of dysuria, low abdominal pain and virginal discharge were routinely enquired. All the CSWs had a complete pelvic examination. Specimens for culture were taken with sterile cotton-tipped applicator from the urethral, vagina and endocervix.

CONTROL GROUP

136 female patients that attended special treatment clinic but without symptoms during the period of study were randomly selected and entered into the study.

LABORATORY PROCEDURES

The cervical and urethral swabs were Gram-stained for intra-cellular Gram-negative diplococci, and cultural onto Modified Thayer-Martin's medium. The plates were incubated at 37"c in an atmosphere of a candle extinction jar. Suspected oxidase-positive colonies were Gram-stained for Gram-negative diplococci and confirmation by Sugar utilization test in serum-free medium (17). Betalactamase activity of the isolates was tested using the Starch-paper technique (18).

Blood samples were taken and tested for HIV antibodies using a commercially available enzyme-linked immunosorbent assay (ELISA) and a Western blot (WB) assay using the procedures recommended by the manufacturer. The sera were also used for VDRL and TPHA tests.

SCREENING FOR TRICHOMONAS VAGINALIS

The high vagina swabs were examined for the presence of Trichomonas vaginalis by agitating the cotton swab in 1 ml of saline in a test tube and a drop of the resulting suspension transferred to a microscope which was covered with a cover slip and then examined at x 100 and x 400 magnification. The swabs were also examined for Candida albicans, Gardnerella vaginalis (Clue cells) and other parasites. Culture for T. vaginalis was performed using Nutrient broth glucose serum medium (19). Making a wet mount of sediment from the bottom of the Bijou bottles containing the medium made examination for growth of Trichomonas vaginalis at 48 hours and 5 days of incubation and motile T. vaginalis were searched for.

DATA ANALYSIS

Applying the t-test and the chi-square test did the statistical analysis.

RESULTS

During the period of study, 169 commercial sex workers (CSWs) randomly selected from 18 brothels and 136 female patients without symptoms were investigated for Trichomonas vaginalis at the Special treatment clinic, University College Hospital, Ibadan. Thirty-seven (21.9%) of the 169 CSWs had Trichomoniasis whilst 26 (19.1%) of the 136 control subjects were positive for Trichomonas vaginalis. There was no significant difference between the CSWs with Trichomoniasis and the control group. The vagina candidiasis was the most common STD diagnosed in both CSWs and the control group. The other STDs in their order of frequency in the CSWs were HIV infection 34%, Non-specific vaginosis (Gardnerella vaginalis infection) 24.9% and Tinea cruris 18.9%. Gonorrhoea and "Genital ulcers" had an incidence of 16.6% each. Syphilis seropositivity (table1). All the 13 CSWs that had scabies, 4 (36.4%) with Genital warts and 19 (67.9%) with "Genital ulcers" had HIV infection. There was no significant difference between the CSWs with vaginal candidiasis, Gonorrhoea, Trichomoniasis and the control group. The HIV positive were significantly higher ($p<0.001$) between "Genital ulcers" among CSWs (16.6%) and the control group (1.5%) was observed. The betalactamase producing strains constituted 97.6% of the gonococcal isolates.

Table 2 shows the age distribution of the CSWs and symptomless women investigated, 63.3% being within the age of 20 to 29 years while only 4.2% were over 50 years of age. Of the control subjects investigated, 58.1% were within the ages of 20 to29years. Table 3 shows that majority of the CSWs (74.5%) were of low parity (0-1).

Of the 169 CSWs seen, 88 (52.1%) were singles, 37 (21.8) separated, 28 (16.6) divorced and 10 (5.9%) widowed. Only 6 (3.6%) were married and these were not living in the brothels (Table 4). Ninety-three (55%) of them engaged in various types of contraceptive devices including the use of condom.

All the CSWs investigated admitted to regular use of prophylactic antibiotherapy in various combinations, common ones being weekly injection of spectinomycin.

Diagnosis	No of subjects	%	Control group	%
Gonorrhea	28	16.6	21	15.4
Candidiasis	99	58.6	70	51.4
Trichomoniasis	37	21.9	26	19.1
Genital warts	11	6.5	3	2.2
Non-specific vaginosis(clue cells)	42	24.9	12	8.8
Scabies	13	7.7	—	—
"Genital ulcers"	28	16.6	2	1.5
Tinea cruris	32	18.9	13	9.6
Syphilis seropositivity	7	4.1	—	—
HIV-seropositivity	58	34.3	3	2.2

TABLE 1
DIAGNOSIS OF STDs IN CSWs AND THE CONTROL GROUP.

Age group (years)	No of subjects	%	Control group	%
10-19	19	11.2	20	14.7
20-29	107	63.3	79	58.1
30-39	23	13.6	16	11.8
40-49	13	7.7	14	10.8
>50	7	4.2	7	5.1
Total	169	100	136	100

TABLE 2
AGE DISTRIBUTION OF CSWs AND CONTROL GROUP EXAMINED.

No. of parity	No. of subjects	%	Control group	%
0-1	126	74.5	52	38.2
2-3	40	23.5	67	49.3
>4	3	1.8	17	12.5
Total	169	100	136	100

TABLE 3
PARITY OF CSWs AND THE CONTROL GROUP INVESTIGATED.

Marital status	NO. of subjects	%	Control group	%
Single	88	52.1	63	46.3
Married	6	3.6	32	23.5
Separated	37	21.8	25	18.4
Divorced	28	16.6	13	9.6
Widowed	10	5.9	3	2.2
Total	169	100	136	100

TABLE 4
MARITAL STATUS OF THE CSWs AND CONTROL GROUP INVESTIGATED.

DISCUSSION

Trichomoniasis remains one of the most common sexually transmitted diseases all over the world and like other STDs constitute a major public health problem in both developed and developing countries (20). Trichomoniasis is more prevalent among patients with higher levels of sexual activity and larger numbers of different sexual partners. It is not surprising, therefore, that CSWs are an important group for the transmission of a number of sexually transmitted diseases including Trichomoniasis.

A considerable number of unmarried unemployed women that engage in commercial sex work live comfortably in many brothels scattered around urban areas of Ibadan and cater for the sexual needs of men who pay cash in exchange for sex (21). The data on the prevalence of STDs such as Trichomoniasis vary around the world, depending on the reliability of the diagnostic test used on them and their response to medical examination. Some of them declared to be examined or investigated and these were the older women who now acts as the "godmothers" to the younger CSWs.

The spectrum of STDs among the CSWs investigated has widened since the previous study done in 1972 by Osoba in our center. While Gonorrhoea and Trichomoniasis were the only STDs diagnosed, a wider spectrum of STDs among the CSWs investigated was demonstrated. In the present study, 37(21.9%) of the 169 CSWs investigated had Trichomoniasis whilst 26(19.1%) of the 136 control subjects were positive for *Trichomonas vaginalis*. There was no significant difference between the CSWs with Trichomoniasis and the control group. The vaginal candidiasis was the most common STD diagnosed in both CSWs and the control group. The other STDs in their order of frequency in the CSWs were HIV infection 34.3%, non-specific vaginosis 24.9%, Tinea cruris 18.9%, Gonorrhoea 16.6%, scabies 7.7%, Genital warts 6.5% and Syphilis seropositivity 4.1%. this achievement was partly due to the fact that majority of the CSWs were examined and investigated in their hotels rooms and there was an improvement in the diagnostic methods used in this study. While Osoba in 1972 used only direct microscopic method in the screening for T.vaginalis, both the direct microscopic and culture methods were used in this study. Our finding is consistent with those of Turner and Morton (1976) in Sheffield, Meheus et al (1974) and many other workers (16, 22-31), who demonstrated a reasonably high incidence of STDs among CSWs.

Most of the CSWs encountered during the study were young, with about three quarters being below 30 years. This is not unexpected as there is usually a higher demand for the younger women by men who patronize commercial sex workers. This is keeping with the findings reported by other researchers in Nigeria and elsewhere (21,32,33). In this study, over half of the CSWs were single whilst another third were either divorced or separated ; a tiny proportion was widowed. This compares favourably with the findings 0f another study on sex in Ibadan in 1998 (32) which observed that over half of CSWs were either separated, divorced or widowed whilst about 40% were single and this was attributed to the traditional extended family system which hitherto had served as a social institution gradually breaking down due to the process of urbanization and hence such categories of women who otherwise would have been taken care of in traditional setting find that they now have to fend for themselves.

It has been reasonably established that the presence of STD in a person is associated with an increased risk of HIV infection following exposure by a factor of three to five and can be as high as 10-300 fold in the presence of a genital ulcer (34). Trichomonas vaginalis has been considered a major risk factor in the transmission of human immunodeficiency virus (HIV) (35). In a study done by Niccolai et al in 2000, a high rate of reinfection with Trichomonas vaginalis in HIV-infected women was demonstrated. We, however, found no association between T.vaginalis and HIV diagnosed in the CSWs investigated. Similarly, there was no significant difference between CSWs with vaginal candidiasis, Gonorrhoea and Trichomoniasis and the control group but a high significant difference between CSWs with HIV infection and control subjects was demonstrated. The implication of this is probably that apart from HIV infection that is presently untreatable, the role of CSWs as vectors of STDs has gradually declined in importance as compared with the promiscuous amateurs who pose a serious threat to

the control of STDs. One of the reasons for failure to interrupt STD transmission could be that these transmissions are frequently as a result of sexual exposures with these promiscuous amateurs who parade themselves as 'responsible' ladies on the street.

The magnitude of the problem of Trichomoniasis in infected individuals need to be considered. Trichomoniasis like other STDs such as Gonorrhoea and Chlamydial infections can be associated with some complications.(4). Trichomonas vaginalis has been identified in pus from the fallopian tubes in patients with acute salpingitis, though; a casual relationship was not demonstrated (37). However, Ogunbanjo et al (1989) in a study on infective factors of male infertility among Nigeria found T. vaginalis to be one of the causative agents of conjugal infertility. A similar study done by el-Sharkawy et al (39) found infertile women with T.vaginalis to have decreased C3 & C4 and increased 1Ga level in the vaginal discharge with serum prolactin incriminating T.vaginalis as one of the causes of their infertility. The control of STDs can best be accomplished by public health programs that commit themselves to finding STD transmitters through persistent efforts to screen, diagnose, treat, and follow-up on high risk individual within communities. Women who exchange sexual services for money are at risk and can no longer be ignored. They should be identified and involved in STD prevention and control efforts (10).

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SEXUALLY TRANSMITTED DISEASES (STDs) AND ACQUAIRED IMMUNODEFICIENCY SYNDROME (AIDS) IN NIGERIA.

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The sexually Transmitted Diseases constitute major public health problems in Nigeria. There is early sexual maturity and considerable sexual activity between 9 and 15 years of age. Although there is a high awareness of the condom, people are unwilling to use them despite evidence of multiple sexual exposures.

The STDs, including HIV infections, are on the increase. Tuberculosis is also on the increase because of a HIV/AIDS epidemic. Both categorical and integrated approach to the management of STDs and AIDS are being recommended. There is a need to step up basic research into the biology of HIV and subsidise the treatment of AIDS. Efforts at vaccine development should be initiated to stem the worsening HIV epidemic.

INTRODUCTION

The sexually Transmitted Diseases (STDs) constitute major public health problems in Nigeria, especially with the advent of HIV infection during the last decade. Failure to diagnose and treat the traditional infections such as syphilis, chlamydia and gonorrhoea can have deleterious effects on pregnancy and the newborn (such as miscarriage, prematurity, congenital and neonatal infections, blindness) and are common (1) other complications particularly in women, such as pelvic inflammatory disease, ectopic pregnancy, infertility and cervical cancer have grave social, demographic and economic consequences.

RISK FACTORS ASSOCIATED WITH STDs AND HIV INFECTION

There are several factors associated with the transmission of STDs and HIV in Nigeria. Sexual maturation of Nigerian boys start between 9 and 15 years (2). In a study on prepubertal gonorrhoea in Ilorin, it was found that there was considerable sexual activity by children aged between 9 and 15 years (3).

The keeping of multiple sexual partners have also been reported among married men and long distance drivers in Nigeria (4,5). This is compounded by the fact that people who have multiple partners are unwilling to protect themselves with the condom. In a study conducted in a rural community in South West Nigeria in 1993, it was found that although 94.7% of 302 candidates aged between 20 and 54 years admitted hearing about the condom, only 51.3% admitted ever using it (6). In the study at Ilorin among long distance truck drivers, 91% of 180 drivers admitted having multiple partners. Casual sex rate was 43%, and commercial sex rate was 30%. Although 50% admitted ever using a condom, only 19% used them regularly (5).

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SEXUALLY TRANSMITTED DISEASES

Table 1 shows the prevalence of STDs in treatment centers in Nigeria. The most common causes of genital discharge were Non-gonococcal genital infections (26.37%), post-pupertal gonorrhoea (18.03%), trichomoniasis (9.78%) and candidiasis (9.62%). The commonest genital ulcers were chancroid (4.28%), primary syphilis (2.28%), and genital herpes (2.2%).

PENICINASE-PRODUCING NEISSERIA GONORRHOEA (PPNG)

There have been several reports that show the high prevalence of PPNG strains in Nigeria and are shown in table 2 (8-12). The figures reported from various centers are 74.2% in Ilorin, 78% in Nnewi, 83% in Jos, 87% in Benin-City and 90% in Lagos. These figures show that penicillin has no more place in the treatment of gonococcal infections in Nigeria.

HEPATITIS B VIRAL INFECTIONS

Routine diagnose of Hepatitis B Viral Infections in Nigeria is often based on the identification of the surface antigen (HbsAg). Until facilities became readily available to detect HbsAg, which are associated with infectivity, we will not know what percentage of these cases are actually infective.

Table 3 shows current carriage rates for HbsAg using antenatal patients (16%), STD patients (36-42.2%) and blood donors (12.3-21.7%) in some centers in Nigeria (13,14).

These high rates should be of great concern because of the dangers of developing liver cirrhosis and hepatocellular carcinoma.

HIV SEROPREVALENCE

Sentinel surveillance shows that there has been on increase in HIV seroprevalence in all the states of Nigeria over the years, with the exception of Kano State, which had as initially high rate of about 9% in 1992. Sentinel surveillance at antenatal clinics

shows HIV prevalence of 1.8% in 1990, 3.8% in 1993, 4.5% in 1995, 5.4% in 1999 and 5.8% in 2001 (National AIDS/STD Control Programme). There is also an increase in HIV seroprevalence among the various population groups such as commercial sex workers, STD patients, patients with tuberculosis, long-distance truck drivers and antenatal patients (15,16,17).

If a prevalence of 5.8% reported for antenatal patients in Nigeria represents what happens in the general population of those considered not to be at high risk of being infected with HIV; it means that about 2-5million Nigerians might have been infected with HIV, if 20% of infected persons develop clinical AIDS within the next 5 years it means, by the year 2005, we may have about 600,000 AIDS cases to treat. The AIDS burden alone would be a serious social and economic burden.

CLINICAL FEATURES OF AIDS CASES IN NIGERIA

Clinical AIDS is a common finding in many health facilities in Nigeria. Although presentation varies from centers to center, the common modes of presentation is shown in Table 4. Progressive weight loss (69%), chronic diarrhoea (61%), skin rashes (38%), generalized lymphadenopathy (37%) and prolonged fever (22%) are the most frequent presenting features. Persistent cough (17%) and tuberculosis (17%) are also significant features (15,18).

AIDS AND TUBERCULOSIS

HIV seropositivity in Nigeria has been significantly associated with tuberculous infection (Table5). The upsurge in tuberculosis infections seen in several health centers in Nigeria is therefore to be explained by the worsening HIV epidemic (19,20). The strains of *Mycobacteria* associated with HIV infection as reported by these authors are however not different from those affecting people without HIV infection. They are *M. Tuberculosis (70%), M. Avium (20%), and M. Kansasii (10%).* 60% and 40% of the HIV strains were HIV-2 and HIV-1 respectively among patients with tuberculosis.

MANAGEMENT OF STDs

By 1992, the National AIDS and STD Control Programme (NACP) found that there were 19 STD treatment centers in the country. Treatment was mainly categorical in nature, being based on specific diagnosis and treatment. The experiences of practitioners at these centers assisted the NACP in producing a "Manual of Sexually Transmitted Diseases" which recommended first and second-line regimen for the various STDs (7).

This specialist based approach is often sited in urban centers with laboratory support that are not available at PHC level. The syndromic approach is therefore now being introduced. This longitudinal or integrated approach recognizes the limitation of resources for health care and is in line with WHO recommendations (21.) It uses algorithms for the treatment of genital ulcers and genital discharge.

The algorithms for genital discharge is based on the premise that the commonest cause of genital discharge are chlamydia and *N. gonorrhoea.* It is then appropriate to give treatment for the two conditions. The algorithms for genital ulcers are based on the fact that chancroid and syphilis are the most common cause of genital ulcers. Treatment is then given for the two conditions.

This integrated approach has not been fully implemented in Nigeria. It has however been found to be very useful in Tanzania where it played a significant role in reducing HIV incidence. It was shown that improved STDD care, integrated at PHC level, resulted in a reduction of HIV incidence by 42% over a 2 years period of study (21).

MANAGEMENT OF AIDS

The management of AIDS has been that of the treatment of the various opportunistic infection. Recently Glaxo Wellcome has introduced two antiretroviral drugs; Retrovir (Zidovudine, AZT) and Epivir (lamivudine) that are used in combination therapy. These drugs have been found useful in prolonging the life f patients but their limitation is their high cost.

STD	PREVALENCE (%)
Non-gonococcal genital infection	26.3
Gonorrhoea Post-pubertal	18.03
(a) Pre pubertal	2.02
Trichomoniasis	9.78
Candidiasis	9.62
Venereophobia	4.28
Syphilis	
(a) Primary	2.28
(b) Post-Primary	2.02
Herpersvirus type II:	
(a) Ulcers	2.2
(b) Seroprevalence	13.0
Genital warts	1.87
Lymphogranuloma venereum (LGV)	1.47
HIV	5

TABLE 1
PREVALENCE OF SEXUALLY TRANSMITTED DISEASES IN TREATMENT CENTRES IN NIGERIA.
*SOURCE: NATIONAL AIDS/STD CONTROL PROGRAMME
STD: SEXUALLY TRANSMITTED DISEASE

AUTHOR	LOCATION	RATE (%)
Odugbemi, et al, 1986	Ilorin	74.2
Ameli and Anyiwo, 1997	Nnewi	78
Bello et al, 1996	Jos	83.3
Obaseki-Ebor et al, 1985	Benin-City	87
Olukoya, et al, 1988	Lagos	90

TABLE 2
PENICILLINASE-PRODUCING NEISSERIA GONORRHOEA (PPNG) IN NIGERIA

GROUP OF PEOPLE	RATE (%) BY AUTHOR	
	Bada, et al 1995	Olaleye, et al 1996
Antenatal Patients	16	n.d
STD Patients	36	42.2
Blood Donors	21.7	12.3

n.d. = not done.

TABLE 3
HEPATITUS B SURFACE ANTIGENAEMIA IN NIGERIA

CONDITION	RELATIVE FREQUENCY
Progressive Weight Loss	69%
Chronic Diarrhoea	61%
Skin Rashes	38%
Generalized lymphadenopathy	37%
Prolonged Fever	22%
Prolonged Cough	17%
Tuberculosis	15%
Oral Thrush	14%
Generalized Kaposi's Sarcoma	5%
Neurological manifestation	5%

TABLE 4
CLINICAL PRESENTATION OF AIDS CASES IN NIGERIA*
*SOURCE: NATIONAL STD/AIDS CONTROL PROGRAMME

DESCRIPTION	GROUP OF PATIENTS TUBERCULOSIS	CONTROL (TB-VE)	TOTAL
Number screened	188	348	536
Number seropositive for HIV & HIV seropositive	10 5.3**	3 0.9**	13 2.4

TABLE 5
PREVALENCE OF HIVANTIBIOTICS IN TUBERCULOSIS PATIENTS AND CONTROLS*
* From Idigbe et al, 1994
** P<0.001

% OF TOTAL TB ISOLATES BY HIV SEROSTATUS MYCOBACTERIAL STRAINS	HIV + VE (N = 10)	HIV + VE (N = 130)
M. Tuberculosis	70	73
M. Avium	20	0.6
M. Kansasii	10	11.8
M. Bovis	-	8.4
M. Fortuitum	-	5.1
M. Xenopi	-	1.1

TABLE 6
MYCOBACTERIAL STRAINS ISOLATED IN NIGERIA BY HIV SEROSTATUS
Adapted from Idigbe et al, 1994

CONCLUSION

STDs and AIDS constitute a major burden for the health sector in Nigeria. Both categorical (specialist based) and longitudinal (syndrome-based) approaches should be adopted for the management of STDs. There is a need to heavily subsidize the treatment of AIDS because the available specific antiretroviral agents are too expensive.

In a dynamic world, little progress can only be made in our efforts to combat the AIDS epidemic in Nigeria without adequate finding for basic research into the pathogenesis of HIV. It is therefore recommended that efforts should be made to concentrate on the basic biology of HIV, vaccine development and clinical trials that have not previously been in focus in Nigeria.

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SERUM PROTEIN FRACTIONS OF NIGERIANS WITH PLASMODIUM INFECTIONS: ILORIN EXPERIENCE.

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Malaria fever is a very common and often severe disease in the tropical countries like Nigeria. Measurement of serum proteins is simple and widespread in developing countries. Thus this study aimed at evaluating the relationship (if any) between plasmodium infection and serum protein. A total of 80 subjects were used for this study, 40 people with confirmed plasmodium infection, and 40 clinically healthy adults as control subjects. Their height, weight, age and sex were recorded. Their serum total protein and albumin were assayed while the serum globulin was obtained from the difference.

We obtained a mean BMI of 20.okg/m² for the control subjects and 21.63kg/m² for patients with plasmodium infection. The serum total protein was 71.25g/L and 60.43g/L for the control and malaria patients respectively. With (P<0.05) and T-value of 6.4. the serum albumin value was 44.24g/L and 36.66g/L for the control and the patients respectively with (P<0.05) and T-value of 6.5. the value for the serum globulin was 27.03g/L and 23.77g/L for the control and the patients respectively with (P<0.005) and T-value of 3.1. these results show that the serum total protein, albumin and globulin are all significantly higher in control subjects.

We conclude therefore that haven't established the existence of these significant differences, we suggest that further studies be done

INTRODUCTION

The protein fractions in the blood are commonly estimated in the serum and does not include fibrinogen that will be precipitated when the blood clots. The main serum protein are albumin and globulin. The total serum protein averages 63.86g/L; albumin 34.53g/L; the remainder being globulin (1). Various biochemical changes are observed in disease condition. It is a known fact that, many disease conditions involved among other things, derangement of protein metabolism.

Understanding the importance of adequate protein in the blood and the high prevalence of certain disease condition in this environment no doubt opens the gate toward the research work. Malaria fever is a very common and severe disease in our immediate environment. The morbidity and mortality resulting from plasmodium infection cannot be over emphasized in a topical country like Nigeria (2).

The importance of establishing the basic bio-chemical data in a particular center in normal subjects for the purpose of comparison in disease states is well established (3). Various biochemical changes are observed in malaria which include inhibition of mitochondrial electron transport activity, change in various serum lipid fraction, serum C-reaction and Amyloid-A-proteins (4,5). There are also reported changes in serum and urinary electrolytes (6). It is quite displeasing that no readily available comprehensive report on the serum protein changes in malaria is available. These findings then raise some vital questions. What is the effect of malaria on principal serum proteins? What is the usefulness of changes in serum protein levels, if any in assessing the severity of malaria. Therefore the present study was designed to answer these questions.

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MATERIALS AND METHOD

This research was carried out in the Chemical Pathology department of University of Ilorin Teaching Hospital (UTH). A total of 80 subjects made up of males and females were used for this study. 40 clinically healthy members of University of Ilorin community comprising of 25 males and 15 females were used as control for this study. These control subjects were not on drug, no recent report of illness or chronic diseases, obese subject were not used. 40 patients with confirmed plasmodium infection were randomly selected from the general Out Patient department f UTH. Fluid was allowed freely. The height and weight of each subject were recorded in meter and kilometer respectively.

About 5ml of venous blood was collected from the anticubital area of each subject without stasis. The blood was allowed to clot and retract before serum was separated into clean covered bottles. The analysis were done on the same day of sample collection. Total protein was determined by the Chemical Biuret method (7). Serum albumin was determined by bromocresol green-dye-binding method. Serum globulin level was determined by subtracting serum albumin from total protein.

Data obtained from this research was grouped and analyzed in a tabular and graphical form using Microsoft excel 2000. The data were presented as mean +SEM statistical analysis was by the unpaired student t-test. Differences were considered significant at P<0.05.

RESULT

Table I shows that the values of the mean weight and mean height of both the control subjects and the patients with confirmed plasmodium infection. The average weight of the control subjects was 58.kg while their mean height was 1.71m. for the patients with confirmed malaria infection the average weight was 64kg while their mean height was 1.72m. there was no significant difference in either their weights or in their heights (P>0.05). The mean Body Mass Index (BMI) for the control group was 20.01kg/m^2 while the the corresponding value for patients with malaria was 21.63kg/m^2

Table II shows the values of different protein fractions of the control subjects and patients with malaria. From this table the mean total protein value for the control subjects was 71.25g/L compared to the value of 60.43g/L for patients with malaria. The difference between these values is significant (P<0.05). the mean serum albumin for the control subjects was 44.24g/L while the corresponding value for patients with malaria was 36.66g/L. The difference between these values is also significant (P<0.05). The mean serum globulin for the control subjects was 27.03g/L, while the corresponding value for the malaria patients was 23.77g/L, the difference between these values is also significant (P<0.05). These shows that there exist a statistically significant difference between the two groups.

	Mean Weight (kg) (SEM)	Mean height (M) (SEM)	BMI (kg/ m^2)
CONTROL n = 40	58.5 (±1.2)	1.71 (±0.01)	20.01
MALARIA n = 40	64.0(±1.08)	1.72 (±0.01)	21.63
P. Value	0.095	0.105	0.780

TABLE 1
ANTHROPOMETRIC DATA OF CONTROL SUBJECTS AND THOSE WITH CONFIRMED PLASMODIUM INFECTIONS

TABLE 2
THE VALUES OF DIFFERENT PROTEIN FRACTIONS OF THE CONTOL SUBJECTS AND PATIENTS WITH MALARIA

	Control			Malaria Patients			T-Value	P-value
	Mean	SD	±SEM	Mean	SD	±SEM		
TOTAL PROTEIN g/L	71.27	6.00	0.95	60.43	8.86	1.40	6.4	<0.05
ALBUMIN g/L	44.24	3.89	0.62	36.66	6.28	0.99	6.5	<0.05
GLOBULIN g/L	27.03	5.04	0.80	23.77	4.20	0.66	3.1	<0.05

DISCUSSION

Malnutrition is a common problem in the tropics. However, the Body Mass Index obtained from this study clearly shows that the result of serum protein analysis for the patients with plasmodium could not be traced to malnutrition. The result obtained showed derangement in all the protein fractions of malaria patients compared to the control subject.

The mean total protein of 71.27g/L in control subjects compared to malaria patients of 60.43g/L show significant difference in the mean total protein (P<0.05). This is in agreement with report of Edozien et al (8). Anorexia often occur in majority of malaria patients, this might lead to rapid gluconoeogenesis and so protein stores may suffer depletion. In addition Edozien et al reported that the anti-malaria drugs used by the malaria patients affects the protein constituents of the blood by decreasing the protein fractions of malaria patients.

In the albumin fraction the result obtained indicated higher mean albumin value in control subjects (44.24g/L) compared to patients with malaria (36.66g/L). The difference between these values is significant (P<0.05). The study further

revealed that the difference between serum albumin is more significantly different than that between the serum total proteins. This is in agreement with earlier reports (9, 10, 11).

Also in the globulin fractions, the result obtained showed a greater mean globulin in control subjects (27.03g/L) when compared to malaria patients' globulin (23.03g/L). This result agrees with earlier work (1,8). Also this study revealed that serum globulin is the less affected compared to serum albumin. Edozien et al in their work documented that prophylactic doses of anti-malaria drugs cause lower mean serum globulin concentration. However the issue of hepatic involvement in plasmodium infection leading to decrease production of the other non-gamma globulin cannot be totally ruled out.

We conclude that all serum protein fractions are significantly low in plasmodium infection and suggests that more work be done to determine the values of serum fractions in patient with plasmodium at various levels of severity with a view to determine the prognostic value of serum protein in plasmodiasis.

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MALARIA CHEMOPROPHYLAXIS AND CHILDBEARING WOMEN IN A PERI-URBAN NIGERIAN COMMUNITY: KNOWLEDGE ATTITUDE AND SOCIO-CULTURAL FACTORS FOR ACCEPTANCE.

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This is a descriptive cross-sectional study, carried on childbearing women in Okelele community of Ilorin West Local Government of Kwara State, Nigeria. The aim was to assess the knowledge and attitude of childbearing women towards antimalaria chemoprophylactics and highlight socio-cultural factors influencing their knowledge and attitude. Three hundred and eighty childbearing women were interviewed but only 375 (98.7%) responded. A systematic sampling method was used to determine the houses where respondents were interviewed after obtaining informed consent; highly structured close-ended questionnaires were administered by trained interviewers. The respondents age range between 15 and 47years. More than half (52.8%) of the respondents were literates. Knowledge of antimalaria chemoprophylactics among the respondents was good 0f375 respondents, 353(94.1%) had knowledge and 317(84.5%) had ever used antimalaria chemoprophylactic drugs. The study revealed that respondents with higher educational level had significantly better knowledge of antimalaria chemoprophylactics and higher level of usage p <O. OS). Positive attitude towards malaria chemoprophylaxis was very high (84.5%) in this study and level of education was found to be an important influencing factor of the positive attitude. Conviction of the benefits of antimalaria chemoprophylactics was the main reasons advanced by the respondents for their positive attitude towards malaria chemoprophylaxis. Despite good knowledge and high level of positive attitude towards malaria chemoprophylaxis, a number of misconceptions and ignorance were still expressed towards malaria chemoprophylaxis by the childbearing women. There is need for regular health education programme especially for childbearing women so as to remove this misconception and ignorance. Health workers should also intensify effort in disseminating information on the benefits of antimalaria chemoprophylactics.

INTRODUCTION

Strategies for reducing the impact of malaria in childbearing women especially during pregnancy have generally relied on chemoprophylaxis. The acquired immunity to malaria is liable to break down under stress condition like pregnancy. Malaria is more pronounced in the second trimester of pregnancy and especially in the primigravidae (1). The actual cause of the increased frequency and severity of ma) aria infection in *pregnancy* is still not fully known. It may be as a result of general pregnancy-associated immuno-supression or due to malaria specific component. Rilley et al (2) has implicated increased steroid hormone as a result of placenta production of cortisol and other steroids known to be immuno-suppressive agents as a possible cause of increase frequency and severity of malaria infection in pregnant woman, which can depress the cellular as well as hormonal immune response of the host. Impaired antibody production and depressed cell mediated immunity have been implicated by Parsed et al (3), as possible factors for increased frequency and severity of malaria in childbearing women during pregnancy. Chemoprophylaxis has been directed almost exclusively towards childbearing women especially during pregnancy because multiple scientific studies (3) have suggested that malaria infection during pregnancy may be associated with maternal morbidity and mortality, first and second semester abortions, still births, premature deliveries, low birth weight and rarely congenital malaria, According to WHO expert Committee (4) on malaria, repeated attacks of malaria during pregnancy result in placental insufficiency and women exposed to infection are at a high risk (particularly during their first pregnancy) of delivering premature or low birth weight infants. Chemoprophylaxis as a malaria control strategy has become progressively more difficult in recent years owing in part to the increase of drug resistance and the increasing recognition of the adverse side effects of several important antimalaria prophylactic drugs. Due to these adverse side effects associated with some antimalaria chemoprophylactic drugs, there is need to be cautious about their use especially in childbearing women and most especially pregnant mothers. The antimalaria drugs that are commonly in use for prophylaxis include Chloroquine, Proguanil, Pyrimethamine, Amodiaquine, and Pyrimethamine/ Dapsone combination (5). Women generally play an important role in determine the health of the family but regrettably their opinion have been traditionally ignored in most communities in developing countries-due to cultural beliefs and practices. Furthermore, women have rarely been asked what they know and feel about antimalaria chemoprophylaxis. The socio-cultural background particularly in developing countries is male dominant and male attitudes and values have strongly influence all women's decisions that are taken in the home including action to take even with regards to their own health. The aim of malaria chemoprophylaxis is to reduce

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morbidity; mortality and antecedent complications associated with malaria in groups that are at high risk especially the pregnant mothers. This study is designed to enhance the level of awareness and understanding of childbearing women to antimalaria chemoprophylaxis thereby promoting and maintain, the quality of life of childbearing women as a whole. It is the complexity of malaria that has enabled it to resist so successfully the many and varied attempts to eradicate or control it. With this in mind, and recognizing that the world wide eradication of the disease is not an attainable goal in the foreseeable future, it therefore becomes imperative to give antimalaria chemoprophylaxis special attention and consideration especially in childbearing women during pregnancy considering their easily susceptibility to malaria as well as the consequences of malaria in pregnancy.(6)

MATERIALS AND METHODS

This is a descriptive cross-sectional study and was carried out in Okelele Community of Ilorin West local government of Kwara state. The community is characterized by poor social amenities and is inhabited by Ilorin indigenes of poor socio-economic status. The community has one Primary Health Care center (PUC), which offers a wide range of services. Traditional healers also provide a wide range of health care services including malaria prevention and treatment. The Ilorin West local government and Department of Epidemiology and community health of the University of Ilorin Teaching Hospital jointly run the PHC center. The geographical climate of the area is similar to the holoendemic region of malaria infection (7). The study was conducted between April and May 1997 and approval was obtained from the community leaders and most importantly the head of the ward (Ajikobi Ward) in which the community belongs. For ethical reason the consent of the childbearing women was properly sought and duly given. Furthermore, their human rights and dignity were respected. Data collection was based on a cross-sectional household survey. Due to lack of resources ten streets were randomly selected for the study. A systematic sampling of every fourth house in the selected streets was carried out to determine the houses in which the respondents would be obtained. The first house was chosen randomly with the use of ballot papers. All the childbearing women between the ages of fifteen and forty-nine found in the selected houses were interviewed until required sample size was obtained. A total sample size of 380 childbearing women was used for the study. Four trained interviewers who were of public health background carried out the data collection and a close-ended questionnaire were used as data collection instrument. The interviewers were properly monitored and supervised so as to strengthen the reliability of the data being collected. The questionnaire was pre-tested in twenty houses outside the study area and standardized before being administered to the study subjects. The items in the questionnaire include the socio-demographic variables as well as knowledge and attitude of childbearing women towards antimalaria chemoprophylactics. Interviews were mainly carried out in the evenings during the working days and throughout the day on weekends to ensure that the women were met at home. The interview was over a period of six weeks and each interview lasted about thirty minutes.

The collected data were coded and analyzed using a computer with EPI-INFO version 6 soft ware packages. Frequency distribution tables including descriptive statistics such as means and standard deviation where applicable were used. Significant levels of association between variables were tested by chi-square test.

This study was not without constraints. Some of the respondents initially thought the interviewers were sanitary inspectors and they therefore felt reluctant until after so much explanations before they could withdraw their initial thought. Some respondents before cooperation requested material or monetary incentives. Even though malaria study has been extensively researched, yet few literatures could be found with regards to the title of this study.

RESULTS

Out of the 380 women of childbearing age interviewed, 375 (98.7%) responded while only 5 (1.3%) did not respond for reasons such as lack of time and interest as well as lack of permission from husbands. The respondents' age ranged between IS and 47 years. The mean (SD) and median (SD) ages were 34.2(12.8) and 36.8 *(13.5)* years respectively (Table 1). As seen in table one, 255 (68%) of the

respondents were Yoruba, 78 (20.8%) were Hausa / Fulani, 17(4.5%) were Igbo while 25 (6.7%) were from other ethnic *groups* like Gambari, Tapa and Nupe. More than half 210 *(56%)* of the respondents were married, 90 (24%) were single and only 13 (3.5%) were widowed. More than half (52.8%) of the childbearing women in this study were literates, I OS (28%) had primary education, 64 (17.1%) had secondary education and 29 (7.7%) had tertiary education. The Table I further shows that 76 (20.3%) of the respondents were civil servants, 102 (27.2%) were traders, 83 (22.1%) were artisans, *62(16.5%)* were farmers, and only 52 (13.9%) were unemployed. Income was generally low and the respondents depended on support, mainly from their husbands and other members of the family.

Of 375 respondents, 359 (95.7%) knew malaria to be transmitted by mosquitoes, 122 ¾-(32.5%) understood malaria to be caused by standing too much in the sun were 290 (77.3%) said germs caused it. Respondents' knowledge on the presentations (signs and symptoms) of malaria are shown in Figure 1.Concerning susceptibility to malaria infection, majority 358 (95.5%) indicated that pregnant women were more at risk than non pregnant ones and no reason was given for their indication. Majority d53 (94.1%) bf the respondents were aware about malaria chemoprophylaxis and their sources of awareness range from Friends (13.6%), Husband (8%), Health workers (56%), and Media (22.4%). The distribution of respondents' awareness status and sources of information to various forms of anti-malaria chemo-prophylactic drugs is shown in Table 2. Knowledge scores on antimalaria chemoprophylactics were not associated ($p>0.05$) with parity. Childbearing women with low parity (mean score =58.4 points out of total 100 points mean scores) were found to be more knowledgeable than those with high parity (mean score = 36 2 points).

Concerning antenatal clinic attendance, there was a statistically significant relationship a ($p<0.05$) between antenatal clinic attendance and knowledge scores (Table 3). Knowledge scores of those who attend antenatal clinic were higher (72.8 points) than those who do not attend (24.5 points). As regards the benefits of antimalaria chemoprophylactic drugs, 272 (72.5%) of childbearing women indicated malaria prevention during pregnancy. Reduction of malaria attacks was indicated by 288 (76.8%), improvement in health status by 155 (41.3%) and assurance of healthy baby by *253(67.5%)* childbearing women. These benefits were recalled from their experiences in the past pregnancies. It was observed in this study that 237 (63.3%) of respondents said both husband and wife should make decision while 77(20.5%) said only husband should be the decision maker with regards to the use of antimalaria chemoprophylactics.

Only 18 (4.8%) claimed that health workers should be the only persons to decide. Table 4 shows no statistical significant relationship between the level of education of respondents and those who should make decision about antimalaria chemoprophylactics usage. In this study, 317 (84.5%) of the respondents had positive attitude towards malaria chemoprophylaxis and use antimalaria chemoprophylactic drugs when they are pregnant. However 58 (15.5%) were not willing (negative). Education wise, while only 5 (17.2%) of respondents without formal education interviewed reacted positively to the use of antimalaria chemoprophylactic drugs, 38 (59.4%) with primary education, 81 (72.1%) with secondary education and 144(81.5%) with tertiary education reacted positively. There was a significant statistical direct relationship ($p< 0.05$) between levels of education of respondents and their possible consideration of the use of antimalaria chemoprophylactic drugs as shown in Table 5. There was however no statistical significant difference in the percentages of respondents attitude religious wise (Table 6) Concerning 58 respondents with negative attitude to malaria chemoprophylaxis, 60.2% did not give any reason for their attitude; while 24.5% claimed fear of side effects and 4.5% mentioned lack of awareness, and 5.6% indicated that it was not necessary due to non-belief in its use. Only 4.2% linked their attitude to poor financial status (lack of money).

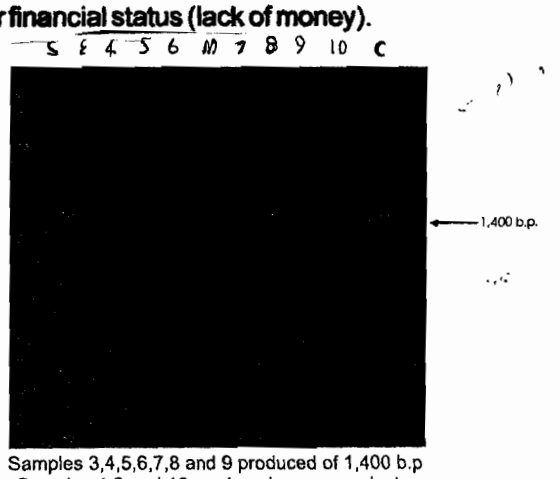
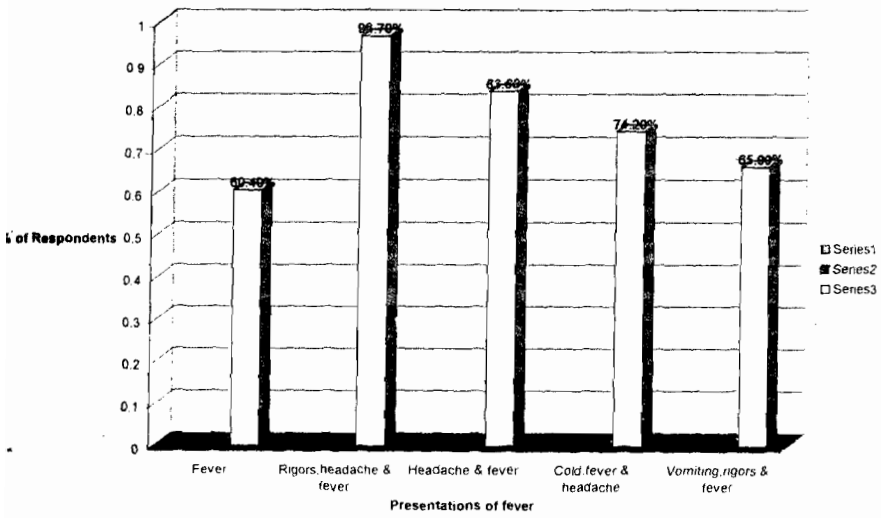


Fig 1

Samples 3,4,5,6,7,8 and 9 produced of 1,400 b.p
Samples 1,2 and 10 produced no pcr products.
C = Positive Control

Fig 1 Respondents knowledge of presentations of fever



Items	Frequency (%)
General Awareness	
Aware	353 (94.1%)
Not Aware	22 (5.9%)
Total	375 (100%)
Awareness on Specific Drugs	
Chloroquine	98 (27.8%)
Fansidar	70 (19.8%)
Pyrimethamine	140 (39.7%)
Proguanil	28 (7.9%)
Amodaquine	11(3.104)
Mefloquine	6(1.7%)
Total	* 353(100%)
Sources of awareness	
Friends	51(13.6)
Husband	30(8)
Media	84 (22.4)
Health workers	208 (55.5)
Others	2(0.5)
Total	375(100)

TabIe2:
AWARENESS STATUS OF RESPONDENTS ON ANTIMALARIA CHEMOPROPHYLACTIC DRUGS
* Total number of awareness to antimalaria chemoprophylactic drugs.

Variable	Freq (%)
Age (years)	
15-24	125 (33.3)
25-34	154 (41.1)
35-44	51 (13.6)
45-54	45 (12)
Total	*375 (100)*
Ethnic Group	
Yoruba	255(68)
Hausa/Fulani	78(20.8)
Igbo	17(4.5)
Others	25(6.7)
Total	*375(100)*
Marital Status	
Single	90 (24)
Married	210 (56)
Separated	27(7.2)
Widowed	13 (3.5)
Divorced	35 (9.3)
Total	*375 (100)*
Education	
No formal education	177 (47.2)
Primary education	105 (28)
Secondary education	64 (17.1)
Tertiary education	29(7.7)
Total	375 (100)
Occupation	
Civil service	76 (20.3)
Trading	102 (27.2)
Artisan	83(22.1)
Farming	62(16.5)
Unemployment	52(13.9)
Total	*375(100)*
Parity*	
1-4	119
>4	256
ANC attendance*	
Yes	294
No	81
Religion	
Christianity	38
Islam	325
Others	12
Total	*375(100)*

ANC means antenatal clinic,
* still has total frequency of 375.

Factors	No	Mean Score	Variance	P-value
Parity				
1-4	119	*58A*	0.97	0.718
>4	256	36.2	1.46	
ANC attendance				
Yes	294	72.8	0.62	0.0165
No	81	24.5	0,34	

Table3: RESPONDENTS KNOWLEDGE SCORES BY PARITY AND ANTENATAL CLINIC ATTENDANCE ON MALARIA CHEMOPROPHYLAXIS
ANC means Antenatal Clinic. Total mean scores 100

Level of Education	Who should make decisions				
	Husband	Wife	Both	H/workers	Total
No. formal Education	18(10.2)	10(5.6)	143(80.8)	6(3.4)	177(100)
Primary Education	20(19.1)	16(15.2)	64(60.9)	*5(4.8)*	*105(100)*
Secondary Education	21(32.8)	14(21.9)	25(39.1)	4(6.2)	64(100)
Tertiary Education	18(62.2)	3(10.3)	5(17.2)	3(10.3)	29(100)

Table4:
LEVEL OF EDUCATION RESPONDENTS VERSUS THEIR VIEWS ON SHOULD MAKE DECISIONS ABOUT THE USE OF ANTIMALARIA CHEMOPROPHYLACTIC DRUGS
df=9, p-value =0.08975.

Level of Education	**Possible use of Chemoprophylactics**		
	YES	**NO**	**TOTAL**
No formal Education	35(19.8)	142(80.2)	177(100)
Primary Education	60(57.1)	55(42.9)	105(100)
Secondary Education	*47(73.4)*	17(26.6)	64(100)
Tertiary Education	*24(82.7r*	5(17.3)	29(100)
df=3, p-value = 0.00027.			

Table5:
LEVEL OF EDUCATION OF RESPONDENTS VERSUS THEIR POSSIBLE CONSIDERATION OF THE USE OF ANTIMALARIA CHEMOPROPHYLACTIC DRUGS

Religion	**Possible use of Chemoprophylactics**		
	YES	**NO**	**TOTAL**
Christianity	22(57.9)	16(42.1)	38(100)
Islam	200(61.5)	125(38.5)	325(100)
Others	9(75)	3(25)	12(100)

Table6:
RELIGION OF RESPONDENTS VERSUS THEIR POSSIBLE CONSIDERATION OF THE USE OF ANTIMALARIA CHEMOPROPHYLACTIC DRUGS
df=2, p-value = 0.6728.

DISCUSSION

The age range of respondents interviewed was between 15 and 47 years. The lowest age of respondents being fifteen years is expected because the area of study belongs to the Northern part of Nigeria where females are contracted earlier for marriage and expected to be having children. There was a preponderance of 25-34 years age group amongst the respondents. The preponderance is in keeping with the population pyramid of most developing countries where there are more young ones compared with the few elderly individuals.

The knowledge of childbearing women on the transmission, presentations and susceptibility of women to malaria was good. The level of awareness of antimalaria chemoprophylactics was generally high as a result all the women interviewed had knowledge of at least one form of antimalaria drugs (chemoprophylactics). This finding is similar to a Lagos study (8) in which women knew that malaria could be prevented by taking Daraprim. Furthermore, the Uganda study (9) showed that the childbearing women afflicted with malaria were quite knowledgeable about antimalaria chemoprophylactic drugs and therefore used them to prevent and treat malaria. In Malawi, Schultz (10) found out that the level of awareness about antimalaria chemoprophylactics was high among childbearing women. This level of awareness was as a result of the women's level of education, which serves as a significant predictor of initiating antenatal care. The decision on malaria chemoprophylaxis sometimes is based on the knowledge of the risk of malaria transmission, effectiveness and side effects of the drugs. This assertion is corroborated by Kenya study (11) in which the major reason for not taking chemoprophylactics was lack of awareness that the service was available. Considering the fact that childbearing women should be properly protected against malaria especially during pregnancy, it. It is equally important that they themselves should be knowledgeable about the epidemiology and chemoprophylaxis of malaria. For effective control of malaria the basic principles of epidemiology by everyone involved is crucial (12, 13).

There was a positive attitude to antimalaria chemoprophylactics in this study because majority (84.5%) of the respondents had ever used them. The study also revealed that husband (63.2%) as well as both husband and wife (20.5%) jointly made decision about using antimalaria chemoprophylactics. All these may be explained by high level of education amongst the respondents as well as the fact that the family structure in most African cultures is patrilinear and patriacal. The social and economic dependence of women including the childbearing ones on their husbands gives men great influence in the house hold, a position that is strengthened by a patrilinearly organized family structure. As a result of this position occupied by men, they often decide the choice of health services women receive during pregnancy. Men in many developing countries place a high premium on children because of the tangible and emotional benefits derived from them. Children bring a high sense of satisfaction or success to a man even if he is materially poor. Children especially sons are agents of continuity for the family name -a characteristic that encourages both polygamy and prolific childbearing to ensure that sons survive to perpetuate the lineage~) It is not surprising to find out that religion has no influence on the attitude of the respondents with regards to malaria chemoprophylaxis because all the practiced religions in the community support prevention against diseases.

CONCLUSIONS AND RECOMMENDATIONS

This study revealed that Okelele Community consists of families belonging mainly to the low socio-economic group, and are multi-ethnic and multi-tribal. The study demonstrated high knowledge of malaria chemoprophylaxis among the childbearing women, which could be due to high level of education among the respondents coupled with increased level of primary health care activities in the community. Education was found to play a very significant role on the respondents' attitude towards malaria chemoprophylaxis by increasing the level of awareness and usage. Decision on the usage of antimalaria chemoprophylactics was observed and greater percentage of husband and wife took decision jointly. It is worth nothing that there was no significant relationship between religion of respondents and the attitude towards malaria chemoprophylaxis. Considering malaria endemicity in the study area and its implications on childbearing women especially during pregnancy, the health workers must increase their effort in dissemination of information about malaria chemoprophylaxis. Greater emphasis should be laid on antenatal clinic attendance for the delivery of antimalaria chemoprophylactics to childbearing women. Factors like efficacy, safety and ease of delivery of the antimalaria chemoprophylactics as well as compliance with the dosage regimen must be considered in selecting a practical antimalaria chemoprophylactics for childbearing women during pregnancy.

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BIOLOGICAL CONTROL AGENTS OF CYCLOPOID COPEPOD, VECTOR OF DRACUNCULIASIS: LABORATORY EXPERIENCE.

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In order to determine the predating capability of some indigenous fishes to Nigeria on cyclopoid copepods, the vector of *Dracunculus medinensis*, species of three genera of fishes were fed experimentally with Cyclops in the presence or absence of alternate food substances as described in the text.

The study revealed that indigenous fishes like *Hemicromis fasciatus*; barbus occidentalis, Tilapia niiotica and T galilea were identified as very effective indigenous biological control agent of Cyclops-the vector of dracunculiasis. The benefits of rearing these fishes in village ponds are highlighted. It is believed that there would be no longer cases of resurgence of dracunculiasis in areas where transmission has been broken when the biological agents are used in concert with filtration method.

INTRODUCTION

Dracunculiasis is primarily a rural problem where there is no supply of portable water, and this account for the relative obscurity of the disease. Until recently the disease had a very high prevalence and scope with the devastating effect on the productivity of the affected.

The efforts of both government agencies and non-governmental organization like Global 2000, UNICEF and Gowon Foundation combining many approaches has yielded some positive results. In fact many countries of the world have eradicated the disease. The combined approach involving health education and distribution of water filters in the endemic villages have reduced the cases in many villages. Since Nigeria has seen a dramatic decrease in reported 98% reduction cases between 1992 and 1997(1), there has been resurgence of cases in some areas in Nigeria where disease was eradicated.

The use of chemicals advocated from some quarters (2) has not brought much success either. Therefore there is need for a search for an alternative model for control, which should be indigenous and should not require much in terms of foreign exchange. Where other approaches have failed it is thought germane that biological method of control could serve as alternative model for control. Since the use of certain small fishes Gambusioa Species. Barbus Species and Rasbora doniconius a biological control agent worked in India (3,4), it could also work in Nigeria as well. The need to search for new biological control agents has become more urgent in view of the fact that most areas where there is frequent resurgence of dracunculiasis after the international target dates for eradication has expire are yet to enjoy potable water. The communities still rely on untreated streams and ponds, which have a large population of the vector cyclopoid copepods for there water needs. Therefore it has become imperative to search for new-bio-control agents to act in synergy with the filtration programme in eradicating the disease in the country. This study was stimulated by an observation made at an endemic village in Oyo State, Nigeria, which indicate a low Cyclops count in ponds where certain fishes were found in large number.

This study therefore reports the effect of some indigenous fishes as biological control agents of vector of dracunculiasis under laboratory conditions.

* *Corresponding Author*

MATERIALS AND METHODS

This study was conducted with pond water collected from an endemic village near Ibadan, capital of Oyo State, Nigeria.

Samples of pond water were periodically collected and filtered through a plankton net made up of monofilamentous nylon gauze with mesh size 75um attached to a plastic tube. Adult and nauplii stages of different species of Cyclops retained on the filter and collected in the tube were transferred to a glass container, their number were counted under the low power objective of a compound microscope and use in the laboratory experiments. The dominant species in this part of Nigeria were shown by Sridhar and Kale (5) to be: *Thermocyclops neglectus decipiens, T. crassus, Afrocyclops gibroni, Mesocyclops major, Tropocyclops prasinus, Microcyclops varicans, thermocyclos species, crytocyclops linjanticus and tropocylcops onabamiroi.*

In order to determine the predating capability of indigenous species in western Nigeria, the following species of fishes obtained from the Oyo State Fishery Department at Agodi Station in Ibadan were used for

the experiment *Hermicromis fasciatus, barbus occidentalis, Tilapia nilotica and T. galilea.*

The physical characteristics of the pond in these experiment was determined as described by Adeyeba (6) before the commencement of the experiment in order to ascertain the prevailing ecological character.

Samples of pond water were collected and filtered as described earlier on, into each 15 liters plastic aquarium containing 10 liters of Cyclops free pond water and 16,000 active Cyclops were introduced 10 active young five of the same species H fasciatus N. occidentalis and Tilapia species. The experiment was done in replicate with a control tank, which contained only pond water and Cyclops but no fish. The experiment was maintained at room temperature (26 28°C). The Cyclops counts were record over a period of hours and days. The Cyclops density per unit time was determined in the system and expressed in percentage.

In another experiment, alternate food was provided for the fish in order to assess the predating effectiveness of the fish on the Cyclops in the presence of alternate food. Into each 15 liter capacity aquarium containing 10 liters of Cyclops free pond water, 16,000 active Cyclops and 10 active fish of the same specie were introduced 4 gramme of fish meal as an alternate food supply to Cyclops meal. The experiment was carried out in replicate with a control aquarium that contained only pond water, Cyclops and fish meal but no fish. The experiment was similarly maintained at room temperature (26 28°C) and observed over a period of hours and days. The Cyclops density per unit was determined and expressed in percentage.

RESULTS

Effect of predating fishes on Cyclops:- The result of the experiment involving H. fasciatus is shown in Figure 1. Data indicate that these relatively big fishes of about 4cm long reduced the Cyclops population by 50% in 60 hours and by 75%, 90% in 72 hours and 84 hours respectively. The Cyclops counts in the control bank did not drop throughout the duration of the experiment.

Figure 2 shows the result of the experiment involving the Tilapia species. Data indicate that Cyclops count dropped by 45% and 90% in 4 days and 9 days respectively when the fish were kept on pure Cyclops diet whereas the fish caused a drop in Cyclops population by 33% and 84% in 4 days and 9 days respectively when there was an alternate food supply, there was no drop in Cyclops count in the control bank. This result shows that the presence of alternate food supply had very little influence on the feeding habit of fish on Cyclops.

The result of the experiment involving barbus occidentalis is shown in figure 3. The result shows B. occidentalis reduced the population of Cyclops by 48% and 90% in 3 days and 6 days respectively when there was no alternate food supply in the medium whereas there was a drop of 38 percent and 52 percent in Cyclops count when there was an alternate food supply. There was no drop in Cyclops count in the control bank. Barbus lived longer than 3 weeks the experiment lasted.

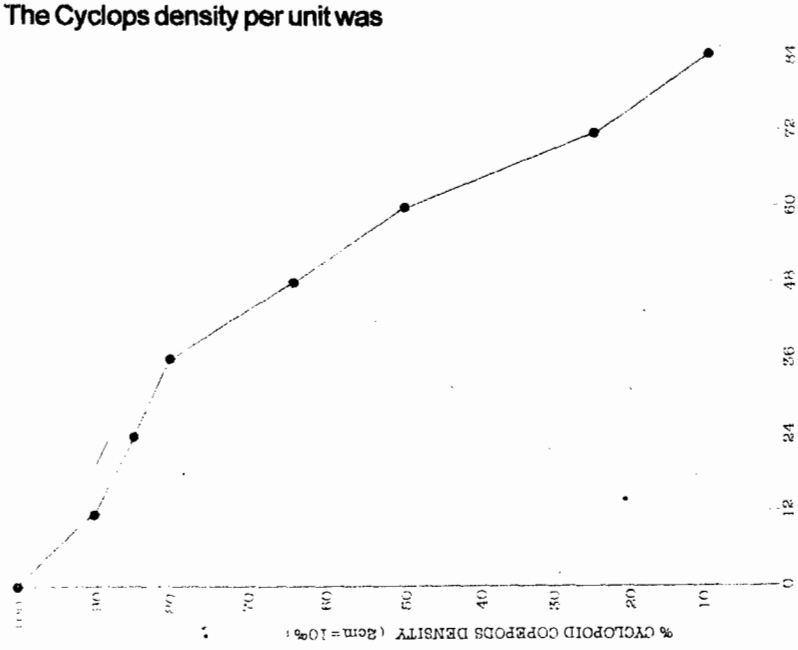

FIG. 1: EFFECT OF PREDATING FISH (*Hemicromis fasciatus*) ON CYLOPOID DENSITY IN POND WATER UNDER LABORATORY CONDITION. (2cm - 12hrs.)

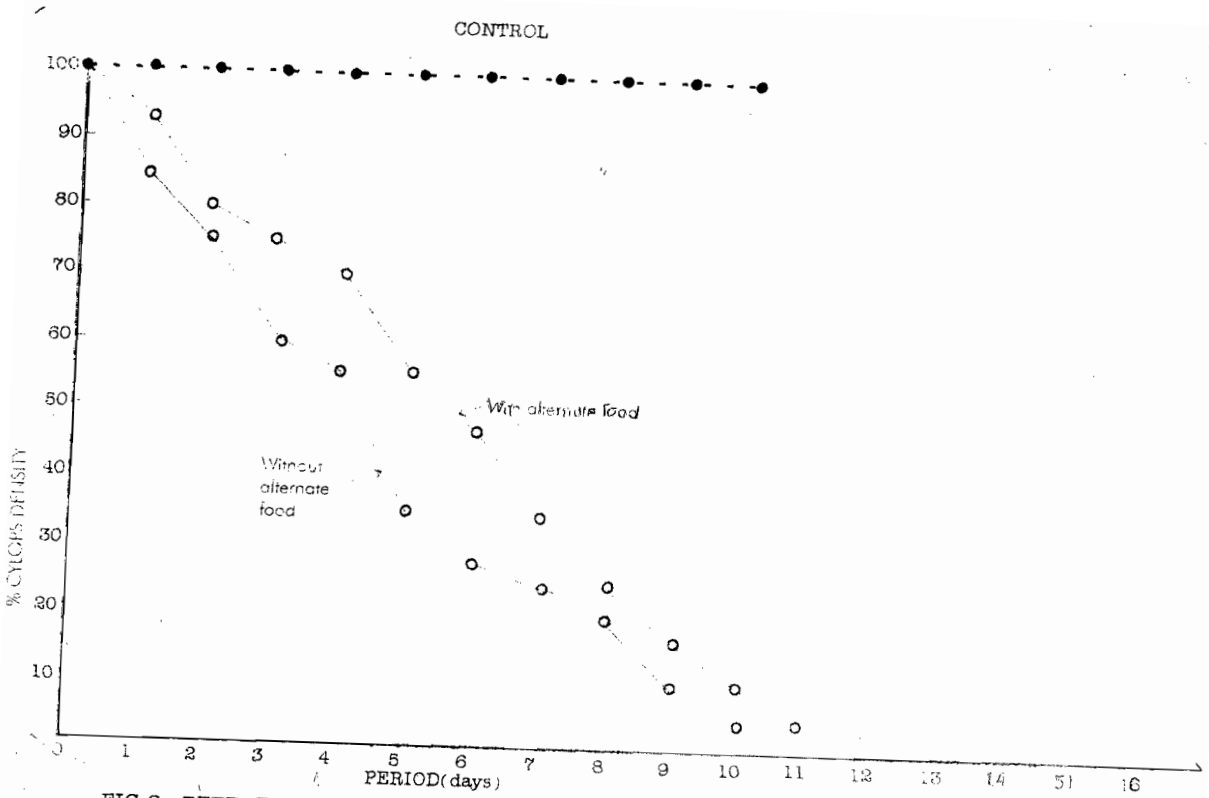

FIG.2 : EFFECT OF PREDATING FISH (Tilapia nilotica x T. galilea) ON CYLOPS DENSITY IN POND WATER UNDER LABORATORY CONDITION.

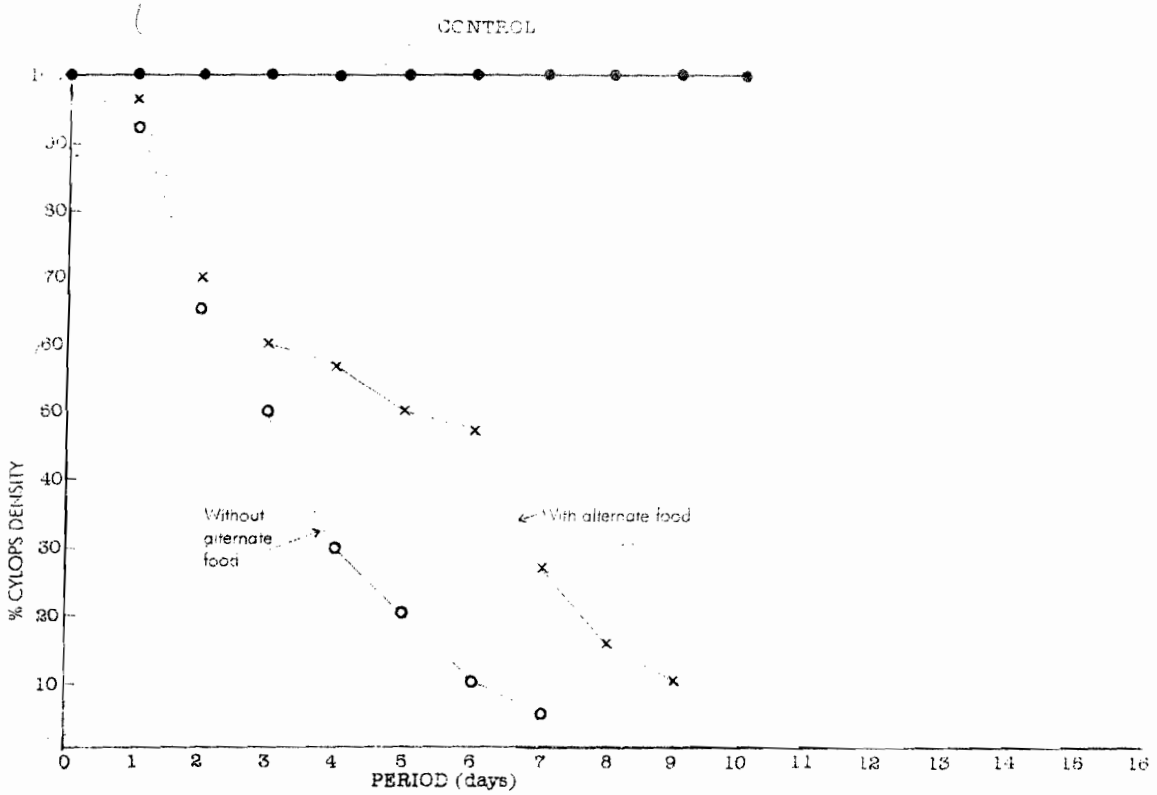

FIG.3 : EFFECT OF PREDATING FISH (Barbus occidentalis) ON CYLOPS DENSITY IN POND WATER UNDER LABORATORY CONDITION.

DISCUSSIONS

The observations reported here seem to be applicable in the control of dracunculiasis in the tropical areas. In most of these areas the ponds are seasonal, with excess water in raining season, which gradually recede and finally dry up.

This present study has shown that certain fishes are efficient biological control agents of the vector of dracunculiasis. Earlier on successful field experiment using small fishes (Gambusia, Barbus species and Rasbora doniconius) reported in India by some workers (3,4) agree with the present findings. This is significant in the sense that for the first time there has been a report of indigenous species of fishes that could serve as biological control agents of cyclopoid copepods in Nigeria.

This method of using biological control agents if employed, may save money for the country. Of all the species of fishes used in this experiment Barbus occidentalis, was the most enduring fish, followed by Tilapia nilotica and T. galilea as they were able to withstand the adverse laboratory conditions which let hemicromis fasciatus dead within 4 days. Despite the inadequate facility for aeration in the course of the experiment Barbus and Tilaia species survived as long as the experiment lasted, and even beyond. Besides, these species were observed to be preponderant in ponds in this part of the country (6). This is an indication that these small fishes would be invaluable tools of biological control in the field, their natural ecological environment. Hemcromis fascinatus, though a very voracious predator of Cyclops could not regarded as a good experimental model animal as it could not withstand the laboratory stress. Besides, it was discovered that the fish did not only prey on cyclopoid copepods but predate also on the other small fishes like tilapia within the same enclosure, a situation that does not augur well for the other mates in the ecosystem that expected to be more efficient bio- control agents.

Tilapia fish could be safely regarded as the most reliable of all the indigenous fishes in villages Southwestern Nigeria. The fish could also endure to a large extent, the adverse conditions occasioned by prolonged dry season. It has been demonstrated that the presence of alternate food supply in the ecosystem had very little negative effect on the predating ability of Barbus and Tilapia fishes on cyclopoid copepods. This further supports the adoption of fish as biological control agents of Cyclops.

Although field trials have not yet been undertaken the prospect of success is very bright. It was discovered from an unpublished observation that those ponds in fishes were more preponderant had lower density of cyclopoid copepods than others in some villages Southwestern part of the country. In addition, it was observed that the level of acceptability of presence of fishes in drinking water supply is very high among all the house heads interviewed [Adeyeba- unpublished observation]. In fact, the fishes are given maximum protection possible by the villagers through legislation against fishing in ponds that are designated for drinking. It is a general belief of the Yoruba nation (occupiers of South-western Nigeria) that the presence of fishes in pond water meant for drinking is n indicator or index of fitness of water for drinking. The water is believed to is believed to be free of all undesirable elements such as poison, etc.

In addition, fishing activities in the pond is strictly forbidden because of the belief that water might go dry as a result.

Although certain fishes had been recommended for biological control in India (3,4), no other report has been published elsewhere. Hence it is believed that this report has made a landmark in the search for new biological control agents of vector of dracunculiasis in Nigeria. Particularly at this time of economic reconstruction in this country, indigenous bio-control agents could be adopted, as an alternative to the expensive imported chemicals used for water treatment with little success in the country.

In view of the identification of some indigenous fishes like Hemicromis occidentalis, barbus fascinatus, Tilapia nilotica and T. galilea as biological control agents of dracunculiasis it would be commendable to encourage the rearing of such fishes in the village pond. This is expected to compliment the use of filter synergistically in the control and eventually eradication of dracunculiasis in the country where sporadic resurgence of cases occur in areas where the disease had been contained. Besides, there is an additional benefit of protein supplement to the community with good management.

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THE PATHOLOGY OF INFETIOUS BURSAL DISEASE IN CROSSBREEDS OF HARCO COCKS AND INDIGENOUS NIGERIAN HENS.

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An outbreak of infectious bursal disease (IBD) occurred in a flock of 11-week old crossbreeds of Harco cocks and indigenous Nigerian hens (referred to as exotic and locals respectively in the text). Clinical signs observed include depression, anorexia, ruffled feathers and diarrhoea. Haemorrhages were present in the bursa of Fabricius, muscles and proventriculus-gizzard junction, while the bursae and kidneys were enlarged. Oedema, lymphocytic depletion and presence of remnants of dead lymphocytes were observed in histopathological sections of the bursae and spleen. IBD viral antigen was detected by the agar gel preciptin test (AGPT) in suspensions of the bursae of dead birds. Sera obtained from survivors were also positive for IBD virus precipitins in the AGPT. These observations appear to be the first description of IBD in crossbreed chickens in Nigeria and confirm that they are susceptible to clinical IBD.

INTRODUCTION

Infectious bursal disease (IBD) which was first described in 1962 (1) was reported and confirmed in Nigeria in 1975 (2). Subsequent studies have shown that the dreaded disease of poultry results in varying mortalities of 50-100%. The disease has been known to affect very young chicks of 9 days old (3) and 16-20 weeks old birds (4,5). However, indigenous birds of less than 8 weeks are most predisposed to this disease (6). In Nigeria, however, the studies on IBD of chickens are mostly on exotic commercial chickens. Few studies have been done on the pathology of IBD in indigenous Nigerian chickens (7) despite the serologic evidences of IBDV infection in the birds as reported by various authors (6,8,9,10,11,12).

The exotics currently in use for commercial poultry production in Nigeria do not perform optimally under the prevailing tropical conditions as temperature, humidity and nutrition while the local chickens, which are well adapted, do not have the genetic endowment for high production performance as the exotics. Hence, the need for selective crossbreeding programme proposed for the development of the indigenous chicken population which is about 124million 913,14,15).

There has been no report on the status of IBD in crossbred chickens in Nigeria. In this paper, the pathology of a confirmed outbreak of IBD in a flock of crossbred chickens is presented.

MATERIALS AND METHODS

Flock History

The affected birds were 11-weeks old crossbreeds of Harco cocks and indigenous Nigerian hens. They were hatched and reared locally under a semi-intensive management system and were not vaccinated against IBD.

Clinical Signs

The birds became sleepy with ruffled feathers. They were anorexic and had watery whitish faeces. Prostration was noticed before death and mortality was 16.7%.

Necropsy and Histopathological Changes

Dead birds were examined for gross pathological changes and the bursa of Fabricius, spleen and kidney were collected and fixed in 10% formalin for histopathology.

Virus Extraction

Unfixed bursae of dead birds were homogenized and 50% suspension prepared in phosphate buffered saline (PBS). These suspensions were tested for IBDV antigens using the agar gel precipitin test. (AGPT)

Serology

Blood collected by jugular venipuncture from the survivors was left lying sideways in universal bottles for about 4 hours to clot and allow for serum separation. Harvested sera were inactivated at 56°C for 30 minutes and tested for IBDV precipitin using AGPT.

Agar Gel Precipitin Test

The agar used was made of 1% purified agar, 8% Nacl and 0.01% sodium azide. The agar dishes were kept in a humid chamber at room temperature. For the detection of precipitin, the positive control was normal serum. In antigen identification, the positive control was a suspension of known infected bursa and the negative control was a normal bursal suspension.

RESULTS

Necropsy Findings

The bursae were swollen and highly haemorrhagic both on the serosal and mucosal surfaces. There were haemorrhages at the junction between the proventriculus and the gizzard. The spleen

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was mottled, while the kidneys were enlarged. The liver was yellowish and there were areas of ecchymotic haemorrhages on the pectoral and thigh muscles.

Histopathology

The bursa showed moderate interfollicular oedema and haemorrhage. The follicles were depleted of lymphocytes and contained remnants of dead lymphocytes (Fig. 1) There were similar lesions in the spleen (Fig 2.). Degeneration was seen in epithelial cells of the renal tubules and ducts, which also contained eosinophilic casts.

Virus Identification

The bursal suspensions examined for IBDV antigen in AGPT gave precipitin lines within 36 hours.

Serology

Eight of the ten serum samples assayed for IBDV precipitin gave positive result within 36 hours.

Fig. 1 Bursa showing moderate interfollicular oedema and hemorrhage with lymphoid depletion and necrosis.

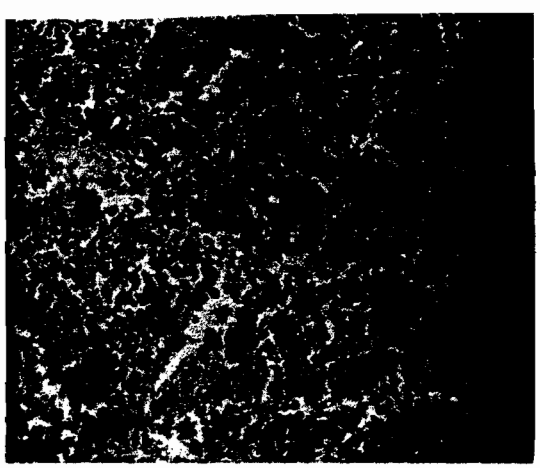

Fig 2. Spleen with marked lymphoid depletion and necrosis.

DISCUSSION

The Nigerian local chickens have been shown to be more resistant to diseases than the exotic ones (16,17) even though it has also been suggested that both locals and exotics are equally susceptible to IBD and could be infected by similar IBDV serotype (18). The necropsy findings and histopathology characterized in this case by classical haemorrhage and marked lymhoid depletion in the bursal follicles were more severe than those reported in the local chickens (7). These might be associated with the pathogenicity of the infecting IBDV strain, the virus dose and probably the contribution of the exotic component to the overall genetic make-up (in terms of resistance to diseases) of these crossbreeds. The mortality recorded is not too different from that observed with the Nigerian local chicken (7) but significantly lower than that of exotics (3,19).

Growth rate and organ weight studies of crossbreeds is advocated since earlier work (20) reported that higher resistance of the locals to diseases is due to earlier growth and higher organ weight of their bursae compared to that of the exotics. It has been noted also (7) that the earlier growth and higher organ weight of the bursa of the locals is likely to make them more susceptible to IBDV infection than the exotics because the bursa is the target organ of IBD. This implies that the bursae of the Harco/local crosses in this study were sufficiently large enough at 11 weeks to make them susceptible to IBDV infection.

The result of this study shows that the crossbreeds of Nigerian local hens and Harco cocks are susceptible to clinical IBD. It is very important that this should be noted if the popular campaign of enhancing the productivity of local chickens by crossing with the exotics is to succeed. Mass vaccination of such crossbreed stock should be encouraged.

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IMMUNE RESPONSE OF BROILER CHICKS TO LOCAL IBD VACCINE USING DIFFERENT ROUTES OF ADMINISTRATION.

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Four groups of ten-day-old broilers each were vaccinated at 7 and 14 days post hatch (PH) against infectious bursal disease IBD, using the local IBD vaccine (VOM & Nigeria). The vaccine was administered using different routes; intramuscular, subcutaneous and oral. 10- day old broiler was randomly sampled from a group of 40 for the presence of IBD antibodies using qualitative and quantitative agar gel precipitation test (AGPT). The maternal antibodies in the chicks were variable, low and waned completely 12 days post hatch (PH). The group that received subcutaneous route of vaccination (sc/sc) gave consistently higher antibody titers than the oral and intramuscular route in post vaccination days. All the groups including the unvaccinated control were challenged 16 days post vaccination with a field strain. All the routes were appreciably protective (90%) against the field strain with the unvaccinated control group recording 30% mortality. However, subcutaneous route had a complete protection (100%). The gross and microscopic lesions seen in the study were consistent with IBDV. This study has shown that the maternal antibodies in the broiler chicks were low, variable and waned completely by 12 days post hatch. The subcutaneous route of vaccination achieved better response and protection when given at day 7 and day 14-post hatch.

INTRODUCTION

Cosgrove first described infectious bursal disease IBD of chickens (1) and it is caused by a birnavirus (2). Although, efforts on its control have been unrelenting, "SD infection has been reported in vaccinated flocks (3), maternally immune chicks (4) and adult flock of 20 weeks (5). There is also the emergence of very virulent strain of IBDV (6) that has been found to be highly pathogenic. Hence it is evident that many factors are militating against success fill vaccination with IRD live vaccines in the field apart from poor and improper handling of the vaccines.

Most of the IBD vaccines are administered intraocularly or orally except for the Vero cells adapted "SD vaccine that was given parenterally (7). Komine et al (8) also showed the efficacy of the subcutaneous route of administration in specific pathogen free (SPF) young chicken and those with maternally derived antibodies. In this report we studied the effect of routes of vaccine administration on the immune response of broilers chicks to our local "SD vaccine.

MATERIALS AND METHODS

(a) Chicks.

A flock of 50-day-old avian breed of broiler chicks was obtained from a local hatchery. The breeders were vaccinated against IBD and boosted at 16 week of age with an IBD oil emulsion vaccine. They were raised from day old until termination of the experiment at the poultry experimental unit of department of veterinary medicine University of Ibadan

(b) Vaccines

The local IBD vaccine produced at the Nigerian Veterinary Research Institute verb (NVRI) Vom was used. The vaccine was constituted with sterile physiological saline by Dissolving a vial in 4Omls and 0.2mls given using different route of administration (oral subcutaneous and intrarnuscular routes).

(c) Field Virus

A 20% suspension in phosphate buffered saline (PBS) of bursae from birds that died in a recent confirmed outbreak of IBD was used. The homogenate was centrifuged at 5OOpgm for 10 minutes and the supernatant harvested and stored at 4°C after the addition of procaine penicillin to prevent bacterial growth. This was tested using Agar gel precipitation test as described by Durojaiye et al. (9).

Experiments Two experiments were performed.

(a) Qualification of material antibody (MA) levels in chicks: -

Sera were collected through the jugular vein from 40 birds randomly selected at day 1 3, 7; and 12 post hatch, 10 chicks were sampled on each occasion and monitored for the presence of IBD antibodies by qualitative and quantitative AGPT after inactivation at 56%.

(b) Vaccination/challenge

40 chicks were divided into 4 groups of 10 each and placed in separate isolation units. Vaccination was carried out on days 7 and 14 using different routes of application as on Table I. Sixteen days after the last vaccination (30 days post hatch, PH) all the birds were challenged using the homogenate of infected bursa of birds from the recently confirmed Field outbreak. The intraocular route was used as described by Adene et al (10). All birds in each of the groups were observed for clinical signs and mortality rates recorded alongside with the presented signs. Chicks that died during the course of the experiment were

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necropsied and tissues were fixed in 10% buffered formalin and processed routinely for histopathology. Section 5u thick were cut, stained with haematoxylin and eosin (H & E) and examined under the light microscope. The various groups were bled weekly for 7 weeks post hatch. Serum samples were collected and tested for the presence of HID antibodies using qualitative and quantitative AGPT as described by Cullen and Wyeth (11)

Statistical Analysis

The data were analyzed by standard Anova procedure and Duncan's multiple - range a Test (a = 0.05)

RESULTS

Antibody Profiles

Table 2 shows that at day *1,* 5 out of 10 chicks sample showed the presence of MA with antibody titer varying from 4 to 32 GMT (Geometrical mean titer) of 8. At day 3, 50% that is, S out of 10 chicks were positive for M& The antibody flue varied from 2 4 Showing decay in the MA3 days post hatch and the GMT was 2.

Seven days post bath, there was a further decay of the MA. Only 10% of the chicks had IBD antibody titer. The GMT had reduced to 0.5 No maternal antibodies were detected on day12 post hatch.

The antibody response of Group B (SC/SC) was higher than those of other groups at the post vaccination days, while ant of group A (oral/oral) was same as that of Group C (IM/IM) route 14 days post vaccination. After challenge, there was a steady increase in antibody response in Group B (SC/SC) while Group A (Oral/Oral and G (IM/IM) showed a decrease 12 days post challenge.

At day l9 post challenge, the values 52 and 59.7 for the subcutaneous and intramuscular routes were significant (P<0.05).

(1) **Clinical Signs**

Morbidity was highest in the control group After the challenge infection and clinical signs were observed only in group A, B and C shows in tables 3 and 4. Table 5 shows that the Group that received subcutaneous route of vaccination at day 7 and 14, Group B had 100% protection while groups that received only oral and intramuscular routes of vaccination had 90% protection with 10% mortality. The control group lost 30% of its chickens. At post mortem examination the carcasses were well-fleshed and showed eccymotic haemorrhages on the leg muscle and the proventriculus - gizzard junction. The lung' were slightly congested while the kidney in the dehydrated carcass was slightly swollen. The bursa was markedly swollen, haemorrhagic and contained some caseous material on incision. Proventicular haemorrhagic and petechial haemorrhages on the duodenum and part of the jejunum were observed in the control birds.

Insert Table 5 and figure 1

The histopathological findings included muscular and proventricular haemorrhages with marked amounts of protein casts in the renal tubules. Although there were no gross hepatic lesions, there was fatty degeneration of hepatocytes with lymphocytic infiltration around some portal veins. The splenic lesions were that of marked heterophilic infiltration with some follicles showing lymphocytic depletion. The bursa showed oedema in the interfollicular spaces with most follicles showing lymphatic depletion and necrosis.

COMPARISON OF ORAL ROUTES TO PARENTERAL ROUTES OF ADMINISTRATION

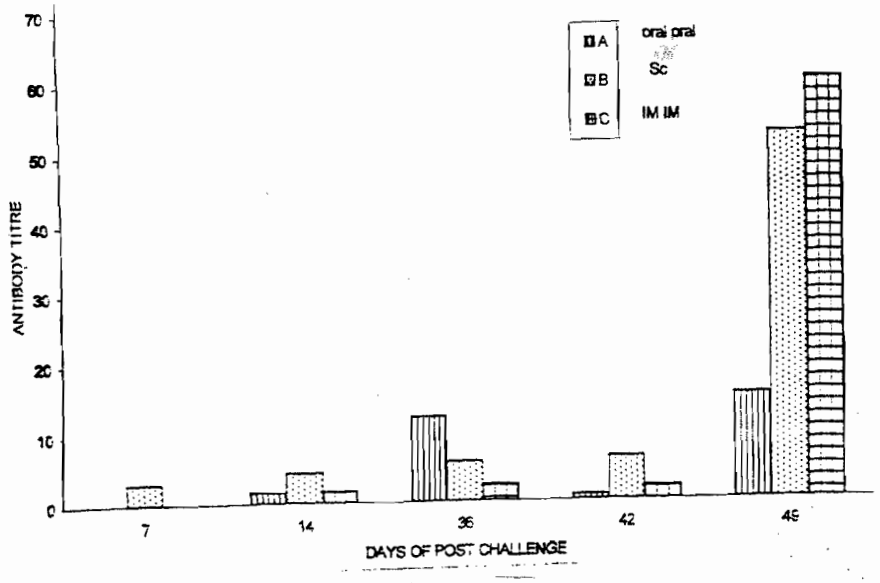

Table 1: **Vaccination groups and routes of administration at different ages.**

Group	Day 7	Day 14
A	Oral	Oral
B	S/C	S/C (subcutaneous)
C	I/M	I/M intramuscular)
D (control)	Distil water	Distil water

Table 2: **Material Antibody Levels of Chicks**

Group	1	3	7	12
A	0	0	2	0
B	4	0	0	0
C	0	0	0	0
D	0	0	0	0
E	0	0	0	0
F	8	4	0	0
G	0	2	0	0
H	16	2	0	0
I	32	4	0	0
J	16	4	0	0
GMT	8.0	2.0	0.5	0
Percentage Positivity	50	50	10	0

Table 3: **Morbidity**

Group	Route	No. In group	No. In group	No. In group
A	Oral/Oral	10	1	10
B	SC/SC	10	None	0
C	I/M I/M	10	1	10
D	Control	10	5	50

Table 4; **Clinical Signs**

Clinical signs	A	C	D
	Oral Oral	I/M I/M	Control
Ruffled feathers	1/10	1/10	5/10
Depression	1/10	1/10	5/10
Greenish diarrhoen	0/10	0/10	3/10
Weight loss or Emaciation	1/10	1/10	3/10
Prostration	0/10	0/10	3/10
Death	1/10	1/10	3/10

Table 5: **Mortality and level of protection against clinical IBD**

Group	Route	Days PC	No Dead	%	Protection %
A	Oral/Oral	2	1	10	10
B	SC/SC	0	0	0	0
C	IM/IM	3	1	10	10
D	Control	6-7	3	30	30

DISCUSSION:

The results of the MA assay showed that antibodies in the broiler chicks waned completely 12 days post hatch. This observation is in arrangement with the report of HOMER et al. (12). This observation is interesting especially when the parent stocks from which the chicks were derived received oil emulsion boosters at 18th week of age before the onset of lay. However, other reports indicated that the MA disappeared at 3-5 weeks post hatch (13), while some workers reported 4 days (14) and 7days post hatch (10).

The GMT at hatch however, was low while individual antibody titer varied between 2-32. The variation is similar to that earlier reported by Winterfield et al, (15), and different front that reported by Vielitz (16) who stated that high and uniform level of MA were observed when oil based vaccines were used as booster in breeders. The difference in MA in chicks showed the variation in seroconversion in the parent stock since it has been reported by Lucio and Hitchner (17), that there was a direct correlation between the antibody titre of the dam and the MA of the chick.

In this Study, only half of the chicks had MA on day 1 PH and the levels were low. Again, this observation agrees with that of Adene et al; (10) who reported low MA levels in chicks horn some of the major commercial hatcheries in Nigeria. This has been attributed to the fact that the parent stocks are rarely given consistent booster doses. Thus, early vaccination or if possible double vaccination in the first 21 days post hatch of such chicks was recommended (10). Precipitating antibodies can be detected early, 14- 25 days (post-*vaccination as* reported by some workers (18). In this experiment, it was detected within 7-14 days post vaccination. This is earlier than reported, and May be associated with the vaccination, which was done twice, at days 7 and 14-post hatch as against single vaccination reported by all the workers mentioned above. In this case, the first vaccination served as primer to the antibody producing cells while the other served as a booster especially when there was no Interference or mopping up of vaccine virus by MA as previously reported by WOOD et al (4), Winterfield and Thatchkel, (19)

The NVRI vaccine has been reported to induce antibody levels that withstood challenge infections when given orally and intramuscularly (20) The results obtained in this study where various vaccination (oral, subcutaneous and intramuscular) routes were used showed that the double subcutaneous route induced more antibodies response than the other route. The parental routes also induce more response than the oral routes at day 49-post hatch. This observation agrees with the reports of some previous workers (8,21).

The antibody response to live vaccines has been reported to correlate with the degree of protection (22). In this study however, the subcutaneous route was found to be more consistent in antibody response and also more protective than the oral and intramuscular routes. The enhanced antibody response observed in chicks vaccinated by the parental route may be associated with the fact that the antibody producing cells were exposed to the vaccine virus earlier than those of chicks vaccinated using the oral routes (23). In the same vein, the enhanced and consistent response in antibody production by the chicks that received subcutaneous vaccination could be explained by the fact that the vaccine virus was slowly release into the blood stream hence the immunopotentiating effect (23).

There is direct correlation between the antibody titer and resistance to IBDV challenge (17). The low morbidity rate found in this study (10 30%) when compared to 44-100% earlier reported by Onunkwo, (25) may be associated with the type of bird used and the presence of antibody in the birds (26). Broilers have been found to have more natural resistance to IBD than other types of chickens (19).

The clinical signs seen which included depression ruffled feathers, greenish diarrhoea, weight loss, prostration and death were more pronounced in the control group than the other groups. Similar clinical sign have been described by earlier workers (25). However, vent pecking and trembling reported by Cosgrove (1) and Hitchner (27) were not observed in this study and other studies so far in Nigeria (28). The mortalities reported in this study were low in vaccinated flocks (10%). This observation is also in agreement with the report of Awolaja and Adene (29). In the control chickens, a mortality of 30% was not too different from the 43% previously reported in exotic chicken (3O). In IBD Mortality do occur on day 3 PI (27),

however in group A which was given oral/oral vaccination, the mortality occurred 2 days; PI corresponding with the timing of marked lymphocytic destruction reported by Resenberger (31). A bird from this group (oral/oral) also showed acute splenitis. Mortality occurred on day 3 in Group C (I/M I/M) and stopped on day 7 in the control group. These are in agreement with the incubation periods and mortality pattern of the disease (32).

However, to further enhance the find of the study, there is need to compare the results obtained through quantitative AGPT and ELISA, which has been known to be sensitive (33). Also there is a need to determine the titer of the vaccine and the challenge virus to check for the reason behind the low titers obtained during post vaccination days. Other works are being carried out in this area.

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FORTHCOMING CONFERENCES

THEME: HIV/AIDS and Sexually Transmitted Diseases

1. Association of Pathologists of Nigeria (ASSOPON) Annual Conference and General Meeting Jos University Teaching Hospital Jos, Plateau State, Nigeria. 24th to 26th July, 2002.
2. National Postgraduate Medical College of Nigeria All-fellows Congress Aminu Kano Teaching Hospital, kano, Nigeria. August 13th - 15th, 2002.
3. National Association of Resident Doctors (NARD) of Nigeria Annual Conference and General Meeting, University College Hospital, Ibadan, Nigeria. September, 2002.

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PARASITIC INFECTIONS OF DRY SEASON FARMERS IN SOME PARTS OF PLATEAU STATE, NIGERIA

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A parasitological survey was conducted among 1080 dry season farmers and controls spread over nine farming locations in the Jos and Barkin-Ladi areas of Plateau State, Nigeria, to provide data or parasitic infections in the area due to waste utilization. Standard laboratory procedures were adopted in the collection, processing and parasite identification in the stool samples. The rates of parasites infections in the farmers were 91.6% for helminthes and 86.4% for protozoa. Helminth infection rates but not those of protozoa, varied significantly between farmers and controls. Average infection rates were. Ancylostoma duodenale 91.9%. Ascaris lumbricoides 84.7%, Trichuris trichiura 74.2%, Strongyloides stercoralis 50.3%, Giardia lamblia 13.3%, Entamoeba coli 28.4%, Chilomastix mesnili 15.4%, Endolimax nana 17.3%, Isospora belli 6.3% and Lodoamoeba butshlii 11.5%.

More males than females were infected. The rates of infection varied among farmers in the different locations, but younger farmers had higher prevalences. Significant correlations between infections and their symptoms were observed with diarrhoea and abdominal pains being most common.

INTRODUCTION

One major aspect of development challenges confronting mankind towards the close of the 20th century was how to achieve a cost effective, technologically appropriate and environmentally benign strategies to deal with the waste crisis. The threat of the waste crisis. The threat of the waste on the assimilative and carrying capacities of our land, water air, and the overall environment is enormous. For this reason, waste utilization in agriculture, aquaculture and biogas production has gained prominence. For this reason also, the application of community/municipal refuse, urban and rural soil, animal manure mainly from cows, pigs, goats, sheep, poultry; crop residues, green manures and some aquatic plants to agricultural land has become a better alternative than the costlier chemical fertilizers in many countries of the developing and industrialized world.

However, wastes from all communities contain parasites. These parasites survive to different degrees as the wastes are transported, treated and applied to land (2). The parasites present in such wastes also constitute a health hazard to agricultural workers, waste handlers and consumers of crops so produced (3,4). The presence, prevalence and distribution of intestinal parasites in wastes have been reasonably reported in different parts of the world (4,5-9). The most important of these parasites among others are ***Ascaris lumbricoides, Necator*** americanus ***Ancylostoma duodenale, Trichuris trichiura, Taenia*** spp and some protozoa.

In Nigeria, the parasitic diseases that have been associated with solid wastes include malaria, myiasis, amoebiasis, filariasis, ascariasis and taeniasis (10,11). Ologhbo (12) demonstrated the role of wastes in the transmission of ***Trichuris trichiura*** and ***Ascaris lumbricoides*** in slums and low-income neighbourhoods, of many towns and cities in Nigeria. Okoronkwo and Onwuliri (13,14) documented significantly high prevalence of protozoa cysts and helminth eggs in municipal waste handlers; and from municipal refuse and abattoir wastes respectively in Jos Metropolis of Plateau State, Nigeria.

A number of intestinal parasites of cattle, pigs, ruminants and poultry which are of public health importance have been reported (5) gastrointestinal nematode eggs have also been regularly demonstrated in dung heaps on farms elsewhere (16-19).

Previous studies indicated that there is an increasing use of waste waters human and animal wastes for irrigation agriculture in Plateau States (20) and that as a consequence of this, there is contamination of vegetables and salad crops as produced by helminth eggs (21). Similarly, studies of irrigation water, as well as the stream and pond sediments also revealed the presence of parasitic helminthes (22,23).

The objective of the present study is to describe the parasite impact and the public health importance of waste utilization on dry-season irrigation farmers in some parts of Plateau State, Nigeria.

MATERIALS AND METHODS

The Study Area:

This study was carried out in six Local Government Areas of Plateau State, Nigeria, including Bassa, Jos North, Jos South, Jos East,

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Riyom and Barkin-Ladi, where intensive dry season irrigation farming is going on. Further details of the study area are shown in Figure 1.

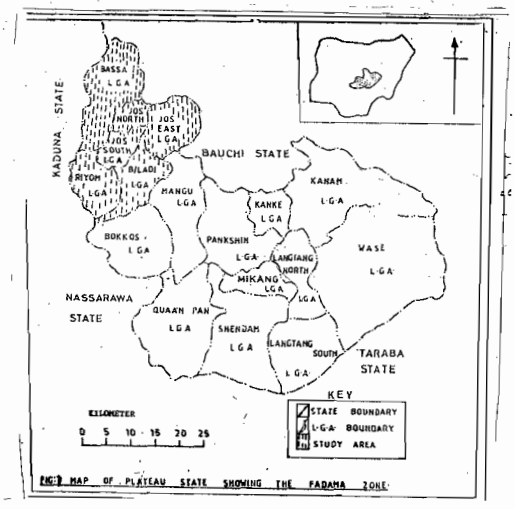

FIG: 1 MAP OF PLATEAU STATE SHOWING THE FADAMA ZONE.

Figure 1: Map of Plateau State Showing the Fadama Zone.

The Dilimi River and its tributaries as well as five other rivers and eight abandoned mine ponds were the major sources of water for crop irrigation in the study area. These water bodies received numerous discharges of raw sewage, animals slurries, night soil, municipal/community refuse from the municipality with some diversions, from where the wastewater laden river water was transported to cultivation areas through irrigation canals.

In the dry season, the irrigation farmers make substantial investments towards the building of canals and appurtenances. The farmer produce a variety of exotic crops, which include tomatoes, carrots, lettuce, cabbage, cucumber, onions and other such vegetable and salad crops meant for the Nigerian market.

In this cross-sectional comparative study, the cluster sampling technique was adopted in the random selection of one hundred and twenty farmers from each of the nine sampling stations (giving a total of 1080 samples). One hundred and thirty five other farmers who used only chemicals fertilizers and matched for age and sex were similarly selected as controls (24).

Stool samples were collected from each of the 1080 dry-season farmers and 135 controls. The samples were quickly preserved in 10% formal-saline and transferred to the Public Health Laboratory of the Department of Community Health University of Jos, for examination for the presence of protozoal cysts and helminthic eggs. Stool concentration was done using the formal-ether concentration technique modified by Allen and Ridley (25). Parasite eggs were quantified by the Stoll egg count technique modified by Fleck and Moody (26) in all the positive stool samples.

The type of cysts and eggs of parasite encountered in the samples were identified to reference to Muller (27) and Soulsby (28).

The results were fed into the computer (Epi Info version 5) and the chi-squared test was used for comparative analysis.

RESULTS

Out of a total 1080 dry-season farmers spread over nine locations in the study area, 989 (91.6%) were infected with one type of helminthic parasite or the other, while 633 (86.4%) were infected with different types of protozoa (Table 1). Similarly, of the 135 farmers in the control group, 104 (76.5%) had helminth infections, while 77 (56.6%) had protozoal infections. There was a significant difference in the combined rates of infection with both helminths and protozoa between the control group and the rest of the farmers (Chi-square=16; df=8 $p<0.05$). There was however, no significant difference between the infection rates of the control and the rest of the farmers at the different locations.

Infection rates between the farmers and control were however, significantly different with ***Schistosoma mansoni*** (476 (44.1%) versus (32 (23.7%); ***Ascaris lumbricoides*** 915 (84.7%) and ***Trichuris trichiura*** 801 (74.2%) versus 66 (48.9%) (Chi-square = 8.7; df = 3 $p<0.05$). ***Enterobius vermicularis*** infection in the two groups was not significantly different. ***Strongyloides stercoralis*** infection however, was significantly different between the farmers 543 (50.3%) and control 24 (17.8%) (Chi-square = 1.6; df = 1 $p<0.05$).

Table 2 shows the prevalence of different parasitic species among the farmers at the different locations. The results show that there was no significant variation between infection rates by protozoa in the farmers and the control. But between locations, ***Entamoeba histolytica*** infection was highest in farmers at Anglo Jos (41.7%) and Zaramaganda (41.7%). Farmers at Anguldi had 50.0% of them infected with ***Entamoeba coli,*** The table 2 further indicated that among the helminths,

infection rates with ***Taenia*** spp; ***Hymenolepis nana, Hymenolepis diminuta, Dipylidium caninum*** and ***Enterobius vermicularillis*** were very low in all the nine locations and even within the control group. The greatest problem of the farmers seemed to be from ***Schistosoma mansoni,*** which infected 81.7% of farmers at Farin-gada, 50.0% at Anglo-Jos, 47.5% at Zaramaganda and 44.2% at Mai-Adiko; ***Ascaris lumbricoides*** infection was high among farmers at all locations, but highest at Mai-Adiko (99.2%), Farin-Gada (90.8%), Korot (89.2%) and Zaramaganda (89.2%). Both ***Necator americanus and Ancylostoma duadenale*** appear to have been evenly distributed at the various farming locations with values ranging from 41.3% to 56.3%. Infection with ***Trichuris trichiura*** was somewhat higher than the hookworms. Thus, farmers at Farin-Gada (82.5%), Mai-Adiko (80.0%), and Anguldi (79.2%) were the worst affected infection rates of farmers at Gada-Biu and Foron/Bisichi can be gleaned from Table2.

The prevalence rates of intestinal parasites in different age groups and in both sexes are presented in Table3 ***Entamoeba histolytica, Entamoeba coli, and Endolimax*** nana were more prevalent in the older age groups. ***Giardia lambia, Iodoamoeba butschlii, Chilomastix mesnili, Isospora belli and balantidium coli*** were highest in the younger age groups (19-39 years olds).

Similarly, ***Ascaris lumibricoides and Tricuris trichiura*** were more prevalent in the younger farmers, while ***Strongyloides stercaralis*** and ***Taenia*** spp were more commonly found in the older age groups. Hookworm and Schistosoma masoni were highly prevalent in all age groups. ***Enterobius vermicularis, Hymenolepsis nana, H. dimunita and Dipylidium caminium*** were too few to be analysed by age and sex.

However, the frequency of infection increased with age to a peak value in the 30 39 and 40 49 year age groups. The farmers in this subpopulation includes young farmers of 30 39, and 40 49 years who constitute up to 60% of farmers harbouring the intestinal parasites encountered at Farin-Gada/Dilimi, Anglo-Jos and Zaramangada. Generally, males had higher worm burdens than females, but there were also differences in sex prevalence rates of the various parasites. Hookworm infection did not show any sex-related differences in prevalence rates, but more females were infected with ***Ascaris, Trichuris and Entamoeba histolytica.*** Infection with ***Taenia*** species was rather diffuse and did not show any sex related differences as well.

Both the farmers and the control group expressed some symptoms during sampling 617 (57.1%) of the farmers compared with 19 (14.1%) of the control group had diarrhoea; 202 (18.7%) of the farmers compared with (1 (5.2%) of the control group complained of fever; 168 (15.6%) of the farmers compared with 4 (3.0%) of the controls said they were having skin itch; while 397 (36.8%) of the farms compared with 12 (8.9%) of the controls reported abdominal discomfort at sampling time.

Location of Farmers	Helminths			Protozoa		
	No examined	No Positive	Infection Rate (%)	No examined	No Positive	Infection Rate (%)
Control	135*	104*	(76.5)	135	77*	(56.6)
Yelwa Mista Bow	120	104	(86.7)	120	62	(51.7)
Farin-Gada/Dilimi	120	120	(100.0)	120	83	(69.2)
Anglo-Jos	120	112	(93.3)	120	81	(67.5)
Zaramaganda	120	117	(97.5)	120	103	(85.8)
Mai-Adiko	120	106	(88.3)	120	92	(76.7)
Anguldi	120	99	(82.5)	120	67	(55.8)
Gada-Biu (B/Ladi)	120	109	(90.8)	120	57	(47.5)
Foron/Bisichi	120	103	(85.8)	120	71	(59.2)
Korot	120	119	(99.2)	120	74	(61.7)
Total	1080	989	(91.58)	1080	633	(86.39)

*Figures for the Control were taken separately.

Table 1 : Prevalence of Helminths and Protozoa among Farmers at Different Locations in the Study Area

Parasite	Yewa Misc Sow	Fann-Gada	Anglo-Jos	Zaramaganda	Ma-Adiko	Anguldi	Gada-Biu	Foron /Bischi	Kirot	Control Group
	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)	No Pos (%)
PROTOZOA										
Entamoeba histolytica	12 (10.0)	18 (15.0)	50 (41.7)	38 (31.7)	18 (15.0)	16 (13.3)	11 (9.2)	16 (13.3)	21 (17.5)	23 (19.3)
Entamoeba col	15 (12.5)	34 (28.3)	31 (25.8)	32 (26.7)	36 (30.0)	60 (50.0)	17 (14.2)	15 (12.5)	31 (25.8)	36 (26.7)
Endolmax nana	13 (10.8)	10 (8.3)	19 (15.8)	24 (20.0)	6 (5.1)	69 (57.5)	5 (4.2)	12 (10.0)	18 (15.0)	10 (7.4)
Iodoamoeba butschli	3 (2.5)	13 (10.8)	11 (9.2)	17 (14.2)	11 (9.2)	9 (7.5)	10 (8.3)	3 (2.5)	18 (15.0)	15 (11.1)
Giardia lamblia	12 (10.0)	14 (11.7)	29 (24.2)	28 (23.3)	14 (11.7)	16 (13.3)	10 (8.3)	7 (5.8)	7 (5.8)	12 (8.9)
Chilomastix mesnil	8 (6.7)	10 (8.3)	25 (20.8)	25 (20.8)	11 (9.2)	31 (25.8)	10 (8.3)	15 (12.5)	15 (12.5)	16 (11.9)
Balantidium col	4 3.3)	0 (0.0)	1 (0.8)	3 (2.5)	2 (1.7)	1 (0.8)	4 (3.3)	5 (4.2)	0 (0.0)	0 (0.0)
Isospora bell	7 5.8)	2 (1.7)	0 (0.0)	1 (0.8)	0 (0.0)	47 (39.2)	3 (2.5)	3 (2.5)	2 (1.7)	3 (2.2)
HELMINTHS										
Taenia spp	8 6.6	11 (9.2)	9 (7.5)	7 (5.8)	15 (12.2)	4 (3.3)	2 (1.7)	10 (8.3)	9 (7.5)	11 (8.1)
Hymenolepis nana	7 5.8	8 (6.7)	5 (4.2)	5 (4.2)	1 (0.8)	5 (4.2)	9 (7.5)	10 (8.3)	4 (3.3)	7 (5.2)
Hymenolepis diminuta	4 3.3	1 (0.8)	2 (1.7)	2 (1.7)	3 (2.5)	3 (2.5)	7 (5.8)	6 (5.0)	12 (10.0)	2 (1.5)
Dipylidium caninum	3 2.5	6 (5.0)	3 (2.5)	2 (1.7)	1 ' 0.8)	2 (1.7)	0 (0.0)	2 (1.7)	7 (5.8)	9 (6.7)
Schistosoma manson	28 23.3	96 (81.7)	60 (50.0)	57 (47.5)	53 (44.2)	45 (37.5)	42 (35.0)	49 (40.8)	46 (38.3)	32 (23.7)
Ascaris lumbricoides	89 74.2	109 (90.8)	100 (83.3)	107 (89.2)	119 (99.2)	104 (86.7)	88 (73.3)	92 (76.7)	107 (89.2)	71 (52.6)
Ancylostoma duodenale	61 50.8	66 (54.6)	71 (59.3)	54 (45.0)	57 (47.1)	63 (52.0)	55 (45.5)	61 (50.9)	64 (53.4)	35 (30.9)
Necator americanus	54 45.0	57 (47.1)	59 (48.8)	61 (52.8)	59 (49.1)	58 (47.9)	50 (41.3)	55 (45.9)	55 (45.9)	30 (22.0)
Trichuris trichura	72 60.0	99 (82.5)	92 (76.7)	90 (75.0)	96 (80.0)	95 (79.2)	90 (75.0)	84 (70.0)	83 (69.2)	66 (48.9)
Enterobius vermicularis	3 2.5	0 (0.0)	0 (0.0)	4 (3.3)	3 (2.5)	6 (5.0)	2 (1.7)	0 (0.0)	2 (1.7)	5 (3.7)
Strongylo stercoralis	19 15.8	79 (65.8)	47 (39.2)	69 (57.5)	65 (54.2)	54 (45.0)	57 (47.5)	74 (61.7)	69 (57.5)	24 (17.8)

TABLE 2: Prevalence of Intestinal Parasites Among Dry Season Irrigation Farmers Using Human and Animal Wastes and Control Farmers Using Chemical Fertilizers

Study Locations

	Age in Years							Sex		
	10-19	20-29	30-39	40-49	50-59	60-69	Above 70	Male	Female	Total
PROTOZOA	n=124	n=156	n=265	n=259	n=197	n=98	n=59	n=810	270	n=1080
Entamoeba histolytica	27(21.4%)	46(29.6%)	99(37.4%)	59(22.9%)	80(40.7%)	53(54.5%)	32(54.5%)	286(35.3%)	95(35.3%)	381(35.3%)
Entamoeba coli	9(7.1)	30(19.0%)	54(20.4%)	51(19.5%)	54(27.5%)	35(35.4%)	27(45.4%)	194(24.0%)	54(20.0%)	261(24.2%)
Endolimax nana	0(0.0%)	0(0.0%)	17(6.6%)	34(13.2%)	58(29.4%)	16(16.3%)	5(9.1%)	16(2.0%)	5(1.7%)	132(12.2%)
Iodoamoeba butschlii	18(14.2%)	11(7.1%)	23(8.2%)	18(6.9%)	23(11.7%)	9(9.1%)	13(21.2%)	79(9.7%)	22(8.0%)	102(9.4%)
Chilomastix mesnil	53(42.8%)	14(9.1%)	46(17.3%)	27(10.4%)	36(18.4%)	14(14.5%)	7(12.2%)	100(12.4%)	43(16.0%)	144(13.3%)
Giardia lamblia	81(65.7%)	67(42.8%)	65(24.4%)	45(17.3%)	11(5.5%)	5(5.5%)	5(9.0%)	167(20.0%)	61(20.6%)	222(20.6%)
Balantidium coli	5(4.0%)	9(5.8%)	0(0.0%)	5(1.9%)	0(0.0%)	0(0.0%)	0(0.0%)	8(1.0%)	11(4.1%)	19(1.8%)
Isospora belli	10(8.1%)	4(2.6%)	0(0.0%)	0(0.0%)	2(1.0%)	6(6.1%)	0(0.0%)	17(2.1%)	5(1.7%)	22(2.0%)
HELMINTHS										
Taenia spp	18(14.2%)	16(10.2%)	23(8.2%)	20(7.6%)	22(11.0%)	0(0.0%)	0(0.0%)	71(8.8%)	29(10.7)	97(9.0%)
Hymenolepis nana	21(16.9%)	12(7.7%)	7(2.6%)	16(6.2%)	0(0.0%)	0(0.0%)	0(0.0%)	42(5.2%)	14(5.2%)	56(5.2%)
Hymenolepis diminuta	0(0.0%)	0(0.0%)	15(5.7%)	3(1.2%)	0(0.0%)	0(0.0%)	0(0.0%)	10(1.2%)	8(3.0%)	18(1.7%)
Dipylidium caninum	19(15.3%)	0(0.0%)	5(3.2%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	19(2.3%)	5(1.9%)	24(2.2%)
Schistosoma mansoni	53(42.8%)	85(56.2%)	129(48.8%)	122(47.7%)	94(47.7%)	53(54.5%)	20(33.3%)	78(46.7%)	151(56.0%)	529(49.0%)
Ascaris lumbricoides	89(71.4%)	88(81.6%)	213(80.2%)	218(84.2%)	177(89.9%)	93(94.5%)	54(90.9%)	761(94.0%)	228(84.5%)	943(87.3%)
Ancylostoma duodenale	106(85.7%)	140(89.6%)	251(94.6%)	252(97.2%)	179(90.4%)	94(96.4%)	48(81.9%)	634(78.3%)	261(96.8%)	1017(94.2%)
Trichuris trichiura	106(85.7%)	112(71.5%)	254(96.0%)	233(90.0%)	152(77.0%)	87(89.1%)	45(75.8%)	486(60.0%)	173(64.0%)	808(74.8%)

Table 3:- Distribution of Intestinal Parasites in Different Groups and Sexes of Dry Season Irrigation Farmers in Jos and Barkin-ladi Areas of Plateau State, Nigeria

DISCUSSION

Parasitic infections of the dry0season farmers and the controls were comparatively high. Among the helminthic parasites, the results show that the most highly prevalent species found in the farmers were the hookworm, ***Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis and Schistosoma mansoni.*** Among the protozoa, ***Entamoeba histolytica, Entamoeba coli, Endolimax nana, Giardia lambia, Chilomastix mesnil and Iodoamoeba butshili*** constitute the major spectrum. These results confirm earlier reports that these same range of intestinal helminthic and protozoa constitute the greatest transmission potential waste re-use schemes (3,6,29,30). These findings also indicate that the use of untreated river or pond water for irrigation can lead to a qualification excess of the parasites listed above among dry season farmers irrespective of their farming location.

The explanation for the observed distribution of infection in this study is not certain, but it is probable that several transmission routes are involved, with dry-season irrigation as only one factor. Principal among the multifactoral reasons for the high prevalence of parasitic diseases in both farmers and control are poor environmental sanitation, poor hygiene habits and lack of health education. In the study area, excreta disposal is practiced on-site i.e. mainly through open defecation and therefore, there is no organised sanitation infrastructure particularly in the metropolitan slums of Kobang, Angwan-Rogo, Dilimi, Nasarawa-Gom, Angwan-Rukuba, Congo-Rissa and Tudun-Wada (20). The habit of the farmers of working barefoot in their farms, and the agricultural practice of using bare hands to construct earth dams for crop irrigation can also be a contributory factor to the high prevalence of intestinal parasites in the dry-season farmers. This creates room for the broken skin of their hands and feet to be penetrated readily by the motile hookworm larvae, ***Schistosoma mansoni*** cercariae and perhaps ***Strongyloides stercoralis.*** A parallel view has similarly been expressed by Krishnomoothi et al (31) and Shuval (32) who noted that sewage farm workers in India exposed to raw wastewater had much higher levels of infection with nematode eggs than other agricultural workers.

As is common with most cultures, several aspects of the farmers culture influence sanitary practice and thereby also the impact of the use of wastes. First, most of the farmers studied were Moslems (61%) from Katsina, Kano and Borno States (20). Anal cleansing is performed with water, applied by hand, and there is no tradition of washing the hands after wards with soap. It would seem likely therefore, for excreta-related diseases to be higher in this group. Any consideration of the impact on health of the use of waste wastewaters in irrigation must take into account other social and cultural factors, which have an influence on the occurrence or transmission of gastro-intestinal infections.

The spectrum and intensity of infections varied according to age and sex; thus the result showed that most infections increased with age up to the age of 50 years, except for ***Giardia lamblia.*** This finding is in agreement with the observations of Yakubu and Bello (33). The higher prevalence rates of ***Ascaris, Trichuris*** and other nematodes in the younger age groups (i.e. 19-39, 30-39, 40-49 age groups) suggests that this age group spent more time in their farms thereby having more frequent contact with the wastes. This pattern of frequency distribution of parasites is widely recognised, and according to Nwosu (34) it is the younger age group that falls within the negative binomial sub-population whose behaviour underlies the maintenance of parasitic infections at high levels.

Males had higher parasite rates for the various intestinal parasites than females. However, females were infected with ***Ascaris lumbricoides, Trichuris Trichiura and Entamoeba histolytica*** than males, but hookworm, and ***Schistosoma mansoni*** did not reveal any sex related differences. These results are similar to those of Nwosu(34).

Diarrhoea disease, abdominal discomfort, skin itch and fever were the major symptoms causing morbidities among the farmers. The result has confirmed earlier reports that gastrointestinal infections are endemic in Plateau State, Nigeria (35,36). Our results further showed that 59% and 42% of the dry-season farmers showed symptoms of diarrhoea and abdominal discomfort respectively. This is in agreement with the work of Krishnonmoorthi et al (31); who correlated the symptoms with exposure to untreated sewage in five farming locations in India. At Yelwa-Mista Bow, Farin-Gada, Dilimi and Anglo-Jos, more farmers showed

symptoms of diarrhoea, abdominal discomfort and skin itch than farmers at Anguldi, Ga-Biu, Fom/Bisichi and Korot. As shown in Table 3, the varying degrees of parasitic infections associated with diarrhoea, are consistent with the work of Yakubu and Bello (33) who observed diarrhoea as a common symptom in amoebiasis, giardiasis, trichuriasis and strongyloidiasis.

In nearly all the irrigation farms, the main crops are vegetable and salad crops. The detection of helminth eggs (***Ascaris, Trichuris***, hookworm and ***Toxocara eggs***) from the irrigation water (24) and even from the vegetable and salad crops grown by irrigation (21) means that thousands of dry-season farmers are at increased risk of intestinal nematode infections, and possibly other parasitic and bacterial infections. In addition, many consumers of vegetable crops may also be at risk. The most effective way to control these risks would be to institute some form of partial wastewater treatment prior to use. A waste stabilisation pond system capable of reducing the concentration of nematode eggs would be most suitable. Alternatively, the risk to irrigation farmers could be reduced by control of human exposure and modification of waste management practices.

Although there is a law prohibiting the application of raw wastewater on salad vegetable, this practice is still going on in Plateau State. Apparently, there is little or no enforcement of the law regulating this practice. Government bodies are not routinely involved in the monitoring and control of the health and agricultural effects of waste re-use. Treatment of the irrigation water is therefore; absolutely necessary in order to reduce the health risks to the dry-season farmers and consumers of the vegetables and salad crops.

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