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Cover Page, Introduction, Contents, Call for Papers, All papers in one file

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Mechanical Stress Analysis By Eddy Current Method

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Abstract: A lot of progress is made in the last decade concerning the theoretical and practical aspects of eddy current testing. In addition to the defect characterization, actual studies deal with the metallurgical evaluation of materials. Surface examination allows the prediction of the material strength and consequently its life time. In order to obtain various microstructures and to modify the mechanical and metallurgical characteristics of materials, samples made from aluminium or steels have been exposed under mechanical stress. It was shown in this work that all microstructure modifications of the samples were detected and they can be quantified by eddy current impedance measurement. The impedance analysis by eddy current will be correlated with the microstructure changes observed in the material because of plastic damage and fatigue. [The Journal of American Science. 2008;4(3):1-6]. (ISSN 1545-1003).

Keywords: mechanical Stress; method; microstructure

Introduction

The ability to control the working stresses level in mechanical components and structures is an important factor in engineering industries. Evaluation and monitoring of the stress state of these elements is time consuming, because of the conventional techniques involved [4].

The characterization of microstructures, mechanical properties, deformation, damage initiation and growth by Non-Destructive Evaluation (NDE) techniques plays a vital role in various industries because of the growing awareness of the benefits that can be derived by using NDE techniques for assessing the performance of various components. Fracture mechanics based analysis of component integrity requires quantitatively characterization of microstructure defects as well as stresses.

Any alteration in the microstructure, which reduces the life or performance, should be predicted sufficiently in advance in order to ensure safe, reliable and economic operation of the components. This prediction is possible with NDE techniques, so far the interaction of the non-destructive probing energy with the material depends on the sub structural / micro structural features such as point defects, dislocations, voids, micro and macro cracks, secondary phases, texture and residual stress. The stress sensitivity plays a very important role with respect to the different material properties.

Physical approach

Various non-destructive techniques are available for the measurement of either applied and/ or residual stresses [5-10]. Eddy current testing is also sensible to changes in micro structural characteristics and the stress state of the material as well and can be used to evaluate these materials characteristics.

Eddy current testing [1, 2] allows to evaluate the state of stress in ferromagnetic material. The method can be used for determining residual stress, also named inner stress, as well as stress induced by external loads. In the study by Dybiec et al. [1] eddy current inspection is used to evaluate the state of stress in ferromagnetic material. Because a notable change in the magnetic characteristics can be observed, even at small values of strain degree, the technique has high sensitivity.

It is known that during plastic deformation microstructure irregularities, i.e. lattice defects, are generated (e.g. microspores, micro cracks, vacancies, etc). The lattice defects are observed even at very early stages of plastic deformation. The formation of discontinuities is combined with partial relaxation of elastic energy [1], which leads to changes in the magneto-elastic energy of the material in regions adjacent to the lattice defects. This phenomenon is likely to affect the magnetic and electric parameters of materials.

Experimental Procedure

A Merlin machine of traction is used for evaluating the solicitation degree of the material in the gauge length of the specific specimen. The effect of traction strain and accumulated damage on magnetic

properties was studied by using standard tensile specimen subjected to elongation. The correlations obtained allowed to evaluate the traction strain and current damage, as well as to estimate the residual lifetime of the specimen under traction.

The equipment applied can be characterized by its high sensitivity to detect changes in the material microstructure. The software allows to continuously control the tensile load or stepwise. The eddy current probe is placed in the predicted zone of failure which was preliminarily determined by tests. At each specific load, five local impedance amplitude or phase values were measured in the rupture zone. (Figure 1).

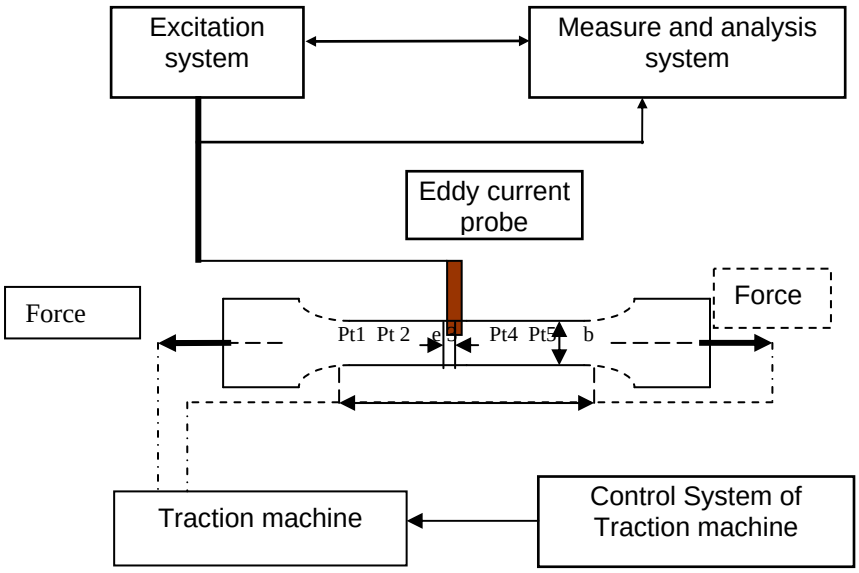

Figure 1: Principle of the measuring system (Traction and eddy current)

Measuring conditions

In order to obtain the required measuring precision the recommendation is:

- to determine the rupture load
- to select an incremental load step of 3KN in case of aluminium alloy 2024 with 3 minutes of maintain time
- to select an incremental load step of 8KN in the elastic regime and 3KN in the plastic regime in case of stainless steel 304L with 2 minutes of maintain time.

At each load step, the impedance amplitude and phase of the eddy current probe impedance in the five selected points was measured.

Results and Interpretation

The objective is to find a relationship between the electric/magnetic and the load parameters. Therefore the sample was exposed to traction while taking measurements of the phase and the amplitude of the impedance in several points of the specimen.

The curves representing these relationships typically show a transition behaviour.

This result is obtained for all of locally selected points chosen on the sample according to Figure1, which indicates that the microstructure presents a priori the same electric and magnetic modification when the sample is exposed to traction. This fact is observed for all of the curves representing the impedance as function of mechanical properties like load, elongation and deformation (Figure 2-Figure 7).

This result can be verified also for the phase behaviour (Figure 12- Figure 17).

The curve representing the impedance as function of the load typically shows two zones of transitions. It is to notice that in case of Aluminium a first transition in the curve is observed in the load

range 9-10 kN corresponding to an impedance of 23.82 Ohm, the second is located at 22-24 kN corresponding to an impedance of 23.78 Ohm.

These transitions are observed in all of the curves representing the impedance as function of the deformation, the elongation, and the load. (Table 1).

Table1: Alumium, amplitude of the impedance and mechanical parameters at the transition points

Aluminium	Amplitude Z(Ohm)	A(%)	DI(mm)	P(kN)
transition1	23.82	2.22	4.82	9-10
transition 2	23.78	4.5	5.2	22

This significant feature is also observed in the functional representations of the phase versus various mechanical parameters (Table 2)

Table 2: Aluminium, phase of the impedance and mechanical parameters at the transition point

Aluminium	Phase (degree)	A(%)	DI(mm)	P(kN)
transition 1	35.32	2.22	4.82	9
transition 2	35.45	4.5	5.2	22

We notice that in case of the austenitic steel, the curve representing the impedance as function of the load also shows two zones of transition (figure 2-4). Table 3-4 gives the values of the impedance and the phase as function of the deformation, elongation, and the load for the two points of transitions
In comparison of table 1 and 3 and table 2 and 4 the different results obtained at austenitic steel and aluminium are visible. The differences allow to individually characterize the materials behaviour under load... The value of the impedance or the phase is different between the first and the second transition points.

Table 3: Steel, amplitude of the impedance and mechanical parameters at the transition points

Steel	Amplitude Z(Ohm)	A(%)	DI(mm)	P(kN)
transition 1	21.910	2.41	4	18
transition 2	21.885	5-6	9	55-58

Table 4: Steel, phase of the impedance and mechanical parameters at the transition points

Steel	Phase (degree)	A(%)	DI(mm)	P(kN)
transition1	27.285	2.4	4	18
transition2	27.300	5	8-9	55-58

These results at Aluminium are confirmed by discussing the mechanical parameters alone where the transition points can be observed too (figure 8-9). (table 5).

Whereas the impedance curve of the austenitic steel reveals two transition points, allows the observation of the mechanical measurements only the detection of the second transition (figure 10-11) (table 6).

Table 5:

Aluminium mechanical measurements	P(kN)	A(%)	DI(mm)
transition 1	9	2.2	4
transition 2	25	4.5	5-6

Table 6

Steel mechanical measurements	P(kN)	A(%)	DI(mm)
transition 2	55	5	9

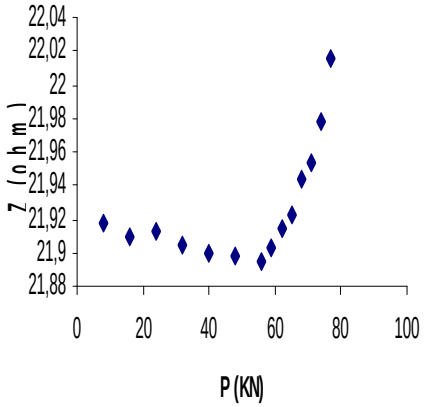

Figure2: impedance as function of the load for steel

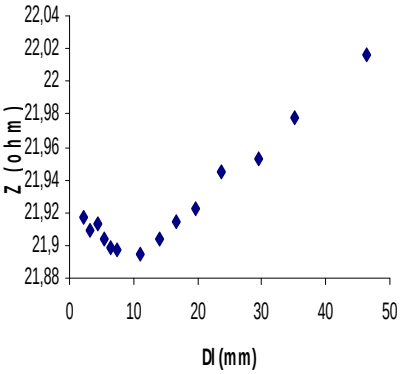

Figure3: impedance as function of the

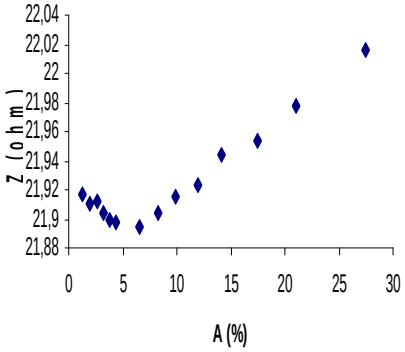

Figure4: impedance as function of the deformation for steel

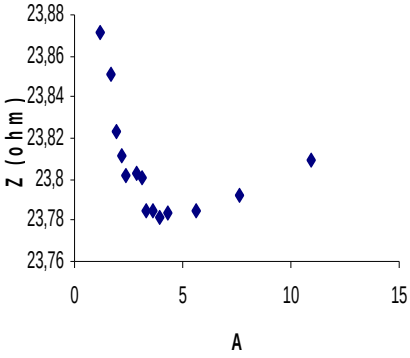

Figure5: impedance as function of the

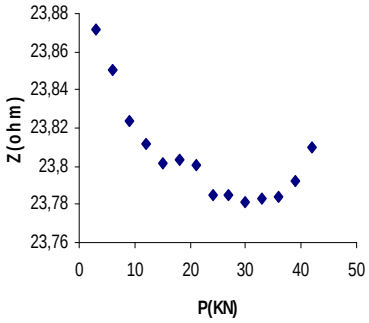

Figure:6 Impedance as function of the load for aluminium

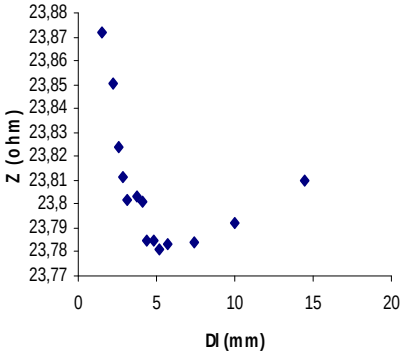

Figure:7 impedance as function of the

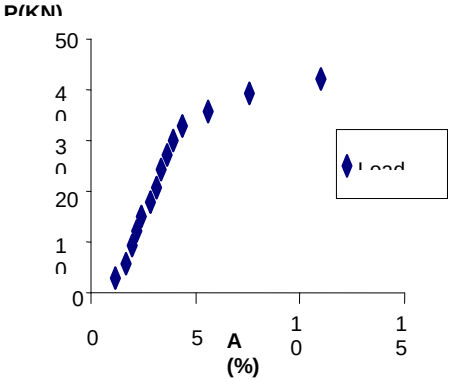

Figure 8. Load as function of deformation for aluminium

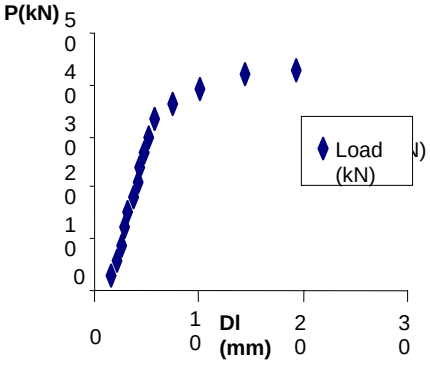

Figure 9. Load as function of elongation for aluminium

These results are significant in order to non-destructively detect the elastic limit and the plasticity of material. In the case of aluminium, the first transition point corresponds to the elastic limit and the second corresponds to the limit of mechanical resistance, a very useful parameter concerning the determination of the endurance against fatigue. For steel we notice two transition points which correspond to the elastic limit and the start of the plasticity. In the case of steel we also observe that the impedance increase and the phase decrease after the second transition point, corresponding to the start of the plasticity. In the austenitic stainless steel the development of a magnetization by increasing the tensile stress can be observed, according to a localized phase transformation of the fcc austenitic phase to bcc α' cold-forming martensitic phase [9].

In case of the aluminium, we notice in the curves (figure 5 to7 and 15 to17) that the impedance and the phase have a relation with the young modulus. This fact is very important because we can measure the elastic parameter only by an impedance amplitude or/and phase measurement. Also the start of the plasticity can be detected.

Conclusion

Lifetime extension of components in technical applications is a general task with tremendous economical benefits. NDT/NDE has developed first attempts for materials characterization taking into account damage assessment as part of the in service inspection. We have shown in this work the relation between parameters obtained by eddy currents measurements and mechanical parameters.

The curve representing the impedance or the phase as function of the elongation or deformation follow a well determined trajectory where the elastic limit and the start of the plasticity of the material can be detected by the impedance amplitude or the phase measurement. In case of aluminium the relations between the impedance phase and the load as function of the elongation has the same shape.
The curve of the phase increases linearly with the load in the elastic regime and therefore it is possible to determine Young's modulus.

This work shows the ability to determine the material behaviour exposed to external loads in the elastic as well as in the plastic regime by analysis of eddy current inspection results only. The elastic limit or the start of plasticity can be detected by the impedance measurement. In the case of aluminium, it is also possible in the future to evaluate Young's modulus by the phase analysis.

The results are very significant for the non-destructive mechanical properties determination and useful to be applied in In Service Inspection.

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Alpha-Smooth Muscle Actin (α-SMA)

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Abstract: Alpha-smooth muscle actin (alpha-SMA) is the actin isoform that predominates within vascular smooth-muscle cells and plays an important role in fibrogenesis. Myofibroblasts are metabolically and morphologically distinctive fibroblasts expressing alpha-SMA, and their activation plays a key role in development of the fibrotic response. In an activated state, myofibroblasts cease to proliferate and start to synthesize large amounts of extracellular component proteins. The expression of alpha-SMA correlates with the activation of myofibroblasts. In contrast, the expression of alpha-SMA in cells made quiescent by cell-cell contact was lower than that in cells made quiescent by serum starvation. [The Journal of American Science. 2008;4(4):7-9]. (ISSN: 1545-1003).

Keywords: Alpha-smooth muscle actin (alpha-SMA); fibrogenesis; myofibroblasts; proliferate; cell

Introduction

Alpha-smooth muscle actin (alpha-SMA) is the actin isoform that predominates within vascular smooth-muscle cells and plays an important role in fibrogenesis (Kawasaki et al., 2008). alpha-SMA was recently shown to be present in mouse subcutaneous tissue fibroblasts in the absence of tissue injury (Storch et al., 2007). Myofibroblasts are metabolically and morphologically distinctive fibroblasts expressing alpha-SMA, and their activation plays a key role in development of the fibrotic response. In an activated state, myofibroblasts cease to proliferate and start to synthesize large amounts of extracellular component proteins. The expression of alpha-SMA correlates with the activation of myofibroblasts. In contrast, the expression of alpha-SMA in cells made quiescent by cell-cell contact was lower than that in cells made quiescent by serum starvation (Nakatani et al., 2008).

Myofibroblasts are a form of fibroblast cell that has differentiated partially towards a smooth muscle phenotype (Elberg et al., 2008). It can contract by using some of the cytoskeletal proteins that are normally found in smooth muscle cells, in particular alpha-SMA (Jiroutova et al., 2005). These cells are then capable of speeding wound repair by contracting the edges of the wound. Early work on wound healing showed that granulation tissue taken from a wound, could contract in vitro in a similar fashion to smooth muscle, when exposed to substances that cause smooth muscle to contract, such as adrenaline or angiotensin (Park et al., 2007). After healing is complete, these cells are lost through apoptosis and it has been suggested that in several fibrotic diseases that this mechanism fails to work, leading to persistence of the myofibroblasts, and consequently expansion of the extracellular matrix and contraction (Darby and Hewitson, 2007). It is generally accepted that fibroblast-to-myofibroblast differentiation represents a key event during wound healing and tissue repair. The high contractile force generated by myofibroblasts is beneficial for physiological tissue remodeling but detrimental for tissue function when it becomes excessive such as in hypertrophic scars, in virtually all fibrotic diseases and during stroma reaction to tumors. Specific molecular features as well as factors that control myofibroblast differentiation are potential targets to counteract its development, function, and survival. Such targets include alpha-smooth muscle actin and more recently discovered markers of the myofibroblast cytoskeleton, membrane surface proteins, and the extracellular matrix. Moreover, intervening with myofibroblast stress perception and transmission offers novel strategies to reduce tissue contracture; stress release leads to the instant loss of contraction and promotes apoptosis (Hinz, 2007).

Actin is a globular structural protein that polymerizes in a helical fashion to form an actin filament. These form the cytoskeleton - a three-dimensional network inside an eukaryotic cell. Actin filaments provide mechanical support for the cell, determine the cell shape, enable cell movements. In

muscle cells they play an essential role, along with myosin, in muscle contraction. In the cytosol, actin is predominantly bound to ATP, but can also bind to ADP. An ATP-actin complex polymerizes faster and dissociates slower than an ADP-actin complex. Actin is one of the most abundant proteins in many eukaryotic cells, with concentrations of over 100 μM. It is also one of the most highly conserved proteins, differing by no more than 5% in species as diverse as algae and humans (Lambert et al., 2005).

Alpha-SMA molecular weight is 42 kD. The individual subunits of actin are known as globular actin, while the filamentous polymer composed of G-actin subunits (a microfilament), is called F-actin. The microfilaments are the thinnest component of the cytoskeleton, measuring only 7 nm in diameter. Much like the microtubules, actin filaments are polar, with a fast growing plus (+) or *barbed* end and a slow growing minus (-) or *pointed* end. ADP-actin dissociates from the minus end and the increase in ADP-actin stimulates the exchange of bound ADP for ATP, leading to more ATP-actin units. This rapid turnover is important for the cell's movement. Many cellular functions depend on rapid cytoskeletal rearrangements localized to specific cytoplasmic domains. End-capping proteins such as CapZ prevent the addition or loss of monomers at the filament end where actin turnover is unfavourable like in the muscle apparatus (DiNubile, 1999).

Actin filaments are assembled in two general types of structures: bundles and networks. Actin-binding proteins dictate the formation of either structure since they cross-link actin filaments. Actin filaments have the appearance of a double-stranded helix. In non-muscle actin bundles, the filaments are held together such that they are parallel to each other by actin-bundling proteins and/or cationic species. Bundles play a role in many cellular processes such as cell division (cytokinesis) and cell movement. Actin, together with myosin filaments, form actomyosin, which provides the mechanism for muscle contraction. Muscular contraction uses ATP for energy. The ATP allows, through hydrolysis, the myosin head to extend up and bind with the actin filament. However ATP is not needed to the attachment of myosin (in muscle it is myosin II) onto the actin filament. The myosin head then releases after moving the actin filament in a relaxing or contracting movement by usage of ADP (Cooke et al., 1994).

In contractile bundles, the actin-bundling protein actinin separates each filament by 40 nm. This increase in distance allows the motor protein myosin to interact with the filament, enabling deformation or contraction. In the first case, one end of myosin is bound to the plasma membrane while the other end *walks* towards the plus end of the actin filament. This pulls the membrane into a different shape relative to the cell cortex. This results in the shortening, or contraction, of the actin bundle. This mechanism is responsible for muscle contraction and cytokinesis, the division of one cell into two (Lewalle et al., 2008).

Actin filaments, along with many actin-binding proteins form a complex network at the cortical regions of the cell. Recent studies have also suggested that actin networks on the cell cortex serve as barriers for molecular diffusion within the plasmic membrane (He et al., 2007; Jaworski et al., 2008).

Principal interactions of structural proteins at cadherin-based adherens junction. Actin filaments are linked to α-actinin and to membrane through vinculin. The head domain of vinculin associates to E-cadherin via α-, β - and γ -catenins. The tail domain of vinculin binds to membrane lipids and to actin filaments (Miyake et al., 2006).

Although most yeasts have only a single actin gene, higher eukaryotes generally express several isoforms of actin encoded by a family of related genes. Mammals have at least six actins, which are divided into three classes according to their isoelectric point. Alpha actins are generally found in muscle, whereas beta and gamma isoforms are prominent in non-muscle cells. Although there are small differences in sequence and properties between the isoforms, all (DeBiase et al., 2006; Lee et al., 2006).

All non-spherical prokaryotes appear to possess genes such as MreB which encode homologues of actin; these genes are required for the cell's shape to be maintained. The plasmid-derived gene ParM encodes an actin-like protein whose polymerised form is dynamically unstable, and appears to partition the plasmid DNA into the daughter cells during cell division by a mechanism analogous to that employed by microtubules in eukaryotic mitosis (Clerici et al., 2005; Stehle et al., 2007).

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INFINITE-TIME And SPACE-TIME

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ABSTRACT: This report will show why photons have duality as a wave and as a particle and will show the origin of atoms and cause of gravity and offer a solution in graphic form that otherwise has remained a mystery. The theory is based on scientific deduction and deemed valid conceptually as outlined in the illustrations giving an understanding of Pi and infinity as it relates to mass in the electro-magnetic and gravitational field. [The Journal of American Science. 2008;4(4):10-13]. (ISSN: 1545-1003).

Keywords: electro-magnetic and gravitational field; energy; time; infinity

INTRODUCTION:

The electromagnetic field is comprised of electromagnetic waves in the opposite direction resulting in standing waves where direction is relative to the source of energy as the waves are without end. The wave duality provides for both the electromagnetic and gravitational forces. A photon is a standing wavelet focused like a particle where the speed of light is relative to infinity and mass is part of infinity. A photon has duality as a wave and as a particle [1]. Energy is equal to frequency of the photons based on Planck's constant.

REPORT:

Electro and magnetic wavelets expand into larger wavelets from the antinodes (displacement) energy of standing waves at twice the wave amplitude of the smaller wave giving distance to the field. The wavelets increase in amplitude to a level causing stress energy from expansion resulting in a coupling link of electro photons to fuse having a strong nuclear force in an open atom with a nuclei and electron(s). The electro field coupling is perpendicular to the inside magnetic field coupling, therefore, the electro link will fuse as the weaker of the two from energy expansion. Atom identity is determined by the life cycle duration of the electro coupling that became mass. Like the life cycle of a star [2], a field coupling having a longer duration to expand will fuse as a lighter atom such as a hydrogen atom. Direction of energy is relative to the source energy of mass and field energy. The field expands into larger wavelets from the displacement energy of standing waves and the energy from mass propagates on the wavelets.

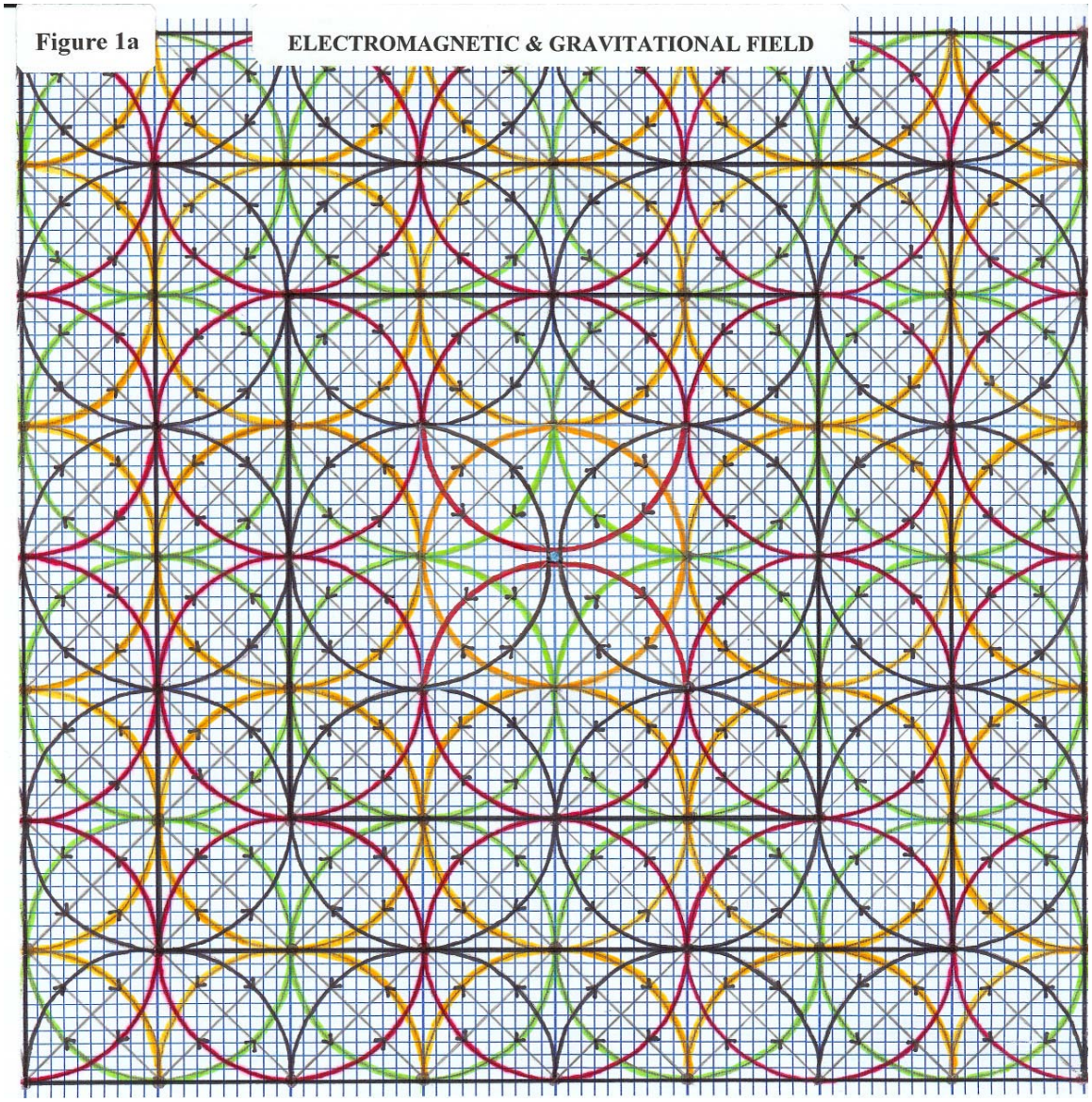
From the field illustration Fig.1a the black-red (dark color) are the electro wavelets perpendicular to the page and brown-green (light color) are the magnetic wavelets on the same plane as the page. The transverse wavelets in the figure must be visualized in three dimensions and the expansion of the wavelets must be visualized in a cone or spiral form. The atom is depicted at center when an electro field coupling link fused from expansion. The coupling link between the open circles is the e coupling constant, like 137.0359 [3]. Due to the expansion of the standing wavelets in a spiral cone (energy displacement of standing waves into larger wavelets), the base or space between the circles give the necessary amplitude for the photons and electron(s) to interact as a coupling and the apex or dead center between the circles in the cone is the nuclei fused in time at much shorter wavelengths. The field wavelets are endless in time; hence the circles are open regardless of the size of the spiral wavelets in the wavelength spectrum.

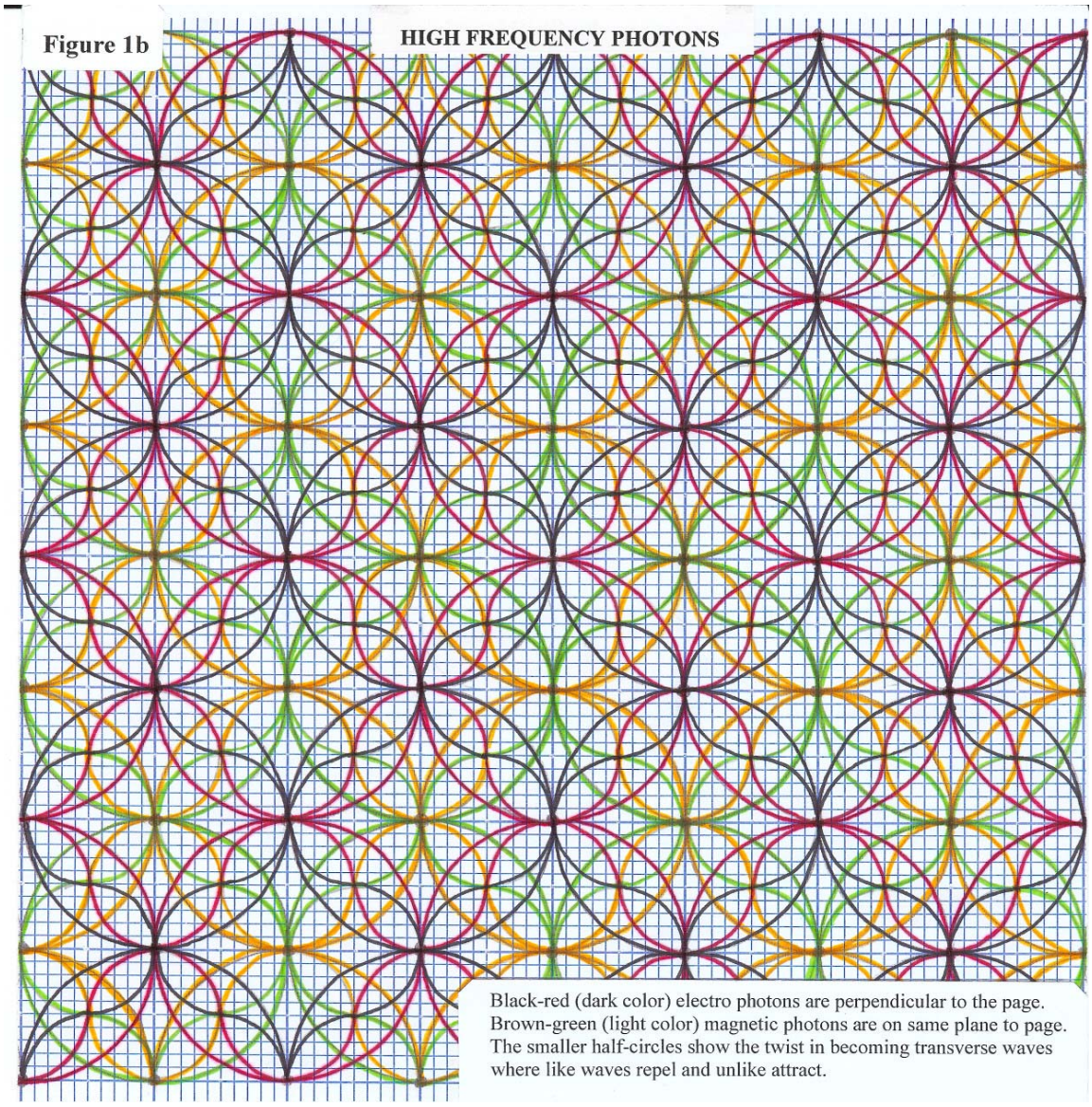
Moving two steps from center and squaring, there are four complete light color circles to the square Fig.1a. On three steps from center and squaring, there are nine complete light color circles to the square and so forth in the inverse-square distance of the magnetic field where the hypotenuse of each square alternate between the electro and magnetic wavelets.

Energy forms matter from an electro coupling link giving a point center to the magnetic circle of photons where the ratio of the diameter in the magnetic circle to its circumference is infinite. Gravity forces come from the magnetic wavelets around the atom Fig.1a which is an inward attraction force at the nodes that help conserve the energy Fig.1b.

The magnetic photon wavelets will adjust gravity forces for any kind of atom as the field tends to restore the coupling that became mass to its original state. The atom and gravity are united and comply with the inverse-square distance and the constant G. It follows that the accumulation of mass includes the

accumulation of gravity as the photon wavelets interact with other field electrons via constructive and destructive wavelet interference from the wavelength spectrum.





CONCLUSION:

	Left Side		Right Side
Einstein's Equation:	E	=	MC^2

Expanded Equation: E = C = ∞ = Open Circle = MC^2

Zero times anything equal zero given mass on the right side of the equation. Zero does not apply on the left side of the equation because there is no mass. As follows:

With Mass	0 x Mass	= 0	(on the right side of the equation).
With Mass	0 x C	= 0	(on the right side of the equation).
Without Mass	0 x C	= ∞	(on the left side of equation, since zero does not apply).
Without Mass	E	= ∞	(on the left side of equation, since zero does not apply).
Therefore	E	= C = ∞	(on the left side of the equation).

Infinity is the field without mass Fig.1b. Infinity is the open circle without mass Fig.1b. Infinity is on the left side of the equation because infinity is not a number. Mathematics has no application on the left side of the equation because there is nothing to measure or count. Photons are counted on the right side of the equation with mass because C is finite (general relativity). Photons are not counted on the left side of the equation because C is infinite (no relativity). In essence, the speed of light is relative to infinity because the speed of light is coming from the same energy.

The idea of speed is relative to moving from point A to B. If there was no point A and B, the waves would be fixed or standing relative to infinity. Moving from point A to B in the field is the propagation of the standing waves at the value of C relative to mass. For mass, C^2 is the offset to the inverse square distance of the field. If there was no mass, there would be no need for C^2 and E would equal hf or C. If there was no mass, there would be no circle center for a diameter as a ratio to the open circumference.

Time is relative only when mass is created where C is finite. Time is without mass in the form of photons always moving. Time is the act of joining the three spatial dimensions together as part of time itself. An observer is part of time and part of infinity from the same source of energy where the speed of light is the same for all observers. General Relativity indicates that everything is connected within the context of curved space-time and motion of mass [4].

Energy-time is a universal constant for time, mass and gravity in a changing field of photons. From the universal constant of energy-time, the vacuum in free space is part of infinity. From this it can be concluded that energy in the form of photons is responsible for time, mass and gravity where energy is equal to the reality of forming mass in infinite time.

ILLUSTRATIONS, color or grayscale:
Figure 1a, Electromagnetic & Gravitational Field.
Figure 1b, High Frequency Photons.

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[2] Ibid, p105.
[3] Feynman, R.P., *QED, The Strange Theory of Light and Matter*, Princeton University Press (1988), p129.
[4] Ibid, Hawking, S., p40.

Emergence of Cross- Resistance to Fluoroquinolones in Gram-Negative Isolates from Cancer Infections in a Tertiary Hospital in Nigeria

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ABSTRACT: Background: This study determined the gram-negative bacilli associated with various cancer infections and defined fluoroquinolone (Ciprofloxacin, Pefloxacin and Ofloxacin) susceptibility of isolated strains. **Methods:** Materials for research were blood culture, urine, aspirates, fluids and swabs from cancer wounds. Samples were cultured and organisms isolated were determined using API system (Bio-Merieux). Antimicrobial resistance was estimated by the disc diffusion method according to NCCLS/CLSI recommendations and ESBL detection was carried out using the Double Disk Synergy Test method. **Result**: Of the 103 strains isolated 22 (21.4%) were found to be resistant to only ciprofloxacin. Only 1 of these resistances to ciprofloxacin was observed to have an accompanying production of ESBL. Of the 7 isolates that had resistance to a combination of two fluoroquinolones, 2 (28.6%) were found to be ESBL-producers. Cross resistance to the 3 quinolones tested, occurred in 40 (38.8%) of the strains isolated. The strains in this group were observed to be associated in most of the cases with MDR [35 (37.5%)] and production of ESBL [16(41%)]. This group was observed to be predominant amongst strains of *E.coli*, *Pseudomonas* spp and *Klebsiella* spp. **Conclusion:** Cross-resistance to fluoroquinolones has emerged amongst our clinical isolates and more worrisome is its association with ESBL-Production and Multidrug Resistance. Antibiotic resistance surveillance is thus of utmost importance for prompt intervention in the spread of emerging resistance. [The Journal of American Science. 2008;4(4):14-20]. (ISSN: 1545-1003).

Keywords: fluoroquinolone resistance, multidrug resistance, ESBL, Cancer, Gram-negative organisms

INTRODUCTION

The introduction of the fluoroquinolones (FQs) in the 1980s provided clinicians with a class of broad-spectrum agents applicable to a range of gram-negative infections (Ball, 1998, Hooper, 1998). The fluoroquinolones were a major therapeutic advance because they have 100-fold greater activity than their parent compound, nalidixic acid (Bauernfeind and Petermuller, 1983). Unlike nalidixic acid, which is used only for urinary infections and occasionally shigellosis, the fluoroquinolones have a broad range of therapeutic indications and are given as prophylaxis, e.g., for neutropenic patients. (Livermore *et al*. 2002).

Early researchers had thought that fluoroquinolone resistance was unlikely to evolve, largely because resistant *Escherichia coli* mutants are exceptionally difficult to select in vitro (Smith, 1986) and because plasmid-mediated quinolone resistance remained unknown even after 30 years of nalidixic acid usage. Nevertheless, mutational fluoroquinolone resistance emerged readily in staphylococci and pseudomonads, and more recently, fluoroquinolone resistance has emerged in *E. coli* and other Enterobacteriaceae, and these are attributable to multiple mutations that diminish the affinity of its topoisomerase II and IV targets in various ways, reduce permeability, and upregulate efflux (Everett *et al*., 1996). Plasmid-mediated quinolone resistance now has also been reported (Paterson, 2006; Martinez-Martinez *et al*., 1998).

Bacterial infections remain an extremely frequent complication of neutropenia caused by cytotoxic chemotherapy and are a major cause of complications and death in patients with hematologic cancers and chemotherapy-induced neutropenia. (Bucaneve *et al*. 2005). Worldwide, the reported mortality rate due to bacterial infection in patients with cancer and neutropenia has been estimated to be approximately 5% (Cometta *et al*., 1996, Del Favero *et al*., 2001). Neutropenic patients are at high risk for various infectious diseases even if cultures of clinical specimens are not positive. However, the choice of empirical antimicrobial therapy should be evaluated periodically to prevent treatment failure due to antimicrobial resistance. (Siu *et al*., 1999) as the emergence of new groups of antibiotic-resistant bacteria is once again threatening the ability to manage these bacterial infections in cancer patients.

Agents such as ciprofloxacin, norfloxacin and ofloxacin are used in most large cancer treatment centres for antimicrobial prophylaxis in high-risk patients with prolonged neutropenia, including patients with acute leukaemia undergoing remission induction chemotherapy, and recipients of bone marrow transplantation.(Dekker *et al*., 1987; Winston *et al*., 1987). Ciprofloxacin and ofloxacin have also been used for the treatment of febrile episodes in neutropenic patients (generally in combination with agents such as the aminoglycosides, beta-lactams, and vancomycin) both in the hospital and in ambulatory settings (Flaherty *et al*., 1989; Rolston *et al*., 1989; Rubenstein *et al*., 1993). The rise in resistance to many antimicrobials has led to the growth of the fluoroquinolones for use in various forms of infection. But since the early 1990s, Rybak (2004) reported that the resistance to this type of drug has increased by as much as 21%, corresponding to a 2.5-fold increase.

This study thus sets out to investigate the current status of FQ activity against prominent Gram-negative species associated with cancer infection and also to determine phenotypically any association between fluoroquinolone resistance and resistance to the broad spectrum antibiotics, ESBL production and multidrug resistance. This is because a decline in the activity of FQs would lead to many clinical therapy failures in view of the ability of gram-negative bacilli to easily acquire resistance to all these other classes of antimicrobials.

MATERIALS AND METHODS

Isolation and Identification

One hundred and three bacteria isolates from 256 patients who attended the Radiotherapy & Radio diagnosis Unit of Lagos University Teaching Hospital, Nigeria between January 2006 and November, 2006 have been included in this study. The identity of the organisms were confirmed with API 20E and API 20NE systems (Bio Merieux, France). These strains were from various clinical sources including blood culture, urine, fluids, aspirates and wounds.

Susceptibility Testing

Susceptibility testing was performed using antibiotic disk testing to 9 antimicrobials: cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), imipenem (30µg), gentamicin (10µg), amikacin (30µg), ciprofloxacin (30µg), ofloxacin (30µg) (Oxoid, UK), and pefloxacin (30µg) (May and Baker, Nigeria). Testing was performed on Mueller-Hinton II agar (Oxoid, Basingstoke, United Kingdom) according to NCCLS guidelines (NCCLS, 2000). The control strains *E. coli* ATCC 25922, *E. coli* ATCC 35218, *Proteus vulgaris* ATCC 13315 and *Pseudomonas aeruginosa* ATCC 27853, obtained from the Research laboratory of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, were run simultaneously with the test organisms. Results were interpreted with the National Committee for Clinical Laboratory Standards now known as Clinical and Laboratory standard Institute (CLSI) criteria for disk diffusion (NCCLS, 2000). Strains with intermediate susceptibility have been included in the 'resistant' category.

Double-Disk Synergy Test (DDST)

All isolates resistant to at least one of the extended-spectrum cephalosporins (ESCs) namely ceftazidime, ceftriaxone and cefotaxime were subjected to Double-disk synergy tests (DDST) as described by Jarlier *et al*. (1988) with modifications suggested by Thomson and Sanders (1992) to detect the presence of ESBL enzyme.

RESULT

One hundred and three non-duplicate gram-negative organisms belonging to 12 different species were isolated from clinical samples of various cancer infections of 256 patients, who attended the Radiotherapy & Radio diagnosis unit of Lagos University Teaching Hospital, Nigeria between January 2006 and November, 2006.

Table 1 shows the list of various cancer infections from which the organisms were isolated. Tables 2 and 3 show the antibiotic resistance patterns of the isolates. The strains were classified into 3 groups based on resistant pattern to the FQs. Group 1 showed strains found to be resistant to only ciprofloxacin amongst the 3 FQs tested. This occurred in 22 (21.4%) of the 103 strains. Only 1 of those resistant to ciprofloxacin was observed to have an accompanying production of ESBL. This occurred in a strain of *Acinetobacter calco iwoffii.* Group 2 showed strains with resistance to a combination of any two of the

fluoroquinolones tested. There were 3 combination types of resistance to 2 fluoroquinolones observed in this study (1.Pefloxacin and Ciprofloxacin resistance; 2.Ofloxacin and Ciprofloxacin resistance and 3. Pefloxacin and Ofloxacin resistance). Of the 7 isolates that had these types of resistance to a combination of two fluoroquinolones, 2 (28.6%) were found to be ESBL-producers (*E. coli* and *K. planticola*) and both of these ESBL-producers had ciprofloxacin resistance. Group 3 highlights strains resistant to all of the 3 quinolones tested. This occurred in 40 (38.8%) of the strains isolated. This group 3 resistance phenotype was found to be associated in most of the cases with multidrug resistance [35 (87.5%)] and production of ESBL [16(41%)] and was observed to be predominant amongst strains of *E.coli* 13(59%), *Pseudomonas* spp 11(64.7%) and *Klebsiella* spp 7(25%) particularly *K. planticola* 5 (45.5%). Other species in which this resistance phenotype was found were *Providencia* spp, *Enterobacter* spp, *Citrobacter freundii*, *Yersinia enterocolitica* and *Stenotrophomonas maltophilia* (Table 2).

Table 1: List Of Various Cancer Infections From Which Organisms Were Isolated

Cancer of the Breast
Cancer of the Cervix
Sigmoid Colon Cancer
Cancer of the Lungs
Cancer of the Oesophagus
Squamous Cell Carcinoma
Basal Cell Carcinoma
Oropharyngeal Cancer
Fibrosarcoma
Histiocytoma
Nasal Cancer
Renal Cell Carcinoma
Cancer of Larynx
Rhabdomyosarcoma
Retinoblastoma

Table 2. Antimicrobial Resistance Pattern of Clinical Isolates To The Three Fluoroquinolones Tested and the Strains in the 3 Different Groups

Organisms	Total No and % isolated	Cip	Ofl	Pef	Grp 1	Grp 2	Grp 3
K. ozaenae (4)		1 (25%)	1 (25%)	1 (25%)			
K.pneumoniae(6)		4(66.7%)	2 33.3%)	2 (33.3%)			
K.planticola (11)		8(72.7%)	5 45.5%)	6 (54.5%)			
K. oxytoca (5)		2 (40%)	0 (0%)	0 (0%)			
K.rhinoscleromatis(2)		2 (100%)	0 (0%)	0 (0%)			
***Klebsiella* spp**	**28(27.2%)**	**17(60.7%)**	**8 8.6%)**	**9 (32.1%)**	**8(28.6%)**	**2(7.1%)**	**7(25%)**
Escherichia coli	**22 21.4%)**	**16 72.7%)**	**16(72.7%)**	**16(72.7%)**	**3(13.6%)**	**3(13.6%)**	**13(59.1%)**
P. alcalifaciens (1)		0 (0%)	0 (0%)	0 (0%)			
P. stuartii (2)		1 (50%)	1 (50%)	1 (50%)			
***Providencia* spp**	**3 (2.9%)**	**1 (33.3%)**	**1 (33.3%)**	**1 (33.3%)**	**0 (0%)**	**0 (0%)**	**1 (33.3%)**
A. baumanii (4)		2 (50%)	0**(0%)**	1 (25%)			
A. calco iwofii (1)		1 (100%)	0**(0%)**	0**(0%)**			
***Acinetobacter* spp**	**5 (4.9%)**	**3 (60%)**	**0(0%)**	**1 (20%)**	**4 (80%)**	**0 (0%)**	**0 (0%)**
S. amnigenus (1)		0**(0%)**	0**(0%)**	1 (100%)			
S.liquefaciens(1)		1 (100%)	0**(0%)**	0**(0%)**			
***Serratia* spp**	**2 (1.9%)**	**1 (50%)**	0**(0%)**	**1 (50%)**	**2 (100%)**	**0 (0%)**	**0 (0%)**
E. agglomerans (2)		0**(0%)**	0**(0%)**	1 (50%)			
E. aerogenes (2)		2 (100%)	1 (50%)	2 (100%)			
E. cloacae (5)		2 (40%)	2 (40%)	2 (40%)			
***Enterobacter* spp**	**9 (8.7%)**	**4 (44.4%)**	**3 (33.3%)**	**4 (44.4%)**	**1(11.1%)**	**1(11.1%)**	**3 (33.3%)**
C. freundii (4)		2 (50%)	2 (50%)	2 (50%)			
C. amaloniticus (3)		1 (33.3%)	0	1 (33.3%)			
***Citrobacter* spp**	**7 (6.8%)**	**3 (42.9%)**	**2 (28.6%)**	**3 (42.9%)**	**0 (0%)**	**0 (0%)**	**3 (42.9%)**
A. caviae (1)		0	0	0			
A. fluvialis (1)		0	0	0			
***Aeromonas* spp (2)**	**2 (1.9%)**	**0**	**0**	**0**	**0 (0%)**	**0 (0%)**	**0 (0%)**
Proteus mirabilis	**5 (4.9%)**	**1 (20%)**	**0**	**1 (20%)**	**0 (0%)**	**1 (20%)**	**0 (0%)**
Yersinia enterocolitica	**1 (0.97%)**	**1 (100%)**	**1 (100%)**	**1 (100%)**	**0 (0%)**	**0 (0%)**	**1 (100%)**
Stenotrophomonas maltophilia	**2 (1.9%)**	**1 (50%)**	**1 (50%)**	**1 (50%)**	**0 (0%)**	**0 (0%)**	**1 (50%)**
P. fluorescens (3)		2 (66.7%)	2(66.7%)	2(66.7%)			
P. aeruginosa (14)		9 (64.3%)	10(71.4%)	12(85.7%)			
***Pseudomonas* spp**	**17(16.5%)**	**11(64.7%)**	**12(70.6%)**	**14(82.4%)**	**4(23.5%)**	**0 (0%)**	**11(64.7%)**

Key:
Cip=Ciprofloxacin, Ofl=Ofloxacin, Pef=Pefloxacin, No=Number

Table 3. Resistance Pattern to Other Antimicrobials and ESBL-Production

Organisms	Imipenem	Amikacin	Gentamicin	Ceftazidime	Cefotaxime	Ceftriaxone	ESBL-Producers
Klebsiella spp (28)	2 (7.1%)	8 (28.6%)	15 (53.6%)	11(39.3%)	16 (57.1%)	11 (39.3%)	4 (57.1%)
E.coli (22)	1(4.6%)	5 (22.7%)	15 (68.2%)	13 (59.1%)	15 (68.2%)	14 (63.6%)	8 (61.5%)
Providencia spp (3)	0 (0%)	0 (0%)	1 (33.3%)	3 (100%)	3 (33.3%)	2 (66.7%)	0 (0%)
Acinetobacter spp (5)	0 (0%)	0 (0%)	3 (60%)	3 (60%)	1 (20%)	1 (20%)	0 (0%)
Serratia spp (2)	0 (0%)	1 (50%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)
Enterobacter spp (9)	0 (0%)	2 (22.2%)	4 (44.4%)	1 (11.1%)	3 (33.3%)	4 (44.4%)	0 (0%)
Proteus spp (5)	0 (0%)	1 (20%)	3 (60%)	2 (40%)	3 (60%)	2 (40%)	0 (0%)
Citrobacter spp (7)	0 (0%)	2 (28.6%)	3 (42.9%)	2 (28.6%)	3 (42.9%)	2 (28.6%)	2 (66.7%)
Stenotrophomonas maltophilia (2)	0 (0%)	1 (50%)	1 (50%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)
Yersinia spp (1)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Aeromonas spp (2)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)
Pseudomonas spp (17)	6 (35.3%)	6 (35.3%)	14 (82.4%)	8 (47.1%)	15 (88.2%)	16 (94.1%)	1 (9.1%)

DISCUSSION

We determined the activities of three fluoroquinolones (ciprofloxacin, ofloxacin and pefloxacin), and six comparative agents against aerobic gram-negative organisms isolated from cancer patients attending the Radiotherapy & Radiodiagnosis unit of Lagos University Teaching Hospital, Nigeria. Ciprofloxacin and other FQs resistance has been focused on in this study, because of the increasingly frequent use of fluoroquinolones for treatment in most large cancer treatment centres for antimicrobial prophylaxis (Rubenstein et al., 1993, Flaherty et al., 1989, Rolston et al., 1989, Dekker et al., 1987).

Notable findings in this study include the presence of a number of gram-negative pathogens not commonly encountered in clinical samples of patients from this environment. These organisms included *Stenotrophomonas maltophilia*, *Aeromonas caviae, Aeromonas fluvialis, Yersinia enterocolitica, Providencia alcalifaciens* and *Providencia stuartii*. This finding is in agreement with the report by Oppeinheim [1998] who reported that cancer patients and more particularly the neutropenic host is extremely vulnerable to a range of bacterial infections. Indeed, for some of the rarer opportunistic organisms, this is sometimes the only setting in which infection occurs.

Secondly, resistance to ciprofloxacin which is known to be one of the most potent FQs (Habib Babay, 2007), and which is also widely used in this environment, was observed to be high amongst the Gram-negative organisms identified in this study except for *Aeromonas* spp. This may be due to the fact that it is one of the commonest FQ used in this environment for therapeutic purposes. The rise in resistance to many antimicrobials has led to the growth of fluoroquinolones use in various forms of infection. Incidence of FQ resistance has now been reported to have increased markedly in recent years due to increase in use in human, agricultural and veterinary sector (Paterson, 2006, Aibinu *et al*., 2004). The most notable resistance to ciprofloxacin in this study occurred in *Yersinia enterocolitica* (100%), *E.coli* (72.7%), *Pseudomonas* spp (64.7%), *Klebsiella* spp (60.7%) and *Acinetobacte*r spp (60%).

Thirdly, there was cross-resistance to other FQs amongst the ciprofloxacin-resistant organisms. 40 (38.8%), of the strains isolated, had resistance to ciprofloxacin and the other 2 FQs used in this study. This development is rather a worrisome trend in this environment. Though cross-resistance had been observed with other newer fluoroquinolones against ciprofloxacin-resistant gram-negative bacteria in other regions of the world (Tankovic et al., 1999) few data exist on such report here in Nigeria.

Fourthly, the most disturbing finding of this work is the occurrence of extended-spectrum beta-lactamase (ESBL)-production and multi-drug resistance amongst the group 3 strains. Of the 40 strains that

had resistance to the 3 FQs tested, 16 (41%) were found to be ESBL-producers and 35 (87.5%) were multi-drug resistant. Production of ESBL enzymes confers resistance on organisms producing this enzyme against the third generation cephalosporins which are amongst the last line drugs available in this environment for treatment of serious infections. Existence of resistance to a combination of 3 FQs in association with ESBL-production and MDR, particularly in cancer patients is a great cause for concern. This may be one of the underlying or contributing factors to the high mortality and morbidity rate observed amongst cancer patients in this environment as therapeutic options available due to these resistances is grossly limited. More investigation needs to be carried out to ascertain this.

The global public health implication of the findings of this work is the potential mobility of these MDR strains and the plasmids that may harbor them, as plasmid-mediated quinolone resistance is being reported now (Paterson, 2006; Martinez-Martinez et al., 1998). Patients move from one health care centre to the other, interchangeably; in both developed and developing countries where adequate and qualitative treatment can be obtained; particularly when it is related to cancer treatment. In essence it is imperative to carefully continue to monitor trends in infection and modify our guidelines for treatment accordingly. This can be achieved by routinely screening patients to be admitted into health-care institutions for possible colonization or infection with a cross-resistant and MDR organism. This will guide the clinicians in the appropriate treatment to be given to such patients and also in the implementation of adequate infection control practices that should be followed for a timely intervention and prevention of spread of such resistance in health-care institutions and amongst health-care workers.

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Interleukin-8 (IL-8) profile in Nigerians with *Schistosoma haematobium* infection

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ABSTRACT: The profile of serum interleukin-8 (IL-8) among 46 Nigerian volunteers with *Schistosoma haematobium* infection was investigated. Significantly elevated levels of IL-8 were seen in heavy infections (2278.33±274.56 pg/ml) than light infections (1738.33±384.83pg/ml) at (χ^2 = 72.6, p<0.05). Mean sera levels of IL-8 for age group <16 years was 2072.86 pg/ml and age group >16 years was 1642.50±274.56 pg/ml. This difference in mean IL-8 levels was statistically significant (χ^2 = 49.84, p<0.05). The intensity of *S. haematobium* infection showed positive correlation with IL-8 cocentration (r = 0.9).The relationship between IL-8 profile and age was negatively correlated (r = -0.9). We deduce that this elevated IL-8 can be used as biomarker of *S. haematobium* in our locality. [The Journal of American Science. 2008;4(4):15-18]. (ISSN: 1545-1003).

INTRODUCTION

Schistosomiasis infection remains an important infection in many tropical areas, especially Africa. Two hundred million are estimated to be infected while 600 million people are thought to be at risk (Chan *et al.,* 1996). Recent analysis estimated that about 280,000 deaths due to schistosomiasis infection occur annually in sub-Saharan Africa (King *et al.,* 2005). *Schistosoma haematobium* infection has been implicated to elicit a range of responses among inflammatory cytokines (Mutapi *et al.,* 2007).

Interleukin-8 (IL-8) is a chemokine and a chemotactic factor secreted by activated monocytes and macrophages that promotes the directional migration of neutrophils, basophils and T lymphocytes (Baggiolini *et al.,* 1989; Rossi and Zlotnick, 2000). This cytokine (IL-8) has been found to play important roles in autoimmune and inflammatory responses and also in infectious disease pathogenicity (Harada *et al.,* 1994; Koch *et al.,* 1992; Smyth *et al.,* 1991). *In vitro* experimentation of the activity of schistosome on the expression patterns of cytokines revealed a down-regulation of IL-8 (Fusco *et al.,* 1993). Schistosomal worm diagnosed among school children in Gabon has been documented to induce the production of IL-8 (van der Kleij *et al.,* 2004). Also, investigation among rural Zimbabweans infected with schistosomiasis showed an increased level of IL-8 with intensity of infection (Erikstrup *et al*., 2006).

Schistosome immuno-epidemiology studies have shown that the development of antigen responses is related to cumulative exposure to parasite antigen (Anderson, 1987; Woolhouse and Hagan, 1999) and the rate of development of different components of these responses, give distinct profiles across host age range (Mutapi *et al.,* 1997). Cytokine responses to *S. haematobium* infection have been reported to show contrasting profiles with age (Mutapi *et al.,* 2007).

Despite the impact of schistosomiasis on public health and the role cytokines play in the immunopathogenesis of the disease, there is dearth of information on the profile of IL-8 responses to *S. haematobium* infection in our locality. In this study therefore, we investigate the profile of serum IL-8 concentration with intensity of infection and establish the relationship between IL-8 and age and intensity of infection.

MATERIALS AND METHODS

This study was carried out in Ihieve-Ogben; a rural community in Owan East local government area of Edo State. The study area is located within the guinea savanna region of the State at latitude 6˚N and longitude 6˚E. Agriculture especially farming and hunting is their predominant activities, while a few of them, mostly women, are traders. The village has a stream which the inhabitants use as their source of water and recreational activities. There are about 1,000 inhabitants in this community.

This investigation commenced during a community mobilization campaign at Ihieve-Ogben. This involved educating them regarding the significance of the study as well as seeking their consent. Ethical permission was obtained from the State Ministry of Health, Benin City, Nigeria. Mid stream urine samples were collected from volunteers between 11:00 and 13:00 GMT after slight physical exercise. The specimen was kept in a wide-mouthed screw capped 50(ml) size container. These bottles containing the urine

samples were immediately transported to our parasitological laboratory for examination for the ova of *S. haematobium.* The ova were quantified and classified as light infection ≤50 ova/10 ml and heavy infection >50 ova/10 ml according to WHO standards (WHO, 1983).

Whole venous blood of individuals positive with *S. haematobium* infection (3 ml) was collected from a peripheral vein by venipuncture in the sterile EDTA bottle. Blood was processed by the centrifugation and the serum was immediately subjected to cytokine assays. The serum IL-8 concentration was determined by a standard Enzyme-Linked Immunosorbent Assay (ELISA) kits obtained from Abcam plc, Cambridge, United Kingdom according to the manufacturer's instructions. From the information supplied by the manufacturer, the upper limit of normal serum IL-8 concentration is 76pg/ml with the mean serum IL-8 level of 44pg/ml.

The data obtained in this study were subjected to statistical analysis, namely, correlation and chi-square tests using Microsoft Excel Statistical package.

RESULTS

Table 1 shows the mean serum IL-8 levels of *S. haematobium* infected volunteers with light and heavy infections. Forty six individuals infected with *Schistosoma* egg were categorized based on their parasite load. Heavy infections of >50 ova/10 ml were observed in 18 volunteers with mean IL-8 level of 2278.33±274.56 pg/ml; while the 28 individuals with light infection of (≤50 ova/10 ml) had a mean IL-8 concentration of 1738.33±384.83 pg/ml. The difference in the IL-8 levels of heavy infection and light infection was statistically significant ($\chi^2 = 72.6$, $p<0.05$).

The mean IL-8 concentration of 14 children (<16 years) with *S. haematobium* infection and 32 adults (<16 years) age groups had mean sera of 1642.50±363.81 pg/ml and 2072.86±400.21pg/ml, respectively (table 2). The difference in the sera levels for age group <16 years and >16 years was statistically significant ($\chi^2 = 49.84$, $p<0.05$). The relationship between IL-8 levels and age was negatively correlated (r = -0.9). The relationship between the intensity of *S. haematobium* infection and the IL-8 concentration was positively correlated (r = 0.9).

Table 1: Intensity of *S. haematobium* infection and mean serum IL-8 concentration

Intensity of infection/μL	Mean IL-8 (pg/ml)	No. infected
Light ≤50 ova/10 ml	1738±384.83	28
Heavy >50 ova/10ml	2278.33±274.56	18

Table 2: Mean IL-8 concentration with age group

Age group (years)	Mean IL-8 (pg/ml)	No. infected
<16	2072.86±400.21	32
>16	1642.50±363.81	14

DISCUSION

We reported significantly elevated IL-8 level in heavy infection. This supports the report of Erikstrup *et al.* (2006) and implicates IL-8 in the immunopathogenesis of schistosomiasis infection. IL-8 has been documented among other stimulants to be induced by lipopolysaccharide (Baggiolini *et al.,* 1994; DeForge *et al.,* 1993). A schistosomal phosphatyldiserine has lipopolysaccharide-like effect (van der Kleij, 2004). Also biochemical analysis of schistosome revealed chemical composition of glycolipids which has been shown to induce the production of IL-8 in *S. haematobium* infected children (van der Kleij, 2004). So the higher the level of infection, the more phosphatyldiserine and glycolipids in circulation, which probably explains the increased level of IL-8 with intensity of infection.

It was observed that serum IL-8 concentration among age group <16 years was significantly higher than age group >16 years. This contradicts the report of Mutapi *et al.* (2007) in the light that schistosome-infection induced the production of IL-10 which has been documented to be potent inhibitors

of IL-8 (Mukaida *et al.,* 1994; Xie, 2001). IL-10 with the intensity of infection peaked in childhood and thereafter declined in adults suggesting that IL-8 increased with age (Mutapi *et al.,* 2007). Previous studies have suggested that antihelminth-immune responses fall into a Th1 (pro-inflammatory) and Th2 (anti-inflammatory) dichotomy with resistance to schistosome infection associated with Th2 responses (Medhat *et al.,* 1998; Wilson, 1993; Capron *et al.,* 1999; Dunne and Mountford, 2001). Our finding suggests a shift from Th1 to Th2 patterns with increase in age which probably elucidates the reduced IL-8 concentration with increase in age and therefore implicates age in conferring immunity.

This study shows that IL-8 is induced by *S. haematobiun* infection which implicates this cytokine in the immunpathogenesis of schistosomiasis infection. Also, we reported a negative correlation of IL-8 levels with age. Our finding implicates age in conferring immunity; and suggests that IL-8 can be used as biomarker of *S. haematobium* infection especially in heavy infection in our locality. Since there is dearth of information on other cytokine responses to schistosomiasis infection, it is recommended that investigation in this regard be carried out in order to establish their roles in the disease pathogenicity, taking into account the role of cytokines in immunopathology of diseases and the global public health significance of schistosomiasis.

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Painful death of an elephant through rumour and gunshots, north-west India

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Abstract: An adult male elephant aged about 35 years was brutally gunned down near to Rajaji National Park area, north-west India. Before approaching to the unnatural death, the elephant moved approximately 12 kilometers in 9 hours in rural and populated areas and killed 2 villagers and injured 7 persons, while passing from different villages and in between the agriculture fields. The victim elephant was a sharp crop raider and non aggressive in nature. A total of 27 bullets were found over to his body whereas about 70 gunshots were fired at the accident site to kill the elephant. Study suggested that huge rumour, misunderstandings and human pressure has caused the sorrowful death of elephant. This was the first case in the history of Uttarakhand state, India in which wild elephant was brutally killed in one way encounter mainly due to rumour spread within Haridwar district administration and forest officials. This study reviews the case history of this tragedy and conservation measure regarding to Asian elephant's conservation. [The Journal of American Science. 2008;4(4):19-26]. (ISSN: 1545-1003).

Keywords: Elephant, gunshot death, developmental activities, north-west India.

Introduction

India has between 21,000 and 25,000 Asian elephants (*Elephas maximus*) in the wild and among them Uttarakhand state harbours 1346 elephants distributed within 14 protected areas. India currently has the largest surviving population of the Asian elephant, approximately 50 % of the total world population of the species (Daniel, 1996). Historically, elephants were known to raid crops but during the recent past because of increasing human population and decreasing forest cover, the conflicts between man and elephant have escalated. A number of wildlife habitats have undergone or are being threatened with fragmentation due to various anthropogenic factors and this has adversely affected the large mammal populations residing in them (Johnsingh et al., 1990). Recently, developmental activities and habitat destruction have caused major decline in the abundance of the terrestrial megafauna. As most of the wild animals are presently categorized under threatened category therefore, there is increasing concern that the area-wise decline of the elephant will have unexpected and grave consequences for the long-term viability of the terrestrial ecosystems.

The Rajaji National Park was established to enhance the long-term survival of the Asian elephant in a sub tropical moist deciduous forest in India. But during the recent past natural continuous forest ranges of India has been broken up into many parts due to agriculture, urbanization, increasing road traffic and development related activities as well as other anthropogenic activities. This situation creates many problems for various organisms living in forests especially for large size mammals like elephant. Genetic isolation, limitation of dispersal, migration and the decline of populations of animals requiring large territories are the most common problems connected with fragmentation of forests and other components of the environment. Shivalik landscape (lesser Himalayan zone) is one of the last few places in the world where elephants exist and offers urgent need for conservation. From conservation point of view Rajaji National Park appears to be India's one of the most successful national park and its development has helped to boost the population of Asian elephant in their natural habitat.

During the past one decade, elephants were observed moving through many intensively cultivated and populated areas, including national highways, railway track and villages. Rapid urbanization and industrialization (as a result of separation of Uttarakhand state from Uttar Pradesh state) around the Rajaji National Park and Hardwar forest division have been major factor that has caused disturbance in elephant's frequent movement. A number of villages were situated around both of these protected areas and grow many potential cash crops to enrich their economy. The major cash crops are *Saccharum officinarum* (Sugarcane), *Oryza sativa* (Paddy), *Triticum* spp. (Wheat) and *Zea mays* (Maize) and few cultivators also cultivate fruit yielding species in their fields like *Musa paradisiaca* (Banana) and *Mangifera indica* (Mango). Elephants traditionally often leave the forest to feed in nearby villages, usually at night period and returned back to forest area at early morning hours. Even before 1998 elephants were reported to be raiding crops but their outside movement was more common from 2001 (Joshi et al., 2001). Currently the

raids have become more frequent and the number of complaints by cultivators has increased. Elephant' stray behaviour was more common in villages those are attached to the boundary of Hardwar forest range of the Rajaji National Park area as anthropogenic disturbance was quite more as compared to other forest ranges (Joshi and Joshi, 2001).

The question arises that why do elephants go into cultivated lands by crossing a large stretch with populated area and with imminent risk to their lives. Generally, it is said that elephant's habitat becomes exhausted and their large home ranges were converted into smaller ones. But in Rajaji National Park and Hardwar forest division situation is totally different as said area is enriched with sufficient fodder species, which the elephants like and it can be said that seasonal food resources are available round the year. Elephants utilize 50 fodder plant species round the year in the Rajaji National Park and their movement was dependent on the seasonal availability of water and fodder species (Joshi and Singh, 2008b). The factors that contribute to the killing of humans by elephants are the presence of people into elephant's habitat to collect firewood and fodder, conflict over water and cultivation of palatable crops near the forest boundary. In between years 1986 to 2004, elephants have killed 47 persons and injured 43 persons in and around the Rajaji National Park area. And in Hardwar forest range, elephants have killed 26 persons and injured 11 persons in between year 1985 to 2001. On the other hand from 1987 to 2004, more than 134 elephants succumbed in the wild to various reasons (train accident, poaching, electrocution, fallen through hillock, disease, bull fight and natural).

Human settlements in and around the park area have created the shrinking of elephant's natural paths. The human population around the Rajaji National Park alone has doubled during past one decade and rapid urbanisation and industrialisation has resulting in the loss of many forestlands to townships and to various development related activities (Joshi and Singh, 2008a). During the recent past several adult male elephants were killed intentionally through poisoning and electrocution but this type of elephant death was observed for the first time after the establishment of Uttarakhand state. An adult male elephant was killed through heavy gun shooting near to Rajaji National Park area and this death incident of elephant was in itself a historical record because this type of one side encounter was not earlier observed from this region. This report deals with the case study of unnatural death of an elephant from north-west India.

The incident and observations

On May 29, 2008 just after releasing the official order (to kill elephant) of Chief Wildlife Warden, Government of Uttarakhand, a adult male elephant aged between 35-40 years was badly gunned down near to Ranipur jhal area, Bahadrabad, Hardwar (Figure 1,2,3,4,5,6,7,8,9,10,11&12). A total of 27 gunshots were observed from his body and surprising thing was that AK-47 rifle was also used for killing the elephant. This historical incident was very painful and caused violenceness among local people. Observations indicated that elephant was killed mainly due to rumour, which was spread like jungle fire among government officials and local people and pressure developed by local villagers regarding to killing of elephant. Information like elephant has killed 11 villagers and now moving towards human habitation area has also pressurised the forest officials to forcefully declare the elephant as rogue.

On the morning of 29th, it came to my notice that an adult bull elephant has killed 2 persons near to Buddhahedi village and was escaped in adjoining area. Quickly, I with my camera moved towards the accidental site. After reaching and looking into the situation it appeared that villagers were too much aggressive and were badly responding to elephant. At the same time forest officials and administrative officers of the district along with police force were trying to convince the local people but serious situation has motivated the villagers and they badly reacted against the administration and few villagers snatched down one of the firearm from a forest official. Besides, huge crowd was also running towards elephant with their firearms to deter away the elephant from village premise. Elephant was running towards Delhi – Hardwar National Highway and at 12.15 pm, elephant crossed the highway and moved towards Ganga canal near to Jamalpur village. During that period elephant also crossed a nallah, a high wall of about 5 feet and a canal. I was looking the incident calmly and concentrated my mind in the route, which was followed by the elephant.

After entering the transparent forest cover situated in between two Ganga canals (one concrete and another rough based), elephant stayed there for about one hour (Ranipur jhal area), however at short intervals he was chased by forest officials and heavy crowd. During the said period elephant was continuously trying to move towards Rawli river, which links the Rawli forest with accidental site (torrential) but tremendous crowd everywhere have not allowed the elephant to move across the area. Rawli river was a traditional seasonal corridor for elephants and elephants has utilized this corridor and performed

their outside journey upto Ranipur jhal area before 2001. But currently elephants were not utilizing this river stretch to move outside from the protected area. One of the fact related to this was presence of small agriculture land near to Ranipur jhal (Subhash nagar area) but after 2001 rapid urbanization and industrialization has led to shrinking of this stretch. Cross-fires were also noted during the said period at short intervals. At 1.15 pm, elephant was shooted twice by a police man and just after that elephant ran away very fast and enter to a plantation site of *Populus ciliata* (popular). Again elephant kept himself in popular site for about one hour. As per the field observations, he was quite irritated and aggressive in nature and broken down a tree from there.

The Incident (29.05.2008)-

S. No.	Time	Area / incident
1.	06.00 am – 07.00 am	Elephant moved around the Bahadarpur village
2.	07.00 am – 08.00 am	Elephant moved adjoining to Sanghipur and Ransura village, one villager was killed by elephant
3.	08.45 am – 09.10 am	Elephant moved peripheral to Sehdevpur and Buddhahedi village, one villager was killed by elephant
4.	09.30 am – 10.40 am	Elephant crossed the Rohalki and Ahmedpur area
5.	10.45 am – 12. 05 pm	Elephant crossed the Bhadrabad area and reached to Hardwar-Delhi National Highway and crossed it
6.	12.15 pm – 12. 25 pm	Elephant entered to Ranipur jhal area
7.	01.00 pm	1st gunshot fired over to his body
8.	01.15 pm	2nd gunshot fired over to his body
9.	01.18 pm	Elephant entered to Popular plantation area
10.	01.20 pm – 02.50 pm	Continuous cross firing on elephant started
11.	02.55 pm	Elephant was killed
12.	03.02 pm	Elephants' dead body was wobbly fell down in Ganga canal
13.	03.05 pm	Elephant was merged in Ganga water

At 2.55 pm forest officials collectivized in circular manner and started cross-firing on elephant. Next movement, elephant started running towards Ganga canal and after 2 minutes when he was crossing the canal very mournful climax was occurred. Official's fired again on the elephant climbing on the slope of canal. Huge amount of bullets were fired to shoot out besides, AK-47 rifle was also used during that moment. Suddenly, elephant fell down adjoining to canal and defecated on sloppy area of canal. After few seconds, he again tried to standup and looking into the same more than a dozen of gunshots were again penetrated over to his body. Concluding point was that his body was strike down in the Ganga canal and after 3-4 seconds he was merged in water (03.05 pm). I was watching whole of the incident sorrowfully and for few seconds, tiers has supported me to overcome from the situation. What a painful death was? His only mistake was that he has followed erroneous way because of human hindrance. I was searching the answers regarding to these questions.

Next day morning forest officers uplifted his dead body and bring it to Rawli forest of the Hardwar forest range of the Rajaji National Park with the help of crane and a truck. I examined the morphological features (tusk size, pigmentation rate, forehead shape, fore foot circumference and tail shape) of the elephant. Besides, gunshots were also counted from his body. From next day (31 May, 2008), I have conducted indepth survey of whole of the area through which elephant enter to the populated area with the point of view to develop a route map (Figure 13) for government so that no such incident will occur in near future and if happened we can return back the animal to adjoining forest area. This way will be a reliable method to safe the elephant as the animal also know the better way to return back from their traditional route. And for this I conducted survey in all the villages from where elephant moved during last night and where possible traced the footprints of elephant in agriculture field with the help of villagers. As most of the areas where elephants raid the crops traditionally and those were attached to the Ganges were well known to me, therefore, it supported me in various ways during the field data collection.

Case history

Field observations and ongoing study has helped me to identify died elephant. I am conducting the research studies on the behavioural biology of Asian elephants in Rajaji National Park and its adjoining

areas since last 10 years. And during the said period I have observed the victim elephant several times in Chilla forest (Rajaji National Park), Shyampur forest (Hardwar forest division) and Laldhang forest (Lansdowne forest division). I have regularly encountered and monitored earlier this elephant in Chilla forest from March to June but his movement was mostly observed in Shyampur forest range at the onset of monsoon and upto the winter (February). The elephant was a sharp crop raider and mostly raided crops in Mishrpur, Ajeetpur, Jagjeetpur and in Jaipota villages, which are adjoining to river Ganges. As far his behaviour was concerned, he was not aggressive at all and before death suffering from musth.

After conducting in-depth survey of the track and looking into the ground situations it seems that on 27th May 2008, he started from Chandi forest towards Anjani forest (Shyampur forest range, Hardwar forest division) and after crossing the Hardwar – Bijnor National Highway and three islands situated in between river Ganges he entered to village Mishrpur and performed his journey towards Jaipota village (towards Laksar area). On next morning (28th May) he returned back to Anjani forest and stayed in the same area upto the sunset. Anjani forest is dominated by *Acacia catechu* – the favourite food item of elephants. The villages along the river Ganges are situated on the land that was once part of the elephant's home range and elephants are utilizing their traditional feeding grounds in few of these areas, which are presently denied to them and are replaced by human settlements (Joshi and Singh, 2007). In evening hours he again started for agriculture fields to feed on cultivated crops and entered to Jaipota village. From Jaipota he moved to Bahadarpur area as whole of the area is dominated by *Saccharum officinarum* (Sugarcane) and from Bahadarpur he walked adjoining to Pathri forest, Tehri Dam colony, Sanghipur village and entered to Ransura village. Finally he entered to Buddhahedi village as his movement was disturbed and has got restricted by the villagers those are deterring and chasing him with loud noise. As per the observations elephant continuously moved in wrong direction mainly because of heavy crowd and obstacles present in his way. During this long journey of about 12 kilometers elephant has killed two villagers in Ransura and Buddhahedi villages. But as per the interviews obtained from local people, in Buddhahedi village a person has made fire on elephant and the bullet penetrated the ear lobe of the elephant and next moment elephant attacked and killed the villager immediately.

While running from these villages stretch, seven people were also injured by elephant not intensely. Next incident when elephant reached to Hardwar – Delhi highway and wants to cross the road, I was there and managed the situation through controlling the heavy traffic running over the highway. Moment when elephant realized that traffic has been stopped, he quickly crossed the road and moved towards Ganga canal near to Jamalpur village. A total of 27 gunshots were found over his body whereas about 70 fires were made at the accident site to kill the elephant. While examining the body, a bullet shot was also found in his upper jaw.

What could be done at the situation?

From early morning to 02.00 pm (9 hours), elephant was continuously running and chased by tremendous crowd along with forest and police officials. Approximately 500 people were continuously watching the incident from all the four directions and in such a situation there was not any chance for elephant to escape out. However few suggestive measures could be considered.

1. Elephant may be tranquilized during early morning hours at any village site and efforts can be made to shift him smoothly to nearby forest area. If impossible mercy death can be provided as this method will be quite appropriate at the situation. The incident which has been occurred may encourage the villagers to react as same in near future if any incident of such kind will occur.
2. Forest officials has not any accurate knowledge about the route from where elephant came, elephant can be forcefully returned back towards forest area but positively during the early morning hours or even during evening hours. Morning and evening hours will be the best timings as elephant perform their outside movement during said period and it will help him to some extent. But during this course, administration should control the crowd of the different villages from where elephant will return back to the forest area.
3. At last, elephant reached to the Ranipur jhal area – one of the denied elephant corridor for outside movement and if strong steps should be considered, he may be forcefully returned to the Rawli forest through Rawli river (torrential and situated near to accident site). For conducting this operation people movement should be stopped near to SIDCUL (State Infrastructure Development Corporation of Uttarakhand Limited) and BHEL (Bharat Heavy Electricals Limited) road area. The distance from accident site to Rawli forest is only 3 kilometers.

It was felt that some efforts could be done before killing the elephant and misunderstandings, rumour and tremendous human pressure has caused the sorrowful death of the elephant.

Because elephant ranges over a wide variety of habitats, the elephant's conservation ensures the survival of less prominent animals. Thus, the elephant has become a flagship species, emblematic of our global conservation movement. In those parts of India where villagers live along-side the elephant habitats, sharing with it the land's resources, the animal has become a source of fear and dread because of the damage it causes to crops and the danger it poses to human life. The arrival of tuskers on the outskirts of a village arouses terror in the residents, whose long experience has forced them to view elephants with skepticism, hostility and deep resentment. Historically, man and elephant engaged in occasional conflicts and recently the number of incidents is escalating. The sources of these conflicts can be traced to historical events, as well as to poor land-use practices, rapid human population growth, inappropriate land policies, poaching, presence of national highways and railway tracks in between the protected areas and other management related deficiencies.

India's elephant populations are currently threatened by habitat deterioration, developmental activities, anthropogenic pressure inside the deeper forest regime and unregulated exploitation of natural resources. Effective human-elephant conflict mitigation cannot take place in the long-term without the involvement and true support of the local communities. Similarly, rural livelihoods depend on a flow of natural resource benefits, many of which cannot be sustained without active protective measures. To build these partnerships requires greater understanding about working with local communities in designing programs to realize joint benefits. It also requires effective community empowerment to allow the communities to plan for wildlife management and conservation.

The Rajaji National Park, Lansdowne forest division and Hardwar forest division are important biological areas and have great potential for wildlife and its conservation. One line of evidence is that the Rajaji National Park harbours important populations of species on the IUCN Red list, the Asian elephant. In recent years, human activities have expanded in the boundaries of the protected areas and as a result most of the wildlife corridors have been shrinking rapidly. In this type of situation elephants are bound to move within protected area limit and if their movement expands as per their traditional home range, there may be the chances of enhancement of man-elephant conflict and even any incident may occur like this. I am concluding my words with the questions like that "*does raiders can co-exist with human beings? And what are the important factors, which are making elephants rogues*".

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THE INCIDENT

Figure 1) Last walk with friends: Victim elephant (center) with his friends during his movement along Hardwar – Bijnor National Highway. 2) Elephant crossing the Delhi – Hardwar National Highway. 3) Elephant at Ganga canal. 4) Heavy crowd at accident site. 5) Elephant near to *Populus* plantation. 6) Elephant was crossing the Ganga canal when firing started over to him.

(7)

(8)

(9)

(10)

(11)

(12)

Figure 7 & 8) Officials were firing over to elephant. 9) Merged dead body of the elephant in Ganga water. 10) Officials uplifting the dead body of elephant with the help of crane. 11) Elephant before the postmortem at Rawli forest. 12) Bullet marks over the dead body of elephant.

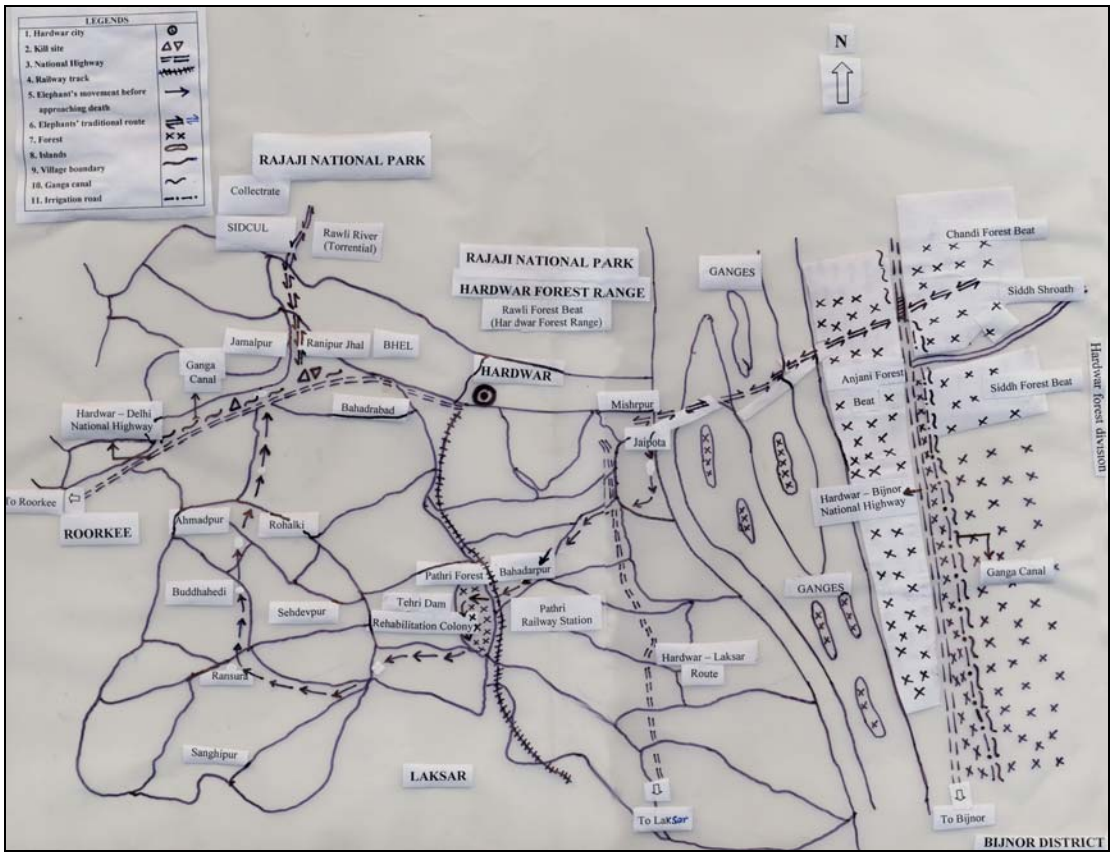

Figure 13. Sketch map of elephant movement from Shyampur forest range to Ranipur Jhal area (where elephant was killed), not to scale.

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Chusua nana: An orchid, new record for Nanda Devi National Park (Nanda Devi Biosphere Reserve), Uttarakhand, India

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Abstract: Nanda Devi National Park (NDNP) is one of the "World Heritage Site" covering an area 624.6 km^2 and forms one of the core zones of Nanda Devi Biosphere Reserve, which was established in 1988 under the MAB programme of UNESCO. Nanda Devi NP was unexplored and naturally protected by high mountains and glaciers until the scaling of Nanda Devi peak in 1934. In 1962 the widely acclaimed "Chipko Movement" brought the National Park into limelight for the efforts of villagers in conservation of natural resources. During 1934-1983 park faced serious threats and environmental degradation due to human activities, particularly mountaineering. [The Journal of American Science. 2008;4(4):27-31]. (ISSN: 1545-1003).

Keywords: *Chusua nana*; Nanda Devi National Park; World Heritage Site

Introduction

A complete ban was imposed in 1983 and a baseline survey was conducted by Botanical survey of India and Zoological Survey of India in the years 1981-1984. Two explorations conducted in the park yielded a total number of 312 plant species distributed over 199 genera and 81 families (Hajra 1983). Since then several efforts has been made to conserve the biodiversity of the park through effective protection, management and people's participation. An assessment of changes in the status of flora and fauna was made in 1993 by a team of scientist from various organizations. A total of 793 plant species belonging to 406 genera and 120 families from Nanda Devi Biosphere Reserve were recorded by Hajra and Balodi (1995), however, Samant (1999) reported 620 plant species belonging to 344 genera and 118 families from Nanda Devi Biosphere Reserve. The difference is mainly due to that in both the studies different areas were visited. According to the study by Samant and Joshi (2005) Nanda Devi National Park harbours 490 species belonging to 281 genera and 89 families of Angiosperms and Gymnosperms.

The orchidaceae family has more than 17000 species belonging to 735 genera, which are cosmopolitan in distribution, chiefly in tropical and temperate regions in the world. Over 1100 species of orchids are occurring in India, of which 30 orchid species belonging to 19 genera and 21 species belonging to 18 genera have been reported from Nanda Devi Biosphere Reserve (Hajra & Balodi 1995, Samant 1999).

To assess the status of decadal change in the flora and fauna another significant effort was made by Forest Department of Uttarakhand in 2003. During the ecological expedition (18 June - 8 July, 2003) various parts inside the core zone of Nanda Devi NP were visited (Adhikari 2004) for sampling and on 28 June, 2003 enroute to Betartoli at an altitude *ca.* 4075-4150m asl, *Chusua nana*, a new record for Nanda Devi National Park was collected and photographed (Plate I & II).

***Chusua nana* (King & Pantling) Pradhan, Indian Orc.: Guide Identif. Cult. 2: 678 (1979)**; Pearce & Cribb, Orch. Bhutan, 134 (2002)

Orchis chusua var. *nana* King & Pantling in Ann. Roy. Bot. Gard. (Calcutta) 8:304, t.402 (1998).

Ponerorchis nana (King & Pantling) Soo, Acta Bot. Acad. Sci. Hung. 12: 353 (1966); Seidenfaden & Arora 2: 24; Deva *et* Naithani, Orch. North-West Himalaya 199. t. 106 (1986).

Type: China (Xizang), Chumbi, Pantling 326 (holotype CAL)

Description

Plant terrestrial, 10-12cm tall; tuber small, ovoid, 7-8 x 7-8mm; *Stem:* Slender, glabrous, bearing a bladeless sheath at base, ebracteate above leaf, 3.5-6.5cm long; basal sheath tubular, 1.3-1.7cm long; *Leaf*: Single, linear to linear-lanceolate, acute, sessile, not sheathing, blotched with maroon patches, 1.7-3.5 x 0.15-0.2cm; *Inflorescence*: 1-2 flowered; rachis short, glabrous, 0.5-1.4cm long; floral bracts lanceolate, acute, margins minutely glandular, 0.9-1.1 x 0.2-0.25cm, longer than ovary; *Flowers:* 5-8mm across; sepals and petals purple, lip purple with red spots; pedicel and ovary fusiform, 7-8mm long; Sepals: subsimilar, lanceolate-ovate, acute, 1-veined, 2.5-3 x 2-3mm; Petals: ovate, acute, 1-veined, 2-3 x 1.5-2.5mm; Lip: obscurely 3-lobed, spurred, base broad, expanding to an equally 3-lobed apex, margins crenulate, 5-6 x 5-6mm; Spur: cylindric, wider at base, apex curved, 4-7mm long; Column: 1-1.5mm tall; Pollinia: clavate; caudicle long.

Flowering: Mid-June to mid-August

Distribution in World: Tibet-Qinghai to Eastern Himalaya (Govaerts 2003) and Bhutan (Pearce & Cribb 2002)

Distribution in India: Himachal Pradesh, Sikkim, Darjeeling and Arunachal Pradesh

Distribution in Uttarakhand: Chuli (alpine meadow in Uttarkashi District) and Betartoli in Nanda Devi National Park (Nanda Devi Biosphere Reserve) in Chamoli district of Garhwal Himalaya

Ecology: It is generally found in rolling grassy meadows**.** The associated species are *Cassiope* on depressed with moist areas and *Poa* and *Danthonia* on exposed with less moist areas.

Nativity: Himalayan region

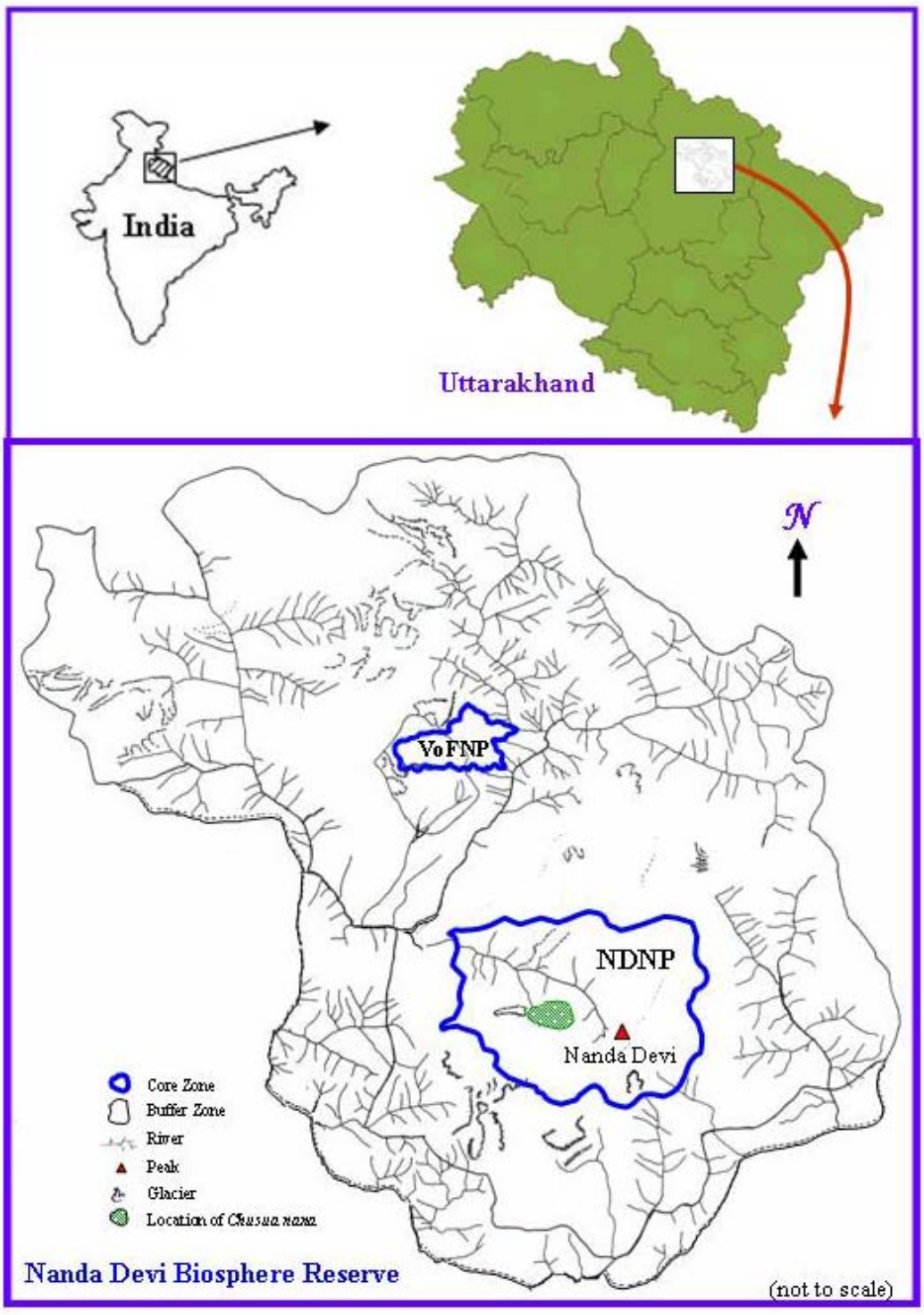

Fig. 1: Map of Nanda Devi Biosphere Reserve (Core zones: Nanda Devi National Park (NDNP) and Valley of Flowers National Park, VoFNP) showing location of *Chusua nana* in Uttarakhand, India.

Plate I. Cluster of *Chusua nana* in an open grassland

Plate II. An individual plant of *Chusua nana*

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6/28/2008

Recognizing Objects by Detecting Multiple Moving Parts

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ABSTRACT: Detecting objects in a given video stream is an important step to understand their types, movements, and activities. Existing object detection algorithms suffer from their inability to detect the components constituting a particular object that may result in classifying such components as standalone objects. Such instances may happen particularly when the colors of some components of the object have colors, which are close to the background. In this paper, we propose a technique to detect such objects by analyzing multiple images for the same object and observing the motion of various components of the object. [The Journal of American Science. 2008;4(4):32-43]. (ISSN: 1545-1003).

Keywords: video processing, detection, tracking, background subtraction, minimum boundary rectangle (MBR).

1. INTRODUCTION

Computer vision research has increasingly focused on recognizing objects. Their main purpose is to track objects especially human and vehicles in order to detect abnormal or desired behaviors. Object detection techniques can be classified into two categories [1, 2]. The first category requires a preprocessing phase, which subtracts the current image from a reference frame in order to isolate moving objects. After detecting and subtraction the background classification of the object performed by applying two approaches: the first is a codebook approach, and the second involves tracking of the object based on color histograms, motion and size of the foreground blob. False alarms due to static oscillatory motions are also detected and removed. Infrared (IR) cameras can be used instead of standard camera for detecting moving objects. The cameras in [3] are mounted close to each other and observe the same scene from a similar viewpoint. Actually, the approach is not human specific, but will detect any moving object. Background subtraction is performed independently by using Gaussian probability distribution to model each background pixel. The detected foreground from the two cameras is registered using a hierarchical genetic algorithm, and the two registered silhouettes are then fused together into the final estimate. On the other hand, IR images can be fused with images from a regular camera [4], Humans display a characteristic signature in IR images due to their skin temperature, but these images typically have low contrast. They can be fused with images from a standard camera to obtain superior detection results. In addition, a shape-based approach can be utilized to determine the object types as human, animal or vehicle based on approximate matching of the object's shape boundary contour to a polygon [5].

The second category detects moving objects by directly observing temporal difference between images to separate the foreground and background using a statistical model to classify human depending on the fact that the relative positions of various body parts are common to all humans, although the pixel values may vary because of the clothes [6]. The technique uses a structure known as the distance map, which is built by taking an image of a human and breaking it into MxN blocks then using distance maps for a large database of human and non-human images. A statistical model is built for distance maps of each type, which consists of the average and covariance matrix for each block. Then, the image is segmented into smaller objects based on threshold of luminance and/or color components of the image. Real-time detecting human system implemented in [7] by combining Viola's face detection framework and the HOG feature pool. The system keeps the discriminate power of Histograms of Oriented Gradients (HOG) features and the real-time properties of Viola's face detection framework. Besides human, the detection framework can be used to detect other objects such as vehicles.

Detect passengers onboard public transport vehicles method proposed in [8] by detecting the passenger head first using the curvature profile of the human head as a cue, followed by applying the geometric blur features which are consistent to affine distortion of the image to keep track of the movement of the head within the vehicle. The profile of moving heads with respect to each other within a length of time can be

used as indicative features to detect the advent of suspicious behavior of the passengers. The two categories differ in speed, memory requirements and accuracy [9]. Most algorithms may fail to detect some objects or separate one object to multiple ones because of false background estimation [10, 11].

In this article, we introduce a new approach, which is a hybrid of both categories. The proposed method recognizes human objects in spite of undetected components by inspecting multiple frames in a video stream and calculating the average speed of the detected components. Consequently, the dimensions of the human object in the image can be determined.

2. OBJECT DETECTION METHODS

Background estimation and subtraction play gigantic role in object detection and may affect the quality of the detection outcome. The big question is what if some parts were undetected from the video frames during the background subtraction process?

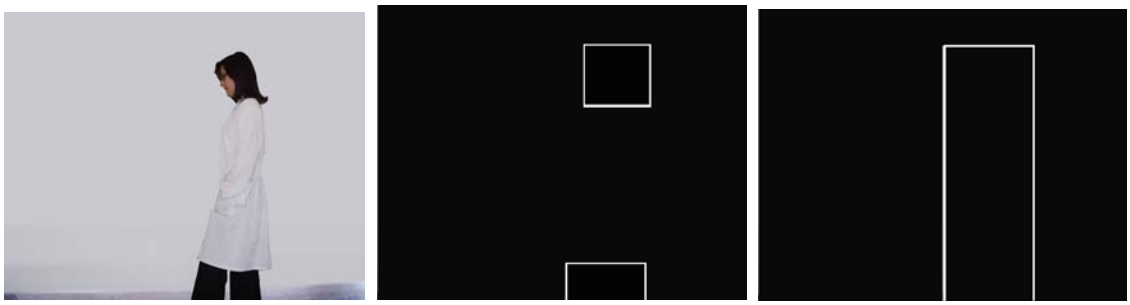


Figure 1: a) An image for a walking human. b) Object detection for the human c) Dimensions of human using average component speed.

Figure 1(a) shows a human dressed in white, walking in front of a white wall. Clearly, applying classical object detection algorithms on such an image leads to undetected parts of the human object as shown in figure 1(b), which may lead to false decision in recognizing the identity of the object. By studying multiple frames, it is quite reasonable to assume that if both rectangles in figure 1(b) move with the same average speed, that the dimensions of the human object be as shown in figure 1(c).

Figure 2 displays a flow chart for the proposed technique. Clearly, considering multiple frames, all components, which lie in the same vertical position and move at the same average speed, may be combined as one object. The proposed approach is also capable of recognizing multiple humans walking at the same speed by calculating their speed, position and direction as shown in figure 3. The components of each object are in the same vertical position and move at approximately the same speed.

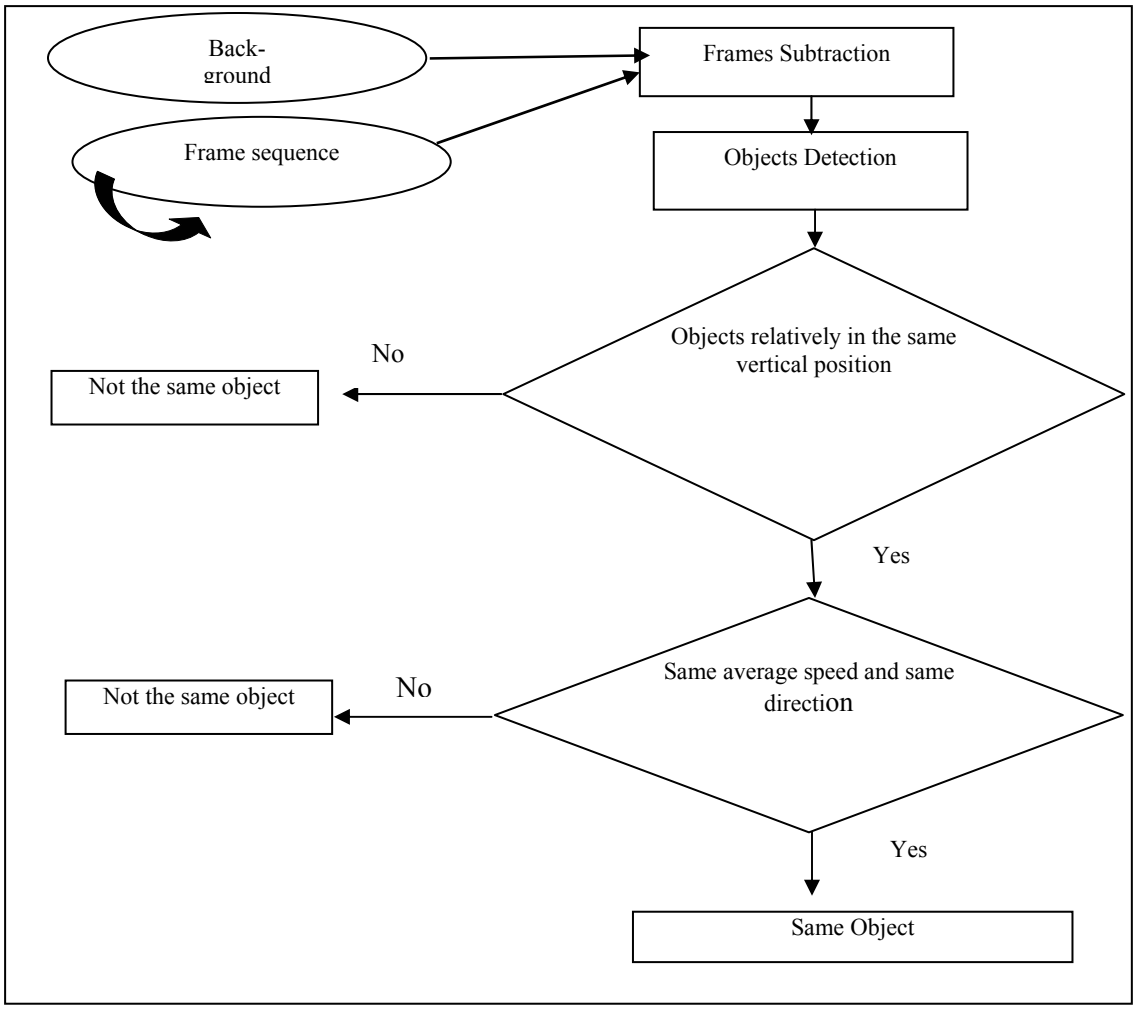
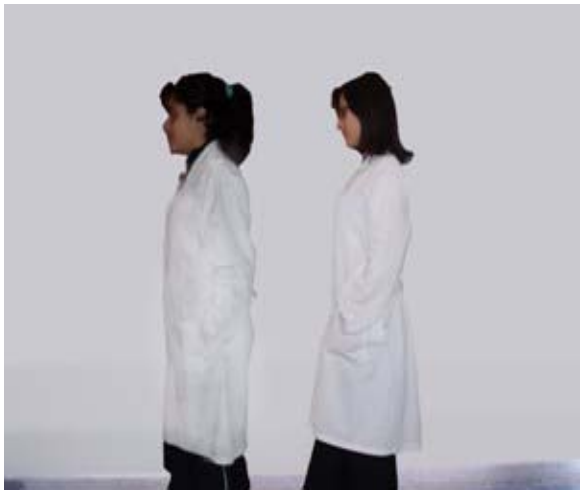
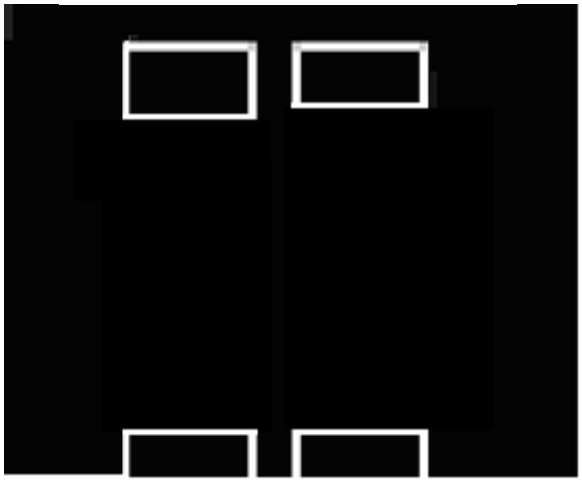


Figure 2: Enhancement flowchart



(a)



(b)

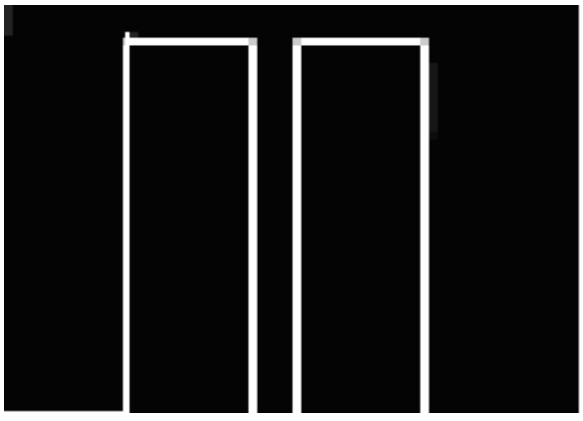


Figure 3: (a) Two walking humans
(b) Object detection for the human
(c) Dimensions of human using the average component speed.

3. ANALYSIS AND EVALUATION

The proposed approach can be used to enhance existing background subtraction [12,13], RGB-difference and absolute difference algorithms [9]. Detecting components of objects using the previous steps and finding their minimum bounding rectangles, (i.e. MBR's) are prerequisites for the proposed enhancement. The absolute difference of two images is performed in a single pass by taking two images P1 and P2 of equal size as input and producing a third image Q of the same size whose pixel values are simply those of the first image minus the corresponding pixel values from the second image as describe in equation 1.

$$Q(i,j) = P1(i,j) - P2(i,j) \quad \ldots\ldots (1)$$

The absolute difference of two images can be achieved using the MATLAB function ***imabsdiff***. The RGB-difference can be computed using the algorithm in figure 4 where the pixel value is divided into three components.

RGB-Difference (A, B)

```
> create a 2-D array called BW.
For i = 1 to row
    For j = 1 to columns
        Diff-Red = A [i, j, 1] – B[i, j, 1] ; Diff-Green = A [i, j, 2] – B[i, j, 2] ; Diff-Blue = A [i, j, 3] – B[i, j, 3] ;
        > row1 represent red-value
        > row2 represent green-value
        > row3 represent blue-value

     Difference = Diff-Red + Diff-Green + Diff-Blue;

                    if (Difference <= th)
                    >th: threshold value

                    {     BW [i, j, 1] = 255;   BW [i, j, 2] = 255; BW [i, j, 3] = 255;
                    }
                    else
                    {      BW [i, j, 1] = 0; BW [i, j, 2] = 0; BW [i, j, 3] = 0;
                    }
        end
end
return BW;
```

Figure 4: RGB-Difference

Combining image subtraction and object detection techniques can be utilized to examine the proposed enhancement. Figure 5 explains the first method, where absolute image subtraction is performed with respect to the reference frame.

Method 1

>All functions are built-in MATLAB
>functions [12, 13]

A: Reference Frame
>A: contains only the background with
>no objects

B: Frames Sequence
> B: *contains the background with /*
>without objects

Resize A, B
> *for Memory Utilization*

Image Subtractions
>*imabsdiff function*

Find threshold value
>*graythresh function*

Convert the image to binary image
>*im2bw function*

Dilation
>*imdilate function*

Filling holes
>*imfill function*

Smoothing
>*imerode function*

Remove all object containing fewer than 50 pixels
>*bwareaopen function*

Finding objects and drawing MBR
> *bwlabel , regionprops, boundaries* functions
> *BoundingBox property*

Figure 5: Method 1

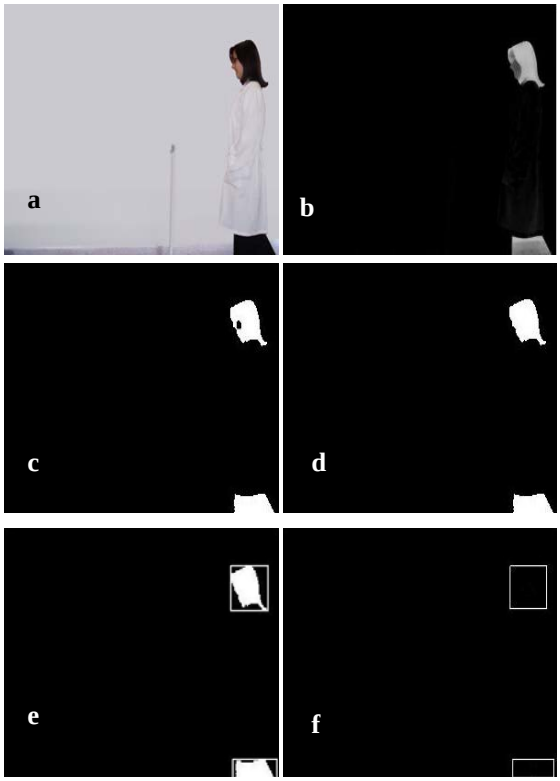


Figure 6: Applying method 1 to the image in figure 3 (a) original image (b) subtracted image (c) binary image (d) smoothing and hole filling (e)(f) minimum bounding rectangle

Figure 6 displays the outcome of applying method 1 on the image in figure 3. The subtracted image is converted into binary and holes are removed through dilation filling. Small objects are totally ignored. Method 2 is displayed in figure 7 with edge detection being employed. The outcome of method 2 with respect to the image in figure 3 is traced in figure 8.

Method 2

>All functions are built-in MATLAB
>functions [12, 13]

A: Reference Frame
>A: *contains only the background with*
>no objects

B: Frames Sequence
> B: *contains the background with /*
>without objects

Resize A, B
> for Memory Utilization

Image Subtractions
>imabsdiff function

Drawing the edges
> edge function

Dilation
>imdilate function

Filling holes
>imfill function

Remove all object containing fewer than 50 pixels
>bwareaopen function

Smoothing
>imerode function

Finding objects and drawing MBR
> bwlabel , regionprops, boundaries

functions
> BoundingBox property

Figure 7: Method 2

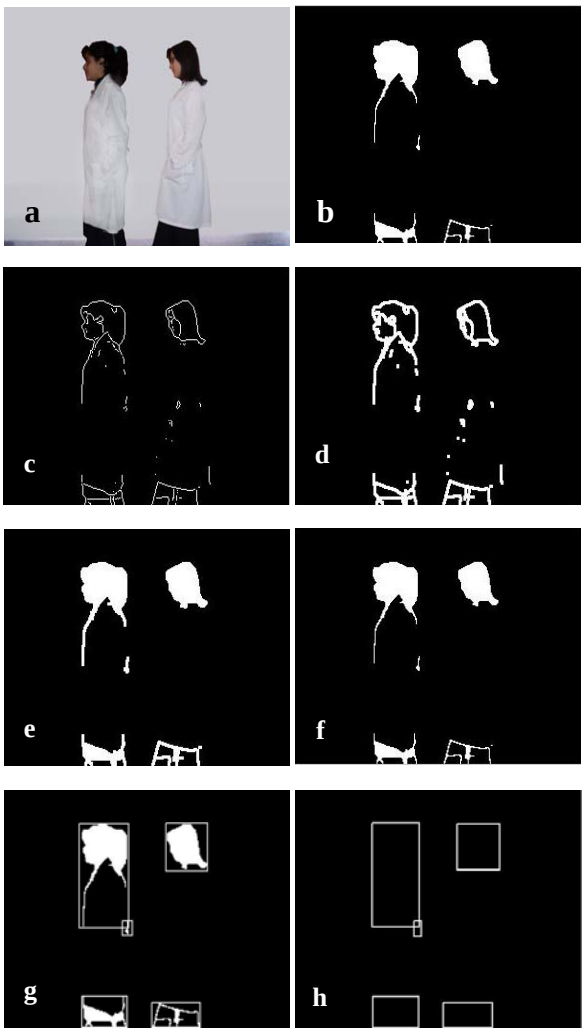


Figure 8: applying method 2
(a) original image (b) subtracted images
(c) edge detection (d) dilation (e) holes filling
(f) smoothing (g)(h) minimum bounding rectangle

Method 3 utilizes the RGB-difference with respect to the reference frame as explained in figure 9. A trace for the method is displayed in figure 10.

Method 3

A: Reference Frame
>A: *contains only the background with >no objects*

B: Frames Sequence
> B: *contains the background with / >without objects*

Resize A, B
> for Memory Utilization

Diff =RGB Difference (A, B)

Smoothing (Diff)

Dilation (Diff)

Filling Holes (diff)

Drawing MBR

Figure 9: Method 3

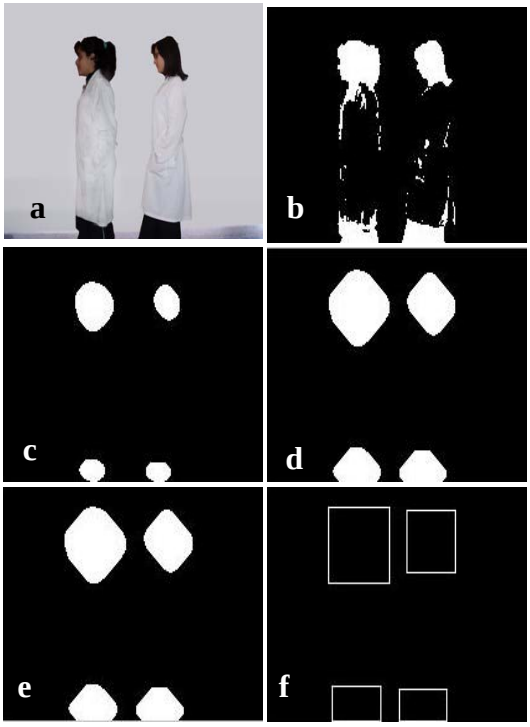


Figure 10: Applying method 3
(a) original image (b) RGB-Difference (c) smoothing (d) dilation (e) holes filling (f) minimum bounding rectangle

The enhancement proposed in this article connects components of objects based on their spatial position in the image and their average speed. Figure 11 presents the pseudo code of the enhancement.

Enhancement Method

>this enhancement can be applied to any human >detection algorithm (considering human >movement is horizontal.
>after detecting MBRs
For number of frames
>in our evaluation =3
For 1 to number of objects in every frame
>take center of each MBR Centroid
>property and compare the X-values
If (difference (X1,X2) ≥ th)
{
> th : in our evaluation =15 , if yes may be they belong to the same object
If (average speed is equal)
{
If (same direction movement)
They are the same object
}
}
Else
They are different objects.
> Average speed is equal: objects are moving in >the same speed ratio (according to the Y- axis)
> Same direction movement: if objects position >is increasing or decreasing.

Figure 11: Enhancement Method

	Frame1	Frame2	Frame3	Frame4	Frame5	Frame6	Frame7
Original images							
Intermediate step (method 1)							
Final Outcome (method 1)							
Applying Enhancement on method 1							
Intermediate step (method 2)							
Final Outcome (method 2)							
Applying Enhancement on method 2							
Intermediate step (method 3)							
Final Outcome (method 3)							
Applying Enhancement on method 3							

Figure 12: Object detection using three methods along with the proposed enhancement for single object frames.

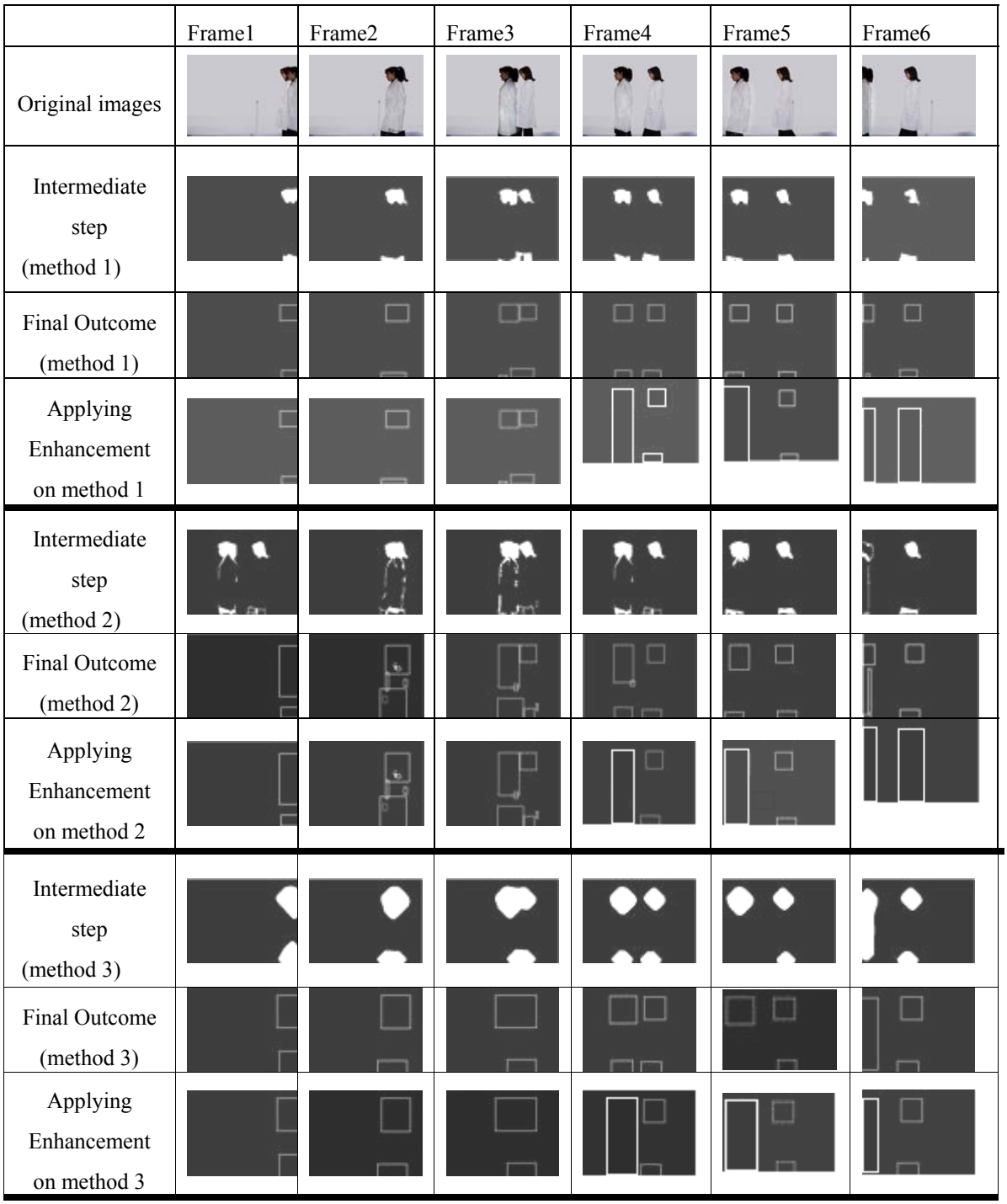


Figure 13: Object detection using the three methods along with the proposed enhancement for single two walking humans with different speed**.**

In order to study the impact of the proposed enhancement, we executed the three methods along with the enhancement on the sequence of frames shown in figure 12. Clearly, the three methods fail to detect the human object in all the seven frames. However, the enhancement in combination with each of the methods correctly generated the MBRs in the fourth frames. Similarly, we analyzed another sequence of six frames with two humans as shown in figure 13. Obviously, none of the three methods successfully detected the two walking humans. Method 1 and 3 counted two to four objects in each frame while method 2 detected

two to six objects in each frame. In combination with the proposed enhancement, method 1 and 2 successfully detected both humans in frame 6 while method 3 failed.

4. CONCLUSION

This paper discusses object detection techniques. It proposes an enhancement based on the average component speed in order to integrate the ingredients of a specific object. To enable object detection, the proposed technique considers a stream of frames and examines the speed of various components appearing in the frames. Combining this approach with classical image processing techniques, demonstrates clear superiority.

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Interactions between *Proteocephalus ambloplitis* and *Neoechinorhynchus* sp. in Largemouth Bass, *Micropterus salmoides*, Collected from Inland Lakes in Michigan, USA.

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Abstract: Largemouth bass *Micropterus salmoides* (L.) is a popular freshwater warm-water sportfish in Michigan. Due to its position in the food chain, largemouth bass (LMB) are plagued with endoparasites belonging to a number of phyla. The bass tapeworm, *Proteocephalus ambloplitis* Leidy 1887 and different species of acanthocephalans are considered among the most common endoparasites of Largemouth bass. Although these parasites usually exist together in the intestine of the largemouth bass, little is known about the correlation among them. Furthermore, bass endoparasites and their potential effects have not been thoroughly investigated. In this study we report the presence of *Neoechinorhynchus* sp. and *Leptorhynchoides* sp. in the intestine and *Proteocephalus ambloplitis* plerocercoids in the visceral cavity of largemouth bass collected from seven inland lakes in Michigan's Lower Peninsula. The presence of the *Proteocephalus ambloplitis* plerocercoids was associated with severe adhesions in the peritoneal cavity with the presence of plerocercoids attached to the surface of the internal organs. On the other hand, infection with *Neoechinorhynchus* sp. and *Leptorhynchoides* sp. was not associated with visible lesions, though mild congestion was noticed in the intestine at the site of attachment. An inverse correlation was also noticed with the number of *Proteocephalus. ambloplitis* plerocercoids significantly increased in the ovary when the number of *Neoechinorhynchus* sp. decreased in the intestine. Possible explanations of the findings are discusses. [The Journal of American Science. 2008;4(4):44-51]. (ISSN: 1545-1003).

Key Words: Interaction - endoparasites - Largemouth bass- Michigan- *Proteocephalus ambloplitis*

1. Introduction:

Largemouth bass (LMB), *Micropterus salmoides*, is considered one of the most popular freshwater sportfish worldwide (Chen, Hunt & Ditton 2003). Due to its position in the food chain, LMB are plagued with endoparasites belonging to a number of phyla (Ingham & Dronen 1980; Szalai & Dick 1990; Banks & Ashley 2000). In the state of Michigan, the spatial and temporal distribution of helminthes and their potential effects on LMB have not been thoroughly investigated. One of the few published studies reported the presence of *Proteocephalus ambloplitis*, *Neoechinorhynchus cylindratus*, *Leptorhynchoides thecatus*, and *Pomphorhynchus bulbocolli* present in LMB caught from three inland lakes in Michigan (Esch 1971; Gillilland & Muzzall 2004; Muzzall & Gillilland 2004).

The bass tapeworm, *Proteocephalus ambloplitis* Leidy 1887, is a common endoparasite of LMB with LMB acting as both second intermediate and final host (Freeman 1973; Amin 1990). No serious pathological lesions have been recorded from infection by adult *P. ambloplitis* in intestine of LMB; however, serious pathological lesions were usually associated with the plerocercoids in the visceral organs. Plerocercoids migration throughout visceral organs is typically associated with spleen damage, hepatic necrosis and gonadal damage that might has the potential to reduce the reproductive capability and survival of affected bass (Esch & Huffines 1973; Joy & Madan 1989; Amin 1990). In the case of acanthocephalans, adult worms are found in the intestine or the pyloric cecae and are usually associated with damage of intestinal mucosa at sites of attachment (Venard & Warfel 1953; Esch & Huffines 1973; Eure 1976; Leadabrand & Nickol 1993)

A few studies reported the presence of an inverse relationship between *Proteocephalus* sp. and acanthocephalans or among acanthocephalan species. For example, a negative correlation between *Proteocephalus exiguus* and an acanthocephalan species in the intestine of Ciscoes fish was recorded by

Cross (1934). Similar correlations were recorded between *Neoechinorhynchus* sp. and adult *Proteocephalus ambloplitis* in the intestine of Largemouth bass (Durborow, Rogers & Klesius 1988) and between *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in the intestine of Rainbow trout (Bates and Kennedy 1990). In these examples, the correlation was drawn between parasites residing in the same organ; i.e., the intestine, a matter that has been attributed to either competition (for food or space) or presence of inhibitors.

In the present study, we report a new geographical location for *P. ambloplitis*, *Neoechinorhynchus cylindratus*, and *Leptorhynchoides thecatus* within the state of Michigan. Additionally, we report the presence of a negative correlation between infection with intestinal acanthocephalans and visceral *P. ambloplitis* infection in adult LMB caught from seven inland lakes in Michigan's Lower Peninsula.

2. Materials and Methods:

2.1 Fish:

A total of 68 Largemouth bass fish were collected from seven different inland lakes in Michigan in the summer of 2002. The fish were euthanized using overdose of Finquel MS-222 (Argent Laboratories**,** Redmond WA). Total length and weight for each fish were recorded prior to dissection. The fish were either subjected for parasitic examination immediately after euthanization or the whole viscera was preserved in 10% formalin-buffered saline for later parasitic examination.

2.2 Parasitological examination:

All acanthocephalans species in the intestine of each fish were counted for individual fish and identified according to the morphological criteria detailed in Hoffman (1999). Acanthocephala were collected and relaxed in water at 4C followed by fixation in10% formalin. Proboscis size and shape, number and arrangement of hooks, and number of cement glands were observed and recorded to reach the genus level of the target acanthocephalans (Hoffman (1999). Preliminary work performed on 281 LMB indicated that the number of plerocercoids in ovaries reflects the severity of parenteric infection with *P. ambloplitis* plerocercoids (Data not shown). Guided by this data and similar observations by Amin (1990) who found that parenteric plerocercoids are localized primarily in the gonads during the summer, we used ovaries as representative organ for parenteric plerocercoids infection. Therefore, number of *P. ambloplitis* plerocercoids was counted in intact, individual ovaries to determine the prevalence and infection intensity of this cestode. Ovaries were collected and preserved in 10% formalin from fish caught in the eight lakes sampled in this study were examined for prevalence, intensity and abundance of plerocercoids. Plerocercoids were removed from the ovaries to 70% ethyl alcohol. 385 plerocercoids were used for morphometric analysis and compared to the measurements of *P. ambloplitis* plerocercoids taken from the ovaries of large and smallmouth bass taken by Amin and Boarini (1992). The following measurements were made on the plerocercoids: accessory sucker diameter, lateral sucker diameter, scolex width, body length and scolex apex to accessory sucker. The ratios of accessory sucker diameter to lateral sucker diameter and lateral sucker diameter to scolex width were calculated. When an *en face* view of the scolex of worm was presented on the slide, the scolex apex to accessory sucker measurement was not able to be made. Two-sample t-tests assuming equal variances were ran on abundance data to make pair-wise comparisons between ovarian plerocercoid abundance between two lakes and this test was ran between all lakes.

3. Results:

In this study, 68 adult female LMB were chosen from 281 LMB collected from 7 inlands lakes in Michigan. Morphometric analysis of a total of 385 plerocercoids from the ovaries of examined fish revealed that plerocercoids belong to *P. ambloplitis*. While analysis of 585 acanthocephalans collected from intestines of the fish revealed that they belong to two acanthocephalans species, *Neoechinorhynchus* sp. and *Leptorhynchoides* sp (Table 1). No adult *Proteocephalus ambloplitis* was found in the intestine of examined fish.

All examined lakes in the current study were infected with *P. ambloplitis* plerocercoids in the ovary and acanthocephalans in the intestine. However the prevalence and intensity of infection varied among different lakes. Devils and Jordan lakes showed the highest prevalence of infection with *P. ambloplitis plerocercoids* (100%) while Norvel lake showed the lowest prevalence (10%). The intensity of infection was consistent with the prevalence results in the examined lakes. The highest intensity of

infection with *P. ambloplitis* plerocercoids was found in Devils and Jordan Lakes respectively, while the lowest intensity was found in Norvel and Orion Lakes (Table 2)

The prevalence of total acanthocephalans infection was highest in Eagle and Jordan lakes (90%) while Independence Lake showed the lowest prevalence (44%). However the intensity of total acanthocephalans infection was highest in Orion and Devils Lakes while Norvel Lake showed the lowest intensity (Table 1).

The highest prevalence of *Neoechinorhynchus* sp. infection was found in Eagle Lake, while the intensity of infection by *Neoechinorhynchus* was highest in Orion Lake followed by Eagle and Jordan Lakes. In contrast, *Leptorhynchoides* sp. infection showed the highest prevalence in Devils Lake while the infection intensity was highest in Randall Lake followed by Devils Lake.

Clinically, fish from all lakes except Norvel showed severe adhesions in the internal viscera. Plerocercoids were usually found attached to the surface of the internal organs, although sometimes they were found loose in the abdominal cavity. Plerocercoids were most commonly observed either migrating under the wall of the ovaries or inside the ovarian stroma among ova developmental stages. On the contrary, infection with *Neoechinorhynchus* sp. and *Leptorhynchoides* sp. was associated with mild or no congestion in the intestine at the site of attachments.

Interestingly, all lakes illustrated a negative correlation between the intensity of infection of *P. ambloplitis* plerocercoids in the ovary and *Neoechinorhynchus* sp. in the intestine. Using one-tailed test, there is about 40% reduction (Statistically significant reduction) in plerocercoids number associated with the presence of *Neoechinorhynchus* sp in the intestine of the fish.

4. Discussion:

The current study reports the prevalence and intensity of infection with three parasites; *P. ambloplitis*, *Neoechinorhynchus* sp., and *Leptorhynchoides* sp. in Largemouth bass from seven inland lakes in Michigan (Table 1). Although *P. ambloplitis* is a common tapeworm parasites of basses in the Great Lakes (Esch 1971; Esch & Huffines 1973; Dexter 1996; Gillilland & Muzzall 2004), little is known about its distribution among LMB in Michigan inland lakes. In one of the few records on *Proteocephalus ambloplitis* in LMB from Michigan, Gillilland & Muzzall (2004) found that prevalence of *P. ambloplitis* plerocercoids from the gonads in the LMB of Gull Lake was 96%, with a mean intensity of (3.7±3.0). Wintergreen Lake and Duck Lake showed a 0% incidence of *Proteocephalus* plerocercoids in the study done by Esch (1971). Similarily, Amin (1990) found that during the summer, parenteric plerocercoids are localized primarily in the gonads, particularly the ovaries. The current study showed that the prevalence of *Proteocephalus ambloplitis* plerocercoids retrieved from the ovary varied between 10% in Norvel Lake to 100% in Jordan and Devils lakes, while the mean intensity varied between 0.2±0.63 in Norvel Lake and 38.5 ±32.6 in Devils Lake.

In a similar study done by Muzzal & Gillilland (2004), the prevalence of *Neoechinorhynchus* sp. and *Leptorhynchoides* sp in LMB from Gull Lake reached 100% with a mean intensity of 42.1±37.9 and 40.0 ± 53.4, respectively, while prevalence of *Neoechinorhynchus* sp in Wintergreen and Duck Lakes were 87.5% and 0%, respectively. No *Leptorhynchoides* sp was recorded from LMB in either Lake (Esch 1971). Prevalence and intensity of both acanthocephalans in the current study varied greatly from those recorded from Gull Lake. The prevalence of *Neoechinorhynchus* sp. ranged from 25% in Randall Lake to 90% in Eagle Lake, while the prevalence of *Leptorhynchoides* sp. ranged from 0% in Eagle and Norvel Lake to 80% in Devils Lake. The intensity of both acanthocephalans in LMB was much lower than that recorded in Gull Lake. Intensity of *Leptorhynchoides* sp. ranged from 0 in Norvel Lake to 10.6±24.8 in Devils Lake, while *Neoechinorhynchus* sp. intensity ranged from 1.01±2.02 in Devils Lake to 12.7±13.06 in Orion Lake. These discrepancies in parasites prevalence and intensity from different lakes were expected rather than surprising. It is well documented that the abundance of parasites in certain ecosystems is controlled by multiple biotic and abiotic environmental factors. The interactions of such factors with the parasite and the host control the prevalence and intensity of specific parasites within certain host. For example, the prevalence and distribution of myxozoan parasites varied among cyprinids fish species which were sampled from 3 different lakes. These variations were attributed to various biotic and abiotic environmental factors in the studied lakes (Koprivnikar, Koehler, Rodd & Desser 2002). Additionally, intermediate host abundance, transmission environment and infection site specificities are all factors which are intertwined and act in concert to control the diversity of parasites within a specific host species inhabiting a particular

environment (Janovy 2002). Other factors relating specifically to the fish host, such as size, age, diet and immune status might also affect the abundance of parasites in certain environments (Lo, Morand & Galzin 1998).

Clinically, largemouth bass from all lakes except Norvel, showed severe adhesion of the internal viscera typically associated with the presence of the proteocephalus larvae. Some of the examined fish exhibited such severe adhesion that the internal organs appeared to be one big mass of tissue. The clinical signs of infected bass correlated well with the prevalence and intensity of *P. ambloplitis* plerocercoids in the ovary. The most severe adhesions were observed in bass from Devils Lake, while Norvel Lake (lowest intensity of plerocercoids infection) showed no adhesion or abnormalities in the internal organs. This adhesion is attributed mainly to the development and migration of plerocercoids in the LMB. After ingestion of copepods containing *P. ambloplitis* plerocercoids I, the released larvae transform into plerocercoids II and migrate from the intestine to extra-intestinal sites, which include the gonads, spleen and liver (Fischer & Freeman 1969; Freeman 1973 & Amin 1990). This mass migration is most likely associated with persistent and chronic irritation, and subsequent severe internal adhesion. Mass migration of plerocercoids on the ovarian surface and within the ovary itself is typically associated with egg destruction was similarly observed from infected fish in the current study. Related pathological changes in the ovarian tissues of infected small and largemouth bass were previously observed and associated with the migration and localizations of plerocercoids. This localization is eventually responsible for damage to the eggs and other pathological changes occurring in the reproductive organs of the affected fish (Esch & Huffines 1973; McCormick & Stokes 1982 & Amin 1990).

The present study demonstrated that an infection with acanthocephalans is usually associated with swelling of the pyloric cecae (in the case of *Leptorhynchoides* sp.) or the posterior portion of the intestine (in the case of *Neoechinorhynchus* sp.), with mild congestion occasionally occurring in the intestinal wall. These signs are believed to be associated with the physical attachment of the parasite to the wall, which results in the disruption of the intestinal wall at the site of attachment. In fact, a previous histopathological study revealed that the mucosa and submucosa were completely disrupted at the sites of the acanthocephalans attachment. This damage was evoked by the insertion of the proboscis into the intestinal wall. Leucocytes and erythrocytes were heavily infiltrated the site of parasitic infestation (Venard & Warfel 1952; Esch & Huffines 1973).

A negative correlation between number of *Neoechinorhynchus* sp. in intestine and the number of *P. Ambloplitis* plerocercoids in the ovary was observed in the present study. As the number of *Neoechinorhynchus* sp. in the intestine increased, the number of *P. Ambloplitis* plerocercoids in the ovary decreased. A similar, yet high negative correlation of -0.94 (compared to a negative value of -0.103 in current study) was reported in an earlier study between the *Neoechinorhynchus* sp. in the intestine and plerocercoids in the viscera of LMB (Durborow et al 1988). The negative correlation could be due to the recruitment of plerocercoids from only one organ “ovary” rather than all viscera as done in Durborow et al (1988). Rationales for these correlations have been a fertile environment of scientific debating for a great deal of time (Holmes 1973; Holmes 1987; Price 1987 & Janovy 2002), yet some arguments for the interactions could be scientifically accepted. For example, physical or nutritional competition could be the underlying mechanism causing the competitive inhibition between parasites residing in the same site within the same host body (Read 1951; Read 1959; Read & Phifer1959; Holmes 1973; Dezfuli et al 2001). In fact, competitive inhibition between parasites is a well-known phenomenon in fish and other animal. For example, competitive inhibition between *Proteocephalus filicollis* and *Neoechinorhynchus rutili* on the site of infection observed in Sticklebacks (*Gasterosteus aculeatus*) caused the displacement of one parasite upon subsequent infection with the other (Chappell 1969). A similar correlation was also recorded in the least cisco *(Coregonus sardine/la)* between *Proteocephalus exiguus* and *Neoicanthorinchus* sp. (Cross, 1934). However, the mechanisms underlying competitive inhibition have yet to be proven scientifically. In one of the few studies investigating the parasites correlation, a negative correlation was recorded between *P. ambloplitis* plerocercoids in the viscera and *Neoechinorhynchus* sp. in the intestine of largemouth bass (Durborow et al. 1988). In order to ascertain the cause of the observed negative correlation, Durborow et al. (1988) immunized LMB with either *Neoechinorhynchus* sp. or adult *Proteocephalus ambloplitis* vaccines and challenged the fish with plerocercoids of *P. ambloplitis*. He found that the fish vaccinated with either vaccine developed smaller plerocercoids compared to the control group. He suggested that a type of cross immunity might be responsible for the competitive inhibition, which subsequently caused the negative correlation between the two parasites. A similar correlation was recorded as well between exoparasites and endoparasites. The skin fluke *Gyrodactylus derjavini* decreased significantly in number among brown trout,

Salmo trutta, fry that were concurrently infected with larval stages of *Anisakis* sp. in the viscera. In this regard, *Anisakis* larvae in the viscera were thought to be responsible for the activation of the skin immune response against *Gyrodactylus derjavini*, which subsequently caused the decrease in *Gyrodactylus derjavini* number (Larsen, Bresciani & Buchmann 2002)

In conclusion, prevalence and intensity of three endoparasites have been reported for the first time from seven inland lakes in Michigan. A negative correlation between number of *Neoechinorhynchus* sp. in intestine and the number of *P. Ambloplitis* plerocercoids in the ovary was observed. However the reason for this correlation needs further investigation.

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Table 1. Mean Measurements in Millimeters of Plerocercoids from Ovaries by Lake. Mean ± Standard Deviation is Listed on Top, Range is Listed in Middle, and Number of Plerocercoids that were Measured are on Bottom. Numbers of Plerocercoids Measured are not the Same for Each Group Because not all Measurements were able to be Done on all Plerocercoids.

Lake	ASD	SA to AS	Scolex Width	LSD	Body Length	ASD/LSD	LSD/SW
Randall	0.29±0.32 0-2.9 n=74	0.16±0.13 0-0.51 n=46	0.73±0.13 0.34-1.38 n=69	.21±0.07 0.06-0.37 n=66	3.9±3.5 0.85-13.62 n=48	1.57±1.35 0.27-11.15 n=66	0.28±0.1 0-0.51 n=63
Orion	0.26±0.05 0.17-0.37 n=17	0.30±0.15 0-0.52 n=11	0.85±0.24 0.4-1.31 n=17	0.24±0.24 0.15-0.3 n=17	3.64±0.05 1.03-6.57 n=5	1.11±2.7 0.78-1.53 n=17	0.30±0.19 0.20-0.44 n=17
Independence	0.27±0.05 0.20-0.34 n=26	0.18±0.14 0.43-2.33 n=15	0.78±0.19 0.37-0.96 n=26	0.22±0.04 0.15-0.35 n=26	3.54±1.90 0.74-1.6 n=11	1.26±0.18 0.74-1.6 n=26	0.33±0.08 0.24-0.52 n=26
Devils	0.25±0.05 0.14-0.40 n=102	0.21±0.12 0-0.42 n=40	0.70±0.16 0.33-1.11 n=102	0.21±0.04 0.11-0.33 n=104	3.84±2.12 0.72-8.6 n=41	1.19±0.32 0-2.36 n=104	0.32±0.08 0.16-0.63 n=102
Eagle	0.27±0.04 0.2-0.34 n=26	0.16±0.16 0-0.43 n=15	0.67±0.15 0.37-0.96 n=26	0.22±0.04 0.15-0.35 n=26	3.54±1.9 1.08-6.54 n=11	1.26±0.18 0.74-1.6 n=26	0.33±0.08 0.24-0.52 n=26
Jordan	0.28±0.05 0.15-0.40 n=87	0.17±0.15 0-0.44 n=36	0.81±0.17 0.36-1.35 n=80	0.23±0.06 0.14-0.64 n=87	3.80±2.4 0.89-11.8 n=39	1.14±.38 0.05-1.89 n=81	.63±1.28 0-6.33 n=80
Norvel	------ 0.23-0.25 n=2	------ ------ n=0	------ 0.62-0.67 n=2	------ 0.18-0.21 n=2	------ 1.25-2.36 n=2	------ 1.19-1.28 n=2	------ 0.27-0.34 n=2

(ASD): Accessory Sucker Diameter(LSD) Lateral Sucker Diameter (SA to AS) Scolex Apex to Accessory Sucker
(SW) Scolex Width (ASD/LSD) ratio between Accessory Sucker Diameter and Lateral Sucker Diameter
(LSD/SW) Lateral Sucker Diameter to Scolex Width.

Table 2: Prevalence (P) and Intensity (MI±SD) of total acanthocephalans, *Neoechinorhynchus* sp., *Leptorhynchoides* sp. and *P. ambloplitis* plerocercoids in Largemouth bass collected from seven inland lakes in Michigan in 2002.

Lake	**Total Acanthocephalans in the intestine**		***Neoechinorhynchus* sp.**		***Leptorhynchoides* sp.**		***P. ambloplitis* plerocercoids in Ovary**	
	P	MI ± SD	P	MI ± SD	P	MI ± SD	P	MI ± SD
Randall	63	7.4 ± 11.8 n*=8	25	1.6 ± 4.2 n=8	38	5.7 ± 11.5 n=8	88	16.5 ± 10.5 n=8
Orion	73	13.7 ± 13.7 n=11	55	12.7 ± 13.06 n=11	55	1 ± 1.09 n=11	55	1.63±1.96 n=11
Independence	44	5.4 ± 9.1 n=9	33	4.0 ± 6.26 n=9	22	1.4 ± 3.97 n=9	78	20.2 ± 20.1 n=9
Devils	82	11.7 ± 26.2 n=11	27	1.01 ± 2.02 n=11	82	10.6 ± 24.8 n=11	100	38.5 ± 32.6 n=11
Eagle	90	8.6 ± 7.02 n=10	90	8.6 ± 7.02 n=10	0	0	90	4.4 ± 3.8 n=10
Jordan	90	9.7 ± 7.8 n=10	70	8.5 ± 8.6 n=10	40	1.2 ± 1.87 n=10	100	35.1 ±21.2 n=10
Norvel	60	2.2 ± 3.8 n=5	60	2.2± 3.8 n=5	0	0	10	0.2 ± 0.63 n=10

n*: Number of fish

Studies on the effects *Cymbopogon citratus, Ceiba pentandra* and *Loranthus bengwelensis* extracts on *species of* dermatophytes.

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ABSTRACT: Alcohol and water extracts of *Cymbopogon citratus, Ceiba* _pentandra and *Loranthus bengwelensis* were investigated for anti-fungal properties and phytochemical constituents. The anti-fungal examination was by disk diffusion and agar dilution techniques, while the phytochemical constituents were investigated using standard chemical methods. Results showed that all the extracts inhibited the growth of standard and local strains of the organisms used, namely *Epidermophyton flocosum, Microsporum canis, Trichopyton rubrum* and *Candida albicans.* Some of the extracts had fungicidal effects while others had fungistatic effect on the organisms. The treatments were significantly different (P = 0.05). The minimum inhibitory concentration of the extracts against the tested microorganisms ranged between 150mg/ml and 50mg/ml. Comparisons were made with ketoconazole at 1mg/ml. The alcohol extracts were found to be generally more effective than the water extract for *C. pentandra* while the reverse was the case for the *C. citratus* and *L. bengwelensis* extracts. (P = 0.05). The phytochemical analysis revealed the presence of saponins, tannins, fats and oils, alkaloids and phenol but absence of cardiac and cyanogenic glycosides. The presence of saponins and phenols were inferred as being responsible for the anti-fungal properties of the extracts. [The Journal of American Science. 2008;4(4):52-63]. (ISSN: 1545-1003).

Keywords: *Cymbopogon citratu; Ceiba pentandra; Loranthus bengwelensis;* dermatophytes.

1. INTRODUCTION

Plants have been know to synthesize a variety of chemical substances, such as phenolic compounds, terpenes, steroids, alkaloids, glycosides, fats and others. The also synthesize secondary metabolites, which are of no apparent importance to the plants life. However, these have been found to have profound effects on animal systems with therapeutic properties (Abdul 1986). With current advanced technology, plants are being analyzed and their therapeutic abilities investigated more intensely. Today these methods have been used to isolate an ever increasing number of medicinal substances from plant sources. These medicinal plants are the sources of important drugs of the modern world. Some of these include guanine from *Cinchonabark*, reserpine from *Rauwoiffia* root, digitoxin from *Digitalis* leaf, atropine from *Belladonna* root and leaf, hyoscyamine from *Hyoscyanamus* and Datura leaves and roots, conine from *Conium* leaf, morphine from Opium capsule, sennosides from *Cassia* leaves, colchicina from *Colchicum* corn and vineristine and vinblastine from *Catharanthus* root are but a few (Abdul 1986). UNCTAD/GATT (1974) showed that about 33% of drugs produced in developed countries are derived from plants. According to some sources, 80% of present day medicines are directly or indirectly derived from plants (Myers, 1982). Astonishingly, these large quantities of modern drugs come from less than 15% of the plants which are known to have been investigated pharmacologically.

Cymbopogon citratus commonly called lemon grass is an aromatic, perennial grass belonging to the family grimneae. It is a tropical plant, grown as an ornamental in many temperate areas with maximum a height of about 1.8m and its leaves 1.9cm wide covered with a whitish bloom. Like other members of the genus, *citratus,* yields citral, a volatile oil with strong lemon fragrance. It is used in manufacture of perfumes, coloured soaps and synthesis of vitamin A. Folk medicine in certain parts of Nigeria use the essential oil as an insect repellant (Soforowa 1970). In certain medications, it is used for mental illness (Ebomoy, 1986). It is an antifungal, antitoxicant and deodorizing agents (Obiora 1986). In combination with other herbs, it has large use as cure for Malaria (Gbile 1986).

Ceiba pentandra is a tree and belongs to the family *Bombaceae*. It is commonly called silk cotton tree or kapok tree. In Nigeria, it is known in Hausa as *rimi* in Igbo as *akpu* and in Yourba as *araba*. It is reputed to be the largest tree of the West African region and originated from tropical America. The light seed was purported to have been blown across the Atlantic. This plant has been used in traditional medicine for several ailments. The bark contains a blackish mucilaginous gum, that swells in water and resembles tragacanth. Folk medicines in Nigeria use the bark for the treatment of infections. It is astringent and is used in India and Malaya for bowel complaints. The bark is believed to also contain tannin. In West Africa, it is generally used in the treatment of diarrhoea.

Loranthus bengwelensis commonly referred to as Mistletoe belongs to the family *loranthaceae*. It is a semi parasitic shrub (Dutta, 1979) growing on citrus trees and developing sucking roots or haustorias. It is used traditionally for the capture of birds by the very sticky gums produced by the seed. In certain parts of Northern Nigeria, it is used for the treatment of various skin diseases and for the extraction of certain oils used in soaps and creams. Several works have highlighted the enormous potentials available in the use of plants and plant products for therapeutic purposes. Unfortunately however many of the reported claims on the efficacy of these plants and plant products are based on the untested word of traditional medicine healers. While efforts have been made to collate such information from these healers and from other sources, available documented evidence to support these claims are scarce and inadequate (Soforowa, 1982). This paucity of scientific evidence has informed the development and execution of this study to analyse *C. Citratus, C. Pentendra* and *L. bengwelensis* phytochemicaly and to determine if the plant extracts possess any inhibitory, action against the selected species of dermotophytes. The dermatophytes are a group of taxonomically related fungi which have affinity for cornified epidermis, hair, horn, nails and feathers where they produce dermatophilic infections.

Much is known about the sources of infection but very little is known of the factors that determine susceptibility or resistance of host, or prevalence. However, Kam *et al* (1997) stated that changes in climatic conditions affect prevalence of Tinea capitis, an infection of the scalp and hair with any of the dermatophytes. It is probably the most common fungal infection occurring before puberty. Tinea cruris a dermatophyte infection of the groin and upper thigh, is commonly seen after puberty especially in men. This may be caused by several species including *Epidermoplyton floccosum, Trichopyton mentagrophytes* and *T.rubrum.* Itching is a very common feature and change secondarily to persistent scratching and

rubbing as in lichenification is commonly observed (David, 1983). Tinea pedis and manum, dermatoplyte infections of the hand and feet are rare before puberty; they are caused by T. *rubrum* and *T.mentagrophytes* with occasional involvement of other dermatophytes like microsporon canis etc.

All these infections are treated with antibiotics, mainly broad-spectrum antimycotics, usually by topical application with various degree of success. Today so many antimycotics have been brought into use, and some abused leading to development of resistance. This is a problem which only development of new drugs can check (Broston, 1988). The skin assumes enormous importance in a person's self image and its importance, as an organ of sexual attraction is well known. May people believe moreover, with some reason that the skin reflects the underling state of health and often seek reassurance on this point. There is the need to therefore give greater attention to developing more anti fungal (anti-dermatophilic) drugs so as to effectively check the increasing prevalence of these infections. Especially in the tropics and subtropics, where the climate make people more susceptible to the infections.

2. MATERIALS AND METHODS

a. Collection of Samples

The plant samples were obtained from Rasau village behind the students village hostel university of Jos, Nigeria, during the mid rainy season. They were kindly identified by professor Akueshi of Botany Department, university of Jos Nigeria and confirmed by Taxonomists at the Federal College of Forestry Jos, Nigeria.

The collected samples which included 400g of *Cymbopogon citratus* excluding roots, 400g of *Loranthus bengwelensis* made up of 200g each of stem and leaves, and 400g of *Ceiba pentandra* made up of 200g each of roots and bark.

b. Preparation of Extracts

The plant samples were oven dried at 50^0C for 48hours. On cooling they were subsequently ground separately by means of a clean pestle and mortar into a very fine powder. These were stored in airtight containers separately and refrigerated until required for use.

Preparation of extracts was done by modifying methods used by Akinyaju *et al* (1986).

Hot Water Extract (HWE) of the respective plant samples was made by dissolving 150g, of the powdered plant sample in 200mls of distilled water for 4 hours. It was then further extracted using the soxhlet apparatus for a further 2hrs. The resulting infusion was filtered using Watman # 1 filter paper. The filtrate was then subjected to gentle evaporation using a hot plate. The resulting paste was then scrapped onto a watch glass where it was allowed to evaporate to dryness in an oven at 60^0C. The HWE was then ground, weighted and stored in the powdered form in an (AE) airtight container in a refrigerator until required.

The Alcohol Extract was made by soaking 100g of each powdered plant material in a solution of 200ml and 100ml of 95% ethanol and methanol respectively. The mixture was allowed to stay for 48hrs. It was stirred at 12hr. intervals by means of sterile glass rod. The resulting liquid was filtered using Watman #

1 Filter paper. The filterate was evaporated gently to dryness and weighed. It was stored in the same condition as HWE. The pH was determined using the pH meter (Phep 3 microprocessor pH meter by Hanna inst. Co). This indicated the level of acidity or alkalinity of the aqueous and ethanol extracts.

c. Phytochemical Screening of Extracts: The standard methods of analysis employed were adapted from those used by Iwu (1982), Emeruwa (1982) and Onwuliri (1996).

d. Isolation and Identification of test Organisms: The test organianisms *microsporum carnis, Trichophyton rubrum and Epidermophyton, floccosum* were isolated from children with dermatophyte infections in Gingiri primary school Jos Nigeria. These were then compared with stock cultures obtained from to dermato philosis research centre, National Institute for Veterinary research Vom Nigeria. The criteria used for identification and isolation were as those described by Rebel and Taplin (1970) and Campbell (1980).

e. Preliminary Screening for Anti fungal properties: Each of the extracts was individually reconstituted using minimal amounts of extracting solvent and further diluted with buffered glycerol. They were tested neat (without dilution), 200mg/ml, 100mg/ml and 50mglml as in Abide and Irobi (1993). These concentrations of the extract were impregnated into 7mm diameter sterile disks punched out of Wattman filter paper No 1. These sterile disks are replaced on sabouraud dextrose Agar plates amended with chloramphenicol at 0.05mg/l. These plates had previously been inoculated with 1ml of standard solution of fungal spore containing approximately 103 spores/1ml of sample organism and incubated at 25^0C for 10 days. The presence of zones of inhibition around each of the discs after the period of incubation was regarded as the presence of antimicrobial action while the absence of any measurable zones of inhibition was interpreted as absence of antimicrobial action.

f. Minimum Inhibitory Concentration (MIC): The MIC of the extracts was determined by incorporating various concentrations of the extracts into the culture media (chloramphenicol amended Sabouraud Dextrose Agar). Final extract concentrations of 150 to 30mg/ml of media, was made and poured. A 1ml Standard solution of fungal spores (103 spores/ml) was added into each of the tubes and incubated for 10days at 25^oC. Positive control tubes containing only the growth medium and a test organism per tube were also set up. The minimum inhibition concentration was regarded as the lowest concentration of extracts that did not permit any visible growth when compared with that of the control tubes.

g. Minimum Cidal Concentration

Samples from tubes used for the MIC assays which did not show growth after the period of incubation was diluted 1:4 with fresh media and 50ml amounts sub-cultured on fresh medium as in Rotimi *et al* (1988). The Minimum Fungicidal Concentrations (MFC) was regarded as the lowest concentration of the extracts that did not permit any fungal colony growths after seven day of incubation.

3. RESULTS AND CONCLUSION

The percentage extractions lied between 11% and 3% for the alcohol extracts and between 6.96% and 5.10 for the hot water extracts. The lowest was 3% for HWE of *C. citratus* (table 1). The result also showed that alcohol extracts of *C. pentandra* and *L. bengwelensis gave* higher yields than their respective water extracts, while for *C. citratus* the reverse was the case. This justifies the use of water as solvent in folk medicine for preparing some decoctions for cure of skin diseases. That *C. bengewlensis* and *C. pentandra* showed more alcohol soluble constituents or secondary metabolites than water soluble ones also supports the use of alcohols in the preparation of certain herb medicaments in folk medicine. The pH values for the water extracts are within the range of 7.4 and 3.7; this variation is large and might be responsible differences in activity observed amongst of the various aqueous extracts on the test organisms. The values of the alcohol extracts are within the range of 6.4 and 4.9; the variation amongst the alcohol extracts is small which limited might account for the limited variation of the effects of the extracts on the test organisms. The pH values of the water extracts show that pH of all the HWE extracts are in the acidic range (Table 1) and it is likely that the antimicrobial activity of the active principle in the plant HWE extracts are effective at an acidic pH.

Phytochemical studies of the plants extracts showed the presence of tannins, saponins, alkaloids, fats an oils, phenol, steroids and carbohydrates in both the water and alcohol extracts. All extracts showed the presence of glycosides, but none showed the presence 0 and C glycosides. (Table 2). Hence Ebana, *et al.* (1991) suggested that antimicrobial activity of plants may be due in part to the presence of phytoalexins.

The results of the antimicrobial sensitivity showed that inhibition of microbial growth was greater at high concentrations of the plant extracts and, less inhibition was observed as the concentration was lowered. This shows that the effectiveness of the extracts is directly related to the concentration of the extracts. The crude extracts of *L. bengwelesis* showed the highest inhibition against the test microorganisms (tables 3-5). This implies that the inhibitory compound in the *L. bengwelensis* extracts are either more efficacious, or exist in higher concentrations. The lowest inhibition was observed for *C. citratus,* although the neat alcoholic extract performed quite impressively. This might be because the inhibitory compound is more soluble in alcohol than water. In all the preliminary screening, the least inhibitory effect was observed on *C. albicans.* The *E. floccosum* showed the highest inhibition for the water extracts of *L. bengwelensis.* In the comparative antifungal susceptibility assay, the extracts were used at concentrations of 150mg/ml for both the alcoholic and water extracts. ketoconazole was used for comparison at 1mg/ml concentration. Ketoconazole produced the highest inhibitory effect on all the test organisms followed by the water extract of *L. bengwelensis* and then the alcoholic extract of *C. pentandra* respectively (table 6). The higher values observed for the ketoconazole could be explained by the pure nature of the chemical compound, as against that of the plant extracts which are still crude and impure. The minimum inhibition concentration levels at which the crude extracts inhibited the test organisms are very important. They are normally used to evaluate the efficacy of chemotherapeutic agents under standard conditions. Inhibitory concentrations of

100mg/ml and below are considered promising in the antimicrobial screening of crude plant extracts. The reasons for the high MIC values of these extracts examined may either be due to the fact that the extracts used in the experiment were in the crude form, or that the active compound(s) are present in very low concentrations. It could also be as a result of antagonistic action of the component compounds in the extracts. Thus retarding their activity in the crude extracts. Purification of the extracts therefore may drastically reduce the MIC values. This therefore gives support to a certain degree, the traditional medical use of the plants evaluated for treating superficial fungal infections. This also reinforces the concept of ethno-botanical approach to the screening of plants as potential sources of bioactive substances against disease causing pathogenic fungi as very promising.

Table 1: Percentage yield and pH of the Plant extracts

	AE		HWE	
	%	pH	%	pH
C. citratus	3.00	4.9	5.10	7.4
C. pentandra	7.98	6.4	5.74	3.7
L. bengwelensis	11.00	5.9	6.96	4.2

Key: AE = Alcohol Extract
HWE = Hot Water Extract

Table 2: Phytochemical Constituents of Water and Alcohol Extracts of *C. citratus*, *C. pentandra* and *L. bengwelensis*

Plant	*C. citratus*		*C. pentandra*		*L. bengwelensis*	
Extracts	AE	HWE	AE	HWE	AE	HWE
Plant constituents						
Fats/oils	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Starch	-	+	-	-	+	+
Reducing sugar	+	-	+	+	+	-
Glycosides	+	+	+	+	+	+
0 and C Glycosides	-	-	-	-	-	-
Flavenoids	+	+	+	+	+	+
Steroids/terpenoids (Libernlans test)	+	+	+	+	+	+
Alkaloids: Dragendoff	-	-	-	-	+	-
Mayers reagent	-	-	-	-	+	-
Wagners reagent	-	-	-	+	+	-
Hagers reagent	-	-	-	-	-	-
Phenol	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+

Key: + = Present
- = Absent
AE = Alcohol extract
HWE = Water extract

Table 3: MIC for *C. pentandra* Extracts

	Concentration of extracts in mg/ml								
	150	100	80	70	60	50	40	30	MIC
Water Extracts									
Microorganisms									
E.floccosum	-	-	+	+	+	+	+	+	80mglml
M.canis	-	-	-	-	+	+	+	+	70
T.rubrum	-	-	+	+	+	+	+	+	100
C. albicans	-	-	-	-	-	+	+	+	60
Alcohol Extracts									
E. floccosum	-	-	+	+	+	+	+	+	100
M. canis	-	-	+	+	+	+	+	+	80
T. rubrum	-	-	-	-	-	-	+	+	50
C. albicans	-	-	-	-	-	-	+	+	50

Key: + = tubes showed at least one colony of growth

\- = tubes that showed absence of growth

Table 4: MIC for *C. Citratus* Extracts

	Concentration of Extracts of mg/ml								
	150	100	80	70	60	50	40	30	MIC
Water Extracts									
Microorganism									
E. floccosum	-	-	-	-	+	+	+	+	70
M. canis	-	-	-	+	+	+	+	+	80
T. rubrum	-	+	+	+	+	+	+	+	150
C. albicans	-	+	+	+	+	+	+	+	150
Alcohol Extracts									
E. floccosum	-	-	-	+	+	+	+	+	80
M. canis	-	+	+	+	+	+	+	+	150
T. rubrum	-	+	+	+	+	+	+	+	150
C. albicans	-	+	+	+	+	+	+	+	150

Key: + = Tubes showed at least one colony of growth

\- = Tubes that showed absence of growth

Table 5: MIC for *L. bengwelensis* Extracts

	Concentration of extracts in Mg/ml								
	150	100	80	70	60	50	40	30	MIC
Water Extracts									
Microorganism									
E. floccosum	-	-	-	-	-	-	+	+	50mglml
M. canis	-	-	-	-	-	-	+	+	50
T. rubrum	-	-	-	-	-	+	+	+	60
C. albicans	-	-	+	+	+	+	+	+	100
Alcohol Extracts									
E. floccosum	-	-	-	-	-	+		+	60
M. canis	-	-	-	-	-	-		+	50
T. rubrum	-	-	-	-	-	+	+	+	6
C. albicans	-	-	-	-	-	+	+	+	60
							+		
							+		

Key: + = Tubes showed at least one colony of growth

\- = Tubes that showed absence of growth

Table 6: Comparative Antifungal susceptibility Assay

Extracts (150mg/ml)	*C. citratus*		*C. pentrandra*		*L. bengwelensis*		Ketotonazole 1mg/ml
Microorganisms	HWE	AE	HWE	AE	HWE	AE	
E. floccosum	9	10	10	8.5	11	6	16
M. canis	7	7	9	8	12	9	17
T. rubrum	6	6	8	12	10	7	19
C. albicans	6.5	5	9	9	6	6	15

Key AE: Alcohol Extract, HWE: Hot Water Extract.

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Groundwater Development and Evaluation of the White Volta Basin (Ghana) using numerical Simulation

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Abstract: Increased exploitation of groundwater resources in recent years in northern Ghana has raised concerns about the natural limit of it, i.e. quantity of renewable resources. In addressing its sustainability, a three-dimensional transient and anisotropic groundwater flow model was developed to aid in the understanding of the groundwater system and the regional effects of groundwater development alternatives in the White Volta Basin. Mean annual data, available well data, general hydrogeological and geological information were used, and the predicted model results for several scenarios tested (i.e. increased population and decreased rainfall) indicated that the extraction rates will still be less than groundwater input. [The Journal of American Science. 2008;4(4):64-71]. (ISSN: 1545-1003).

Keywords: Ghana, Groundwater, Hydrogeology, Simulation, Model.

1. Introduction

The White Volta Basin is located in northern Ghana in the West African sub- region. Its total area is approximately 48800km^2 and is located between latitudes 8.5^{o}N and 11^{o}N, and longitude 0^o and 2.5^{o}W (Fig.1). Annual rainfall is (1000-1200 mm) with 75% of it occurring in July and September (Van de Sommen and Geinaert, 1988). Groundwater in recent years has become a premium source of portable water supply for most communities in Ghana. An improved economy in recent years coupled with campaigns on the imminent hazards of relying on sources of surface water, and factors such as: ease of developing hand pumps in remote communities, availability during protracted drought, superior chemical and biological quality (compared with surface water sources) and relatively low prices compared with methods of treating surface water (Dapaah-Siakwan and Gyau-Boakye, 2000; Gyau-Boakye, 2001) accounts for the increasing demand for safe water supply. The population growth rate in northern Ghana is 2.5% per year (Van de Giesen et al, 2001) (Gyau-Boakye, 2001), by March 1998, a total of 11,500 boreholes had been drilled, providing 52% of the rural population with potable water (up from 41% in 1984) (Gyau-Boakye, 2001).The White Volta Basin has low and unreliable rainfall pattern with protracted drought. Surface flow is usually ephemeral. Open well drying during dry season is a common feature whilst during the drought of the 1980s, reduced yield in deeper wells and drop in groundwater levels were experienced (Wardrop Engineering, 1987; Thiery, 1990). A phenomenon Gyau-Boakye and Tumbulto (2001) attributed to increasing abstraction and depletion of the groundwater resource.

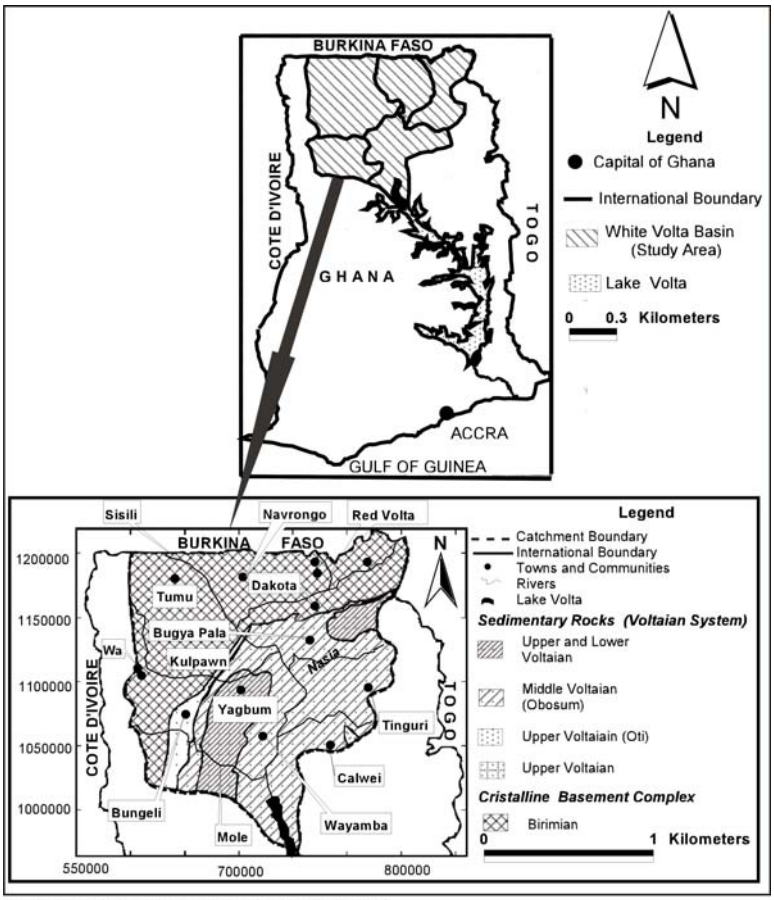
2. Purpose and Scope

(1) The aim is to simulate the groundwater flow in the weathered rock and sandstone aquifers of the White Volta Basin.

(2) Evaluating potential groundwater recharge and discharge rates.

(3) estimating source and quantity of groundwater and ascertaining the effects of current pumping on groundwater resources applying a three-dimensional numerical groundwater flow model taking into consideration population change and drought.

The presence of fluoride, iron and nitrate concentrations exceeding the World Health Organization (WHO) recommended level of 1.5 mg/l, 0.3 mg/l and 10 mg/l respectively are worth mentioning in this paper. A data base of 14 monitoring wells analyzed for various water quality constituents (WRI, 2006) revealed high fluoride concentrations in communities around Tumu, Bongo-Nayare and Tinguri. High concentrations of iron exceeding 0.3 mg/l were recorded in wells at Bongo-Nayire, Bungeli and Galiwei, whilst nitrate concentrations higher than 10mg/l were recorded at Bugya-Pala due to the application of organic manure for farming activities. Groundwater quality in the basin is generally considered acceptable by World Health Organization standards (WRI, 2006).



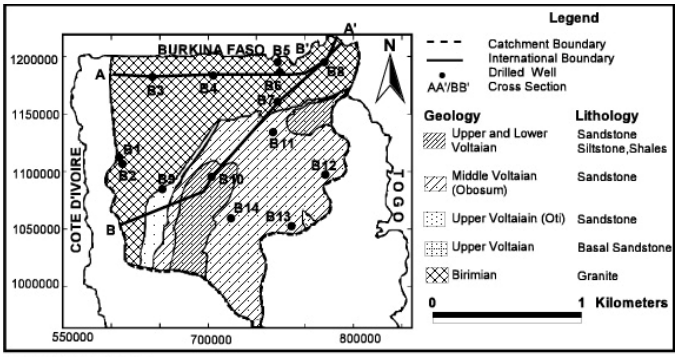
Source:Water Resources Institute (WRI)
Fig.1:Location Of the White Volta Basin(WVB)

3. Materials and methods

3.1. Geology and Hydrogeology

The White Volta Basin is partly underlain by aquifers of the crystalline basement complex (weathered rocks) and aquifers of the consolidated sedimentary (sandstone) rocks. The crystalline basement complex is composed of gneiss, phyllite, schist, granite-gneiss and quartzite which occupy an area of 21,960km^2 with the consolidated sedimentary formation which consists mainly of sandstone, shale, arkose, mudstone, sandy and pebbly beds and limestone occupying 26,840 km^2. Detailed geology and hydrogeology is contained in reports by (Junner and service 1936; Junner and Hist, 1964), (Acheampong; 1969) and (Kesse; 1985). Drilling projects and hydrological investigations indicate that shallow potential aquifers capable of delivering water of sustainable quantities for domestic consumption and for industrial use exist in the basin (Kortatsi; 1984) and (Dapaah-Siakwan; 2006). Previous studies indicate that primary porosities are very low due to the impervious nature of the rocks. However, where secondary porosity imposed by the fracturing and weathering of rocks occurs, the hydrogeological properties of these rocks are very much enhanced. Therefore, the hydrogeological parameters are based on secondary permeabilities in the form of joints developed after the primary porosities had been destroyed in the wake of rock compaction and slight metamorphism (Acheampong and Hess; 1988: Yidana et al; 2007). When secondary porosities have not been imposed to provide ingresses for infiltration and recharge, the aquifer properties are very poor. Where weathering of rocks is intense, secondary permeability is enhanced and they serve as better aquifers. Recharge ranges between 3.8 and 5 % of annual precipitation (Ricolvi, 1999; Ampambire,

200; Martin and van de Giesen, 2005). There has not been any known recharge from surface water bodies but water from aquifers in this basin has been noted as a source of recharge of the Volta Lake.



Source : Kesse (1985)
Fig.2 : Map Showing Geology and Cross Sections Of the White Volta Bassin

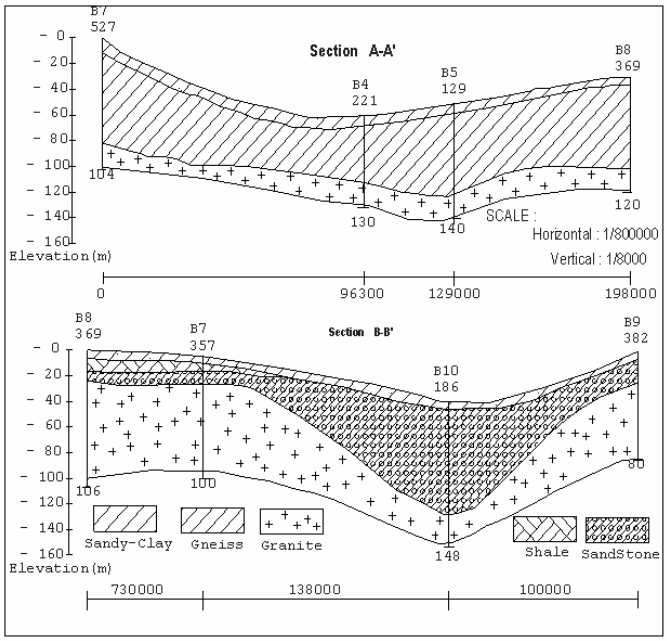


Fig.3: Cross Sections of the Crystalline Basement Complex and the Weathered Sedimentary Rocks

4. Data collection and analysis

The data used for this research was collected during drilling projects conducted during the periods of 1984 and 2006 by World Vision International and the Water Resources Institute, Ghana. The hydrogeological data used in the study include information on aquifer hydraulic conductivity, well yield and depths, specific capacity and the general geology of the hydrostratigraphic units. Well log data was used to help determine the thickness of the hydrostratigrahic units at several locations in the area.

5. Conceptual Model

There are no obvious physical and geographical boundaries in any direction of the model domain, except the White Volta River which traverse the model. The basin is predominantly underlain by granite, granitic gneiss, hard sandstone, shale, siltstone and mudstone as revealed by shallow high and moderate yielding wells in the area. These lithologies can be separated into distinct hydrostratigraphic units on the basis of the hydraulic conductivity (fig.3). The wells in the study area do not expose all the hydrostratigraphic units and information from literature was used to cover the entire stratigraphy. The upper 20-100 m of materials reveals various materials with different hydraulic properties. There is also a mixture of different lithologies with the hydraulic conductivities ranging from 0.057-4.38 m/day.

The conceptual model (fig.4) was converted to a numerical model using Groundwater Modeling Software (GMS) (EMRL 2004). The study area was discretized into 80 cells in the x- direction by 80 cells in the y- direction. The hydrostratigraphic units were converted to three layers on the basis of available hydraulic conductivities. All the vertical boundaries of the model were assumed to be no- flow boundaries because they either coincided with flow symmetry below the major water bodies of the basin or were far enough from the main recharge areas of interest of the model domain.

The bottom boundary was also assumed to be a no-flow boundary because the hydraulic conductivity had decreased significantly relative to the value in the top model layers. The entry and exit boundaries of the river were modeled as constant head boundaries, using the river and the Lake Volta water level as the constant head for the top layer. This is because the water level in both the river and the lake must be maintained at a constant level of 90-300m to achieve ecological stability.

Recharge in the study area is principally by precipitation. Annual recharge was computed as between 3.8-5% of the annual precipitation of 1,000 to 1,200mm.Maximum recharge values were assigned to cells in the top layer located in the low lying areas of the model. Other cells in the top layer located around highland areas were assigned reduced recharge values.

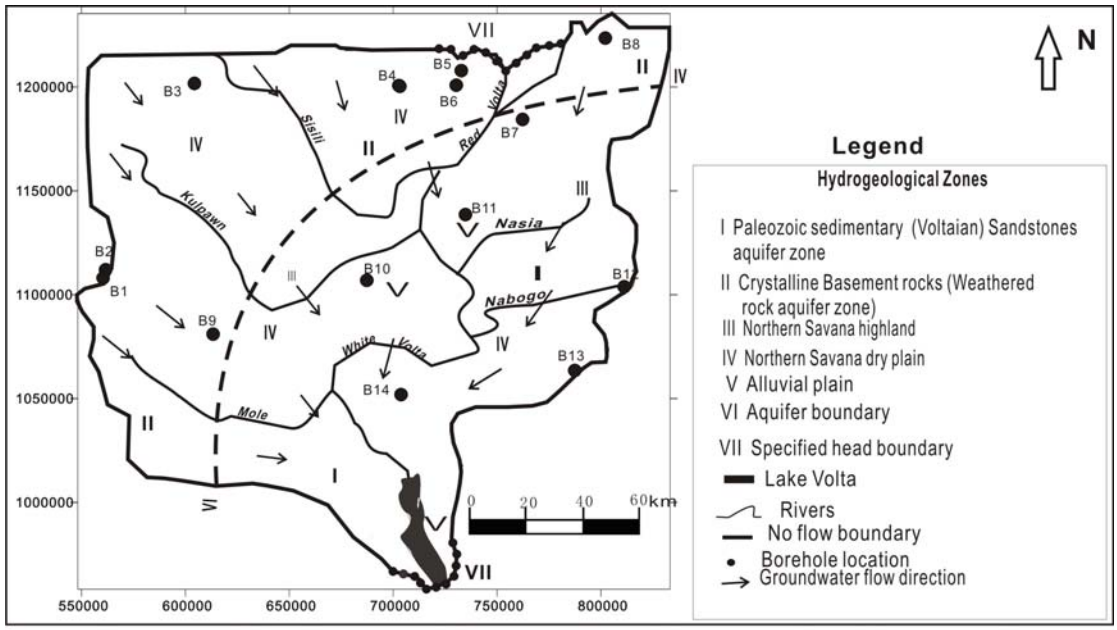


Fig.4 : Conceptual Model Map of the White Volta Basin

6. Numerical Model

The aquifers in the study are of varying depths and are widely apart. They are thick and complex in nature with very vibrant horizontal flow and less active flow in the vertical direction. The hydrogeologic parameters of the aquifers are complex, they vary with time and in space, allowing the model medium to be described as heterogeneous, anisotropic, with a transient groundwater movement. The equation that describes this condition of flow of the groundwater system can be mathematically

$$\frac{\partial}{\partial x}\left(K_{xx}\frac{\partial h}{\partial x}\right)+\frac{\partial}{\partial y}\left(K_{yy}\frac{\partial h}{\partial y}\right)+\frac{\partial}{\partial z}\left(K_{zz}\frac{\partial h}{\partial z}\right)-w=S_s\frac{\partial}{\partial t} \quad (1).$$

$$h\,(x, y, z, 0) = h_0\,(x, y, z)$$

$$h\big|_{B1} = f(x, y, z, t)\big|_{B1}\ ;\quad K\frac{\partial h(x, y, z, t)}{\partial h}\Big|_{B2} = q(x, y, z, t)\big|_{B2}$$

Where $K_{xx,}$ K_{YY} and K_{ZZ} are values of hydraulic conductivity along x, y and z coordinate axes, which are assumed to be parallel to the major axes of hydraulic conductivity (LT^{-1}); h is the hydraulic head (L),

w is the volumetric flux per unit volume and represents sources or sinks of water (T^{-1}), S_S is the specific storage of the

porous material (L^{-1}), and t is time. *h* is the initial hydraulic head (L), $f(x, y, z, t)\big|_{B1}$, is the first boundary; $q\ (x, y, z, t)\big|_{B2}$ is the second boundary. In general, S_S, K_{xx}, K_{YY}, K_{zz} may be functions of space ($S_S = S_S\ (x, y, z)$, $K_{xx} = K_{xx}\ (x, y, z)$, etc,) and W may be a function of space and time $W = (W(x, y, z, t)$. Equation (1) describes groundwater flow under nonequilibrium conditions in a heterogeneous anisotropic medium, provided the principal axes of hydraulic conductivity are aligned with the coordinate's directions (McDonald and Harbaugh, 1988).The finite difference code, MODFLOW (McDonald and Harbaugh; 1988) in the GMS package was chosen to solve Equation (1) for hydraulic heads in the area. Transient conditions were assumed in other to make limited predictions, and to further evaluate the combined long-term effects of increased pumping, the suitability and the dynamic response of the system for average groundwater flow conditions. The simulation period was defined for January 1st 2007 to December 31st 2007. Each month was considered a stress period so the total stress period was twelve months with a time step of ten days.

7. Model Calibration

The model developed under transient conditions was calibrated using water levels in 14 observation wells monitored between the periods of 2005 and 2006. Calibration was achieved varying the hydraulic conductivities of each of the layers defined in the model. The sources and sinks (recharge) was also adjusted within the ranges of (3.8- 5%) of the annual precipitation for the purpose of the calibration. There were no significant changes in groundwater extraction in the study area between 2005 and 2008, therefore the same transient conditions were assumed during the calibration. Recharge and hydraulic conductivity values were adjusted until a reasonable match was obtained between the heads of the 14 wells and model calculated heads.

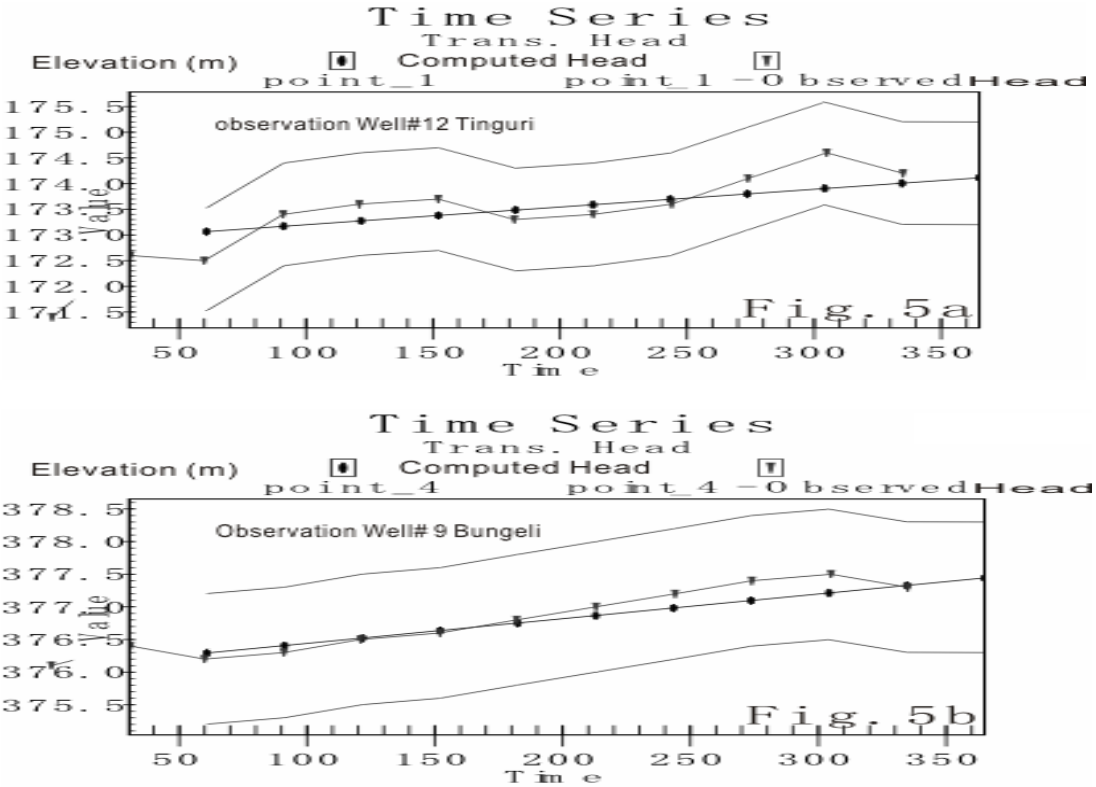


Fig. 5a&5b Fitting graph of time series of groundwater levels from January 2004 to December 2004.

8. Discussions and conclusion

In the transient flow simulation, a reasonable match between the model calculated hydraulic heads and water levels observed in the 14 observation wells after several adjustments in hydraulic conductivity of each layer, and recharge (Fig. 5a and 5b) shows a reasonable fit between the computed heads and the observed heads for two of the 14 monitored wells used in the calibration process. The calibrated simulation model indicates that groundwater generally flow from the northern savanna highlands (high topography) of the model to the northern savanna plains (low topography) recharging the Lake Volta in the process (fig.4). Hydraulic heads generally range between 520m in the recharge areas to 60m at the boundary to the Lake Volta. Well yields in the recharge areas, thus towards the fringe of the model are believed to be lower than those in the discharge areas. This is because in the recharge areas, groundwater flows is directed downwards (Freeze and Cherry; 1979) and upwards flow is limited. However, groundwater flow at discharge areas is directed upwards and flows into wells is comparatively easier at these locations in groundwater flow system.

The pattern of flow and distribution of hydraulic heads in the model domain indicates that groundwater in the aquifers of the basin has great potentials to be developed to meet domestic, industrial and other needs of the area. In other to evaluate and predict the groundwater potentials of the basin, a transient simulations was performed using six stress (management periods), (2006-2012) with ten time steps under each stress period. Each represents a management period. Thirty six hypothetical wells were cited on the basis of the hydraulic head distribution.

Total water demand was computed using the per capita water demand and population increase. A 2.5% annual population increase with its corresponding economic activities were taken into consideration. The projected daily water demand requirements were apportioned to the 36 hypothetical wells to simulate the dynamic response of the hydraulic system to these extraction scenarios. These scenarios of increased population and decreased rainfall show extraction rates still remain relatively low to estimated recharge of the basin. Current extractions, however, may be too small to play a significant role in the regional water balance, though concentrated pumping in local areas may result in water level declines.

There are many reasons why groundwater resources in the area offer the best solution to the water delivery situation. First, the aquifers in the basin have been well investigated and their properties are well known. In addition, groundwater for most part is protected from surface activities and thus cleaner than most surface water sources. Above all, groundwater is much protected from the high temperatures and evaporation conditions and if properly developed and managed, can last the entire year. The proper management of groundwater resources in rural communities requires a good understanding of the dynamics of the resource. It is for this reason that communities in the study area have seen the influx of many governmental and non-governmental organizations with focus on developing groundwater resources for various uses. The government of Ghana intends to investigate the possibility of using groundwater resources in the area for irrigation to supplement rainfed agricultural activities. This would offer all year round employment for the youth and raise the standard of living.

Groundwater flow simulation models have not been extensively used in Ghana to advise management decisions on the allocation of groundwater resources. However, globally, groundwater flow simulation models have thus gained global acceptance as good decision support tools. Numerical modeling of groundwater is an attempt to simplify the physical hydrogeological system using physical equations and governing boundary conditions. The effectiveness of a flow simulation model as management support tool depends among other things on the expert knowledge of the local hydrogeological system, and the boundary conditions. Therefore, groundwater flow modeling procedure begins with a proper understanding of the physical system or problem to be investigated. Once the system or problem is well understood, the next step is to translate it into solvable mathematical equations. This has resulted in the familiar groundwater flow and transport equations in common use today.

Simulation models have been used effectively in such countries as India, China and Pakistan among others, to influence groundwater management paradigms. Don et al. (2006) coupled a numerical simulation model with an optimization model to predict groundwater response to settlement and determine the optimal yield for groundwater without violating physical, environmental and socio-economic constraints in Shiroishi area in the Saga Plain in Japan. This model enabled them to determine the effects of climatic and various pumping scenarios on the aquifer system.

This project presents a description of the groundwater flow pattern in the crystalline basement complex, and the weathered sedimentary rock aquifers of the White Volta Basin, using a three-dimensional flow simulation model, for management purposes.

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Characterization Of The Gene Encoding 28 S Large Subunit Ribosomal RNA Of The Lung Stage Of Schistosoma Mansoni (7-Days Schistosomula)

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ABSTRACT: The parasitic helminth ***Schistosoma mansoni*** is a major public health concern in many developing countries. Over 200 million people have, and another 600 million are at risk of contracting, schistosomiasis, one of the major neglected tropical diseases. For this dangerous disease the development of long - lasting immunity through vaccination may be the real solution to control the spread of the disease. As molecules on the surface or associated with the tegument of Schistosoma ***mansoni*** are a major focus as potential vaccine candidates, but in the present study we screened surface and internal proteins to increase the chances for the discovery of a unique protein of the parasite to be targeted by the immune system of the host and used as a vaccine candidate. In the recurrent study pooled sera collected from ***Schistosoma mansoni*** chronically infected patients was purified over a column made of soluble extract of lung stage (7-days schistosomula) of ***Schistosoma mansoni*** , then , used to immunoscreen λgt11 cDNA library of 7-days schistosomula, a number of cDNA clones were identified after three rounds of immuno-screening and plaques purification. The phage DNAs of the isolated clones were amplified with polymerase chain reaction (PCR) using λgt11 forward and reverse primers, then, cloned in PCR™II plasmid vector. The isolated clone 4-65 was fully sequenced and found to encode the gene of 28S ribosomal RNA of 7-days schistosomula of Schistosoma ***mansoni.*** The 0.9 kb cDNA clone was found to have a single open reading frame (ORF) encoding 269 amino acids exhibited 97% identity to the gene of 28S large subunit ribosomal RNA of Schistosoma ***mansoni*** and a number of eukaryotic species. [The Journal of American Science. 2008;4(4):72-81]. (ISSN: 1545-1003).

Key Words: ***Schistosoma mansoni;*** 7-days schistosomula***;*** immuno-screening

INTRODUCTION

Schistosoma mansoni is a blood-dwelling parasitic worm that causes schistosomiasis in humans throughout Africa and parts of South America. A vaccine would enhance attempts to control and eradicate the disease that currently relies on treatment with a single drug (**Gary *et al.*, 2008**). An effective schistosome vaccine is a desirable control tool but progress towards that goal has been slow (Alan *et al.,* 2006). An effective vaccine would be a useful adjunct and if sufficiently potent, a replacement for chemotherapy but the development of such a product has proved elusive (Alan *et al.,* 2006). The first attempts to develop a schistosome vaccine began half a century ago. By analogy with successful microbial and viral vaccines, they involved the vaccination of mice with crude worm extracts or purified components, followed by a cercarial challenge (Hayunga *et al.,* 1985)**.** The results were equivocal with 20, 30, and even 50% reduction in worm burden recorded, but there was a lack of consistency, even in the same laboratory and it seemed apparent that crude extracts were inadequate vaccines. Perhaps there were a few key antigens that needed to be identified (Alan *et al.,*

2006). So, a particular attention was thus given to identify and characterize sensitive and specific ***Schistosoma mansoni*** antigens to obtain better diagnostic tool and vaccine development (Valili *et al.,* 1990**).** A few defined soluble antigens were separated to show high sensitivity and specificity in endemic areas (Valili *et al.,* 1990).

Schistosomes are truly a formidable adversary that won't easily be beaten. It has been shown that hosts can develop an acquired immunity against challenge infection either after primary infection, immunization with irradiated larvae, or with defined antigens (Smithers and terry 1990; Soisson *et al.,* 1969). The skin and lung stages of the parasite, termed schistosomula, from a challenge infection are the target of these immune responses (Capron *et al.,* 1987**)**.

In the present research we reported the result of a cDNA clone isolated from 7-days schistosomula cDNA library which could be a target for immune attack. DNA, the genetic material, carries the information to specify the amino acid sequences of proteins. It is transcribed into several types of RNA functioning in protein synthesis , including messenger RNA (mRNA) which ultimately direct the process of protein synthesis , transfer RNA (tRNA) form the adaptor that select amino acids and hold them in a place on a ribosome for incorporation into protein and ribosomal RNA (rRNA) which has the dominant role in translation forming the core of ribosomes , determining the overall structure of the ribosome , forming the binding sites for the tRNAs , matching the tRNAs to codons in the mRNA, and providing the peptidyl transferase enzyme activity that links amino acids together during translation (Gesteland *et al.,* 1999).

Approximately 80% of the total RNA in rapidly growing mammalian cells is rRNA. The primary transcripts from most rRNA genes undergo extensive cleavage, processing and modification, so that the mature functional form of rRNAs contained within the precursor are liberated and incorporated into the correct ribosomal subunit (Von 1998; Kressler *et al.,* 1999; Venema *et al.,* 2000). Eukaryotic ribosomes contain four RNAs: 28S, 18S, 5.8S and 5S. The 5S rRNA is transcribed by RNA polymerase III, whereas the three remaining rRNA are transcribed as 45S polycistronic precursor by RNA polymerase I contained within the 45S precursor of the 18S rRNA , the RNA component of the small ribosomal subunit and the 5.8s and 28s rRNA which are the components of the large ribosomal subunit. Many of the processes leading to maturation of rRNA are carried out in the nucleolus by myriad of small nucleolar RNPs, maturation of 18S, 5.8S and 28S rRNA requires the box C/D small nucleolar RNPs, the box H/ACA small nucleolar RNPs and RNAse mitochondrial RNA processing (Watkins *et al.,* 2000). To meet the need for transcription of large numbers of rRNA molecules, all cells contain multiple copies of rRNA genes. The human genome for example, contains about 200 copies of the gene that encodes the 5.8S, 18S and 28S rRNAs and approximately 2000 copies of the gene that encodes 5S rRNA. The genes for 5.8S , 18S and 28S rRNAs are clustered in tandem arrays on five different human chromosomes (chromosomes 13 , 14 , 15 , 21 and 22), the 5S rRNA genes are present in a single tandem array on chromosome 1 (Gesteland *et al.,* 1999). The major goal of the present study is the identification of some cDNA clones from the lung stage of ***Schistosoma mansoni*** and the study of their vaccine potential in the future for finding out the possibility of using one/or all as vaccine candidates against schistosomiasis.

MATERIALS AND METHODS:

Soluble extract of 7- days schistosomula.

Schistosomula mansoni NMRI strain was maintained in the laboratory of Theodore Bilharz Researsh Institute using Biomphalaria glabrata snails, cercariae were obtained from infected animals (Fletcher *et al.,* 1981). Schistosomula were obtained by mechanical transforming cercariae where cercarial bodies were separated from tails by centrifugation 2000 rpm for 15 min over 70% percoll gradient (Lazdins *et al.,* 1982). Cercarial bodies were recovered from tube bottom and washed three times with Minimum Essential Medium (MEM) containing 10% fetal calf protein, then, incubated in Modified MEM at 37^0C in a humidified 5% CO_2 incubator for 7 days. Finally, the medium was collected and living schistosomula were separated by centrifugation at 2000 rpm for 15 min over 60% percoll gradient (Besch 1981**)**. The soluble extract was made by sonication of the parasites in a buffer containing 20 mM Tris, pH 7.2 and 2 mM phenyl methyl sulphonyl fluoride (PMSF) , then , centrifuged at 6000 rpm for 20 min. The supernatant was removed and stored at -70^{o}C.

Affinity purification of sera:

Sera used in immunoscreening experiment were pooled from schistosomiasis chronically infected patients admitted to Department of Tropical Medicine, Zagazig University Hospitals. Cyanogen bromide – activated Sepharose 4B was used to purify sera according to instructions of manufacturer by coupling 6-8 mg of 7- days' schistosomula soluble extract to the column. Pooled sera were precipitated with 45% ($(NH_4)_2SO_4$, the precipitate was re-dissolved in phosphate buffered saline (PBS) (0.4 g NaCl, 1.44g Na_2HPO_4 and 0.24 g KH_2PO_4/L) and dialyzed against PBS overnight. The dialysate was, then, passed onto the column containing the NP-40 schistosomular extract. The flow through from the column was collected and tested using ELISA for reactivity to the extracted proteins. The column was washed with 30 ml PBS. Antibodies bound to the column were eluted by 0.1 M glycine–HCl, pH 2.6 and collected as 1 ml fractions. The pH of the elute was immediately adjusted to 7.0 with 100 µl 1M Tris-base., then, they were dialyzed against PBS over night to be ready for immunoscrening.

Screening of 7-days schistosomula λ gtll cDNA Library with antibody (Huynh *et al.*, 1985):

To grow cells for transfect ion with schistosomula library, a single colony of ***E.Coli Y1090*** was incubated in 50 ml LB-ampicillin medium (LB-amp) (10 g Bacto-tryptone, 5 g Bacto-yeast extract, 10 g NaCl, and distilled H_2O up to 1L, pH 7.0) containing 0.2% maltose and ampicillin 100 mg/ml allowed to grow overnight with good aeration at 37^{o}C, to used as hosts for plating the library. For the primary screening of the library, 150 mm LB-amp plates were used and 90 mm plates were used for secondary and tertiary screenings. An overnight bacterial culture, about 0.6 ml for each large plate and 0.2 ml for the small one , was incubated with 0.1 ml of SM medium (5.8 g NaCl, 2.0 g $MgSO_4$.7 H_2O, 50 ml 1M Tris ; pH 7.5 and 5 ml of 2% gelatin solution/ L). The cell suspension was incubated at 37^{o}C for 15 min to allow the adsorption of the phage to the bacterial cells. Molten top agar, cooled to 50^{o}C was added to the infected cells, 7 ml / large plate and 3.5 ml / small plate are poured onto the LB-amp plates pre-warmed to 37^{o}C, then, and the plates were incubated at 40^{o}C for 3-4 hours (hrs). Dry nitrocellulose (132 mm and 82 mm) circular filters were used for large and small plates, respectively. The filters were saturated in 10 mM IPTG and air dried, then, placed onto the plates. The plates were transferred to a 37^{o}C incubator for another 3 hrs. then, removed from the plates and transferred to the Blotto buffer (non fat dry milk 5g in 100 ml PBS-0.05% Tween-20) to block the non-specific binding protein sites and shook at room temperature for 30 min. The filters were then washed 3 times in TBST (37.5 ml 4M NaCl, 10 ml 1M Tris; pH 8.0, double distilled H_2O up to 1L and 0.05% Tween-20) for 10 min each, followed by incubation for 3 hrs with primary antibody (the purified sera over schistosomula soluble extract column), then, washed 4 times at room temperature in TBST for 20 min each. The anti-rabbit IgG alkaline phosphatase conjugate, diluted in TBST according to the data sheet, was used to bind the primary antibody-antigen complex. Following 1 hr incubation at room temperature in the secondary antibody, the filters were washed 4 times in TBST as before for 10 min each, dried and transferred to the color development substrate solution [33 µl of 50 mg/ml Nitro Blue Tetrazolium (NBT) + 16.5 µl of 50 mg/ml BCIP per ml AP buffer (10 ml of 1 M Tris; pH 9.5, 2 ml of 5 M NaCl 0.5 ml of 1 M MgCl, distilled H_2O up to 100 ml)]. The filters were incubated in dark until the desired color intensity had been developed, then, rinsed in distilled water. The developed filters were used to pick up agar plugs containing phage particles corresponding to the signals on the filters (the positive plaques) to be suspended into 0.5 ml of SM medium (5.8 g NaCl, 2 g $MgSo_4$.7 H_2O, 50 ml 1 m Tris, pH 7.5, 5 ml 2% gelatin solution and distilled H_2O up to 1 L) and placed on a shaker for 1 hr at 37^{o}C. The purified phage plaques were used for the next round of screening.

Polymerase Chain Reaction (PCR) (Saiki *et al.*, 1988):

The isolated phage DNA from plaques was amplified using a pair of primers, λ gt11 forward (5$^/$-GGTGGCCACGACTCCTGGAG GCGG-3$^/$) and λ gt11 reverse (5$^/$-TTG AC AC CAGACCAACTGGTAATC-3$^/$). Taq DNA polymerase (Perkin-Elmer Cetus and Stratagene) was used in this reaction to synthesize the new strands generated by that process. A typical PCR reaction mix (100 µl reaction) was prepared (10 µl 10 X Taq DNA polymerase buffer, 16 µl of 1.25 mM dNTP, 5 µl forward primer, 5 µl reverse primer, 2 µl (100 ng) phage DNA template, 0.5 µl Taq DNA polymerase, sterile distilled H_2O up to 100 µl) .The reaction components were mixed in 0.5 ml microfuge and a drop of mineral oil was added on top of the reaction mix. The samples were amplified using a programmable thermal cycler Gene Amp 9600, Perkin-Elmer, using a 3-file program. Samples were denatured in

the first file at 94°C for 1 min, then, the primers were annulled to the denatured templates at 55°C for 2 min and finally extended at 72°C for 10 min. The samples were maintained at 4°C. The amplification products (amplicons) were withdrawn from underneath the oil and 10 µl aliquots were separated on 1% agarose gel.

Subcloning of the recombinant gene in PCR™II plasmid vector (Maniatis *et al.*, 1982):

The original TA cloning Kit (Invitrogen) was used for direct insertion of the amplicon into PCR™II vector at EcoR1 site. A typical ligation reaction was prepared as follows (1 µl PCR product , 1 µl of 10X ligation buffer, 2 µl plasmid vector, sterile H_2O up to 9 µl , 1 µl DNA ligase). The ligation reaction was incubated overnight at 15°C till ready for transformation. The readymade INV competent cells of the original TA cloning kit were used. The vial containing the ligation reaction was spun down briefly and placed on ice. Two µL of 0.5M β- mercaptoethanol (β-ME) and 2 µl ligation reaction were pipetted into each vial of the competent cells and mixed by gentle stirring with the pipette tip , then, the vial was incubated on ice for 30 min , and exactly 30 sec in 42°C water bath. The vial was removed from the water bath and placed on ice for 2 min 450 µl of SOC medium were added to the vial which was shaked at 37°C for 1hr . Aliquot of 50 µl was spread onto LB-amp plate and the plate was placed inverted at 37°C for at least 18 hrs finally the plate was shifted to 40°C for 2-3 hrs for the proper color development. Positive transformants can be selected by using Cracking gel procedure, where the non-recombinant transformants migrate faster than the recombinant ones when checked by 1% agarose gel electrophoresis.

Small scale preparation of plasmid DNA (Sambrook *et al.*, 1989):

A single bacterial colony that contains the desired plasmid was used to inoculate 100 ml of LB-amp medium incubated at 37°C with vigorous shaking overnight (O/N). The bacterial cells were harvested by centrifugation at 10000 rpm for 10 min. The cells were lysed using solution I (50 mM glucose, 25 mM Tris HCl, pH 8, 10 mM EDTA, pH 8), freshly prepared lysozyme was added , then , followed by solution II [0.2 M NaOH , 1% sodium dodecyl sulphate (SDS)] , the suspension was incubated at room temperature (RT) for 10 min. 20 ml of solution III was added (3 M potassium acetate, 2 M glacial acetic acid). DNA can be recovered by adding equal volume of isopropanol and precipitated by centrifugation at 10000 rpm for 10 min at (RT). The pelleted DNA was dissolved in 100 µl distilled H_2O to which RNase (10 mg/ml) was added, then, left for incubation at 37°C for 2 hrs. The DNA solution was, extracted with phenol-chisam, then, precipitated by ethanol 2.5 volumes and 0.1 volume of 3 M sodium acetate and dissolved in 50 µl distilled H_2O. The plasmid DNA was quantitated by determining the $\mathbf{O.D_{260}}$, then, stored at – 20°C.

DNA sequencing using fmol DNA (Promega) Sequencing System (Moran *et al.*, 1990):

In four microfuge (0.5 ml) labeled (G, A, T, C), 2 µl of the appropriate d/dd NTPs. Then, to the 4 tubes, 1µg DNA template, 25 µg primer (M13 at 5′-end and T7 a 3′-end) 1µl of α-^{35}S, 5 µl sequencing buffer and dd H_2O up to 16 µl, then, to each tube 1µl of sequencing grade Taq DNA polymerare was added to the template/primer mix. The reaction was placed in a thermal cycler. The profile used in the reaction was 2 min at 95°C, 30 sec at 90°C ,then, 1 min at 70°C for 30 cycle, then, the reaction was stopped by adding 3 µl stop solution to each tube. 3ul of each tube were loaded on a sequencing gel. After the electrophoresis, the gel was fixed, dried by heating, then, exposed to an X-ray film for 24 hrs at –70°C. The film was developed and read from bottom. The informations obtained from DNA sequence were analyzed using the Genetics Computer Group Sequence analysis Software package.

RESULTS

Sera obtained from ***Schistosama mansoni*** chronically infected patients was purified over an antigen column made from soluble extract polyadenylation signals AAATAA , AATTA and ATAA located +114 , +22 and +6 bp, of 7-days schistosomula coupled to Sepharose-4B beads. The affinity purified eluted antibodies were, then, used to immunoscreen 7-days schistosomula λgt11 cDNA library. One of the isolated cDNA clones (clone 4-65) which was identified by affinity purified antibodies obtained from serum of the chronically infected patients contained a 0.9 kb insert. The full DNA sequence of the insert identified a single open reading frame (ORF) of 269 amino acids (aa) that showed high identity with the 28 rRNA gene of the large ribosomal subunit from a number

of eukaryotic species (the % of homology was 97 , 97, 92, 93 , 93 , 94 , 94 , 93 , 94 and 93 to gene for 28S rRNA of *Schistosama mansoni, Schistosama rodhaina*, *Schistosama spindale, Schistosama hippopotami, Schistosama edwariense, Schistosama margrebowiei, Schistosama incognitum, Schistosama intercalatem Schistosama mattheei* and *Schistosama hematobium,* respectively . The 0.9 clone was completely sequenced in both directions after being inserted into a plasmid vector (PCRTMII); it did not contain the entire coding region. The 5/- upstream region in the sequence we had gut considering the first initiation codon (ATG) is located -201 bp from the beginning of this region without any transcription activation TATA and CAAT boxes. There are three putative, respectively from the 3/-downstream region, there is no polyadenylation site (poly A tail) (Fig. 1). The original TA cloning Kit (Invitrogen) was used to provide a quick, one-step cloning strategy for direct insertion of the PCR products into a plasmid vector (PCR™II vector) at EcoR1 site. Some of the isolated clones were checked for size after being inserted in the desired plasmid vector using two restriction enzymes EcoR1 and BamH1 (Fig. 2), which showed no BamH1 site in the insert , while the plasmid DNA was digested by EcoR1 giving the actual size of each insert . The cloned insert was sequenced using two oligonucleotides (primers), M13 from the 5/- end and T7 from the 3/- end followed by another two pairs of primers till reaching the overlapping region for completing the sequence of the isolated clone, each sequence gel was exposed to an X- ray film for 24 hrs, then, developed and read from the bottom of the autoradiogram (Fig.3).

```
1   CCGAAGUCUAAGCACGCAUGCAAGCGCCAUCUCCGACACACCGCACAGAUCCACUACAGC 60
      P  K  S  K  H  A  C  K  R  H  L  R  H  T  A  Q  I  H  Y  S
 61 CAGUUAGUAACACUCACGGCCCAUAAACUGAGCCAAAGAGCCACCUACCGACCCAACCAA 120
      Q  L  V  T  L  T  A  H  K  L  S  Q  R  A  T  Y  R  P  N  Q
121 GACCGACAGAAAUCAGACCUGAUCAGUUUGACCAUAGCAGACAGGCAGCAGUCGUCGUGG 180
      D  R  Q  K  S  D  L  I  S  L  T  I  A  D  R  Q  Q  S  S  W
181 UGCACACCUGAGAAAGUGCACAUGACAAGCAGACCCUCACACCAACAGUGCGCGCGUCGU 240
      C  T  P  E  K  V  H  M  T  S  R  P  S  H  Q  Q  C  A  R  R
241 AAGCAAACGAUUCACACAUACCACCCAAAAAGGAAGGCACCUGCGGUUAAACAGACACUG 300
      K  Q  T  I  H  T  Y  H  P  K  R  K  A  P  A  V  K  Q  T  L
301 AAGGCGGCCAGUAUGCCCAAGUACAAAUCACACUCAUCAGCUGAACUCCCAGAGCUUGCA 360
      K  A  A  S  M  P  K  Y  K  S  H  S  S  A  E  L  P  E  L  A
361 GUUCAACUCCACCCGUUUACCUUUGAGCGGUUUCACGCACUGUUUACUCUCUCUUCAAAG 420
      V  Q  L  H  P  F  T  F  E  R  F  H  A  L  F  T  L  S  S  K
421 UACUUUUCAACUUUCCCUCACGGUACUUGUUUGCUAUCGGACUCGUGUAAGUAUUUAGCC 480
      Y  F  S  T  F  P  H  G  T  C  L  L  S  D  S  C  K  Y  L  A
481 UUGGAUGGAGUUUACCACCCACUUUGGGCUGCAUUCACAAACAACCCGACUCCAAGGGUA 540
      L  D  G  V  Y  H  P  L  W  A  A  F  T  N  N  P  T  P  R  V
541 GCUCAGAGCAAAACUGUCACACUUGAUCUCUGCCCCCACGGGCCUUUCACCCUCUUUGGG 600
      A  Q  S  K  T  V  T  L  D  L  C  P  H  G  P  F  T  L  F  G
601 CCAGGAUGGGAAGCCGUACUCAUUGCUGGACUUGGGACAGAGCAGGUAAUGCCUGAAGCC 660
      P  G  W  E  A  V  L  I  A  G  L  G  T  E  Q  V  M  P  E  A
661 ACCCUAAACACCACAUUGCUUUACGAUCAAAUAACGGCAGGCUUCGGUGUUGGGCUAAUC 720
      T  L  N  T  T  L  L  Y  D  Q  I  T  A  G  F  G  V  G  L  I
721 CCUGUUCACUCGCAGUUACUAGGGGAAUCCUUGUUAGUUUCUUUUCCUCCCCUGAGUAUA 780
      P  V  H  S  Q  L  L  G  E  S  L  L  V  S  F  P  P  L  S  I
781 UGCUUAAGUUCAGCGGGUAAUCACGCCUGAUCGAGGUCGGGGUCAAUUAAAUAAUUCGUG 840
      C  L  S  S  A  G  N  H  A  *  S  R  S  G  S  I  K  *  F  V
841 AUCAUACACACACAAUCGGUACAAACCAUAGACCAAACCAGAGACAAGAUCAAGUGAUUA 900
      I  I  H  T  Q  S  V  Q  T  I  D  Q  T  R  D  K  I  K  *  L
901 ACGUAGCAUACGAUAGGUGCGAAUUAUCCCGAGGAUGUAUAAUGUCAG 948
      T  *  H  T  I  G  A  N  Y  P  E  D  V  *  C  Q
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Fig. 1: The Complete nucleotide sequence and deduced amino acids sequence of the gene encoding 28S large subunit rRNA isolated from λ g11 cDNA library of 7-days schistosomula, start codon (ATG), stop codon (TGA), three polyadenylation signals (AAATAA), (AATTA) and (ATAA) are underlined.

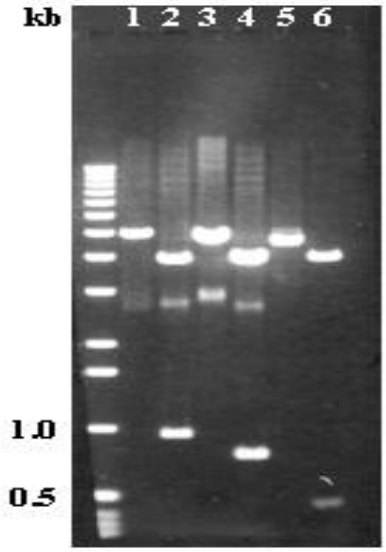

Fig. 2: 1% agarose gel showing the digestion pattern of three isolated inserts from λgt11 cDNA library of 7-day schistosomula, cloned in PCR^{TM}II plasmid vector , digested by two restriction enzymes EcoR1 and BamH1, the plasmid DNA samples were arranged in double, each represents from left to right, EcoR1 digested and BamH1 digested DNA. 1kb ladder was indicated on the left side of the gel. The selected clone (4-65) was run in lanes 1 and 2, the arrow points at the size of the insert (0.9 kb).

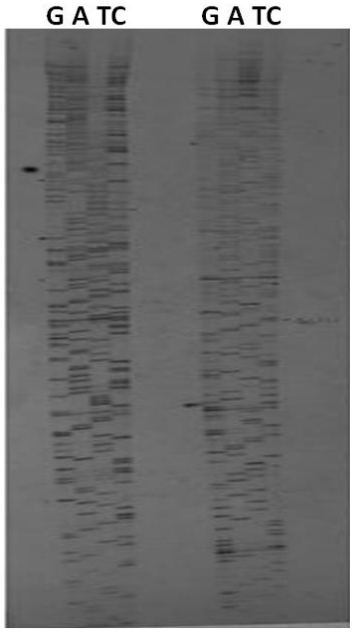

Fig 3: An autoradiogram showing sequence of the gene encoding 28S large subunit rRNA isolated from λ gt 11 cDNA library of 7-days schistosomula cloned in PCR^{TM}II plasmid vector from 5$^{/}$- and 3$^{/-}$ends using M13 and T7 primers.

DISCUSSION:

Over 200 million people have and another 600 million are at risk of contracting schistosomiasis. Transmission of infection which is caused by helminth parasites of genus ***schistosoma*** depends upon release of eggs from the human host (Tori *et al.,* 2007) .The recent studies on schistosomiasis have focused on identification and characterization of defined antigens that may have vaccine and/ or diagnostic potential. The development of vaccine against schistosomiasis would provide a powerful tool for the control of this important parasitic disease and it must be effective which should be confirmed by protection test (Thomas *et al.,* 2006). Vaccine development for complex parasite as *Schistosoma mansoni* is a great challenge. Given the persistant lack of an effective immunogen. Several vaccine strategies have been tried such as the use of synthetic peptides **(Fonseca *et al.,* 2004).** The tegument assoicated with certain antigens which are likely to be involved in important host-parasite interactions and may be important candidates for vaccine development.These antigens expressed on newly transformed and developing schistosomules have been shown to be protective (Miller *et al.,* 1989; Capron M and Capron D , 1986). In the recurrent study , we did not work on identification , characterization and study the vaccine potential of of tegumental antigens of 7-days schistosomula but, we tried to increase the possiblity for identifying new antigens (tegumental and/or internal) which could be vaccine candidates against schistosomiasis , this is why we did not apply the technique of extracting surface proteins , but we did sonicate all the parasite to be able immunoscreen most of the proteins f the parasite either those proteins are surface or internal. After three rounds of immunoscreening of λgt 11 cDNA library of 7-days schistosomula by affinity purified antibodies obtained from serum of the schistosomiasis chronically infected patients, a number of cDNA clones were isolated, one of them (clone 4-65) was amplified by PCR using λgt 11 forward and reverse primers , then , cloned in the EcoR1 site of PCR^{TM}II plasmid vector. The size of the selected clone was shown to be 0.9 kb by checking the pattern of restriction endonuclease digestion using two enzymes EcoR1 and BamH1, then , the result of digestion by the two enzymes was run on 1% agarose gel, the digestion by EcoR1 proved the size of the insert. The DNA sequence revealed that it did not contain the entire coding region which appears clear in both 5$^{/}$-upstream and 3$^{/}$-downstream regions, in the upstream region no transcription activation boxes (TATA and CAAT) can be found , the intiation codon (ATG) is located at -201 bp from the 5$^{/}$-upstream region. The stop codon is located +138 bp , there are three putative three polyadenylation signals (AAATAA), (AATTA) and (ATAA) located + 114 , +22 and +6 bp, respectively from the 3$^{/}$-downstream region , there is no polyadenylation site.

The DNA sequence using fmol sequencing system showed high identity with the 28 rRNA gene of the large ribosomal subunit from a number of eukaryotic species (the % of homology was 97 , 97, 92, 93 , 93 , 94 , 94 , 93 , 94 and 93 to gene for 28S rRNA of *Schistosama mansoni, Schistosama rodhaina*, *Schistosama spindale, Schistosama hippopotami, Schistosama edwariense, Schistosama margrebowiei, Schistosama incognitum, Schistosama intercalatem Schistosama mattheei* and *Schistosama hematobium,* respectively , and with other eukaryotes. The genes for 18S, 5.8S and 28S ribosomal RNA first sequenced and characterized by Ellis *et al.,* (1986) are found in a large tandem repeat of 100-150 copies on the right end of chromosome 1, each repeat contains one copy each of 18S , 5.8S and 28S genes (*C. Elegans* Sequencing Consortium, 1998). 18S, 5.8S and 25-28S ribosomal RNAs are transcribed in nucleolus of eukaryotic cells by RNA polymerase I in the form of a long precursor rRNA (pre-rRNA) that subsequently undergoes a number of processing cleavages to remove the external transcribed spacer sequences (EST) internal transcribed spacer sequences (ITS) and release the mature rRNAs involving a number of small nucleolar RNAs (Sno RNAs) (Borovjagin and Gerbi 1999).

In eukaryotes, nucleolus is the centre of ribosomes biogenesis, important proteins associates with rRNA and the mature ribosomal subunits are, then, exported to cytoplasm (Hughes *et al.,* 1991)**.**

Cloning and sequencing of DNA encoding pre-rRNA from many species showed that this DNA shares several properties in all eukaryotes. First, the pre- rRNA genes are arranged in long tandem arrays separated by non transcribed spacer regions ranging in length from 2 kb in frog to 30 kb in humans. Second, the genomic regions corresponding to the three finished rRNAs are always arranged in the same 5$^{/}$---3$^{/}$ order: 18S, 5.8S and 28S. Third, in all eukaryotic cells (and even in bacteria), the pre-rRNA gene as well as the primary transcript, is considerably

longer than the sum of the three finished rRNA molecules (Venema and Tollervey 1995). Once again new technologies and information open up the prospect of progress towards the elusive goal of an effective schistosome vaccine. Access to the genome, transcriptome and proteome provide a fantastic opportunity to search for new vaccine candidates. However, we should always bear in mind that schistosomes are not stupid. They have had tens of millions of years to evolve mechanisms that help them survive immune attack from the mammalian host, even an attack orchestrated by our vaccine strategies. The next step in this research is to apply technique of DNA hybridization using the isolated clone as a probe to screen genomic library of *Schistosama mansoni* for picking up the full length gene of 28S rRNA of the large ribosomal subunit, then, expressing it in an expression vector to obtain its protein product and to study its vaccine potential.

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MAASTRO Lab is a basic and translational research laboratory embedded within the GROW research institute of the Faculty of Health, Medicine and Life Sciences at Maastricht University. Research carried out in the past has been focused on the tumour microenvironment and EGFR signalling pathways, both of relevance to radiation oncology. MAASTRO Lab has made several important discoveries in these fields, including demonstration that EGFR is up regulated by radiation and that hypoxia inhibits the initiation step of mRNA translation. In addition, we have initiated translational and clinical studies based on these results including both phase I novel treatment and molecular imaging trials as well as a Biobank project with more then 1500 patients included.

The lab has 4 permanent scientists, 5 technicians, more then 5 PhD students and is fully equipped for cell culture, molecular biology, flow cytometry, hypoxia, gene expression, proteomics and microscopy. Maastro lab has set up the necessary infrastructure for controlled exposures to hypoxia and hypoxia/reoxygenation, including development of novel equipment that allows rapid and precise changes in oxygenation. Access to expertise, equipment and resources within the much larger GROW research institute and other facilities in the University are also readily available, including the genome centre, advanced microscopy, and the animal facility with its imaging facility (Optical imager, MRI 7Tesla and micro CTPET to come). MAASTRO has a structural collaboration with the VU in Amsterdam on molecular PET biomarkers, with the TU/Eindhoven on Systems Biology and is initiating a new collaboration with the University of Toronto on research related to the Unfolded Protein Response and tumour hypoxia.

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