Journal of Parasitology and Vector Biology

Volume 5 Number 7 July 2013
ISSN 2141-2510

Academic Journals

ABOUT JPVB

The **Journal of Parasitology and Vector Biology (JPVB)** is published monthly (one volume per year) by Academic Journals.

Journal of Parasitology and Vector Biology (JPVB) provides rapid publication (monthly) of articles in all areas of the subject such as Parasitism, Helminthology, Cloning vector, retroviral integration, Genetic markers etc.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jpvb@academicjournals.org.
A manuscript number will be mailed to the corresponding author shortly after submission.

For all other correspondence that cannot be sent by e-mail, please contact the editorial office (at jpvb@academicjournals.org.

The Journal of Parasitology and Vector Biology will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Editorial Board

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JPVB to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

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

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

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

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.
Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Parasitology and Vector Biology is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Parasitology and Vector Biology

Table of Content: Volume 5 Number 7 July 2013

ARTICLES

Urinary schistosomiasis among pre-school and school aged children in two peri-urban communities in Southwest Nigeria 96
Babatunde, T. A, Asaolu, S. O and Sowemimo, O. A.

Entomological baseline data on malaria transmission and susceptibility of Anopheles gambiae to insecticides in preparation for Indoor Residual Spraying 102
Rock Aïkpon, Razaki Ossè, Renaud Govoetchan, Arthur Sovi, Frédéric Oké-Agbo And Martin C. Akogbéto

academicJournals

Vol. 5(7), pp. 96-101, July, 2013
DOI: 10.5897/JPVB 2013. 0113
ISSN 2141-2510 © 2013 Academic Journals
http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Urinary schistosomiasis among pre-school and school aged children in two peri-urban communities in Southwest Nigeria

Babatunde, T. A., Asaolu, S. O. and Sowemimo, O. A*.

Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 21 May, 2013

A cross-sectional study was conducted between March and April, 2010 among pre-school and school aged children in two peri-urban communities in Osun State, Southwest Nigeria. Urine samples were collected from the pre-school and school aged children, tested for microhaematuria using reagent strips, processed and examined for *Schistosoma haematobium* ova. Out of 274 pupils examined, 132 (48.2%) had infection, with no statistically significant difference (P > 0.05) in infection between male (48.6%) and female pupils (47.6%). The prevalence of infection increase significantly with age (P < 0.05), with the peak (93.3%) of infection recorded in pupils aged 15 to 16 years and the lowest infection (10.0%) in pupils aged 3 to 4 years. There was no statistically significant association (P > 0.05) between intensity in male pupils (156.0 ± 34.5/10 ml) and female pupils (141.7 ± 29.5/10 ml). The prevalence of pupils with microhaematuria was 65.0% and it increased significantly with age (P < 0.001). The conclusion drawn from the study is that to reduce the transmission of *S. haematobium* in endemic communities, health education and provision of potable water are advocated.

Key words: *Schistosoma haematobium*, microhaeamturia, prevalence, urinary schistosomiasis, school-aged children, Nigeria.

INTRODUCTION

Schistosomiasis remains an important public health problem globally, with approximately 779 million estimated to be at risk (Hotez, 2009). Within sub-Saharan Africa, Nigeria is the country with the highest prevalence of human schistosomiasis; about 29 million in 2008 (Hotez and Kamath, 2009). Urinary schistosomiasis is a human disease condition which is caused by infection of the trematode *Schistosoma haematobium*. The parasite is found in the venous plexus draining the urinary bladder of humans (World Health Organization (WHO), 2002). During infection, the parasites deposit terminal spined eggs which clog the venous plexus, impeding blood flow. The eggs of *S. haematobium* provoke granulomatous inflammation, ulceration and pseudo-polyposis of the vesical and ureteral wall. Haematuria is a very common sign of infection but other signs include dysuria, pollakisuria and proteinura. Kidney failure deaths due to urinary tract scarring, deformity of ureters and the bladder caused by *S. haematobium* infection have become less common due to the use of effective drug praziquantel (Gryseels et al., 2006). In Nigeria, urinary schistosomiasis is widespread in both rural and urban communities;

*Corresponding author. E-mail: yomi_showemimo@yahoo.com, osowemimo@oauife.edu.ng. Tel: +2348034425965.

with prevalences ranging between 2 and 90% and the vast majority of cases occurring among the poor and marginalized (Ugbomoiko, 2000; Mafiana et al., 2003; Oladejo and Ofoezie, 2006; Opara et al., 2007). Several studies on schistosomiasis in Nigeria have focused on school-aged children and adults, with little or no information on pre-school children. Recently, urinary schistosomiasis infections have been reported in pre-school children in settlements in Ogun State and Cross River State, all in Nigeria (Opara et al., 2007; Ekpo et al., 2010). However, information on the endemicity of this disease is still lacking in some parts of the country and data collected is still grossly inadequate for planning a credible control programme.

The study was planned with the aim of providing epidemiological data on the status of schistosomiasis infection among pre-school and school aged children of Akinlalu and Ogbagba communities of Osun State Southwest Nigeria. It is hoped that the results to be generated will complement the existing baseline information on the epidemiology of this infection in the country.

MATERIALS AND METHODS

Study area

This cross-sectional study was carried out in the peri-urban/rural communities Akinlalu and Ogbagba both situated in Osun State in Nigeria. Akinlalu (07° 28´N, 04° 15´E) is situated in Ife North Local government Area and Ogbagba (07° 42´N, 04° 15´E) is situated in Ife East Local Government Area both in Osun State. The two communities are in the rainforest belt of Nigeria and the annual rainfall in the region ranges from 1,000 to 4,000 mm. The average maximum and minimum daily temperatures are 32 and 20°C, respectively (Asaolu et al., 1991). The climate is tropical with distinct dry (November to March) and rainy (April to October) seasons (Asaolu et al., 1991). The ecological characteristics of, as well as the socio-cultural and daily economic activities in both communities are similar. Farming and petty trading are the main occupations, and most residents are of Yoruba ethnicity. Neither of the communities has reliable pipe-borne water supply. However, both communities have shared community wells and rivers which are frequently visited for domestic use as well as leisure and religious beliefs.

Study population and design

A school-based cross-sectional study was conducted between March and April, 2010. The study samples were taken by random sampling from pre-school and school age children population attending Surajudeen Primary School, Akinlalu and Sacred Heart Catholic Mission School, Ogbagba Osun State, Nigeria. For schistosomiasis control, WHO recommends that 200 to 250 school aged children should be sampled in each ecological zone (Montresor et al., 1998). A total of 51 pupils from Ogbagba community and 223 pupils from Akinlalu, making a total of 274 children took part in the study. The permission of the community leaders, local government and school authority was obtained before the commencement of the study. In addition, the consent of the parents of the children was also obtained. The study protocol was approved by the Ethical Committee of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC).

Data collection

Each child was provided with a pre-labelled, wide-mouthed, screw capped container for the collection of a mid-day urine sample. Each urine sample was divided into two portions: a portion of the urine sample was tested for microhaematuria using commercial reagent strips (Medi-test Combur-9; Analyticon Biotechnologies, Lichtenfels, Germany) in accordance with the manufacturers' instructions. The other portion of the urine samples were transported to the parasitology laboratory at the Obafemi Awolowo University, Ile-Ife's Department of Zoology and preserved by adding two drops of formaldehyde. A pretested structured questionnaire was used to collect demographic and socio-economic data.

Laboratory analysis

A 10 ml of the preserved urine samples were passed through a 8 µm pore membrane filter, so that any *S. haematobium* eggs could be trapped on the filter and counted under a light microscope and recorded as eggs/10 ml of urine (WHO, 1983).

Statistical analysis

Version 16.0 of the statistical package for social sciences (SPSS) for windows software package (SPSS Inc, Chicago, IL) was used for all the data analysis, comparisons of prevalence by subject age and gender was made using χ^2 tests. Differences in mean egg counts between dichotomous variables and variables with more than two levels were explored using Student's t-tests and one-way analysis of variance (ANOVA), respectively.

RESULTS

A total of 274 pre-school and school aged children were enrolled for this study and they all submitted urine samples. The age of the children ranged from 3 to 16 years, with a mean (standard deviation, SD) of 9.0 (3.4) years and a median of 9 years. 148 (54.0%) of them were boys while 126 (46.0%) were girls.

Prevalence and intensity of *S. haematobium* infection by age and sex

The overall prevalence of *S. haematobium* infection was 48.2%. The prevalence pattern showed an increasing trend with increasing age, and peaked around 15 to 16 years. The lowest prevalence of 10.0% was recorded among the pre-school children aged 3 to 4 years. There was a significant difference in prevalence of infection between ages ($\chi^2 = 59.085$, df = 6; P = 0.000). However, there was no statistically significant difference ($\chi^2 = 0.029$, df = 1; P = 0.865) in prevalence between the boys 72

Table 1. Prevalence and intensity of *S. haematobium* among the children in the two communities.

Age (years)	No. Examined	No. Infected	Percentage (%)	Mean ± (SEM)
3-4	30	3	10.0	4.13±2.46
5-6	42	13	31.0	183.64±74.36
7-8	57	17	29.8	126.70±54.46
9-10	55	33	60.0	158.93±54.59
11-12	40	27	67.5	215.65±66.79
13-14	35	25	71.4	191.66±55.00
15-16	15	14	93.3	120.20±31.69
Total	274	132	48.2	149.41±23.00
P-value		<0.05		<0.05
Sex				
Male	148	72	48.6	156.00±34.50
Female	126	60	47.6	141.73±29.51
P-value			>0.05	>0.05

SEM : Standard error of mean.

(48.6%) and girls 60 (47.6%) (Table 1). The overall mean intensity of infection was 149.41 ± 23.00 eggs/10 ml. Pre-school children aged 3 to 4 years had the lowest intensity of 4.28 eggs/10 ml while the highest mean intensity of 221.8 eggs/10 ml was recorded in pupils aged 11 to 12 years. There was a significant difference between age and intensity of infection ($P < 0.001$). The male pupils have a higher intensity (156.00 ± 34.50 eggs/10 ml) than the female pupils (141.73 ± 29.51 eggs/10 ml), however there was no statistically significant difference between age and intensity of infection ($P > 0.05$) (Table 1).

Micro-haematuria and visible haematuria

Micro-haematuria was detected in 178 (65.0%) of the 274 urine samples, out of which 47(92.2%) were from Ogbagba community while 131 (58.7%) were from Akinlalu. Out of the 178 with micro-haematuria, 86 (31.4%) had gross haematuria, 48 (17.5%) moderate haematuria and 44 (16.4%) mild haematuria. The prevalence of microhaematuria by age showed that the lowest prevalence of 13.3% was recorded in pupils' age 3 to 4 years while the highest of 100% was recorded in the pupils' age 15 to 16 years (Table 2). The prevalence of female pupils 82 (65.1%) with microhaematuria was higher than that of male pupils 96 (64.9%) but there was no statistically significant difference in the prevalence of micro heamaturia between the sexes ($P > 0.05$) (Table 2). Visible haematuria was observed in 44 (16.1%) of the 274 urine samples, out of which 9 (17.6%) pupils from Ogbagba community showed visible haematuria while 35 (15.7%) pupils were from Akinlalu. Male pupils had a higher prevalence (21.6%) of visible haematuria than female pupils (9.5%). There was a significant difference between the prevalence of visible haematuria between the two sexes ($\chi^2 = 7.30$, df = 1, $P = 0.007$). The lowest prevalence (6.7%) of visible haematuria was recorded in pupils aged 3 to 4 years while the highest prevalence (46.7%) was recorded in pupils aged 15 to 16 years. There was a significant differences between the prevalence of visible haematuria among the various age groups ($\chi^2 = 14.804$, df = 6, $P = 0.022$) (Table 3).

DISCUSSION

This study showed that urinary schistosomiasis is endemic in some communities in Osun State and it corroborates with findings of some earlier studies in this geographical region (Oladejo and Ofoezie, 2006; Ugbomoiko et al., 2010). The overall prevalence of urinary schistosomiasis recorded (48.2%) in this study is about four times higher than the National Nigerian mean of about 13% (Ofoezie, 2002). This result is comparable to 47% reported in settlements near Erinle River Dam, Osun State, Nigeria (Oladejo and Ofoezie, 2006) and lower than 62% reported from two communities in Southwest Nigeria (Ugbomoiko et al., 2010), 71.8% from settlements near a dam reservoir, Ogun State, Nigeria (Mafiana et al., 2003) and 58.1% from Ilewo-Orile community in Ogun State (Ekpo et al., 2010). From the results obtained in this study and previous studies as highlighted above, it appears that urinary schistosomiasis is particularly common in the southwest region of Nigeria. This study showed that there was an increasing trend of

Table 2. Prevalence (%) of micro-haematuria in relation to age and sex of children in Akinlalu and Ogbagba communities.

Age (years)	No. Examined	No. and (%) with no haematuria	No. and (%) with mild haematuria	No. and (%) with moderate haematuria	No. and (%) with gross haematuria	No. and (%) with positive haematuria
3 – 4	30	26 (86.7)	1 (3.3)	1 (3.3)	2 (6.7)	4 (13.3)
5 – 6	42	24 (57.1)	6 (14.3)	4 (9.5)	8 (19.0)	18 (42.9)
7 – 8	57	23 (40.4)	12 (21.1)	10 (17.5)	11 (19.3)	33 (57.9)
9 – 10	55	13 (23.6)	11 (20.0)	10 (18.2)	21 (38.2)	42 (76.4)
11 – 12	40	3 (7.5)	5 (12.5)	12 (30.0)	20 (50.0)	37 (92.5)
13 – 14	35	6 (17.1)	5 (14.3)	8 (22.9)	16 (45.7)	29 (82.9)
15 – 16	15	0 (0.0)	4 (26.7)	3 (20.0)	8 (53.3)	15 (100)
P-value		<0.05	<0.05	<0.05	<0.05	**<**0.05
Sex						
Male	148	52 (35.1)	17 (11.5)	31 (20.9)	48 (32.4)	96 (64.9)
Female	126	43 (34.1)	27 (21.4)	17 (13.5)	38 (30.2)	82 (65.1)
P-value			<0.05	<0.05	>0.05	>0.05
Community						
Akinlalu	223	92 (41.3)	39 (17.5)	28 (12.6)	64 (28.7)	131 (58.7)
Ogbagba	51	3 (5.8)	5 (9.8)	20 (39.2)	22 (43.1)	47 (92.2)
Total	274	95 (34.7)	44 (16.4)	48 (17.5)	86 (31.4)	178 (65.0)

prevalence infection among children from three years to sixteen years. This is in conformity with the general pattern of *S. haematobium* infection in endemic areas. Similar findings were reported by previous workers who studied *S. haematobium* infection among school children and stated that individuals aged 5 to 15 years were more likely to be infected with *S. haematobium*. (Okoli and Odaibo, 1999; Ejima and Odaibo, 2010). However, some studies reported a decline in infection from 14 years and attributed the declining trend in prevalence of infection among children aged 15 years and above to probable age acquired immunity (Satayathum et al., 2006).

In this study, the prevalence of infection was higher among the male pupils (48.6%) than in female pupils (47.6%), however, no statistically significant difference in the prevalence of infection between both sexes. This may be an indication that both male and female pupils are equally exposed to infection through water contacts. Previous studies have also reported higher prevalence among male pupils than among their female counterparts (Akinwale et al., 2010; Ejima and Odaibo, 2010; Ekpo et al., 2010). The intensity of infection was also higher among male pupils; this is suggestive that male pupils carry a greater worm burden than the females. Similar findings were reported in previous studies (Ariyo et al., 2004; Ejima and Odaibo, 2010).

The prevalence of microhaematuria in this study (65.3%) shows no statistically significant difference between the sexes. This is due to the fact that microhaematuria is a characteristic symptom of urinary schistosomiasis in endemic communities where its prevalence correlated positively with urinary schistosomiasis infection (Anosike et al., 2001). Visible haematuria was recorded in this study and was an on the spot indication of the

Table 3. Prevalence (%) of visible haematuria in relation to age and sex of the children in the two communities.

Age (years)	No. examined	No. and (%) with visible haematuria
3 – 4	30	2 (6.7)
5 – 6	42	6 (14.3)
7 – 8	57	7 (12.3)
9 – 10	55	7 (12.7)
11 – 12	40	9 (22.5)
13 – 14	35	6 (17.1)
15 – 16	15	7 (46.7)
P-value		< 0.05
Sex		
Male	148	32 (21.6)
Female	126	12 (9.5)
P-value		**<**0.05
Community		
Akinlalu	223	35 (15.7)
Ogbagba	51	9 (17.6)
Total	274	44 (16.1)

presence of urinary schistosomiasis in the study communities. The prevalence of visible haematuria recorded in this study (16.1%) compares favourably with 17.9% from Ishielu Amagunze, Enugu state Nigeria (Nwaorgu et al., 1998). It was noted that even though the disease is associated with river water, a high level of illiteracy, ignorance and traditional beliefs as well as unavailability of pipe borne water and insufficient boreholes have contributed to the endemicity of *S. haematobium* in the two communities. In both communities it is still believed by some that rivers “Amula” and “Ooye” situated in Akinlalu and Ogbagba, respectively have healing and medicinal powers. Provision of basic health education and portable water in the study communities will reduce contact of humans with infested water and thereby reduce transmission. Periodic mass treatment will reduce morbidity and gradually eradicate the disease in the two communities.

ACKNOWLEDGEMENTS

The authors wish to thank the community heads, parents/ guardians and the school authorities of Akinlalu and Ogbagba communities for their willingness to participate in the study and cooperation during the collections of the urine samples.

REFERENCES

Akinwale OP, Ajayi MB, Akande DO, Gyang PV, Adeleke MA, Adeneye AK, Adebayo MO, Dike AA (2010). Urinary Schistosomiasis around Oyan Reservoir, Nigeria: Twenty years after the first Outbreak. Iranian J. Pub. Health 39(1):92-95.

Anosike JC, Nwoke BEB, Njoku AJ (2001). The validity of haematuria in the community diagnosis of urinary schistosomiasis infections. J. Helminth. 75:223-225.

Ariyo O, Olofintoye LK, Adeleke RA, Famurewa O (2004). Epidemiological study of urinary Scistosomiasis among primary school pupils in Ekiti State, Nigeria. Afr. J. Clin. Exp. Micr. 5(1):20–29.

Asaolu SO, Holland CV, Crompton DWT (1991). Community control of *Ascaris lumbricoides* in rural Oyo State, Nigeria: Mass targeted and selective treatment with levamisole. Parasit. 103(2): 291–298.

Ejima IAA, Odaibo AB (2010). Urinary Schistosomiasis in the Niger – Benue Basin of Kogi State, Nigeria. Int. J. Trop. Med. 5(3):73–80.

Ekpo UF, Laja-Deile A, Oluwole AS, Sam-Wobo SO, Mafiana CF (2010). Urinary Schistosomiasis among preschool children in rural community near Abeokuta, Nigeria. Parasite, Vec. 3:58 doi: 10.1186/1756-3305-3-58.

Gryseels B, Polman K, Clerinix J, Kestens L (2006). Human Schistosomiasis. Lancet 368(9541):1106 – 1118.

Hotez PJ (2009). “Mass drug administration and integrated control for the world’s high- prevalence neglected tropical diseases. Clin. Pharm. Therap. 85: 659-664.

Hotez PJ, Kamath A (2009). Neglected tropical diseases in Sub – Saharan Africa: review of their prevalence, distribution, and disease burden. PLoS Neglect. Trop. Dise. 3,e412.

Mafiana CF, Ekpo UF, Ojo DA (2003). Urinary Schistosomiasis in preschool children in settlements around Oyan reservoir in Ogun State, Nigeria : implication for control. Trop. Med. Inte. hlth. 8, 78 – 82.

Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L (1998). Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. World Health Organisation, Geneva.

Nwaorgu OC, Okeibunor J, Madu E, Amazingo U, Onyegegbu N, Evans D (1998). A school – based Schistosomiasis and intestinal helminthiasis control program in Nigeria: acceptability to community members. Trop. Med.Int. Health 3(10)842-849.

Ofoezie IE (2002). Human health and sustainable water resources development in Nigeria: Schistosomiasis in artificial lakes. Natur. Resou. For. 26: 150-160.

Okoli EI, Odaibo AB (1999). Urinary Schistosomiasis among school children in Ibadan, an urban community in Southwestern Nigeria. Trop. Med. Int. Health 4: 308-315.

Oladejo SO, Ofoezie IE (2006). Unabated schistosomiasis transmission in Erinle River Dam, Osun State, Nigeria: evidence of neglect of environmental effects of development projects. Trop. Med. Int. Health 11: 843-850.

Opera KN, Udoidung NI, Ukpong IG (2007). Genitourinary schistosomiasis among pre – primary school children in rural community within the Cross River Basin, Nig. J. Helmin. 81:393 – 394.

Satayathum SA, Muchiri EM, Ouma JH, Whalea CC, King CH (2006). Factors affecting infection or reinfection with *Schistosoma haematobium* in coastal Kenya: survival analysis during a nine year, school – based treatment program. Am. J. Trop. Med. Hyg. 75(1): 83-92.

Ugbomoiko US (2000). The prevalence, incidence and distribution of human urinary Schistosomiasis in Edo State, Nigeria. Nig. J. Parasit. 21: 3-14.

Ugbomoiko US, Ofoezie IE, Okoye IC, Heukelbach J (2010). Factors associated with urinary schistosomiasis in two peri – urban communities in Southwestern Nigeria. Ann. Trop. Med. Parasit. 104(5): 409-419.

World Health Organization (1983). Diagnostic Techniques in Schistosomiasis Control. Document WHO/SCHISTO/83.69. Geneva: WHO.

World Health Organisation (2002). Prevention and Control of Schistosomiasis and Soil-transmitted Helminthiasis, Report of a WHO Expert Committee. Technical Report Series No. 912. Geneva: WHO.

academicJournals

Vol. 5(7), pp. 102-111, July, 2013
DOI: 10.5897/JPVB 2013.0118
ISSN 2141-2510 © 2013 Academic Journals
http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Entomological baseline data on malaria transmission and susceptibility of *Anopheles gambiae* to insecticides in preparation for Indoor Residual Spraying (IRS) in Atacora, (Benin)

Rock Aïkpon[1,2]*, Razaki Ossè[1,2], Renaud Govoetchan[1,2], Arthur Sovi[1,2], Frédéric Oké-Agbo[1] and Martin C. Akogbéto[1,2]

[1]Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin.
[2]Faculte des Sciences et Techniques de l'Université d'Abomey Calavi, Benin.

Accepted 22 June, 2013

To implement indoor residual spraying (IRS), the department of Atacora was selected in Benin. Entomological surveys were performed before IRS implementation. Mosquitoes were sampled by Pyrethrum spray catch and were identified morphologically and by molecular methods. The *Plasmodium falciparum* circumsporozoite indices were measured by enzyme linked immunosorbent assay (ELISA). Molecular detection of pyrethroid knock down resistance and that of insensitive acetylcholinesterase were performed. Susceptibility status of *Anopheles gambiae* was determined using World Health Organization (WHO) bioassay tests to various insecticides. *A. gambiae s.l.* was the main species harvested in houses (81.71%) and *A. gambiae s.s* is practically the only member that was found. Both M and S forms were in sympatry, but the molecular S form was predominant (94.42%). *A. gambiae s.l* were susceptible to bendiocarb but fully resistant to organochlorine (DDT), permethrin and deltamethrin. Entomological inoculation rate vectors (EIR) was 6 infectious bites per man per month on average during the study period. The average of *kdr* and *Ace-1* allelic frequency were 78 and 3%, respectively. *A. gambiae s.l* is characterized by a high endophilic behavior in Atacora, which is a good criterion for IRS implementation. The susceptibility to bendiocarb add to the low *Ace-1* mutation frequency found in *A. gambiae* populations could lead to the use of bendiocarb for IRS.

Key words: Entomological baseline data, IRS implementation, *Anopheles gambiae,* Atacora, Bénin.

INTRODUCTION

Malaria remains a major cause of morbidity and mortality in sub-Saharan Africa and represents one of the most critical public health challenges for Africa. More than two billion people around the world, particularly people living in South America, south-eastern Asia and sub-Saharan Africa, are at risk of contracting malaria. Besides, one million deaths are recorded yearly of which, 91% occur in sub-Saharan Africa (WHO, 2011). In Benin, in 2011, malaria was responsible for more than 1,753 deaths (MS, 2011). However, it incidence in Atacora in 2011 was

*Corresponding author. E-mail: rockypremier@yahoo.fr. Tel: (229) 97434183. Fax: (229) 21308860.

18.5%, which is higher than the national average (15.7%). The main malaria parasite was *Plasmodium falciparum* and was mainly transmitted by *Anopheles gambiae* s.*l*. This disease representing the primary cause of mortality and morbidity in health centres caused an enormous burden to health and economy in developing countries (WHO, 2011). Insecticide Treated Nets (ITNs) use represent the main approaches of malaria control (WHO, 2009). Household ITNs ownership reached more than 50% in several high burden African countries (WHO, 2009). Pyrethroids are the only insecticides used for net impregnation because of their strong efficacy, their fast acting effect at low doses and their low toxicity for mammals (Zaim et al., 2000). Unfortunately, resistance to pyrethroids in malaria vectors has spread across Africa and is now present in most of the countries where national malaria control programmes (NMCP) are implementing large scale distribution of long lasting insecticidal nets to populations at risk, that is children under five and pregnant women (Santolamazza et al., 2008).

There are numerous reports of resistance to pyrethroid throughout Africa (Elissa et al., 1993; Vulule et al., 1999; Koekemoer et al., 2002; Etang et al., 2006; Abdalla et al., 2008; Nwane et al., 2009; Bigoga et al., 2012). In Benin, the resistance of malaria vectors to pyrethroids observed first in Cotonou spread not only to central and southern regions of the country, but also in the northern localities (Akogbéto et al., 1999; Chandre et al., 1999; Corbel et al., 2007; Yadouleton et al., 2010).

The high level of resistance to pyrethroid in *A. gambiae* in Africa, particularly in Benin (Corbel et al., 2007; Yadouleton et al., 2010) is one of the major challenges being faced by malaria vector control programmes. It is then urgent to find ways to manage this resistance. In this context, an alternative insecticide to pyrethroids in IRS strategy supported by the President Malaria Initiative launched since 2008 in 14 African countries including Benin could be on the way to resolve the problem of resistance to pyrethroids in *A. gambiae*. After experimental hut evaluation (phase II), the non-pyrethroid that is effective seems to be bendiocarb (carbamate) (Akogbéto et al., 2010).

A study conducted in Ouémé, in southern Benin showed that the first and second rounds of IRS using bendiocarb were successful with a drastic decrease in malaria transmission in areas under IRS (Akogbéto et al., 2011). In spite of this success, NMCP decided to move IRS from Ouémé to Atacora (northern Benin). As matter of fact, Ouémé is characterized by two periods of transmission which last all the year. Implementation of IRS in such area needs two rounds of intervention. Contrary to Ouémé is characterized by a single transmission period. This means that just one spray round per year would be sufficient to cover the period of transmission. However, no entomological data from Atacora was available. In order to collect baseline data relating to diversity and abundance of mosquitoes, malaria transmission and the prevalence of insecticide-resistance alleles in malaria vectors, entomological surveys were performed between September and October, 2010 in all the districts of Atacora before IRS implementation. The above mentioned baseline data are reported in this paper.

METHODOLOGY

Study area

The study was carried out in Atacora, a department located in north-west of Benin (Figure 1). This department covers an area of 31,665 km^2 and counts a total of 735,845 inhabitants including 146,309 children under 5 years old in 2011 (INSAE, 2009). It is located at 10° 18' 46'' N and 1° 23' 19'' E. There are fifty health facilities for nine districts. This department is characterized by a sub-equatorial climate, with only one dry season (December to May) and only one rainy season (July to November). The annual mean rainfall is 1,300 mm and the mean monthly temperature varies between 23 and 31°C. The department is irrigated by three major rivers: the Mekrou, the Pendjari and the Alibori. The major economic activity is agriculture and it is characterized by the production of cotton and millet, where various classes of pesticides are used for pest control.

Mosquito sampling

Larvae collection

Mosquito larvae were collected from September to October, 2010 at the end of the rainy season. All instars of larvae were collected using dipping method from a wide range of breeding sites (puddles, shallow wells, gutters and rice fields). Then all larvae were brought back to the laboratory of Centre de Recherche Entomologique de Cotonou (CREC) for rearing. After that, emerging adult female mosquitoes were used for insecticide susceptibility tests and finally a susceptible strain of *A. gambiae* (Kisumu) was used as reference strain for bioassays.

Pyrethrum spray catch (PSC)

Adult mosquito collections were carried out from September to November, 2010 in all the nine districts in Atacora. In addition to larvae collection, mosquitoes resting in the house were collected through morning spray catch (MSC) from 7 a.m. to 9 a.m. In all the nine districts, we collected mosquitoes resting indoors. In each district, two areas were selected: a central area more or less urbanized and rural areas. In each area**,** some ten homes were randomly selected for mosquito collection. Three sessions were performed per month. Morning pyrethrum spray catches were performed using pyrethrum spray Rambo® and white canvas spread on the floor to collect knocked down mosquitoes. Knocked down mosquitoes falling on white bed sheets were kept separately and later, kept in labeled tubes containing silica gel and frozen at -20°C for further laboratory analysis. This sampling method led to an accurate estimation of the total density of mosquito species in the houses and the proportion of female mosquitoes resting from the

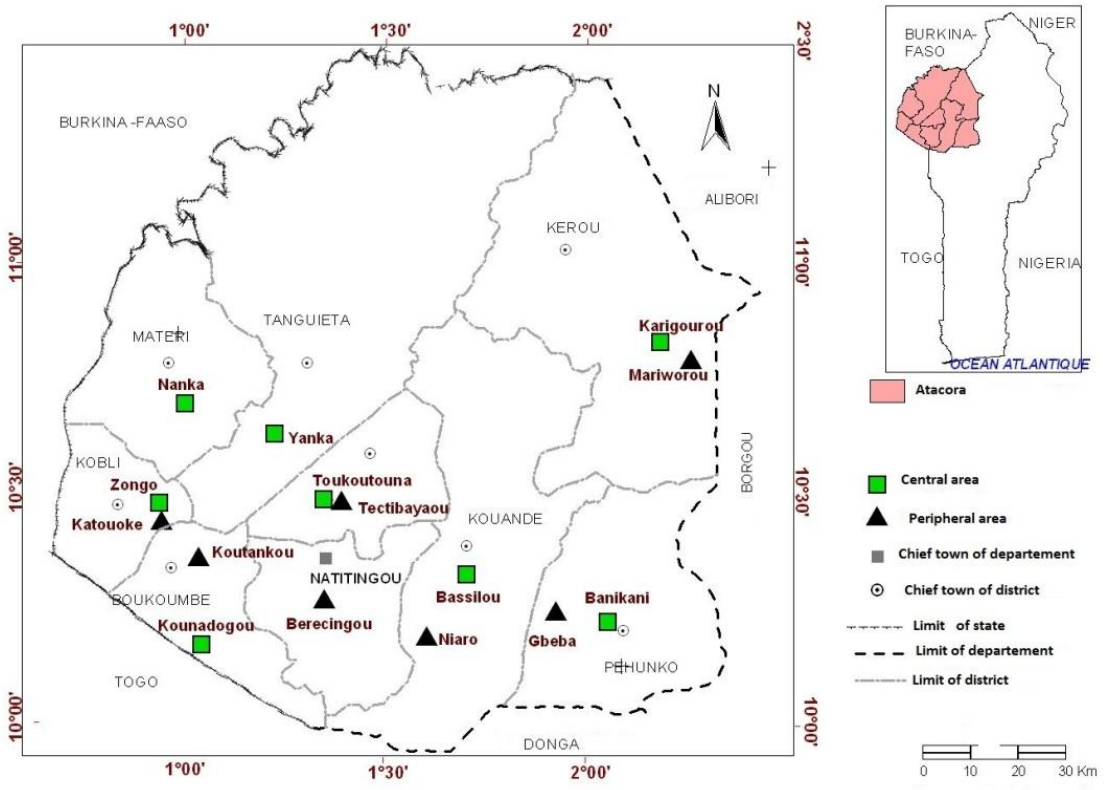


Figure 1. Map of study area.

houses. The recorded data were used to assess the human biting index (ma) and the entomological inoculation rate vectors (EIR).

Insecticide susceptibility tests

WHO insecticide susceptibility test-kits and standard procedures (WHO, 1998) were used to monitor the susceptibility of wild *A. gambiae* populations to the chemical groups of insecticides commonly used in public health and agriculture. Batches of 25 non-blood fed, 3 to 5 days old adult females were exposed to filter papers impregnated with 4% organochlorine (DDT), 0.1% bendiocarb (carbamate), 0.75% permethrin and 0.05% deltamethrin (pyrethroids). The number of mosquitoes knocked down was recorded every 5 min during exposure. After exposure, mosquitoes were supplied with glucose solution as food, and mortality was recorded 24 h post-exposure. Tests with untreated papers were systematically run as controls. WHO criteria were respected to classify populations as 'resistant' if less than 80% mortality was observed, as ''suspected resistant'' if mortality rates were between 80 and 97% and as 'susceptible' for mortality > 97% (WHO, 2001).

Laboratory processing

After each indoor pyrethrum spray, mosquitoes were sorted by genus as anopheline and culicine and counted. Anophelines were morphologically identified to species using taxonomic keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). Ovaries from randomly selected female *A. gambiae s.l.* specimens captured by PSC were dissected to determine parity rate, by observing the coiling degree of ovarian tracheoles (Detinova and Gillies, 1964). Then, mosquito infectivity rates were determined from head and thorax of all female anopheline specimens by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against *Plasmodium falciparum* circumsporozoite protein (CSP) as described by Wirtz et al. (1987). The carcasses of these females (abdomens, wing and legs) were stored in individual tubes with silicagel and preserved at −20°C in the laboratory for identification of species and characterization of molecular forms within the *A. gambiae* complex as previously described (Scott et al., 1993; Favia et al., 2001).

Data analysis

The human biting index (ma) or *Anopheles* density (number of *Anopheles* per person and per night) and the circum sporozoite protein positive rate were calculated. The human biting rate was determined by dividing the number of mosquitoes fed and half gravid collected within a room by the number of sleepers in this room the night before morning spray catching. The circum sporozoite protein positive rate (% CS+) was calculated as the proportion of mosquitoes found to be positive for CSP. The entomological inoculation rate (EIR) was defined as *Anopheles* density by the CSP and estimated as the number of infectious bites per human per month. A Chi-square test with the MINITAB statistical software (Version 12.2) was used to compare the mortality rates among the

Table 1. Mosquito species caught by PSC from September to October, 2010 in the study area.

Species	Nb	%
Aedes aegypti	11	1.04
Aedes gr. Palpalis	5	0.47
Aedes longipalpis	2	0.19
Aedes vittatus	5	0.47
Anopheles broheri	6	0.57
Anopheles funestus	13	1.23
Anopheles gambiae	862	81.71
Anopheles pharoensis	7	0.66
Culex annulioris	3	0.28
Culex fatigans	45	4.27
Culex gr decens	9	0.85
Culex nebulosus	3	0.28
Culex quinquefasciatus	72	6,82
Mansonia africana	12	1,14
Total	1055	

localities. The genotypic differentiation of *kdr* and *Ace1* loci was tested using the Fischer exact test implemented in GenePop software (Raymond and Rousset, 1995), and the Fisher test was used to compare these frequencies. An analysis of variance (ANOVA) was performed to compare the entomological estimates (ma, EIR, CSP) among the sites.

Ethical consideration

Permission was sought from inhabitants to perform collections in their rooms. In addition, community consent had been obtained beforehand in all the villages. This study was approved by the Ethical Committee of the Ministry of Health in Benin.

RESULTS

Culicidae diversity and endophily

A total of 1,055 mosquitoes belonging to 14 different species were caught. They included 84.71% (888) *A. gambiae s.l.*, 2.18% (23) *Aedes* spp., 12.51% (132) *Culex* spp., and 1.14% (12) *Mansonia africana* (Table 1). During the period of study, anopheline species were the most important species collected in all the districts except Tanguiéta where *Culex* spp. was rather very important. The ovary physiological state of *A. gambiae s.l* collected from PSC were mostly fed, half-gravid and gravid, reflecting a strong endophily of this species (Table 2). The percentage of female fed, half-gravid and gravid, was higher than 80% in most of the districts ($p > 0.05$). Vector density by human habitation was on average 10 females in the nine districts.

Vectors infection to CSP and malaria transmission risk

Table 3 shows the circumsporozoite protein positive rate (%CS+) and the EIR at the study sites. Results from this section showed that the risk of malaria transmission was very high in the nine districts. In fact, the average of the sporozoite rate in the nine districts is 6.63% (53 positive thoraces on 800). Among the nine districts, the highest rate was obtained in Natitingou (55.6%) ($p < 0.05$). The average of EIR was 6 infectious bites per person per month (6 bi/p/m). The highest EIR was found in the districts of Cobly and Matéri, with 10 bi/p/m, which means that in these localities every inhabitant receives on infected bite every three days during the study period.

Resistance status

Figure 2 shows the resistance status of *A. gambiae s.l* populations collected in the various districts at Atacora. From this study, it appears that results are similar in the nine districts. *A. gambiae s.l* populations were susceptible to bendiocarb, with mortality rates ranging between 95 and 100%. For this carbamate, no significant difference was noticed from a district to the other ($p > 0.05$). On the other hand, *A. gambiae* was resistant to deltamethrin with mortality rate ranging between 27 and 54%. Moreover, *A. gambiae* was fully resistant to DDT and permethrin with a mortality rate which did not exceed 18%.

Species and molecular forms of *Anopheles gambiae*

Mosquitoes from PSC were analysed by polymerase chain reaction (PCR) for identification of sibling species among *A. gambiae* complex and molecular M and S forms of *A. gambiae s.s*. In all districts, *A. gambiae s.s* was predominant (99.49%). Only one specimen of *A. arabiensis* was found in Kérou (Table 4). Both M and S forms were found in sympatry, but the molecular S form was predominant (94.42%).

Kdr resistance gene status in *A. gambiae*

The average of *kdr* allelic frequency from September to October, 2010 was 78%. *kdr* allelic frequency was very high (98%) in Cobly and Kouandé.

Insensitive acetylcholinesterase gene status in *An. gambiae s.l*

In all the nine districts, the allelic frequency of this gene

Table 2. Vectors distribution and the physiological state of their abdomen.

Districts	Villages	Nb of houses sprayed	*A. gambiae*						*A. funestus*				
			Unfed	Fed	Half Gr	Gr	Total	Density/House	Unfed	Fed	Half Gr	Gr	Total
Pehunco	Gbéba	7	8	64	3	7	82	11.71	-	1	-	-	1
	Banikani	6	4	11	3	5	23	3.83	-	-	-	-	-
Kouandé	Niaro	5		70		1	71	14.20	-	3	-	-	3
	Bassilou	5		8	1		9	1.80	-	-	-	-	-
Cobly	Zongo	7	10	122	30	19	181	25.86	-	2	-	-	2
	katouokè	6	20	120	28	5	173	28.83	-	-	-	-	-
Boukoumbé	Coutankou	5	5	111	30	29	175	35	-	1	1	-	2
	Kounadogou	2	1	4	2	5	12	6	-	1	-	2	3
Tanguiéta	Yanka	7	3	21	1		25	3.57	-	-	-	-	-
	Thanwassaka	5	2	17		1	20	4	-	1	-	-	1
Toukountouna	Sanga	6	8	22		3	33	5.50	-	-	-	-	-
	Tectibayaou	4	13	15			28	7	-	1	-	-	1
Natitingou	Bérécingou	5	1	8		2	11	2.20	-	-	-	-	-
Matéri	Nanka	10		17		2	19	1.90	-	-	-	-	-
Kérou	Karigourou	4		1			1	0.25	-	-	-	-	-
	Maréworou	5		16			16	3.20	-	-	-	-	-
Total		89	75	610	98	79	862	9.69	0	10	1	2	13

was low. The average of *Ace-1* allelic frequency from September to October, 2010 was 3% and no homozygous individuals RR were found (Table 4 and Figure 3).

DISCUSSION

The present study provides entomological base-line data in Atacora for the first time. 14 different species of mosquito were collected during our investigation. A similar result was obtained by Huttel (1950) in southern Benin. As a matter of fact, he got the same species by using the same

Table 3. *A. gambiae* infectivity to circumsporozoïtic antigen (CS) and Entomological Inoculation Rate (EIR) in Atacora.

Locality	N tested	CS$^+$	% CS+	Nb of Sleepers	ma	EIR (bi /p/m)
Pehunco	93	2	2.15^a [00.26-07.55]	43	2.16^b [01.75-02.65]	1.4^a [01.06-01.80]
Kouandé	79	9	11.39^b [05.34-20.53]	45	1.76^b [01.49-02.19]	6^c [05.30-06.76]
Cobly	330	15	4.55^a [02.57-07.39]	44	7.5^c [06.71-08.36]	10.23^d [09.30-11.22]
Boukoumbé	153	3	1.96^a [00.41-05.62]	22	6.95^c [05.89-08.15]	4.09^b [03.29-05.03]
Tanguiéta	44	4	9.09^b [02.53-21.67]	20	2.2^b [01.60-02.95]	6^c [05.35-07.18]
Toukountouna	58	5	8.62^b [02.86-18.98]	24	2.42^b [01.83-03.12]	6.25^c [05.29-07.34]
Natitingou	9	5	55.56^d [21.20-86.30]	18	0.5^a [00.23-00.95]	8.33^d [07.46-09.78]
Matéri	17	5	29.41^c [10.31-55.96]	15	1.13^a [00.66-01.32]	10^d [08.46-11.74]
Kérou	17	5	29.41^c [10.31-55.96]	34	0.5^a [00.29-00.80]	4.41^b [03.73-05.18]
Total	800	53	6.63	265	3.02	6

%CS+: the circum sporozoite protein positive rate; bi/p/m: infective bites per person per month; ma: Number of bites per person per night.

method. However, more species were supposed to be collected if the PSC method was associated with other catch methods such as Human Landing Catch (HLC), Center for Disease Control (CDC) and BG sentinel traps. In fact, a study conducted in southern Benin the same year by Lingenfelser et al. (2010), using HLC with conventional aspirators, showed the presence of 24 species belonging to 6 genera of mosquito. Therefore, the choice of sampling method influenced the entomological data recorded. A report conducted in Senegal by Ndiath et al. (2011) on the sampling method concluded that the sampling technique has to be chosen according to the vector studied and the aim of the study.

A. gambiae, the main malaria vector in sub-Saharan Africa, is the main species harvested in houses (81.71%). This predominance of *A. gambiae* is probably due to the low urbanization level of the districts. Indeed, unlike urban areas, in rural areas, larval habitats are less polluted and therefore, favorable for the production of anophelines; hence the higher production of anopheline fauna in rural areas. *A. funestus* was also present in the department but in small proportion (1.23%), probably due to the presence of dams which surface was covered by vegetation suitable to *A. funestus* development.

Anopheles arabiensis was also very poorly represented in our samples; one specimen was collected in Kérou whereas fifteen years before Akogbeto and Di Deco (1995) reported its presence in sympatry with *A. gambiae* s.s in northern Benin. The absence of *A. arabiensis* during the study period may appear strange. Indeed, the long drought that characterizes Atacora (Sudanian climate), and the presence of a large dry savannah area, satisfy the conditions for the development of *A. arabiensis*. In an article, Djogbénou et al. (2010) reported *A. arabiensis* disappearance in Atacora, especially in Tanguiéta and Natitingou between 2006 and 2007, without explaining the causes of this disappearance. In reality, it was not a problem of disappearance, but a problem of sampling and duration of the study instead.

Regarding our study, the catches were performed inside human dwellings while *A. arabiensis* endophilic trends is low. However, it is possible that the action of man on the environment be unfavorable to the development of *A. arabiensis* in this area. A study on the dynamics of *A. arabiensis* and its distribution showed that within 25 years, *A. arabiensis* moved from latitude 10° 10' N (northern Benin) to latitude 6° 40' N (southern Benin). This change of environment is made possible through deforestation, thus creating favorable conditions for the development of *A. arabiensis* in the central and southern Benin. As a matter of fact, shelters for its development are threatened in the north by a severe drought. During this period, the absence of pasturage in the north led to the movement of this species to the center and the south of Benin through transhumance. Climate change, therefore, favored the movement of *A. arabiensis* to the south. This new distribution of *A. arabiensis* should help the NMCP to adapt to its vector control programme. Molecular analyses performed on the same samples showed that the S form is more represented. This confirms that the S form of *A. gambiae* depends on dry savannah areas as pointed out by studies carried out in West Africa and which reported similar results in similar bioecological zones in Nigeria, Cameroon and Burkina Faso (Wondji et al., 2002; Onyabe et al., 2003; Dabiré et al., 2009). IRS implementation as vector control strategy is based on endophilic behavior of vector populations and their preference to rest inside houses. This is why the high vectors population collected indoors is an asset for the IRS implementation in Atacora. Indeed, the endophilic behavior of vectors facilitates their contact with the insecticide. The second argument for the need to strengthen

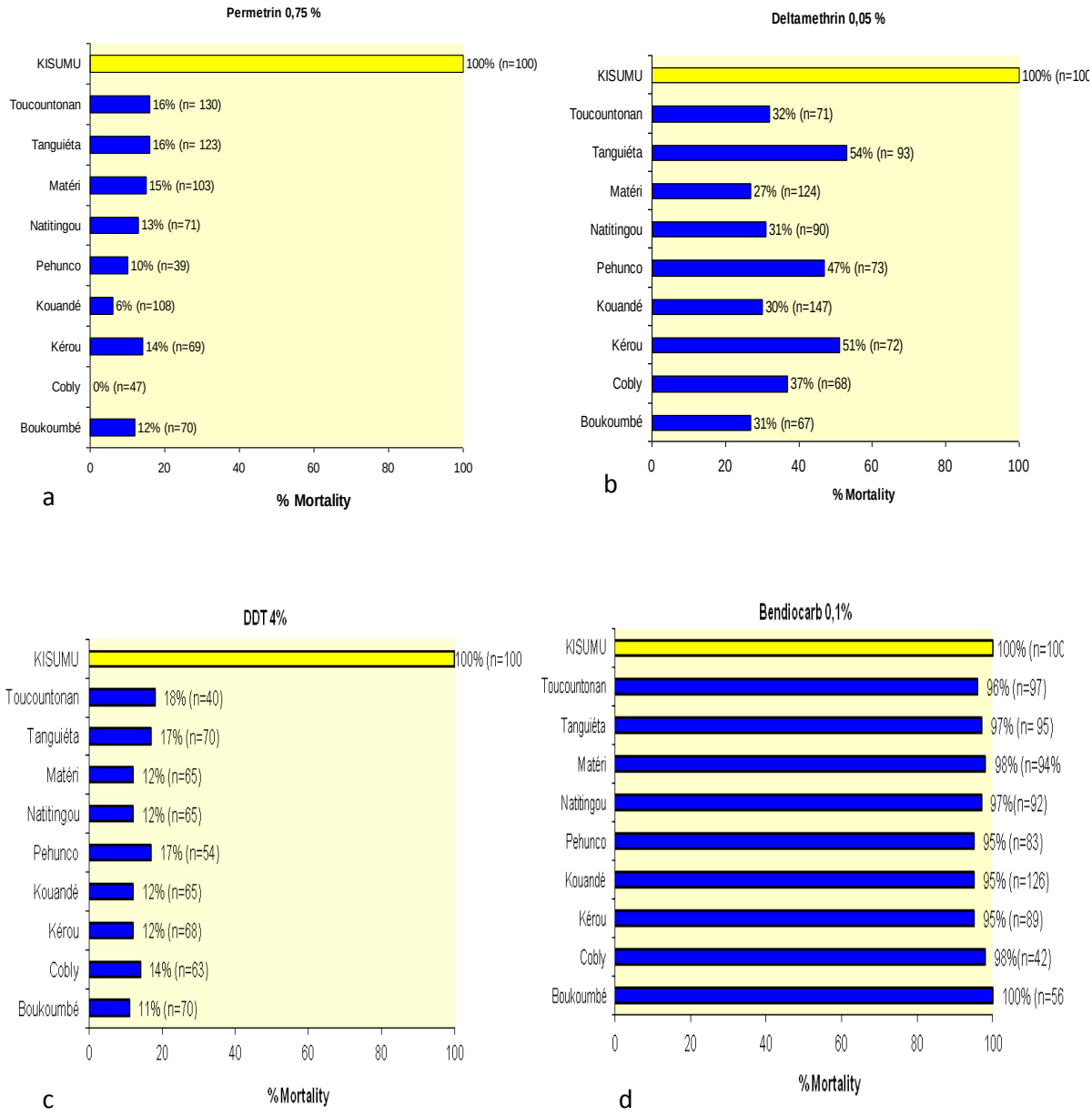


Figure 2. Insecticide susceptibility status of *An. gambiae s.l.* in the nine districts in Atacora.

the fight against malaria in Atacora by a rapid intervention effect on the vectorial capacity can be justified by the very high circumsporozoitic index in Atacora. The entomological inoculation rate recorded during the study (6 infected bites per man per month) was higher than that obtained in southern Benin (Djènontin et al., 2010) and in north-east of Benin (Yadouleton et al., 2010). The high level of this EIR could be explained by the low coverage of LLINs. In fact, a survey conducted in Atacora in 2010 on the use of bednets by the populations showed that very few people had a bednet (1 net for 5 people) and those who had it, would not use it frequently because of the strong heat (Aïkpon, personal communication).

Data recorded from the susceptibility test performed on *A. gambiae* showed a high level of resistance to permethrin, deltamethrin and DDT but susceptibility to bendiocarb. The emergence of resistance to pyrethroid in *A. gambiae* has become a serious concern for the success of malaria control in the last decades. In fact, in Benin, pyrethroids have been extensively introduced in

Table 4. Species and molecular forms of *An. gambiae* and *kdr* and *Ace.1* allelic frequency.

Locality	Species		Molecular forms		*kdr mutation*				*Ace-1* mutation			
	Aa	Ag	M	S	RR	RS	SS	F(*kdr*)	RR	RS	SS	F(*Ace-1*)
Pehunco	0	28	1	27	10	18	0	0.68	0	2	26	0.04
Kouandé	0	27	0	27	26	1	0	0.98	0	1	26	0.02
Cobly	0	21	2	19	20	1	0	0.98	0	1	20	0.02
Boukoumbé	0	27	0	27	10	17	0	0.69	0	0	27	0.00
Tanguiéta	0	26	6	20	20	4	2	0.85	0	0	26	0.00
Toukountounan	0	26	0	26	13	11	2	0.71	0	1	25	0.02
Natitingou	0	9	0	9	3	3	3	0.50	0	1	8	0.06
Matéri	0	17	0	17	10	5	2	0.74	0	1	16	0.03
Kérou	1	16	2	14	11	2	3	0.75	0	3	13	0.09

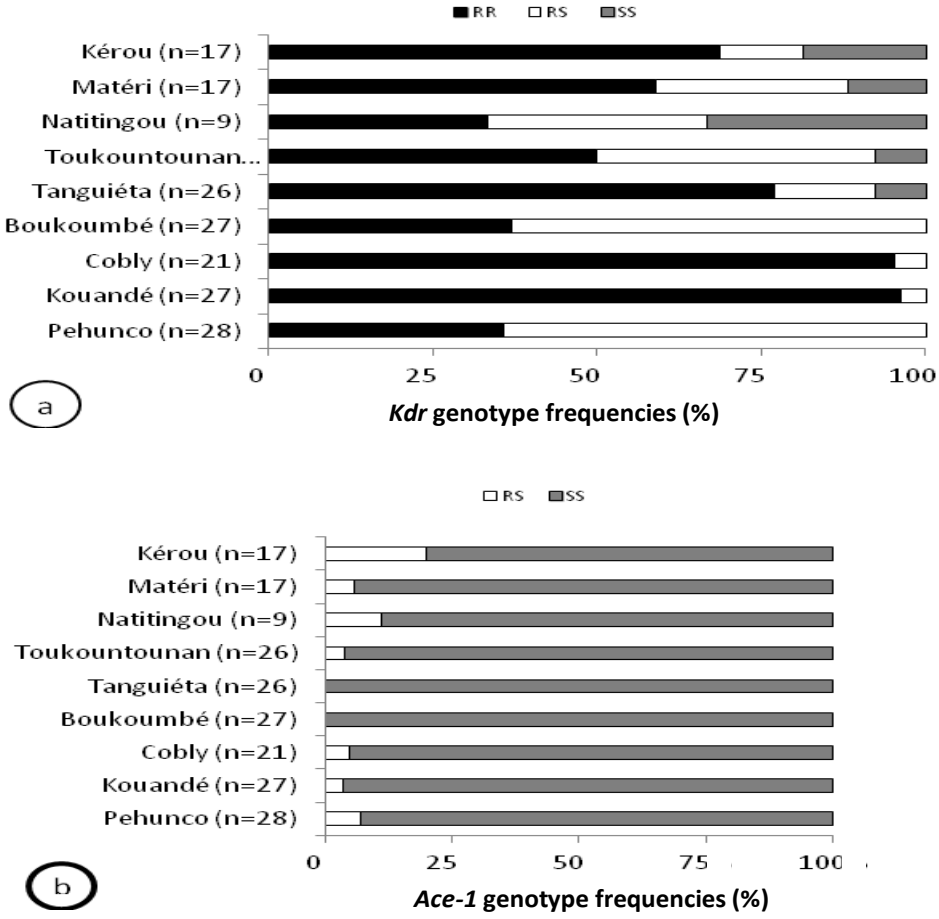


Figure 3. *kdr* (a) and *Ace-1* (b) allelic frequency.

agriculture since 1980s (Prudent et al., 2006). This factor is probably one of the causes of the selection of high resistance in *A. gambiae* to pyrethroids, with a high allelic frequency of the knock down resistance (*kdr*) mutation level. The *kdr* which seems to be the main mechanism conferring resistance in *A. gambiae* to pyrethroids (Leu-Phe *kdr* mutation) in West Africa was found in mosquito samples collected in different sites. The allelic frequency found in our study area was higher than what was reported in southern Benin by Djènontin et al. (2010), and

similar to those reported in Parakou, a town in the north-east of Benin by Yadouleton et al. (2011). This would certainly explain the fact that most of the localities in northern Benin are cotton growing and use many pesticides. This massive use of insecticide selects the resistance of malaria vectors (Akogbéto et al., 2005; Dabiré et al., 2008; Yadouleton et al., 2009).

The *Ace-1* mutation was also found but at a very low frequency (< 0.1). Several reports in southern Benin (Yadouleton et al., 2009; Djènontin et al., 2010; Padonou et al., 2012) confirmed the low allelic frequency of this mutation. Basing on the information above related to the susceptibility tests, and for better management of resistance to insecticide in *A. gambiae* populations, the low frequency of *Ace-1* recorded in all populations encourages the use of bendiocarb as an alternative insecticide to pyrethroids for IRS in the study areas. A previous study conducted in southern Benin (Akogbéto et al., 2010) had shown bendiocarb as alternative to pyrethroids. The author showed that the use of this insecticide for IRS had drastically reduced malaria transmission.

Conclusion

This study shows that *A. gambiae s.l* was the most abundant species found in the nine districts. *A. gambiae s.s* was practically the only member of the complex found responsible for malaria transmission in Atacora. Besides, the high antigen CSP positivity rate observed proves the vulnerability of populations to malaria transmission and constitutes challenge not only for the reinforcement of existing control strategies (LLINs), but also resorting to other complementary strategies such as IRS. The susceptibility to bendiocarb added to the low frequency of *Ace-1* mutation found in *A. gambiae* populations could lead to the use of bendiocarb as a potential alternative against resistance of *A. gambiae* to pyrethroid in the nine districts for IRS.

ACKNOWLEDGEMENTS

This work was financially supported by PMI (President's Malaria Initiative) through USAID. We thank the Ministry of Higher Education and Scientific Research (MESRS) and the team of CREC for their technical assistance during field work and the laboratory. We also thank the people of Atacora for their collaboration.

REFERENCES

Abdalla H, Matambo TS, Koekemoer LL, Mnzava AP, Hunt RH, Coetzee M (2008). Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. Trans. R. Soc. Trop. Med. Hyg. 102: 263-271.

Akogbeto M, Di Deco MA (1995). Répartition des membres du complexe *Anopheles gambiae* et de leurs variants chromosomiques au Bénin et au Togo, Afrique occidentale. J. Afr. Zool.,109: 443–454.

Akogbéto M, Yakoubou S (1999). Résistance des vecteurs du paludisme vis-à-vis des pyréthrinoïdes utilisés pour l'imprégnation des moustiquaires au Bénin, Afrique de l'Ouest. Bull. Soc. Path. Exo. 92: 123–130.

Akogbeto MC, Djouaka R, Noukpo H (2005). Utilisation des insecticides agricoles au Bénin. Bull. Soc. Pathol. Exot. 5: 400-405.

Akogbéto M, Padonou G, Gbénou D, Irish S, Yadouleton A (2010). Bendiocarb, a potential alternative against pyrethroid resistant *Anopheles gambiae* in Benin West Africa. Malar. J., 9:204.

Akogbéto M, Padonou G, Bankolé H, Kindé Gazard D, Gbédjissi G (2011). Dramatic decline of malaria transmission after implementation of large-scale Indoor Residual Spraying using bendiocarb in Bénin, West Africa, an area of high *Anopheles gambiae* resistance to pyrethroids. Am. J. Trop. Med. Hyg. 85(4): 586–593.

Chandre F, Manguin S, Brengues C, Dossou Yovo J, Darriet F, Diabate A, Carnevale P, Guillet P (1999). Current distribution of a pyrethroid resistance gene (kdr) in *Anopheles gambiae* complex from West Africa and further evidence for reproductive isolation of the Mopti form. Parassitologia., 41: 319–322.

Corbel V, N'guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbéto M, Hougard JM, Rowland M (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta. Trop. 101(3): 207-16.

Dabiré KR, Diabaté A, Agostinho F, Alves F, Manga L, Faye O, Baldet T (2008). Distribution of the members of *Anopheles gambiae* and pyrethroid knock-down resistance gene (kdr) in Guinea-Bissau, West Africa. Bull. Soc. Pathol. Exot. 101(2): 119-23.

Dabiré KR, Diabaté A, Namountougou M, Toé KH, Ouari A, Kengne P, Bass C, Baldet T (2009). Distribution of pyrethroid and DDT resistance and the L1014F *kdr* mutation in *Anopheles gambiae s.l.* from Burkina Faso (West Africa). Trans. R. Soc. Trop. Med. Hyg. 103: 1113–1120.

Detinova TS, Gillies MT (1964). Observations on the Determination of the Age Composition and Epidemiological Importance of Populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in Tanganyika. Bull. World. Health. Organ. 30: 23–28.

Djènontin A, Bio-Bangana S, Moiroux N, Henry MC, Bousari O, Chabi J, Ossè R, Koudénoukpo S, Corbel V, Akogbéto M, Chandre F (2010). Culicidae diversity, malaria transmission and insecticide resistance alleles in malaria vectors in Ouidah-Kpomasse-Tori district from Benin (West Africa): A pre-intervention study. Parasit. Vectors.3: 83.

Djogbénou L, Pasteur N, Bio-Bangana S, Baldet T, Irish SR, Akogbeto M, Weill M, Chandre F (2010). Malaria vectors in the Republic of Benin: distribution of species and molecular forms of the *Anopheles gambiae* complex. Acta. Trop.114(2): 116-22.

Elissa N, Mouchet J, Rivière F, Meunier JY, Yao K (1993). Resistance of *Anopheles gambiae s.s.* to pyrethroids in Côte d'Ivoire. Ann. Soc. Belge. Med. Trop. 73: 291–294.

Etang J, Fondjo E, Chandre F, Morlais I, Brengues C, Nwane P, Chouaibou M, Ndjemai H, Simard F (2006). First report of knockdown mutations in the malaria vector *Anopheles gambiae* from Cameroon. Am. J. Trop. Med. Hyg. 74: 795–797.

Favia G, Lanfrancotti A, Spanos L, Siden Kiamos I, Louis C (2001). Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. Insect. Mol. Biol. 10: 19–23.

Gillies MT, De Meillon D (1968). The Anophelinae of Africa South of the Sahara. Publ. South. Afri. Inst. Med. Res., 54:343.

Gillies MT, Coetzee MA (1987). Supplement to the Anophelinae of Africa South of the Sahara, 2nd edn. Publ. South. Afri. Inst. Med. Res. 55: 143.

Huttel J: Note sur la répartition des moustiques dans le Bas-Dahomey.

Bull. Sot. Path. Exot., 43: 563-566.
INSAE (2004). Projection démographique révisée. Atacora. 29.
Koekemoer LL, Kamau L, Hunt RH, Coetzee M (2002). A cocktail polymerase chain reaction assay to identify members of the Anopheles funestus (Diptera: Culicidae) group. Am. J. Trop. Med. Hyg. 66(6):804-811.
reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am. J. Trop. Med. Hyg. 66: 804-11.
Lingenfelser A, Rydzanicz K, Kaiser A, Becker N (2010). Mosquito fauna and perspectives for integrated control of urban vector-mosquito populations in Southern Benin (West Africa). Ann. Agric. Environ. Med. 17(1): 49-57.
Ministry of Health (2011). Health Statistics Yearbook 2011. Cotonou: Department of Programming and Prospective.
Ndiath OM, Mazenot C, Gaye A, Konate L, Bouganali C, Faye O, Sokhna C, Trape JF (2011). Methods to collect Anopheles mosquitoes and evaluate malaria transmission: A comparative study in two villages in Senegal. Malar. J. 10: 270.
Nwane P, Etang J, Chouaibou M, Toto J C, Kerah-hinzoumbe C, Mimpfoundi R, Awono-Ambene HP, Simard F (2009). Trends in DDT and pyrethroid resistance in *Anopheles gambiae* s.s. populations from urban and agro-industrial settings in southern Cameroon. BMC. Infect. Dis. 9: 163.
Onyabe DY, Vajime CG, Noc IH, Ndams IS, Akpa AU, Alaribe AA, Conn JE (2003). The distribution of M and S molecular forms of *Anopheles gambiae* in Nigeria. Trans. R. Soc. Trop. Med. Hyg. 97: 605–608.
Padonou GG, Sèzonlin M, Ossè R, Aizoun N, Oké-Agbo F, Oussou O, Gbédjissi G, Akogbéto M (2012). Impact of three years of large scale Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) interventions on insecticide resistance in *Anopheles gambiae s.l.* in Benin. Parasit. Vectors. 10: 72.
Prudent P, Loko S, Deybe D, Vaissayre M (2006). Factors limiting the adoption of IPM pratices by cotton farmers in Benin: a participatory approach. Cambridge. University. Press. 10: 10-17.
Raymond M, Rousset F (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity. 86: 248-249.
Santolamazza F, Calzetta M, Etang J, Barrese E, Dia I, Caccone A, Donnelly MJ, Petrarca V, Simard F, Pinto J, della Torre A (2008). Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. Malar. J. 7: 74.
Scott JA, Brogdon WG, Collins FH (1993). Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49: 520–529.
Vulule JM, Beach RF, Atieli FK, McAllister JC, Brogdon WG, Roberts JM, Mwangi RW, Hawley WA (1999). Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-impregnated nets. Med. Vet. Entomol. 13: 239-244.
Wirtz RA, Ballou WR, Schneider I, Chedid L, Gross MJ, Young JF, Hollingdale M,Diggs CL, Hockmeyer WT (1987). *Plasmodium falciparum*: immunogenicity of circumsporozoite protein constructs produced in *Escherichia coli*. Exp. Parasitol. 63: 166–172.
Wondji C, Simard F, Fontenille D (2002). Evidence for genetic differentiation between the molecular forms M and S within the Forest chromosomal form of *Anopheles gambiae* in an area of sympatry. Insect. Mol. Biol. 11: 11–19.
World Health Organization (WHO) (1998). Tests procedures for insecticide resistance monitoring in malaria vector, bio- efficacy and persistence of insecticides on treated surfaces. Document WHO/CDS/CPC/MAL/98.12 Geneva, Switzerland 1998 [http://whqlibdoc.who.int/hq/1998/ WHO_CDS_CPC_MAL_98.12.pdf].
World Health Organization (WHO) (2001). Supplies for monitoring insecticide resistance in disease vectors. WHO/CDS/CPE/PVC/20012 2001.
World Health Organization (WHO) (2009). World Malaria Report. Geneva.
World Health Organization (WHO) (2011). World Malaria Report. Geneva, Switzerland.
Yadouleton AW, Asidi A, Djouaka RF, Braïma J, Agossou CD, Akogbeto MC (2009). Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin. Malar. J. 14: 103.
Yadouleton AW, Padonou G, Asidi A, Moiroux N, Banganna S, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Gazard K, Akogbeto MC (2010). Insecticide resistance status in *Anopheles gambiae* in southern Benin. Malar. J. 9: 83.
Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, Djogbenou L, Dabiré R, Aïkpon R, Boko M, Glitho I, Akogbeto M (2011). Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in northern Benin. Parasit. Vectors. 4: 60.
Zaim M, Aitio A, Nakashima N (2000). Safety of pyrethroid-treated mosquito nets. Med. Vet. Entomol. 14:1-5.

UPCOMING CONFERENCES

24th International Conference of the World Association for the Advancement of Veterinary Parasitology, Perth, Australia, 25 Aug 2013

XXI Latin American Congress of Parasitology, Guayaquil, Ecuador, 6 Oct 2013

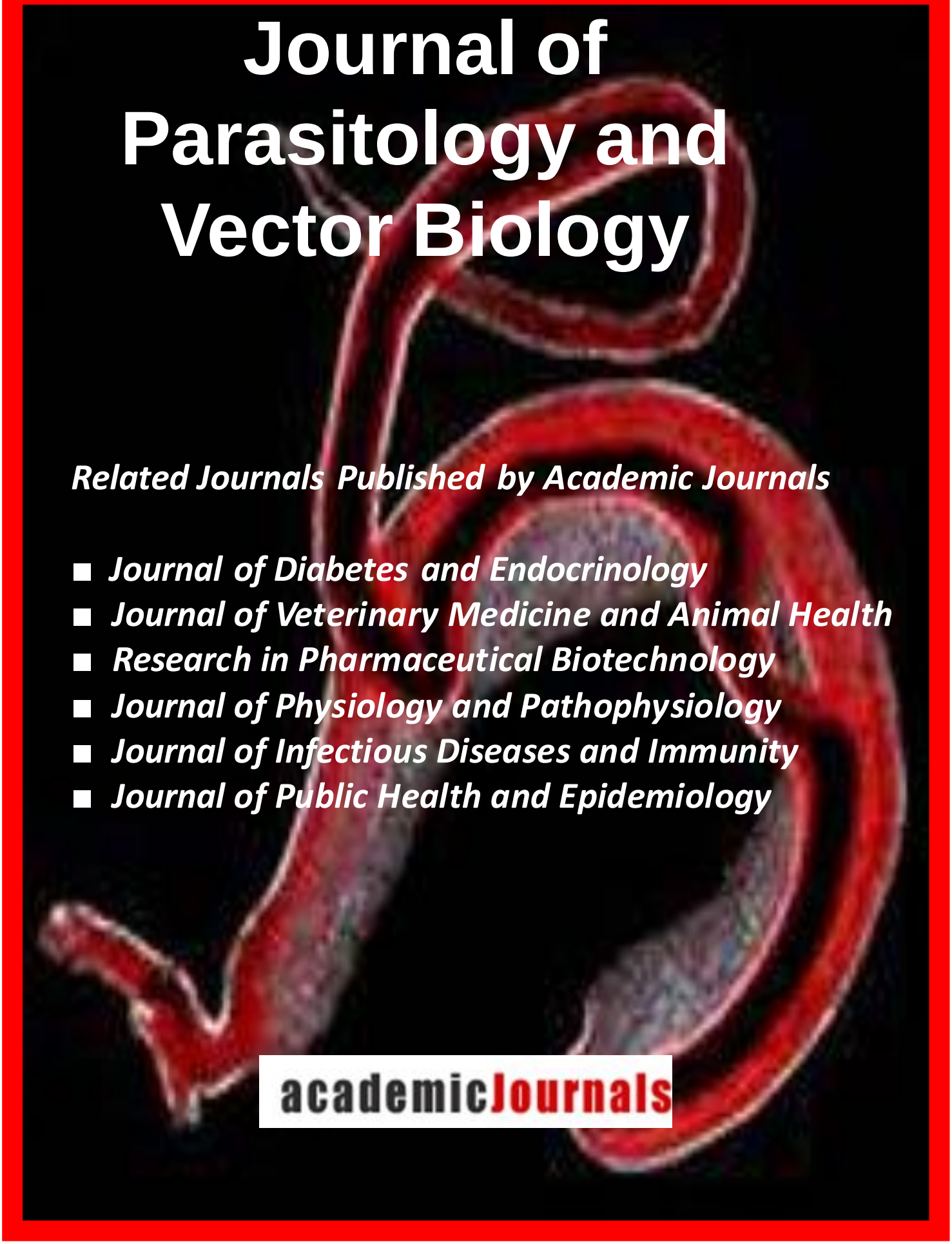
Conferences and Advert

August 2013
24th International Conference of the World Association for the Advancement of Veterinary Parasitology, Perth, Australia, 25 Aug 2013

September 2013
5th European Congress of Virology, Lyon, France, 11 Sep 2013

October 2013
XXI Latin American Congress of Parasitology, Guayaquil, Ecuador, 6 Oct 2013

Journal of Parasitology and Vector Biology



Related Journals Published by Academic Journals

- *Journal of Diabetes and Endocrinology*
- *Journal of Veterinary Medicine and Animal Health*
- *Research in Pharmaceutical Biotechnology*
- *Journal of Physiology and Pathophysiology*
- *Journal of Infectious Diseases and Immunity*
- *Journal of Public Health and Epidemiology*

academicJournals