ISSN: 2583-6455 (Online)

JOURNAL OF MEDICAL ARTHROPODOLOGY & PUBLIC HEALTH

Society
of
Medical
Arthropodology
(SOMA)

J. Med. Arthropodol. & Public Health | Vol. 4 | No. 1 | June 1, 2024 | Page 1–124

Journal of Medical Arthropodology & Public Health, an open access journal available both in electronic and print versions, is an official organ of SOCIETY OF MEDICAL ARTHROPODOLOGY (SOMA) which is published semiannually on June 1 and December 1. It is general policy that all manuscripts are critically peer-reviewed. The Society of Medical Arthropodology is a non-profit, scientific society interested in promoting the science of medical arthropodology in public health (www.soma16.org).

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1st June, 2024

From the Editors' Desk

Dear Medical Arthropodologists &
Public Health Scientists,

We are back again, on time, with a new set of research publications based on a variety of subjects so important to public health managers and policy makers. These papers, however, represent only a fraction of the vast and unexplored spectrum of disciplines within the unfathomable depths of the integrated science of medical arthropodology and public health which are to gradually and periodically come to surface in the future issues, in a way so as to generate irrefutable scientific knowledge toward the varied and diverse world of entrepreneurship! Thus, we are endeavouring hard to publish on the pages of *Journal of Medical Arthropodology & Public Health* – a broad-scope, open access-cum-print journal publishing basic and applied research that has a positive impact on translation of sophisticated data-based scientific studies into usable products by the end-user – the research papers that truly serve the science and society.

Our aim at *Journal of Medical Arthropodology & Public Health* is to maximize the global visibility and impact of your published articles. The *Journal of Medical Arthropodology & Public Health* is for all those men and women who are interested in scientific discovery, and in its industrial, commercial and social consequences. It will report, explore and interpret the results of human endeavour set in the context of science and society. Through *Journal of Medical Arthropodology & Public Health* scientists will be motivated to think beyond their discipline and believe that collaborative science and interdisciplinary ideas can advance national policies related to the control of vector-borne diseases, on one hand, and bring other biomedical concerns under thorough scanning and surveillance, on the other, to inspire new thinking. Thus, in this issue, our authors describe some of the challenges in controlling the various vector-borne & zoonotic diseases, besides exploring new horizons in the biology of medically important arthropods and pave pathways to consolidate new ideas toward their control. The issue, as always before, additionally offers its readers with fresh food for thoughts under the *Perspective Section* and an uncustomary, and yet innovative, motivational and elegantly archivable *Scientists' Biobibliography* Section which are unique in the world of scientific journals. Your satisfaction is our gain. We are experimenting with a new section: *Graphical Abstracts*, wherein, as an encouragement to young and budding medicoarthropodologists who presented their works as a poster during the latest SOMA Conference, such presentations are selectively incorporated to designate their research an undeniable scientific citation!

Soliciting your continued support and patronage in our comprehensive evolution together both as the journal and the authors, we remain, as heretofore,

Yours cordially,

Prof. Dr B.K. Tyagi & **Dr Rina Tilak**

June 1, 2024 Chief Editor Executive Editor

★ ★ ★ ★ ★

We deeply mourn passing away of

Padmashri Dr P.K. Rajagopalan

(27.10.1930 - 29.04.2024)

and

Prof. Karamjit Singh Rai

(1931 - 2024)

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Journal of Medical Arthropodology & Public Health
(Volume 4 • Number 1)

1st June, 2024

Perspective

'ARTHROPODYOGY' OR ARTHRPOD-MOTIVATED YOGIC EXERCISES TO KEEP HEALTHY

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Received : 19th April, 2024

Accepted : 17th May, 2024

ABSTRACT

History is witness that humans are greatly influenced by the behaviour of animals which they have emulated in developing varied designs, tools, machines and, of course, life style. One such array of behaviour is representation of the time-tested Yogic exercises for sustaining good health, vitality, and long age, which many of the arthropods of medical significance are found to depict in their manner of roosting or resting, exhibition of defense or offence tactics, and while at the wing etc. have not only been.

Keywords: Yog, arthropods, health

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Cite this article as:

Tyagi, BK 'Arthropodyogy' or arthrpod-motivated yogic exercises to keep healthy. *J Med Arthropodol & Public Health.* 2024; 4(1): 1-6.

DESCRIPTION

Arthropods are the most successful and diverse animal phylum, species of which make up more than 80% of all known species on Earth, with an estimated 1,170,000 species globally.[1] Modern molecular time-trees calibrated with fossils estimate the origins of arthropods to be in the Ediacaran (i.e., a geological period of the Neoproterozoic era that spans 96 million years from the end of the Cryogenian period at 635 Mya to the beginning of the Cambrian period at 538.8 Mya.) while most other deep nodes date to the Cambrian. The earliest stem-group arthropods were lobopodians, worm-like animals with annulated appendages.[2] They contain a wide diversity of animals with hard exoskeletons and jointed appendages. Many familiar groups of arthropod animals belong to insects, spiders, scorpions, centipedes, and millipedes on land, while crabs, crayfish, shrimp, lobsters, and barnacles in water. Arthropod species make up more than 80% of all known animal species on our planet, with a counted number of 1,170,000 species globally[3] and even estimated about 30 million extant species of arthropods[4].

On a rather conservative note, a global arthropod species richness of 5–10 million species of arthropods seems to be quite realistic. The dominance of arthropods, particularly insects and arachnids, among the world's animals is a fundamental scientific insight, yet one not widely appreciated. This dominance means that in numbers of species beyond our comprehension arthropods permeate diverse and essential natural processes in Earth's terrestrial, aerial and freshwater ecosystems, contributing to the function of the natural world as a self-sustaining biological system. They are, in fact, an integral and complex part of the terrestrial and freshwater ecosystems with which the past, present and future of humans are inextricably linked. Much of humans' vital knowledge has been inculcated from the varied arthropod lives and their behaviour to acquire yogic postures.

'*Yog'*, a life-revitalizing process or phenomenon originated and practiced since ages by sages, saints and priests in Bharat (India) as its birthplace, is derived from the Sanskrit root '*Yuj'*, meaning 'to join' or 'to yoke' or 'to unite' the worldly outer forces with the spiritual inner conscience. So, as per yogic scriptures of yore the practice of '*Yog*' leads to the union of individual consciousness with that of the Universal Consciousness, indicating a perfect harmony between the mind and body, Man and Nature. Thus, *Yog* is a way of living physically, mentally, psychologically and, above all, spiritually elevated, sound and healthy life. Yog has its cradle in

India. Yoga's origins can be traced to northern India over 5,000 years ago. The word yoga was first mentioned in ancient sacred texts called the *Rig Veda*. The Vedas are a set of four ancient sacred texts written in Sanskrit. Yoga is an ancient practice focusing on breathing, flexibility and strength to boost mental and wellbeing. It is composed of a group of physical, mental, and spiritual practices or disciplines. The main components of yoga are breathing and postures (a series of movements designed to increase strength and flexibility. In course of evolution man emulated many yogic exercises from the varied behavioural vicissitudes of animals including arthropods.

In this brief communication we are venturing to highlight the connect between man and arthropods such as arachnids (e.g., spiders, scorpions), and insects (e.g., dragonflies, mantis, ladybird and other beetles, bees, butterflies and their caterpillars, ants, (Figs. 1, 2) and even mosquitos (Fig. 3) – unequivocally the deadliest foe of man on Earth), in seeking various different postures on the prescription of our daily yogic exercises.

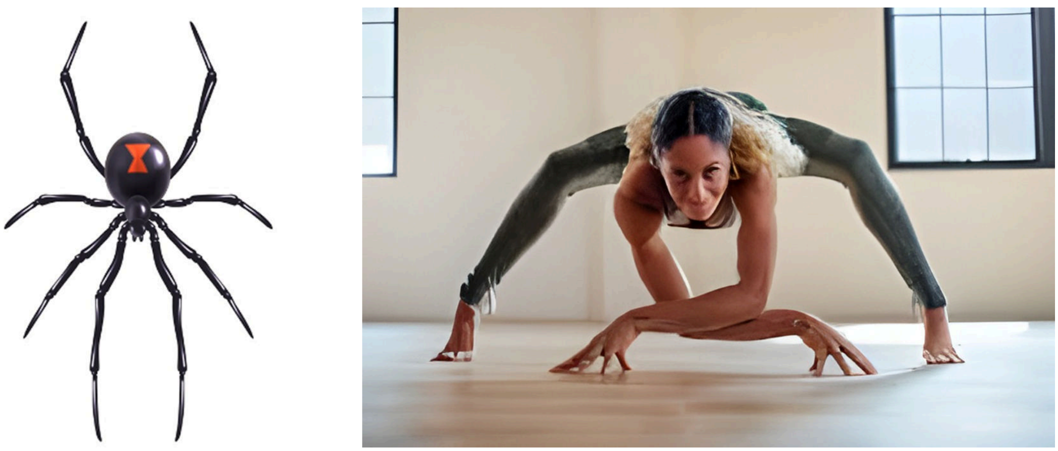

Fig. 1. *Utkata Konasana* – a Spider Pose.

Fig. 2. *Shirshaasan – a Dragonfly (Trithemis festiva)* Pose.

In chronological history mosquitoes were born far more earlier in the pre-Mesozoic era than even the oldest roots of modern man (*Homo sapiens*), only a few thousand years into the much recent Coenozoic Era. This implies that man always had before him the mosquito in all its historical glory of matchless behaviouristic mastery.

It is quite obvious to believe that man in yore had imbibed in him a lot more habits and actions, including that of *Yog*, from the various animals, e.g., *Mandooka-asan*, based on frog's sitting posture, Sarpa-asan adopted from cobra's posture etc.) and plants (e.g., *Taada-asan*, based on posture of the palm tree) in Nature. Many yogic postures are quite famously depicted by arthropods, especially insects (e.g., *Ashtanga-anasan*, based on butterfly caterpillar etc.) and spiders (e.g., *Uttana-asana*). But, there is no record of documentation of any kind so far where a mosquito has been a cynosure of the yogic exercise, albeit the fact that mosquitoes construct some of the yogic asans with great perfection and elan. In fact, there is no other group among insects where three different poses or postures of '*Yogaasan*' are presented as perfectly as by the three phenotypically different allies within an insect family as manifested by the species of the three scientifically well-acknowledged mosquito genera, *Anopheles*, *Culex* and *Aedes* (Fig. 3).

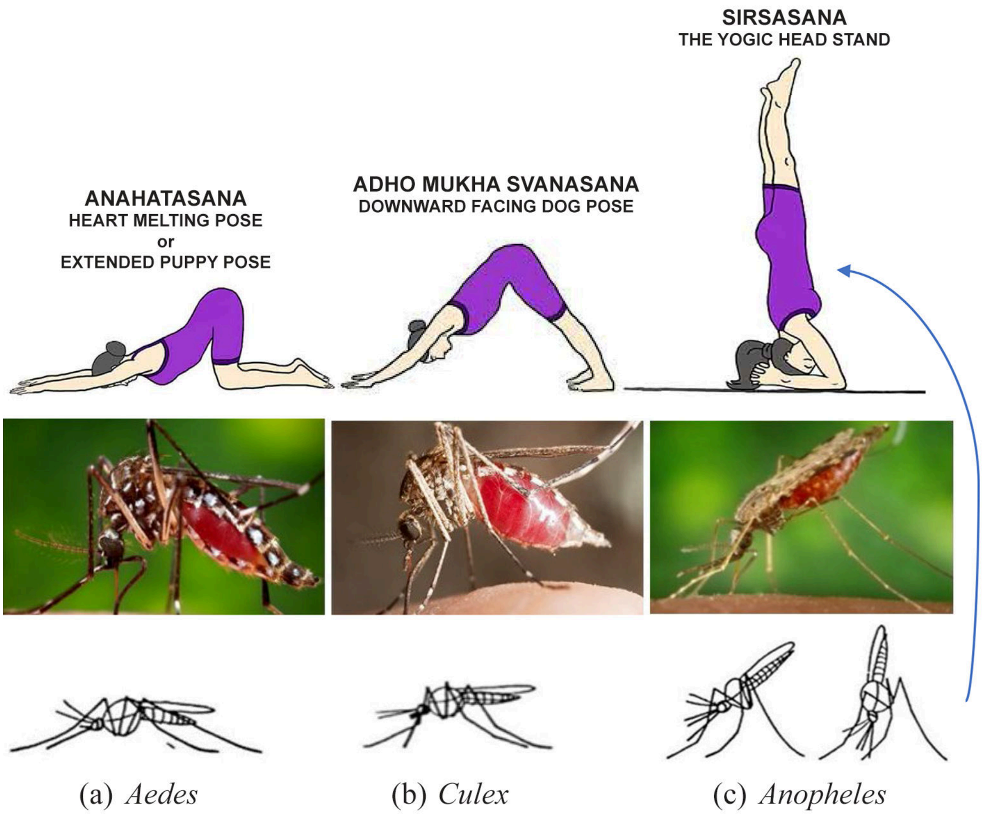

Fig. 3. Amazing natural yogic postures or '*Asans*' by different groups of mosquitoes, (a) *Aedes* (b) *Culex*, and (c) *Anopheles*.

In conclusion, a medically important arthropod's life can present splendours of varied hues, several of which may in fect prove highly motivational in improving values of human life as well such as, for example, their multiple posturing depictions emulated in Yog – an ancient *Bhartiya* practice focusing on breathing, flexibility and strength to boost energy and wellbeing.

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1st June, 2024

Original Article

LABORATORY EVALUATION OF LEMONGRASS OIL-BASED SILVER NANOPARTICLES COMBINED WITH BORIC ACID TOXIC BAIT AGAINST *AEDES AEGYPTI*

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Received : 16th March, 2024

Accepted : 30th May, 2024

ABSTRACT

Background: Recently, the eco-friendly plant-based synthesis of Silver Nanoparticles (AgNPs) has gained prominence as a promising approach for mosquito control. Combining Lgeo-AgNPs with Boric Acid Toxic Bait (BATB) represents a novel approach exploiting adult mosquitoes' feeding behaviour and utilizing boric acid as a potent toxin.

Material & Methods: Lemongrass essential oil-based Silver Nanoparticles (Lgeo-AgNPs) combined with Boric Acid Toxic Bait (BATB) were evaluated

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Cite this article as:

Naik BR, Blore K, Qualls WA, Aryaprema V and Xue Rui-De. Laboratory evaluation of lemongrass oil-based silver nanoparticles combined with boric acid toxic bait against *Aedes aegypti*. *J Med Arthropodol & Public Health*. 2024; 4(1): 7-18.

against *Aedes aegypti* mosquitoes. *Ae. aegypti* mosquitoes, with 15 females per 200mL paper cup, were subjected to various combinations of Lgeo-AgNPs and BATBs in bioassay treatments. Bioassays were carried out at 24.6°C ± 1°C, 60% ± 5% relative humidity, and a 14L:10D photoperiod. Mosquito mortality /grounded / knockdown was recorded every 3, 6, 12, and 24 hours. Each experiment was quadruplicated and repeated thrice.

Results: A multiple comparisons test, such as the Least Significant Difference (LSD), was conducted to assess mean differences among various exposure durations (e.g., 3 hrs, 6 hrs, 12 hrs, and 24 hrs) for the "mortality /grounded / knockdown count" variable. The results revealed significant mean variations across all exposure time intervals ($p < 0.001$), indicating a substantial impact of exposure duration on mosquito mortality rates. The combination of 0.25% Lgeo-AgNPs with 1% BATB demonstrated significant efficacy, resulting in rapid knockdown and mortality of *Ae. aegypti* mosquitoes within a short exposure time.

Conclusion: A combination of 0.25% Lgeo-AgNPs with 1% BATB was effective against *Ae. aegypti* adults. Further optimization and long-term evaluation of this formulation are recommended to establish sustainable adulticidal mosquito control strategies for mitigating the transmission of vector-borne diseases.

Keywords: *Aedes aegypti*, Boric acid Toxic Bait, Lemongrass essential oil, Nanoparticles

INTRODUCTION

With its worldwide distribution, *Aedes aegypti* (L.) (Diptera: Culicidae) is crucial in transmitting arboviral diseases such as dengue, chikungunya, yellow fever, and Zika. The success of this species can be attributed to its behavioural plasticity, rapid development, desiccation-resistant eggs, resistance to typical insecticides, preference for urban environments, proximity to humans, and a tendency to bite humans during daylight hours while seeking resting places in moist vegetation and indoors[1]. Ensuring mosquito populations remain below the tolerable threshold necessary to prevent or control arboviral transmission is a critical challenge in combating arboviral disease prevention. A growing number of studies aimed at controlling Aedes species by mass-trapping using diverse approaches to lure, capture and eliminate, or to use mosquitoes to disseminate control agents to decrease their population[2]. The World Health Organization has launched the Global Arbovirus Initiative, recognizing the increasing global threat posed by arboviruses transmitted by Aedes mosquitoes. This initiative aims to monitor, prevent, and respond to the growing risk of arbovirus disease by fostering collaboration among key partners, strengthening capacity-building, conducting research, and enhancing preparedness and response strategies[3].

Control strategies for managing *Aedes* mosquitoes and preventing arboviral transmission include source reduction, larviciding, and adulticiding measures[4]. Developing effective adulticidal strategies becomes imperative in addressing the challenges of mosquito-borne diseases. However, adulticidal chemical control strategies have diminished effectiveness due to growing obstacles, such as insecticide resistance and the widespread availability of potential developmental sites in urban areas. These factors have contributed to increased vector dispersal and the global spread of disease epidemics[1]. Moreover, despite various existing vector control tools, they have yet to achieve complete success. Consequently, this lack of effectiveness has resulted in multiple issues, such as insecticide resistance, resurging mosquito populations, adverse effects on humans and non-target organisms, and disruption of natural ecosystems. Alternative approaches are necessary to overcome these challenges and improve the efficacy of mosquito control measures[5].

The initial studies by Xue and Barnard[6] on boric acid as an active ingredient of the Attractive Toxic Sugar Baits (ATSB) provided valuable insights into their effectiveness for attracting and controlling *Ae. albopictus* Skuse populations. This has contributed to our understanding of boric acid toxic bait (BATB) that may have potential as a mosquito control agent. The ATSB research has led to active and exciting areas of research on targeted sugar baits for mosquito control[7-8]. Here, we attempted to evaluate Lemongrass essential oil-based Silver Nanoparticles (Lgeo-AgNPs) combined with Boric Acid Toxic Bait (BATB) as an effective toxic substance against *Ae. aegypti*. This innovative approach, specifically targeting the elimination of adult mosquito populations, has the potential to become a successful tool in effectively controlling mosquito-borne diseases and addressing the public health concerns associated with such diseases.

MATERIAL AND METHODS

***Mosquitoes*:** Three- to five-day-old adult *Ae. aegypti mosquitoes (Orlando strain) were used in* the experiment. The mosquitoes were reared at AMCD (Anastasia Mosquito Control District) insectary under controlled conditions at a temperature of 26.6°C ± 1°C, 70% ± 10% relative humidity, and a 14L:10D photoperiod.

Chemicals: Lemongrass essential oil was obtained from Majestic Pure Cosmeceuticals (San Diego, CA, USA). Silver nitrate was obtained from Sigma Aldrich (St. Louis, MO, USA). Tween 20 was used to emulsify lemongrass essential oil into the silver nitrate solution and was obtained from Sigma Aldrich (St. Louis, MO, USA).

Treatment solution: Lemongrass essential oil-based Silver nanoparticles (Lgeo-AgNPs) were synthesized via lemongrass essential oil (% v/v) emulsified into an equal volume of *Tween 20* and added dropwise to a 0.31 mMol $AgNO_3$ solution. The reactant mixture was heated under constant agitation at 100°C for one hour. Nanoparticle formation was confirmed by a visual change of colour followed by subsequent characterization. The synthesised Lgeo-AgNPs solution was mixed with boric acid (BA) as bait in different concentrations, as specified in Table 1.

Experiment: *Aedes aegypti* mosquitoes were placed in 200mL paper cups (15 female mosquitoes per cup). Several experimental combinations of Lgeo-AgNPs and BATBs were prepared and used for bioassay treatments. The control solution consisted of a 10% sucrose solution. The cups were covered with nylon mosquito netting, and a cotton ball saturated with treatment solution was placed on top of each cup to feed at *ad libitum*. Bioassays were conducted at room temperature of 24.6°C ± 1°C, 60% ± 5% relative humidity, and a 14L:10D photoperiod. The dead and grounded mosquitoes were recorded at 3hr, 6hr, 12hr and 24hrs intervals. Each experiment was in quadruplicate and replicated three times.

Fig. 1. Dead mosquito showing an engorged abdomen due to a dyed blue colour after exposure of blue dyed toxic baits.

An additional experiment was conducted to confirm that 90% of dead female mosquitoes took in the Lgeo-AgNPs and boric acid toxic baits by observing their abdomens with blue food dye (Fig. 1).

Data Analysis. A comprehensive result analysis was conducted in the study evaluating the effectiveness of lemongrass oil-based silver nanoparticles combined with boric acid toxic bait against *Ae. aegypti* mosquitoes. The analysis involved performing normality testing, ANOVA, the Duncan test, multiple comparisons, and descriptive statistics to assess the treatment's efficacy. Firstly, normality testing was performed using the Kolmogorov-Smirnov test, which indicated that most experiments (Experiments Nos: 1, 2, 3, 7, 5, 6, 12, 8, 9, 11, and 13) followed a normal distribution ($p > 0.05$). However, experiments No. 4 and 14 (control group) demonstrated non-normal distribution patterns ($p < 0 .05$).

RESULTS

Analysis of variance (ANOVA) of the data, it was found that both the “Experiments” and “Exposure time” factors had a significant effect on the “mortality count” variable ($p < 0.001$). Additionally, a significant interaction effect was observed between the factors “Experiments” and “Exposure time” ($p < 0.001$), indicating that the combined impact of these factors influenced mosquito mortality (Fig.2).

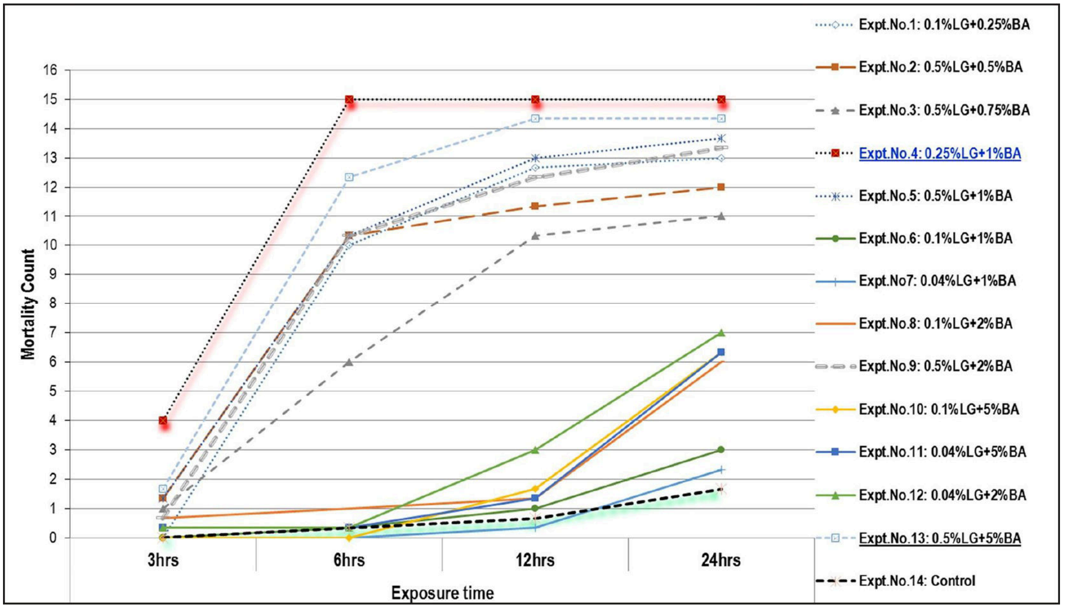

Fig. 2. Efficacy of Lgeo-AgNPs combined with BATB against *Aedes aegypti* (n=15)

The Duncan test was then utilized to identify homogeneous subsets based on the mean values of the "mortality count" variable. The test revealed six subsets with distinct mean values: Subset 1: Control and Expt. No.7 (0.04%Lgeo-AgNPs+1%BA) had similar mean values ($p = 0.051$). Subset 2: Expt. No. 6, 10, 11, and 8 showed identical mean values within the subset ($p > 0.05$). Subset 3: Expt. No.12 (0.04%Lgeo-AgNPs+2%BA) and Expt. No.3 (0.5%Lgeo-AgNPs+0.75%BA) displayed similar mean values ($p > 0.05$). Subset 4: Expt. No. 2, 1, 9, and 5 demonstrated similar mean values within the subset ($p > 0 .05$). Subset 5: Expt. No.13 (0.5%Lgeo-AgNPs+5%BA) showed noticeable mean values compared to all other subsets ($p < 0.05$). Subset 6: Expt. No.4 (0.25%Lgeo-AgNPs+1%BA) stood out prominently with distinct mean values that significantly differed from all other subsets ($p < 0.05$).

A multiple comparisons test (e.g., Least Significant Difference - LSD) was performed to compare the means of different exposure times (hrs) for the "mortality count" variable. The results indicated significant mean differences between all pairs of exposure time intervals (3hrs, 6hrs, 12hrs, and 24hrs) ($p < 0.001$) (Table 1). This finding suggests that the exposure time significantly affected the mosquito mortality rate.

Additional tests with blue food dye with the Lgeo-AgNPs and boric acid toxic baits showed that the dead adult mosquitoes caused by the different toxic baits were not the result of starvation or contact poisoning after exposure to such low concentrations of Lemongrass oils.

Finally, descriptive statistics were calculated, providing mean and standard deviation values for the "mortality count" variable, grouped by the factors "Experiments" and "Exposure time." The analysis offered an overview of observed mortality counts' central tendency and variability across different experimental conditions and exposure times.

Table 1. Evaluation of Lemongrass Essential oil- based Silver Nanoparticles (Lgeo-AgNPs) combined with Boric Acid Toxic Bait (BATB) against *Aedes aegypti* (n=15)

Exp vs Mortality count	Exposure Time								
	3hrs		6hrs		12hrs		24hrs		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Expt.No.1: 0.1%Lgeo-AgNPs+0.25%BA	0.00	0.00	10.00	2.65	12.67	1.15	13.00	1.00	.000
Expt.No.2: 0.5% Lgeo-AgNPs +0.5%BA	1.33	1.53	10.33	4.62	11.33	3.06	12.00	2.65	.010
Expt.No.3: 0.5% Lgeo-AgNPs +0.75%BA	1.00	1.00	6.00	0.00	10.33	1.53	11.00	1.73	.000
Expt.No.4: 0.25% Lgeo-AgNPs +1%BA	4.00	2.00	15.00	0.00					.000
Expt.No.5: 0.5% Lgeo-AgNPs +1%BA	1.33	0.58	10.33	2.52	13.00	1.00	13.67	1.15	.000
Expt.No.6: 0.1% Lgeo-AgNPs +1%BA	0.00	0.00	0.33	0.58	1.00	1.73	3.00	1.00	.031
Expt.No.7: 0.04% Lgeo-AgNPs +1%BA	0.00	0.00	0.00	0.00	0.33	0.58	2.33	1.15	.006
Expt.No.8: 0.1% Lgeo-AgNPs +2%BA	0.67	1.15	1.00	1.00	1.33	2.31	6.00	1.73	.012
Expt.No.9: 0.5% Lgeo-AgNPs +2%BA	0.67	1.15	10.33	4.51	12.33	2.52	13.33	1.53	.002
Expt.No.10: 0.1% Lgeo-AgNPs +5%BA	0.00	0.00	0.00	0.00	1.67	2.08	6.33	3.21	.011

Exp vs Mortality count	Exposure Time								
	3hrs		6hrs		12hrs		24hrs		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Expt.No.11: 0.04% Lgeo-AgNPs +5%BA	0.33	0.58	0.33	0.58	1.33	0.58	6.33	1.53	.000
Expt.No.12: 0.04% Lgeo-AgNPs +2%BA	0.33	0.58	0.33	0.58	3.00	2.00	7.00	0.00	.000
Expt.No.13: 0.5% Lgeo-AgNPs +5%BA	1.67	2.08	12.33	4.62	14.33	0.58	14.33	0.58	.001
Expt.No.14: Control 10% Sucrose solution	0.00	0.00	0.33	0.58	0.67	1.15	1.67	1.53	.274
P-value	**.005**		**.000**		**.000**		**.000**		

In summary, the comprehensive result analysis demonstrates the effectiveness of lemongrass essential oil-based silver nanoparticles combined with boric acid bait in controlling *Ae. aegypti* mosquitoes. The study revealed standard distribution patterns for most experiments, significant effects of experimental factors and exposure time on mosquito mortality, distinct subsets based on mean values, important differences between exposure time intervals. It provided descriptive statistics to understand the mortality count - the standout performance of Expt. No.4 (0.25%Lgeo-AgNPs+1%BA) within a shorter exposure time frame highlights its potential as a promising approach in mosquito control. Further research can focus on optimizing the formulation and understanding its mechanisms to enhance mosquito control strategies and mitigate the spread of mosquito-borne diseases.

DISCUSSION

One of the latest alternatives to conventional insecticides is plant-based metal nanoparticles, which have demonstrated toxicity against all life stages of mosquitoes at different concentration levels. The unique properties of nanoparticles are a high surface area-to-volume ratio, facilitating the efficient delivery of active ingredients to the target site[5]. These nanoparticles' size, shape, and stability are influenced by the concentration of the plant extract or metabolite and the concentration of metal ions in the substrate[9]. These nanoparticles have been screened for various mosquito control activities, including ovicidal, larvicidal, pupicidal, adulticidal, and repellent properties[10]. The phytochemical compounds can disrupt the receptor site of the nervous system through different metabolic pathways, such as monoterpenes acting on the Na+/K+ ion channel, while flavonoids target as Acetylcholinesterase inhibitors[11]. The plant essential oils significantly inhibited both cytochrome P450 and glutathione S-transferase activities, suggesting that inhibiting detoxification contributes to the enhancement or synergism of plant crucial oils for permethrin and pyrethroids[12-14]. This innovative and environmentally friendly approach involves synthesizing plant-based metallic nanoparticles that contain bioactive phytochemicals that are safe, biodegradable, and hold promise as a strategy for controlling mosquito-borne diseases[15-18]. However, most studies have focused on the larvicidal efficacy of metal nanoparticles, with few studies examining their adulticidal potential[19]. The growing challenges posed by mosquito-borne diseases have generated our interest in exploring effective control strategies.

Our study findings demonstrated that the combination of 0.25% Lgeo-AgNPs and 1% BATB was the most effective, leading to 100% mosquito mortality within 6 hours of exposure. This combination proved highly effective in controlling *Ae. aegypti* mosquitoes, highlighting the importance of optimising the concentration of nanoparticles and toxic bait for effective mosquito control.

Comparisons with other published studies on Attractive Toxic Sugar Bait (ATSB) ingredients and mosquito species revealed varying results. Xue and Barnard6 tested 1% boric acid in a 10% sucrose solution for *Ae. albopictus*, achieving ≥98% mortality after 48 hours. Xue *et al.*[20] performed semi-field trials with *Ae. albopictus* and *Culex nigripalpus*, using 1% boric acid in a 5% sucrose solution, resulting in ≥96% mortality after 48 hours. Muller *et al*.[21] conducted outdoor field trials using a mixture of nectarines, red wine, brown sugar, red food dye, spinosad, and BaitStab sprayed on plants, showing 91% mortality for *Anopheles sergentii* and 67% for *Ae. caspius* after 30 days. These studies demonstrate the varied effectiveness of different formulations and ingredients against different mosquito species in laboratory and field conditions. Studies of Kumar *et al*.[22] have reported that the Toxic Sugar Baits (TSB) induced complete mortality of *Ae. aegypti* and *An. stephensi* at a 4% concentration of boric acid. Meanwhile, *An. culicifacies*, 100% mortality was attained at a 3% concentration of TSB solution. Moreover, the TSB solution containing 2% boric acid resulted in 99.1% mortality in *An. culicifacies*, with approximately 95% mortality observed in both *An. stephensi* and *Ae. aegypti.*

The findings of our study demonstrate significant efficacy within a short exposure time of 6 hours and a higher percentage of mosquito mortality using the specific combination of 0.25% Lgeo-AgNPs and 1% BATB in laboratory conditions, marking a noteworthy advancement in mosquito control strategies. However, a deeper understanding of the mechanism by which Lgeo-AgNPs and BATBs induce mortality is required, and further research is needed to validate their effectiveness. Field assessments are essential to evaluate this approach's efficiency, feasibility, and potential impact on adulticidal mosquito control strategies. Additionally, a comprehensive investigation is necessary to evaluate the long-term effects on non-target organisms and assess the environmental implications of this approach.

CONCLUSION

This study extensively evaluated the impact of lemongrass essential oil-based silver nanoparticles (Lgeo-AgNPs) combined with boric acid (BA) bait on the mortality of *Ae. aegypti* mosquitoes. The analysis of variance (ANOVA) results indicated that both the experimental conditions and exposure time significantly influenced mosquito mortality. A notable interaction effect between these factors further emphasized their combined impact on mortality rates.

The Duncan test identified six homogeneous subsets of mean mortality counts, with Experiment No. 4 (0.25% Lgeo-AgNPs + 1% BA) standing out as significantly different and more effective compared to other subsets. Additionally, a multiple comparisons test revealed significant differences in mortality across various exposure times (3, 6, 12, and 24 hours), reinforcing the importance of exposure duration in mosquito control strategies.

Descriptive statistics provided a comprehensive overview of mortality counts' central tendency and variability across different experimental setups and exposure times. The findings highlight the standout performance of Experiment No. 4 within a shorter exposure time frame, showcasing its potential as a promising approach for mosquito control.

Furthermore, additional tests confirmed that the observed mosquito mortality was due to the toxic baits rather than starvation or contact poisoning. This reinforces the efficacy of Lgeo-AgNPs combined with boric acid in mosquito control.

In conclusion, this study demonstrates the significant potential of Lgeo-AgNPs combined with boric acid bait in controlling *Ae. aegypti* mosquitoes. The results underscore the importance of optimizing nanoparticle concentration and exposure time for effective mosquito control. Future research should focus on understanding the mechanisms underlying the observed mortality, optimizing formulations, and conducting field assessments to validate these findings. Additionally, evaluating the long-term effects on non-target organisms and environmental implications is crucial for developing sustainable mosquito control strategies.

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1st June, 2024

Original Article

CULICOIDES SPP. (DIPTERA: CERATOPOGONIDAE) AND THEIR ASSOCIATED BACTERIAL COMMUNITIES, ENDOSYMBIONTS: A REVIEW

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Received : 17th April, 2024

Accepted : 28th May, 2024

ABSTRACT

The biting midges, *Culicoides* spp. (Diptera: Ceratopogonidae) are small haematophagous dipterans, demonstrated as vectors of multiple pathogens such as arboviruses, protozoa, fungi, and nematodes of veterinary and public health importance. Bacteria-specific information is far less despite its relevance in spreading arboviral diseases, these flies still need to be addressed. This article deals with the data on the association of bacteria and endosymbionts harboring in different biting midge species belonging to different subgenera. The bacteria influence insects' physiology, such as digestion and oviposition, and their hosts' survivability and vectorial efficiency. Bacterial strain-

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Cite this article as:

Sarkar A, Banerjee P, Mazumdar A. *Culicoides* spp. (Diptera: Ceratopogonidae) and their associated bacterial communities, endosymbionts: a review. *J Med Arthropodol & Public Health*. 2024; 4(1): 19-34.

specific information is needed to correlate with these midges. However, few studies recorded pathogenic bacteria's association with vector species' life stages. Along with this, our analysis also corroborates with possible routes of pathogens' transmission and organ-specific localization. Future studies will be required to generate information on developing some putative bio-control strategies by utilizing these bacterial species or strain-specific midge-associated bacteria. The zoonotic potential of bacterial pathogens associated with the vector species requires attention.

Keywords: Biting midges, bacteria, endosymbionts, vectors

INTRODUCTION

Adult individuals belonging to *Culicoides* spp. (Diptera: Ceratopogonidae) are the smallest haematophagous flies[1]. Worldwide, 1347 species have been identified and distributed from the tundra to the tropics and from sea level to 4000 m, except Antarctica and New Zealand[1]. Several species have achieved notoriety for inflicting nasty bites, causing severe arboviral disease outbreaks, and transmitting protozoa and filarial worms affecting livestock, birds, and even humans[1]. More than 50 viruses such as African horse sickness virus (AHSV), Akabane virus (AKAV), Aino virus (AINOV), Chuzan virus (CHUV), D'Aguilar virus (DAGV), Ibaraki virus (IBAV), bluetongue virus (BTV), epizootic haemorrhagic disease virus (EHDV), have been isolated from them[1]. Most notably the BTV, which has gained prime significance in the Indian scenario[1,2]. Of the 29 BTV serotypes, 23 were recorded from this country[2]. The following seven *Culicoides* species vectoring BTV belong to three subgenera, i.e., i) *Avaritia* (*Culicoides actoni* Smith, *Culicoides fulvus* Sen and Das Gupta, *Culicoides brevitarsis* Kieffer, *Culicoides imicola* Kieffer, *Culicoides orientalis* Macfie), ii) *Hoffmania* (*Culicoides peregrinus* Kieffer), and iii) *Remmia* (*Culicoides oxystoma* Kieffer) documented from India[3,4]. Besides BTV, all seven species are also implicated as vectors of other pathogens. A summary table depicting their wide distribution within India and pathogen-specific information are mentioned[5]. Besides that, nematode: *Onchocerca gibsoni*, the causative agent of filaria of cattle, and following protozoa: *Leucocytozoon* sp., an intracellular haemosporidian blood parasite, *Leishmania* (*Mundinia*) *martiniquensis*, *L.* (*M.*) *orientalis* and *Crithidia* spp. were detected from various midges[5]. Data regarding the host ranges of these vector species were also collected, and it was recorded that these species were generally mammalophilic and ornithophilic[5]. Earlier review articles focused primarily on arboviruses, not on the bacteria. Bacteria can impact vectoring efficiency and provide several beneficial services to their hosts, such as nutrition, developmental time, reproduction, blood meal digestion, and egg production in haematophagous vectors[6,7,8,9,10]. These bacterial communities can emanate vertically from their parental origin and congeners or may be acquired via horizontal transmission through larval feeding and via ingestion of blood meal in adult stages[11,12,13]. Few bacteria residing within the development habitat mediate attraction and elicit oviposition responses[14]. In addition, the significance of the bacterial communities during larval development has been well studied in mosquitoes and sandflies. When antibiotics were used, mosquitoes' delayed growth and decreased larval survival were noted; however, these were retrieved by inoculating certain bacteria[9,15]. Likewise, incomplete larval development of *Culicoides stellifer* (Coquillet) was observed when rearing medium containing autoclaved tap water[16]. Antibiotic-treated blood-fed *Aedes aegypti* decreased the lysis of red blood cells and reduced the rate of digestion of blood proteins and production of viable eggs in the first gonotrophic cycle[8]. Blood meal digestion leads to oxidative stress by increasing reactive oxygen species (ROS) levels in mosquitoes, and metabolites or antioxidants synthesized by them play crucial roles in heme-induced redox homeostasis[17]. The gut bacterial composition changes due to antibiotic treatment lead to an increased infection rate of *Culicoides nubeculosus* Meigen with Schmallenberg virus[18]. Arthropod-borne bacteria will constitute an essential reservoir of emerging diseases in the future[19]. So, information regarding the load of pathogenic bacteria within these vectors still needs to be included. Only one study regarding pathogenic bacteria documented the blood-borne pathogen, *Bartonella* sp., within *Culicoides* spp. indicating bacterial zoonosis infecting humans, domestic and wild animals[20]. Horizontal transmission includes regurgitation, salivation, through the cuticle, stercoration, and in rare cases, ingestion of the vector via blood feeding[21]. In addition, endosymbiotic bacteria can reduce the longevity of the vectors and can be used to interrupt onward viral transmission as they may affect vector competence by decreasing host susceptibility to viruses[7,22]. This article combines information on the association of bacteria and endosymbionts with the vector species.

Subgenus *Avaritia*

The impact of biotic and abiotic factors on bacterial composition was recorded in *C. imicola*[12]. Shared core microbiomes included *Pseudomonas*, *Escherichia*, *Halomonas*, *Candidatus*, and *Propionibacterium* among the populations of this vector species. Unique bacterial genera were found in *C. imicola* trapped from each location, indicating similarities and divergences in microbiome composition between the two populations[12].

Our study identified bacterial species belonging to two major phyla, i.e., Firmicutes and Proteobacteria, from gut and salivary glands of these vector species: *C. actoni*, *C. fulvus*, *C. imicola*, *C. orientalis*, and *C. jacobsoni* Macfie. Bacterial communities were compared across sexes and physiological ages of females. Within the salivary glands of these midges, the following bacteria: *Bacillus* sp., *Brevibacillus parabrevis*, *Paenibacillus lautus*, *Enterococcus faecium*, *Serratia marcescens*, *Pseudomonas stutzeri* were retrieved, and from the gut, *Bacillus* spp. *Brevibacillus parabrevis*, *Cytobacillus kochii*, *Lysinibacillus* spp., *Paenibacillus lautus*, *S. marcescens*, *Pseudomonas stutzeri* were identified. *Bacillus* spp. was the most abundant, followed by *Lysinibacillus* spp.

The gut microbiota of field-collected adults of four species, i.e., *C. chiopterus* (Meigen), *C. dewulfi* Goetghebuer, *C. obsoletus* (Meigen), and *C. scoticus* Downes and Kettle belonging to *Obsoletus* group from Sweden, Netherlands, and Italy were studied[23]. The gut bacterial communities of *C. chiopterus* differed from the other three species. Bacterial communities were similar for *C. obsoletus* across adults trapped in three countries, while it varied in the case of *C. scoticus*. These bacteria, namely, *Bacillus*, *Pseudomonas*, Enterobacteriaceae, and *Sphingomonas*, were commonly retrieved across the species of this group[23].

The endosymbionts, *Cardinium*, and *Wolbachia*, were detected from *C. brevitarsis* and *C. imicola*, while only *Cardinium* was identified from *Culicoides wadai* Kitaoka[24,25]. Previously, *Cardinium* spp. was not detected in species of the *obsoletus* group, the primary vector of bluetongue and Schmallenberg viruses in northern Europe[26]. After that, records on occurrences of *Wolbachia* and *Cardinium* in *C. obsoletus*[27]. Furthermore, another endosymbiont, *Rickettsia*, was recently found in field-collected adults of *C. dewulfi*, *C. obsoletus*, and *C. scoticus*. *Cardinium* was found in field-collected adults of *C. scoticus*, and *Wolbachia* was found in field-collected adults of *C. dewulfi*, *C. obsoletus*, *C. scoticus*, *and C. chiopterus*[23].

Subgenus *Hoffmania*

The first bacterial study was performed in engorged and nulliparous females of *C. peregrinus* to isolate and identify two haemolytic bacterial strains[28]. The strains, CU1A, CU1B of *Bacillus pumilus* and CU2B of *Bacillus licheniformis* were identified. They suggested that the shortening of blood meal digestion time was due to the presence of these haemolytic bacteria. The quantitative enzyme assay recorded that CU1A synthesized protease, and CU1B and CU2B produced amylase and protease[28]. Furthermore, fourteen culturable haemolytic bacterial strains were identified from different life stages (eggs, four larval instars, pupa). They were compared between reared and field-collected adults, including age-graded females (nulliparous, engorged, and parous)[29]. Most of the bacteria belonged broadly to these phyla, Firmicutes and Proteobacteria. Four strains of *Bacillus cereus* (CU2B, CU3G, CU6A, and CU1E) and two strains of *B. licheniformis* (CU7C and CU1A) were isolated from life stages reared in the laboratory. From eggs to lab-emerged adults, *B. cereus* (CU6A, CU1E), *Paenibacillus* sp. (CU9G), *Alcaligenes faecalis* (CU5A), *Brevundimonas* sp. (CU3C), and *Enterococcus faecium* (CU4B) were recovered. Males and nulliparous females shared four bacterial strains, while engorged and parous females also had four strains in common. The most exciting observation was that except for *B. licheniformis* (CU7C), all other isolated bacterial strains were commonly detected from eggs and post-oviposited females. All bacterial strains were beta haemolytic except *Alcaligenes faecalis*, showing alpha haemolysis on blood agar. Besides that, bacterial strains' colony morphology i.e., shape, color, margin, elevation, consistency, and opacity, was recorded. In vitro biochemical characterization tests including catalase, oxidase, citrate utilization test, indole, growth on Triple Sugar Iron (TSI) agar, methyl red, and carbohydrate fermentation test on six sugars (trehalose, sucrose, arabinose, mannitol, mannose, lactose) were carried out. Along with this, antibiotic susceptibility tests of these isolates were done on different wide-range antibiotics, namely, ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, and tetracycline. All of the identified bacterial strains could synthesize amylase and protease. Throughout the life history of the vector, two strains of *B. cereus* (CU6A and CU1E) and one strain of *Paenibacillus* sp., i.e., CU9G, were detected, hinting towards their probable role in ingested blood meal digestion[29]. Similarly, metagenomic analysis of bacterial communities associated with the life stages of this vector species depicted these two predominant phyla, Firmicutes and Proteobacteria[30].

The bacterial composition was much more diverse in the pupa than in the other life stages of this vector species[30]. *Pseudomonas* spp. was (26-37%) identified throughout all life stages. The abundance of *Bacillus* spp. was slightly increased within the 2nd, 3rd, and 4th instars, with a decrease of *Stenotrophomonas* sp. However, *Stenotrophomonas* and *Pseudomonas* were abundant in this vector species' lab-emerged and field-collected females. High relative abundance of the following bacterial genera, *Stenotrophomonas*, *Pseudomonas*, and *Bacillus*, were seen throughout life stages, which might indicate transstadial transmission[30]. Furthermore, *Alcaligenes faecalis* (CU5A), *Bacillus* sp. (CU5D), *B. cereus* (CU6A, CU3G, CU1E), *B. flexus* (CU8B), *B. licheniformis* (CU1A), *Brevundimonas* sp. (CU3C), *Enterococcus faecium* (CU4B), *Paenibacillus* sp. (CU9G), *Proteus terrae* (CU3B), and *Proteus vulgaris* (CU3A) were isolated throughout the life stages and assumed to be possible transstadial transmission[29].

Culicoides ohmorii Takahashi (synonym of *C. sumatrae* Macfie) and *C. peregrinus* had infections with *Cardinium*[31]. The following endosymbionts *Rickettsia* was noticed in the eggs, 4th instar larva, pupa, and lab-emerged females, while *Wolbachia* was detected only from the 4th instar larva of this vector species[30].

Subgenus *Remmia*

Culicoides oxystoma is a significant species of this subgenus due to its worldwide distribution, high abundance near livestock, and vectoring of various arboviruses, nematodes, and protozoa[5,32,33]. The first study was performed to identify haemolytic bacterial strains and found two similar strains of *Bacillus pumilus* and one strain of *Bacillus licheniformis* from engorged and nulliparous females of *C. oxystoma*[28]. After that, our unpublished data observed that Firmicutes and Proteobacteria are the predominant Phyla, of which *Bacillus* spp. was the most abundant across the life stages. Across the life history (egg, larval instars, pupa, adults), strains of *B. cereus*, *B. pumilus*, *B. tropicus*, *Lysinibacillus* sp., *Paenibacillus* sp., and *Pseudomonas* sp. were retrieved routinely.[34] Besides that, it was observed that *B. cereus* and

Alcaligenes faecalis were shared between natural breeding sites and rearing medium.[34]

Only one endosymbiotic bacteria, *Cardinium* (31.4%), was detected from *C. oxystoma*, and they assumed that the variation of the infection rate of this endosymbiont within *Culicoides* spp. influenced by environmental conditions[24].

Subgenus *Monoculicoides*

The microbial communities of colonized and field-collected pupae, adults and also compared between the microbial communities identified from colonized rearing medium and natural breeding site of *Culicoides variipennis* (Coquillett)[35]. Along with this, bacterial composition of antibiotic treated and untreated midges of two vector species such as *Culicoides nubeculosus* (Meigen) and *Culicoides sonorensis* Wirth and Jones were investigated and it was observed that *Asaia* sp. was dominant among untreated *C. nubeculosus* while relative frequency of *Sphingomonas* sp. was increased within antibiotic treated flies[18]. In contrast the relative frequency of bacteria belonged to family Acetobacteraceae was abundant within untreated *C. sonorensis*, however it was decreased within antibiotic treated flies and *Delftia* sp. was increased[18]. Subsequent changes in the gut bacterial communities within the post antibiotic treated flies of *C. nubeculosus* recorded with increased infection rate of Schmallenberg virus (SBV)[18].

Two endosymbionts namely *Cardinium* sp. and *Rickettsia* sp. were detected from *C. sonorensis* and *C. nubeculosus*[18,36]. Bacterial communities and endosymbionts identified from other *Culicoides* spp. belonging to other subgenera and species groups were summarized into Table 1, Table 2 and Figure 1[12,18,23,24,25,26,27,29,30,31,34,35,37].

CONCLUSION

Firmicutes and Proteobacteria are the predominant phyla among these vector species. *Bacillus* spp. in particular several strains of *B. cereus* was the most abundant in all of the species of these subgenera, indicating their close association with these midges and their in vivo role besides blood meal digestion. *Pseudomonas* and *Lysinibacillus* are the important genera identified from species of *Avaritia*, *Remmia*, and *Monoculicoides* indicating their significance within them.

Table 1. Bacteria identified from various *Culicoides* spp.

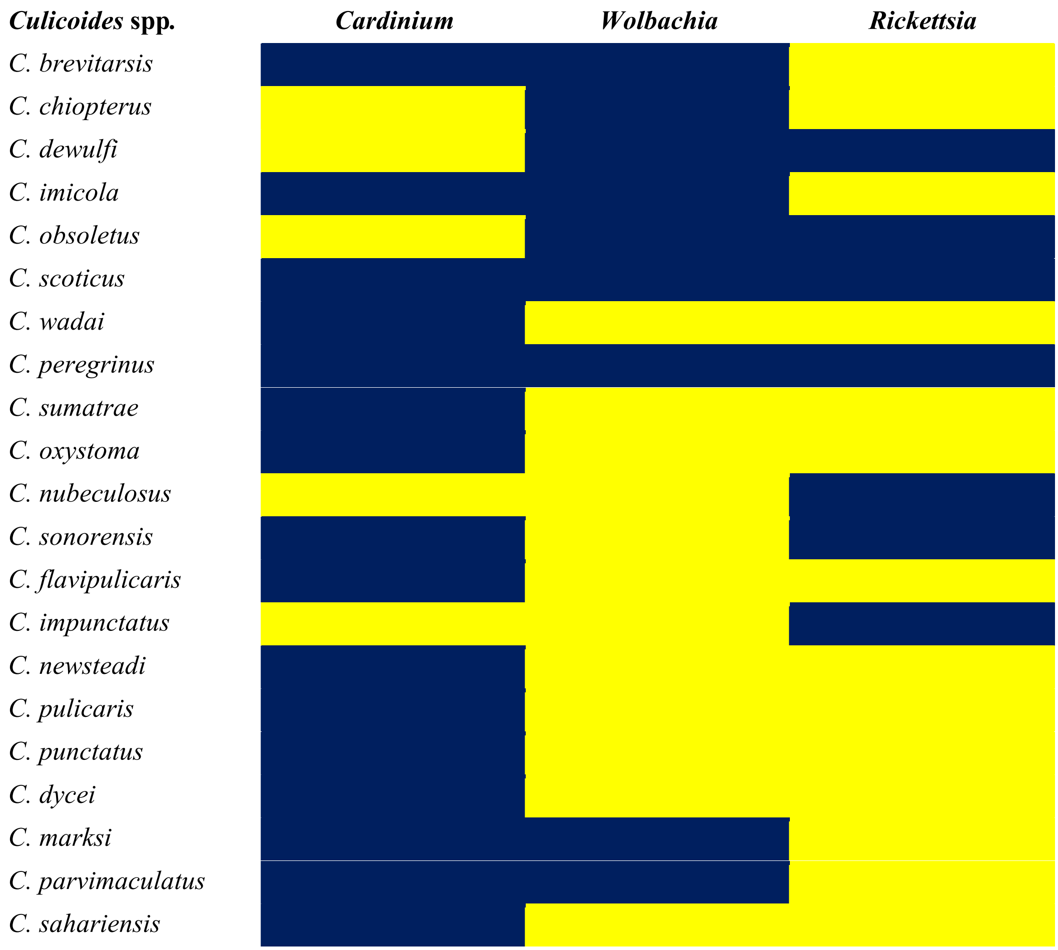
Subgenus	Species	Bacterial families/genera/species
Avaritia	*Culicoides actoni*	*Bacillus cereus*, *Bacillus paramycoides*, *Bacillus pumilus*, *Bacillus flexus*, *Bacillus megaterium*, *Lysinibacillus* spp., *Paenibacillus* sp., *Serratia marcescens*
	Culicoides chiopterus	*Pseudomonas* sp., Enterobacteriaceae
	Culicoides dewulfi	*Asaia* sp., Acetobacteraceae, Burkholderiaceae, Enterobacteriaceae, *Sphingomonas* sp.
	Culicoides fulvus	*Bacillus cereus*, *Bacillus tequilensis*, *Bacillus paramycoides*, *Cytobacillus kochii*, *Enterococcus faecium*, *Lysinibacillus* sp., *Pseudomonas stutzeri*
	Culicoides imicola	*Bacillus cereus*, *Bacillus haynesii*, *Bacillus paramycoides*, *Bacillus tropicus*, *Escherichia* sp., *Lysinibacillus mangiferihumi*, *Brevibacillus parabrevis*, *Enterococcus faecium*, *Halomonas*, *Paenibacillus* sp., *Propionibacterium* sp.
	Culicoides jacobsoni	*Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus paramycoides*, *Bacillus velezensis*, *Enterococcus faecium*, *Paenibacillus* sp.
	Culicoides obsoletus	*Asaia* sp., Acetobacteraceae, Anaplasmataceae, *Aquaspirillum* sp., Burkholderiaceae, Enterobacteriaceae, *Sphingomonas* sp. *Bacillus* sp., *Pseudomonas* sp., Enterobacteriaceae, *Sphingomonas* sp.
	Culicoides orientalis	*Bacillus cereus*, *Bacillus paramycoides*, *Brevibacillus parabrevis*, *Enterococcus faecium*, *Paenibacillus* sp., *Pseudomonas* sp.
	Culicoides scoticus	*Asaia* sp., Acetobacteraceae, Anaplasmataceae, Burkholderiaceae, Enterobacteriaceae, *Sphingomonas* sp. *Bacillus* sp., *Pseudomonas* sp., Enterobacteriaceae, *Sphingomonas* sp.

Subgenus	Species	Bacterial families/genera/species
Hoffmania	*Culicoides peregrinus*	*Acinetobacter* sp., *Alcaligenes faecalis*, *Arcobacter* sp., *Bacillus cereus*, *Bacillus flexus*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacteroides* sp., *Bifidobacterium* sp., *Brevibacillus* sp., *Brevundimonas* sp., *Brevifilum* sp., *Clostridium* sp., *Chryseobacterium* sp., *Enterococcus faecium*, *Flavobacterium* sp., *Lysinibacillus* sp., *Lactobacillus* sp., *Microcystis* sp., *Paenibacillus* sp., *Pelolinea* sp., *Proteus vulgaris*, *Proteus terrae*, *Pseudomonas* sp., *Ralstonia* sp., *Serratia* sp., *Stenotrophomonas* sp.
Remmia	*Culicoides oxystoma*	*Alcaligenes faecalis*, *Bacillus cereus*, *Bacillus flexus*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus paramycoides*, *Bacillus tropicus*, *Bacillus thuringiensis*, *Lysinibacillus* sp., *Enterococcus faecium*, *Paenibacillus* sp., and *Pseudomonas* sp.
Monoculicoides	*Culicoides nubeculosus*	*Asaia* sp., *Brevundimonas* sp., *Chryseobacterium* sp., *Delftia* sp., *Leucobacter* sp., *Sphingomonas* sp., *Pseudomonas* sp.
	Culicoides sonorensis	*Aeromonas hydrophila*, *Bacillus* sp., *Bacillus thuringiensis*, *Bacillus cereus*, *Chryseobacterium* sp., *Lysinibacillus* sp., *Acinetobacter* sp., *Providencia* sp., *Brevudimonas* sp., *Flavobacterium* sp., *Morganella morganii*, *Comamonas* sp. Acetobacteraceae, *Asaia* sp., *Delftia* sp., *Leucobacter* sp., *Sphingomonas* sp., *Pseudomonas* sp.
	Culicoides variipennis	*Achromobacter* sp., *Acinetobacter* sp., *Aeromonas* sp., *Bacillus* sp., *Edwardsiella* sp., *Enterobacter* sp., *Escherichia* sp., *Flavobacterium* sp., *Micrococcus* sp., *Proteus* sp., *Providencia* sp., *Pseudomonas* sp., *Zoogloea* sp.

Table 2. Endosymbionts recorded from various *Culicoides* spp.

Subgenus/Species group	Species	Endosymbionts
Avaritia	*Culicoides brevitarsis*	*Cardinium* sp., *Wolbachia* sp.
	Culicoides chiopterus	*Wolbachia* sp.
	Culicoides dewulfi	*Rickettsia* sp., *Wolbachia* sp.
	Culicoides imicola	*Cardinium* sp., *Wolbachia* sp.
	Culicoides obsoletus	*Rickettsia* sp., *Wolbachia* sp.
	Culicoides scoticus	*Cardinium* sp., *Rickettsia* sp., *Wolbachia* sp.
	Culicoides wadai	*Cardinium* sp.
Hoffmania	*Culicoides peregrinus*	*Cardinium* sp., *Rickettsia* sp., *Wolbachia* sp.
	Culicoides sumatrae	*Cardinium* sp.
Remmia	*Culicoides oxystoma*	*Cardinium* sp.
Monoculicoides	*Culicoides nubeculosus*	*Rickettsia* sp.
	Culicoides sonorensis	*Cardinium* sp., *Rickettsia* sp.
Culicoides	*Culicoides flavipulicaris*	*Cardinium* sp.
	Culicoides impunctatus	*Rickettsia* sp.
	Culicoides newsteadi	*Cardinium* sp.
	Culicoides pulicaris	*Cardinium* sp.
	Culicoides punctatus	*Cardinium* sp.
Marksomyia	*Culicoides dycei*	*Cardinium* sp.
	Culicoides marksi	*Cardinium* sp., *Wolbachia* sp.
	Culicoides parvimaculatus	*Cardinium* sp., *Wolbachia* sp.
Oecacta	*Culicoides sahariensis*	*Cardinium* sp.
	Culicoides vexans	*Wolbachia* sp.
Sensiculicoides	*Culicoides festivipennis*	*Cardinium* sp.
	Culicoides haranti	*Cardinium* sp.
	Culicoides kibunensis	*Wolbachia* sp.
	Culicoides maritimus	*Cardinium* sp.
Trithecoides	*Culicoides paraflavescens*	*Wolbachia* sp.
Wirthomyia	*Culicoides minutissimus*	*Cardinium* sp.
antennalis species group	*Culicoides antennalis*	*Cardinium* sp., *Wolbachia* sp.
molestus species group	*Culicoides* spp.	*Cardinium* sp.

Subgenus/Species group	**Species**	**Endosymbionts**
ornatus species group	*Culicoides marmoratus*	*Cardinium* sp., *Wolbachia* sp.
	Culicoides bundyensis	*Cardinium* sp., *Wolbachia* sp.
victoriae species group	*Culicoides henryi*	*Cardinium* sp., *Wolbachia* sp.
	Culicoides victoriae	*Cardinium* sp., *Wolbachia* sp.
	Culicoides multimaculatus	*Cardinium* sp.
williwilli species group	*Culicoides austropalpalis*	*Cardinium* sp., *Wolbachia* sp.
	Culicoides narrabeenensis	*Cardinium* sp., *Wolbachia* sp.
	Culicoides williwilli	*Cardinium* sp.



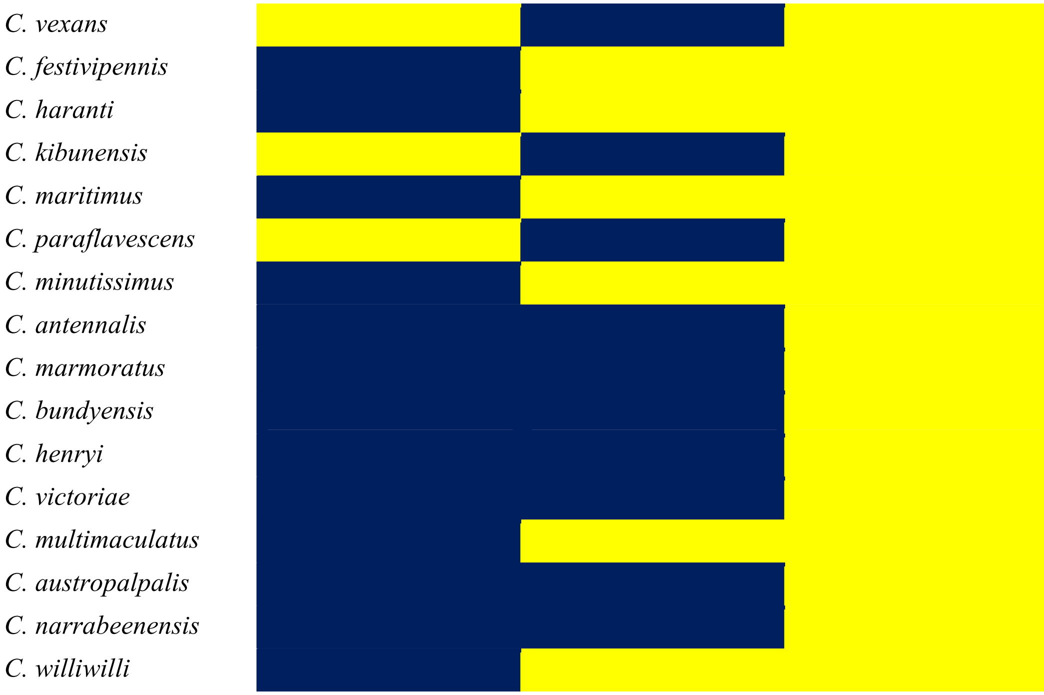


Fig. 1. Heatmap depicting endosymbionts detected in different *Culicoides* spp. ■ present, □ absent)

Bacillus cereus causes endophthalmitis, septicemia and food poisoning in humans and other mammals and acute gangrenous mastitis in dairy cows[38,39,40]. Another, ubiquitous bacterium, *Pseudomonas stutzeri* has been reported as a causative agent of some infections, particularly in immune-compromised patients but has rarely been reported as a cause of infective endocarditis[41]. *Lysinibacillus fusiformis* can cause tropical ulcers, severe sepsis, and respiratory illnesses in humans[42]. *Brevibacillus* species are rarely implicated as human pathogens, causative agents of meningitis, and bacteremia[43]. *Serratia marcescens* is an opportunistic nosocomial pathogen that causes a wound, urinary tract, bloodstream, ocular infections, meningitis, endocarditis, pneumonia, and other respiratory diseases. Infection with *S. marcescens* has been associated with mastitis outbreaks in dairy cattle herds in two dairy farms in Finland[44,45,46,47]. The following pathogenic bacteria, *Bacillus cereus*, *Serratia marcescens*, *Pseudomonas stutzeri*, *Brevibacillus parabrevis* were detected in salivary glands and gut of midge species, raising a chance of zoonotic transmission towards their hosts. The transmissional efficiency of these midges via blood feeding need to be further addressed. Some of these midge-associated strains may be targeted further to develop control strategies by alternating the vector physiology. Some of the endosymbionts, *Wolbachia*, *Cardinium*, and *Rickettsia* were detected. These endosymbionts along with the bacteria such as *Bacillus*, *Pseudomonas*, *Lysinibacillus* may be symbiotically associated with these midges. These bacteria along with endosymbionts may alter competence for disease transmission and affect gene flow by altering cross-compatibility, as reported in other haematophagous vectors. The tripartite interactions among host, vector and pathogens need to be further explored in these vector species of *Culicoides*.

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1st June, 2024

Original Article

SPECIES COMPOSITION AND HABITAT DISTRIBUTION OF HAEMATOPHAGOUS MUSCID FLIES (DIPTERA: MUSCIDAE) IN WEST BENGAL, INDIA

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Received : 17th April, 2024

Accepted : 28th May, 2024

ABSTRACT

Haematophagous or blood-feeding muscid flies belonging to the family Muscidae (Diptera) are potential mechanical and biological vectors for several bacterial, viral, protozoan and helminths diseases associated with livestock, pets, wild animals, and humans. An entomological survey was conducted from October 2019 to June 2023 across various geographical

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Cite this article as:

Jana, N, Mazumdar, A, Sinha, SK, Mandal A. Species composition and habitat distribution of haematophagous muscid flies (Diptera: Muscidae) in West Bengal, India. *J Med Arthropodol & Public Health*. 2024; 4(1): 35-52.

regions of West Bengal, India, to explore species composition and habitat distribution of haematophagous muscid flies. Sampling techniques included sweep nets collected from host animals and visual surveys. Flies were morphologically identified into 16 species within the subfamilies Muscinae and Stomoxyinae, which are prevalent having significant veterinary and medical importance. The survey revealed a diverse community of haematophagous muscid flies with several prevalent species, including *Stomoxys calcitrans* (Linnaeus, 1758); *Stomoxys indicus* Picard, 1908; *Haematobia exigua* de Meijere, 1906; *Musca crassirostris* Stein, 1903; *Musca convexifrons* Thomson, 1869; *Musca ventrosa* Weidemann, 1830; *Musca conducens* Walker, 1859; *Musca inferior* Stein, 1909. The findings addressed a foundation for future research, offering insights into the presence of blood-feeding muscid species in the area and their potential implications for livestock and public health.

Keywords: Haematophagous, muscid flies, Muscinae, Stomoxyinae, West Bengal

INTRODUCTION

Several muscid flies are found to fly around livestock, pets, wild animals and even humans, possess specialised mouthparts adapted for sucking blood by directly penetrating the host skin and licking serum or oozing blood from sores and wounds considered as haematophagous nature[1,2,3,4]. Haematophagous muscid flies belong to the subfamilies Muscinae and Stomoxyinae[1,2,27]. However, many taxonomists have classified these flies under the single subfamily Muscinae, encompassing the tribes Muscini and Stomoxyini[5,6,7,28]. The most well-known haematophagous muscid flies around the world are members of the subfamily Stomoxyinae, which comprises ten genera, including *Bruceomyia*, *Haematobosca*, *Haematobia*, *Haematostoma*, *Neivamyia*, *Parastomoxys*, *Prostomoxys*, *Rhinomusca*, *Stygeromyia* and *Stomoxys*[2]. On the other hand, the subfamily Muscinae includes only one genus *Musca*, recognised as having haematophagous tendencies (not all species, though the status of haematophagy of certain species remains controversial)[1,3]. In India, four genera of Stomoxyinae flies (*Stomoxys*, *Haematobia*, *Haematobosca*, and *Stygeromyia*) and some species of *Musca* in the subfamily Muscinae are known for their haematophagous feeding habits[1]. Both adults and larvae of these flies serve as potential mechanical and biological vectors for several viral, bacterial, protozoan and helminths diseases affecting livestock, wildlife and companion animals[8,9]. Besides their indirect effects, the painful bites and annoyance directly cause blood loss, reduced food intake, and decreased milk and meat production in livestock, leading to significant economic losses in livestock industries[18].

West Bengal harbours a large number of livestock compared to other states in India, with nearly 37.5 million animals (20th livestock census in 2019)[33]. Farmers in the state primarily rear livestock in backyard systems, where management practices often fail to meet recommended standards, particularly regarding disease vector control. The state also has many protected forest areas with abundant wildlife. Several diseases in livestock and wild animals transmitted by haematophagous muscid flies have been frequently reported in India[25,29]. One of the early measures to support vector control efforts, involves monitoring fly presence and identifying their habitats[30]. Although many researchers have expanded the data and knowledge on Muscidae in West Bengal[1,10,11,12,13,14,15,24], information regarding the species composition and habitat distribution of haematophagous Muscidae remains scarce. This study aims to fill the knowledge gap and provide updated insights into the species composition and habitat distribution of haematophagous muscid flies in West Bengal, India, associated with livestock, wildlife and companion animals.

MATERIAL AND METHODS

Study area

The survey was carried out in several regions of West Bengal, India (Fig. 1) from October 2019 to June 2023. Flies were usually collected from various habitats, including livestock farms, open grazing lands, local forest patches, village residential places, animal dung sites, and garbage areas.

Collection and identification methods:

Flies were collected using sweep nets (15 & 30 cm diameter). After collection, the flies were euthanized by exposing a small amount of liquid benzene vapour in a killing jar[15] and brought to the laboratory for further investigation. The flies were identified under a binocular microscope (SYS-45ETR) in the laboratory by studying chaetotaxy and morphological descriptions[1,2,16,17]. Male terminalia dissection was performed under a dissecting binocular microscope (SYS-45ETR) and glass slide mounted for species confirmation.

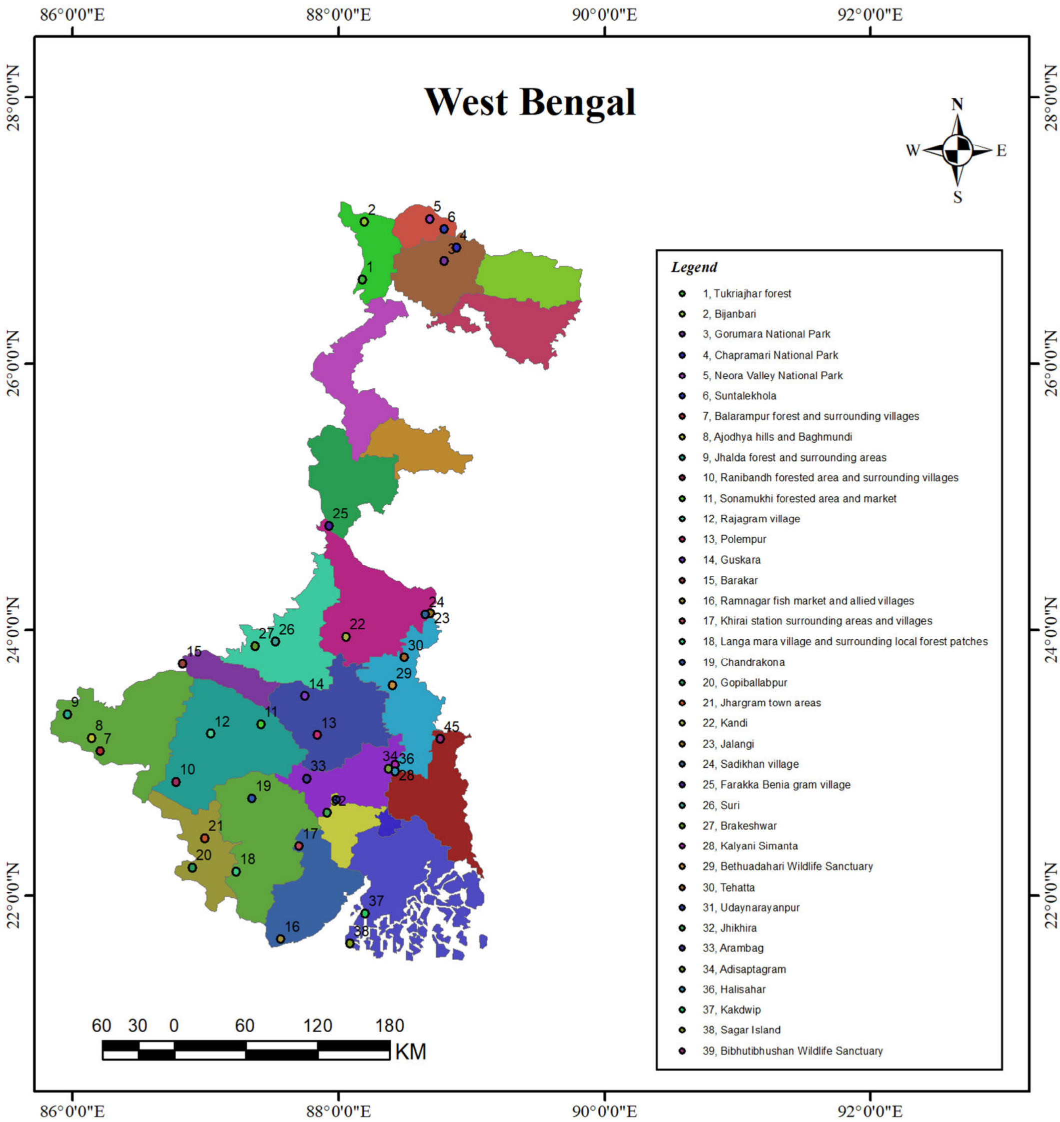

Fig. 1: Study map of West Bengal with sampling sites (map prepared using ArcMap 10.8 software).

RESULTS

Out of the entire collection, a total of 16 species of Muscidae were identified as haematophagous (Table 1; Fig. 2). Flies belonging to the subfamilies Muscinae and Stomoxyinae were identified as haematophagous. The subfamily Stomoxyinae comprises six species in three genera: *Stomoxys* (3 species), *Haematobia* (2 species) and *Haematobosca* (1 species). Conversely, the subfamily Muscinae includes ten species within the genus *Musca*. A brief account of these 16 species follows:

Fig. 2. Haematophagous muscid flies recorded from West Bengal, India. (A) *Stomoxys calcitrans*, (B) *Stomoxys sitiens*, (C) *Stomoxys indicus*, (D) *Haematobia sanguinolenta*, (E) *Haematobia exigua*, (F) *Haematobia minuta*, (G) *Musca bezzii*, (H) *Musca conducens*, (I) *Musca convexifrons*, (J) *Musca crassirostris*, (K) *Musca formosana*, (L) *Musca inferior*, (M) *Musca pattoni*, (N) *Musca sorbens*, (O) *Musca ventrosa*, (P) *Musca* sp.

Table 1: List of haematophagous muscid flies recorded in West Bengal, India

Sl. No.	Species
Subfamily Muscinae	
1	*Stomoxys calcitrans* (Linnaeus, 1758)
2	*Stomoxys indicus* Picard, 1908
3	*Stomoxys sitiens* Rondani, 1873
4	*Haematobosca sanguinolenta* (Austen, 1909)
5	*Haematobia exigua* de Meijere, 1903
6	*Haematobia minuta* (Bezzi, 1892)
Subfamily Stomoxyinae	
7	*Musca bezzii* Patton and Cragg, 1913
8	*Musca conducens* Walker, 1860
9	*Musca convexifrons* Thomson, 1869
10	*Musca crassirostris* Stein, 1903
11	*Musca formosana* Malloch, 1925
12	*Musca inferior* Stein, 1909
13	*Musca pattoni* Austen, 1910
14	*Musca sorbens* Wiedemann, 1830
15	*Musca ventrosa* Wiedemann, 1830
16	*Musca* sp.

1. SUBFAMILY STOMOXYINAE

(i) ***Stomoxys calcitrans*** **(Linnaeus, 1758)**

Habitat: Flies of this species were collected from diverse habitats, including livestock farms, pasturelands, forest areas and dung sites.

Distribution in India: Widespread and found in all the geographical regions within West Bengal.

Diagnosis: Head with a strong, elongated and highly sclerotized proboscis; dorsum of the thorax displays four distinct longitudinal stripes; wing vein M_{1+2} slightly rounded at apex; abdomen checkered marking with one median and two lateral spots on second and third segments[1,2].

Bionomics: This species is commonly called stable fly, and the only blood-sucking Stomoxyinae fly found worldwide. Both males and females primarily target warm-blooded vertebrate hosts for blood meals, including cattle, buffaloes, goats, sheep, pigs, dogs, wild animals, and occasionally humans when other hosts are scarce. Although they feed on diverse hosts, they notably engage in mass attacks on cattle, mainly targeting their lower legs. They are diurnal, with feeding activity occurring during the daytime and taking approximately 5–6 minutes to engorged under undisturbed conditions. Females mainly lay their eggs in cattle dung and decaying vegetation. They transmit pathogens of several livestock diseases, including trypanosomiasis, anthrax, habronemiasis, lumpy skin disease and bovine leukosis[8].

***(ii) Stomoxys indicus* Picard, 1908**

Habitat: This species is found in and around cattle sheds, pasturelands, and forest areas.

Distribution in India: Assam, Bihar, Kerala, Karnataka, Madhya Pradesh, Uttar Pradesh and West Bengal (districts covering the lower Gangetic plain, coastal belt and Chotanagpur plateau regions).

Diagnosis: The morphological characteristics of the head, thorax and wings closely resemble those of *S. calcitrans*. A key distinguishing feature is the presence of transverse dark posterior marginal bands on the second, third and fourth abdominal segments, instead of rounded spots typically seen in *S. calcitrans*[1,2].

Bionomics: Adult flies of this species are potent haematophagous on cattle. The peak activity of adult flies mostly occurs in the evening. Females deposit their eggs on cattle dung.

***(iii) Stomoxys sitiens* Rondani, 1873**

Habitat: Flies of this species were collected from forested areas in Neora Valley National Park, Kalimpong.

Distribution in India: Odisha, Tamil Nadu, Uttar Pradesh, and West Bengal (Kalimpong).

Diagnosis: The morphological characteristics of the head, thorax and wings closely resemble those of *S. calcitrans* but the lateral spots on the abdominal segments of this species appear to be more elongated[1,2].

Bionomics: This species prefers forested environments, and its adults are supposed to target wild animals and other vertebrates. However, previous studies have indicated a preference for donkeys over cattle and buffaloes for feeding[2]. Females oviposit on the dung of cattle, buffaloes, and donkeys.

***(iv) Haematobia exigua* de Meijere, 1903**

Habitat: Adult flies of this species were primarily gathered on domesticated animals, including cattle and buffaloes.

Distribution in India: Punjab and West Bengal (encompassing the regions of lower Gangetic plain and Chotanagpur plateau).

Diagnosis: Small-sized body with length measure is about 2.5-4.0 mm; palpi as long as proboscis; arista with only dorsally plumose; male hind leg second tarsal segment contains 4-6 long curled hairs[1].

Bionomics: Despite being commonly referred to as buffalo flies, this species is predominantly found on cattle. Studies have also reported their feeding activity on pigs and horses[2]. They usually remain active on their hosts throughout the day. Unlike *Stomoxys* spp. adult flies feed on blood with their heads oriented downwards. They are primarily responsible for causing skin lesions in cattle[25].

***(v) Haematobia minuta* (Bezzi, 1892)**

Habitat: Adult individuals of this species were captured in and around animal sheds, especially cattle and buffaloes.

Distribution in India: Chhattisgarh and West Bengal (Himalayan Mountain regions, districts of Darjeeling and Kalimpong).

Diagnosis: The morphological characteristics are similar to the preceding species, but the key differences of this species include prosternum setulose; male hind leg second tarsal segment simple; sixth wing vein relatively short[1].

Bionomics: Adults persistently irritate and bite host animals with their strong proboscis. They mostly hover around the neck and hump of cattle. In Oriental regions, cattle and buffaloes serve as the primary hosts for this species[2]. They share a similar ecology with *H. exigua*.

***(vi) Haematobosca sanguinolenta* (Austen, 1909)**

Habitat: Flies of this species were collected from pasturelands around grazing cattle.

Distribution in India: Arunachal Pradesh, Assam, Bihar, Karnataka, Maharashtra, Tamil Nadu, and West Bengal (Nadia and Kalimpong).

Diagnosis: Palpi as long as proboscis; arista both dorsally and ventrally plumose; dorsum of the thorax with four longitudinal stripes; third and fourth abdominal tergites contain a pair of rounded spots[2].

Bionomics: Adult flies of this species feed on the blood of cattle, horses and wild animals by directly penetrating the host skin. In this study, adult flies of this species were found in much smaller numbers than other Stomoxyinae flies. Larvae and other preimaginal stages have been reported in herbivorous animal dung[16].

2. SUBFAMILY- MUSCINAE

***(vii) Musca bezzii* Patton and Cragg, 1913**

Habitat: Adult flies of this species were collected from wounds and sores and around the eyes of cattle, buffaloes, and other domestic animals in animal sheds and pasturelands.

Distribution in India: Arunachal Pradesh, Assam, Jharkhand, Jammu & Kashmir, Kerala, Karnataka, Nagaland, Odisha, Punjab, Uttar Pradesh, Uttarakhand, Sikkim, Tamil Nadu and West Bengal (Kalimpong).

Diagnosis: Large-sized fly with body length is about 7-9 mm; arista with dorsally and ventrally long plumose; dorsum of the thorax covered with four dark longitudinal stripes; suprasquamal ridge hairy; wing vein M_{1+2} strongly upcurved at apex; abdomen yellowish-orange with a narrow median dark line extending to the last segment; dorsum of the first abdominal segment wholly dark brown[1].

Bionomics: Adults of this species are commonly found around open wounds, sores, and the eyes of host animals, particularly cattle. Their abundant presence around the eyes of cattle suggests a potential association with vectors of *Thelazia* spp. (eye worms, nematode parasites) responsible for causing thelaziasis disease in livestock[16]. They lay eggs on freshly deposited cow dung, and larvae are coprophagous in nature.

(viii) Musca conducens Walker, 1860

Habitat: Adult flies of this species are extensively collected from various habitats, including cattle sheds, pasturelands, and forest areas, especially near dung sites and garbage areas.

Distribution in India: Arunachal Pradesh, Assam, Chhattisgarh, Madhya Pradesh, Nagaland, Punjab, Uttarakhand, Tamil Nadu, and West Bengal (widespread, covering all geographical regions).

Diagnosis: Body medium-sized, measuring 4-5 mm in length; dorsum of the thorax characteristics with four dark longitudinal stripes; suprasquamal ridge bare; second abdominal tergite dark brownish; third and fourth tergites yellowish-orange with a narrow dark median stripe reaching to the last segment[16].

Bionomics: Adults are often attracted to open wounds and sores on cattle and other animals, where they suck or lick mucus and blood. Flies of this species have been reported to have a biological association with *Stephanofilaria assamensis*, which is known to cause hump sores in cattle[13,16,20].

(ix) Musca convexifrons Thomson, 1869

Habitat: Flies of this species were collected from cattle sheds, pasturelands, forest habitats and around human settlements.

Distribution in India: Arunachal Pradesh, Assam, Karnataka, Kerala, Tamil Nadu, and West Bengal (widespread, covering all geographical regions).

Diagnosis: Large fly with length of about 6.5-8.0 mm; thorax blackish with four longitudinal stripes; suprasquamal ridge hairy; abdomen yellowish-silver checkered pattern; dorsum of the first abdominal segment pale yellowish; second to fifth tergites feature a dark longitudinal stripe; stem vein of wing with 1-3 setulae on the posterior margin[16].

Bionomics: This species is frequently encountered near the habitats of cattle sheds, pasturelands and forested regions. Adult flies are often seen around animal wounds, sores and the oozing blood punctures caused by other biting flies such as Tabanids. Gravid females deposit their eggs in the dung of cattle.

***(x) Musca crassirostris* Stein, 1903**

Habitat: Flies of this species were extensively collected in cattle sheds, pasturelands and forest habitats.

Distribution in India: Arunachal Pradesh, Bihar, Chhattisgarh, Jharkhand, Karnataka, Madhya Pradesh, Odisha, Punjab, Rajasthan, Uttar Pradesh, Tamil Nadu, and West Bengal (widespread, covering all geographical regions).

Diagnosis: Greyish-brown colour median-sized fly; proboscis stout and highly sclerotized; dorsal surface of the thorax displays four narrow dark longitudinal stripes; suprasquamal ridge bare; abdomen greyish with triangular spots on intermediate segments[1].

Bionomics: Adult flies of this species possess a robust and stout proboscis used to draw blood by scratching the skin of animals. They are primarily seen near cattle sheds, perching on either the bodies of cattle or nearby vegetation. Studies have documented their ability to also feed on horses and donkeys[1,3]. They lay eggs in patches on freshly deposited cow dung.

***(xi) Musca formosana* Malloch, 1925**

Habitat: Adult flies were collected from local forest patches within Gopegarh Eco Park and pasturelands.

Distribution in India: Bihar, Karnataka, Kerala, Uttar Pradesh, Tamil Nadu, and West Bengal (Murshidabad and Paschim Medinipur districts).

Diagnosis: The head and thorax morphology closely resemble those of *M. convexifrons*. A key distinguishing feature of this species is the presence of 3-7 setulae on the posterior margin of the stem vein of the wing, instead of 1-3 seen in *M. convexifrons*[1].

Bionomics: Adult flies of this species feed on blood or serum from open wounds and sores on animals.

(xii) *Musca inferior* Stein, 1909

Habitat: Flies were collected near livestock farms in urban and rural areas, as well as local forest patches surrounding Wildlife Sanctuaries.

Distribution in India: Arunachal Pradesh, Assam, Andaman Islands, Odisha, Tamil Nadu and West Bengal (widespread, covering all geographical regions).

Diagnosis: Body greyish-brown, large fly with measures 6-8 mm in length; arista with long plumose hair dorsally and ventrally; proboscis stout and sclerotized; dorsum of the thorax characterized by four dark longitudinal vittae; suprasquamal ridge hairy; dorsum of the lower calypter hairy[1].

Bionomics: This species is potent haematophagous on livestock, feeding by puncturing host skin with their fully developed prestomal teeth[3]. Adults are commonly observed around cattle dung.

(xiii) *Musca pattoni* Austen, 1910

Habitat: Found on and around cattle sheds, pasturelands and local forest patches.

Distribution in India: Arunachal Pradesh, Assam, Chhattisgarh, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Uttar Pradesh, Tamil Nadu, and West Bengal (regions of the lower Gangetic plain, coastal belt and the Chotanagpur plateau).

Diagnosis: Median-sized fly with body length is about 5-7 mm; thorax shiny blackish with greyish pollen and features four longitudinal stripes on the dorsum; suprasquamal ridge bare; abdomen yellowish-orange and a median stripe gradually narrows toward the posterior margin[1].

Bionomics: Flies of this species usually feed blood or serum on wounds and sores and/or nasal secretions of animals. Females lay eggs on freshly deposited animal dung[1].

(xiv) *Musca sorbens* Wiedemann, 1830

Habitat: Adults of this species are found in and around cattle sheds, pasturelands, local forest patches, dung sites, and garbage areas.

Distribution in India: Arunachal Pradesh, Chhattisgarh, Karnataka, Kerala, Maharashtra, Madhya Pradesh, Mizoram, Manipur, Odisha, Punjab, Rajasthan, Uttar Pradesh, Tamil Nadu, and West Bengal (widespread, covering all geographical regions).

Diagnosis: Body blackish-orange colouration; face and facial parts usually black with greyish to silvery pollen; dorsum of the thorax displays two broad dark longitudinal stripes; abdomen usually yellowish-orange with silvery pollen; dorsum of the second tergite brownish; a median longitudinal stripe extends across the third and fourth segments[1,2].

Bionomics: Adult flies are found around wounds and the eyes of cattle, feeds on oozing blood and tears. Adults serve as significant vectors for trachoma disease in cattle. Flies of this species have been reported as vectors for various bacteria such as *Shigella dysenteriae, Shigella flexneri, Haemophilus influenzae, Streptococcus* sp. and *Staphylococcus* sp. They serve as essential mechanical carriers by transferring germs from diseased animal hosts to other animals through open wounds or skin lesions[21].

(xv) *Musca ventrosa* Wiedemann, 1830

Habitat: Cattle sheds, pasturelands, local forest patches, dung sites, and garbage areas.

Distribution in India: Arunachal Pradesh, Jharkhand, Karnataka, Madhya Pradesh, Odisha, Punjab, Tamil Nadu, and West Bengal (widespread, covering all geographical regions).

Diagnosis: The head and thorax morphology closely resemble those of *M. conducens*. A notable distinguishing feature of this species is the abdomen uniformly orange[1].

Bionomics: Adults of this species usually feed on blood or serum from open wounds and sores. This species has been reported to serve as biological vectors for *Habronema megastoma* and *Habronema muscae*, parasitic nematodes mainly affecting horses[22].

***(xvi) Musca* sp.**

Habitat: Pasturelands, cattle sheds, local forest patches and flowering plants.

Distribution in India: West Bengal (regions of the lower Gangetic plain and the Chotanagpur plateau covering districts of Purba Medinipur, Paschim Medinipur, Jhargram, Purba Bardhaman, Paschim Bardhaman and Bankura).

Diagnosis: Body bluish colour; dorsum of the thorax with two dark longitudinal stripes; first abdominal segment wholly dark brownish to blackish; a dark median stripe extended from second to fourth segments; fourth abdominal segment with silvery margin.

Bionomics: Feed on blood from wounds and sores of domestic cattle.

DISCUSSION

Several studies on Muscidae have been conducted in India, but few are concerned about these pestiferous flies of their impacts on hosts. In our study, 16 species of haematophagous muscid flies of significant medico-veterinary implications for livestock, companion animals and wildlife were identified from the state of West Bengal, India. These flies are known to transmit various pathogens, including viruses (Equine Infectious Anaemia Virus; Lumpy Skin Disease Virus; Bovine Leukosis Virus; Bovine Herpes Virus), bacteria (*Bacillus anthracis*; *Shigella dysenteriae*; *Dermatophilus congolensis*), protozoa (*Trypanosoma evansi*; *Leishmania tropica*), helminths (*Habronema microstoma*; *Habronema megastoma*; *Stephanofilaria assamensis*; *Heterotylenchus autumnalis*; *Thelazia* spp.) and rickettsia (*Anaplasma marginale*)[8,9,16,18,19,26]. Among the identified species, several Stomoxyinae and Muscinae flies including *S. calcitrans*, *S. indicus*, *H. exigua*, *M. crassirostris*, *M. convexifrons*, *M. ventrosa*, *M. conducens* and *M. inferior* were found to be widely distributed and abundant around livestock farms and pasturelands. The presence spans across diverse ecosystems of these identified species, including animal sheds, pasturelands, and forest landscapes, making them severe pests to livestock, pets, and wild animals in the state. The absence of some *Stomoxys* flies in our study, such as *Stomoxys bengalensis* Picard, 1908 which was previously reported in West Bengal, may be due to the use of only a single collection trap (sweep nets). Various studies in Thailand have reported this species using Vavoua traps[31,32], suggesting that alternative trapping methods may be necessary for collecting blood-feeding muscid flies. Our study is preliminary and findings addressed a foundation for future research, offering insights into the presence of blood-feeding muscid species in the area and their potential implications for livestock, wildlife, and companion animals.

CONCLUSION

The survey reflects the overall presence, habitat distribution and bionomics of haematophagous muscid flies in the state of West Bengal, India. Sixteen species of haematophagous muscid flies within the subfamilies Muscinae (ten species) and Stomoxyinae (six species) were documented. Among them species like *S. calcitrans*, *S. indicus*, *H. exigua*, *M. crassirostris*, *M. convexifrons*, *M. ventrosa*, *M. conducens* and *M. inferior* were found to be highly abundant in and around livestock farms and pasturelands. The findings serve as baseline information for future research on haematophagous Muscidae in West Bengal associated with livestock, wildlife, and companion animals.

ACKNOWLEDGEMENTS

The authors (NJ & SKS) are grateful to the West Bengal Biodiversity Board for financial help from the project (sanction No. 399/3k(Bio)-1/2019) and would like to thank the Principal and Head of the Department of Zoology at Sreegopal Banerjee College for providing laboratory facilities. They are also thankful to the Head of the Department of Zoology at the University of Burdwan for laboratory assistance. Their thanks are also due to Bhatta Charan Murmu, M.Sc. in Geospatial science, The University of Burdwan for helping in preparation of study area map. They extend heartfelt thanks to the anonymous reviewers for their invaluable suggestions and corrections on the manuscript.

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1st June, 2024

Original Article

OBSERVATION ON THE ROLE OF TEMPERATURE AND SALINITY ON THE DEVELOPMENT OF *CULICOIDES* SPECIES (DIPTERA: CERATOPOGONIDAE) IN LABORATORY

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Received : 16th March, 2024

Accepted : 30th May, 2024

ABSTRACT

This article reviews the influence of various substrate salinity and temperature ranges on oviposition, larval survivability, and adult emergence during laboratory rearing. Habitat selection by the gravid females *Culicoides* spp. (Diptera: Ceratopogonidae) is influenced by physicochemical parameters such as temperature, pH, salinity, moisture, conductivity, and organic and inorganic compounds of substrates. These climatic and habitat related factors influence vectors' life history traits. The species-specific information will be useful in establishing a laboratory colony thereby contributing to our understanding of the vectorial capacity and competenceamongst different species of *Culicoides*.

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Cite this article as:

Banerjee, P, Sarkar, A, and Mazumdar, A, Observation on the role of temperature and salinity on the development of Culicoides species (Diptera: Ceratopogonidae) in laboratory. *J Med Arthropodol & Public Health*. 2024; 4(1): 53-61.

Keywords: *Culicoides*, salinity, temperature, vectors

INTRODUCTION

Hematophagous biting midges of genus *Culicoides* Latreille (1809) (Diptera: Ceratopogonidae) are distributed mainly in the tropics and temperate regions of the world, except a few scattered geographical areas. These tiny flies are implicated as vectors of at least 50 arboviruses, several protozoans, and nematodes. The livestock population is affected worldwide by epizootic haemorrhagic disease virus, bluetongue virus (BTV), epizootic hemorrhagic disease virus and African horse sickness virus[1].In India, lack of life history-related data, larval food, captive mating, and artificial blood feeding are the main bottlenecks impeding the studies on vector competence as well as the establishment of successful laboratory colonies of the vector species[2,3]. Naturally, the immatures of these flies prefer areas like muddy fringe areas of stock ponds, shaded muddy pool margins, muddy areas of paddy fields, and cattle manure[4,5,6,7]. Very few species have so far been laboratory-reared, and colonies maintained within the laboratory. Out of the 23 species attempted to rear under laboratory conditions[2,3,8,9,10,11] only colonies exist for only two species of, *Culicoides sonorensis* Wirth and Jones and *Culicoides nubeculosus* (Meigen) are maintained[2]. The rearing procedure and conditionalities of the most prevalent vector species, *Culicoides peregrinus* Kieffer, were performed in the laboratory with a good amount of success[3].The growth and development of this vector species were studied mainly by following habitat-specific temperature ranges and other physicochemical parameters. However, information about species-specific rearing temperature ranges and suitable substrate salinity was unavailable. At different rearing temperature ranges, the rearing of several *Culicoides* spp. was examined, such as *Culicoides variipennis* (Coquillett)[12], *Culicoides arakawae* (Arakawa), *Culicoides maculatus* (Shiraki)[8], *Culicoides brevitarsis* Kieffer[13], *Culicoides (Avaritia) imicola* Kieffer[14]. Besides, the oviposition site selection of these vectors depends on the suitable temperature and the physicochemical factors of these habitats[14,15,16]. Therefore, the peak abundance of these vectors and the appearance of bluetongue disease are related to suitable habitats as well as climatic variations[17]. Thus, in the southern states of India, peak abundance of *Culicoides* and BTD outbreaks was reported in the monsoon period[18], but became low in abundance during post-monsoon[19]. The primary purpose of this article is to explore the tolerance temperature range and substrate salinity of *Culicoides* spp. and how it affects oviposition, larval survivability, and adult emergence. This information provides evidence about the characteristics of the breeding sites and suitable parameters for their adequate survivability.

MATERIAL & METHODS

Investigation on the influence of rearing temperature was performed in laboratory and on field collected species[19,22,26,27]. In laboratory different temperatures was selected for that purpose[12,13,14,21,23]. To observe the effect of substrate salinity, gravid individuals were placed in oviposition glass vials and oviposition beds were prepared with different concentration of saline water[3,30,33,34,35]. This review was on considered field collected species from different habitat with analysis of physiochemical parameters[15,16,26,27,28,29].

OBSERVATIONS

(i) Rearing Temperature

According to the preference performance hypothesis[20], gravid females can detect the ambient temperature to determine maximum larval survivability. It was observed that the development success and duration of different life stages of *C. variipennis* were influenced by rearing temperatures such as larval development was completed within 11-24 days at 25°C, 27°C and 30°C whereas fast development within 8-18 days at higher temperature (35°C). The larval development was delayed (23-48 days) at the low temperature of 20°C[12]. In *C. brevitarsis* more than 80% of fourth instar larval survivability was recorded between 26°C and 30°C[13]. The development and duration of *C. arakawae* and *C. maculatus* were recorded at different rearing temperatures[8]. A higher percentage of pupation was observed in both species at 22.5°C. The duration from egg to adult in both species was prolonged (> 40 days), with a higher mortality rate of 20°C. Larval development ceased for both species in low temperatures (15-16°C), and *C. arakawae* and *C. maculatus* pupation did not occur at 35°C and above 30°C, respectively. The highest percentage of egg hatching and survivability occurred in *Culicoides circumscriptus* Kieffer and *Culicoides paolae* Boorman with a 1:1 sex ratio at 25°C and 30°C, respectively[21]. The most suitable rearing temperature for *Culicoides obsoletus* (Meigen)with the highest developmental success was 24°C, but the sex ratio was 3:1, male: female[22]. This sex ratio became 1:1 at 16-20°C with decreased pupal development. *Culicoides imicola* Kieffer showed the highest larval survivability at a lower temperature 20°C, with delayed development time[14]. Such an experiment was performed with vector species of *C. peregrinus,* and it was noted that 26°C was the ambient temperature for the highest oviposition (70%) and survivability. Duration of pupation was extended (25-39 days) in lower temperatures; 15°C, and 20°C[23]. From these observations, it can be stated that a non-linear relationship exists between rearing temperature and larval survivability. At low-temperature pupation was recorded as 0% and it peaked up to 81% at 26°C later when the temperature increased to 35°C the pupation dropped to 4%. This information could give insight into the possible thermal preference range (20-56°C) of populations to environmental conditions, thereby increasing the understanding of vector distribution and population changes over time. The thermal tolerance in the field conditions may vary greatly depending on the environmental conditions, and geographic location determines the abundance of these vectors.

(ii) Substrate Salinity

Immature distribution of *Culicoides* spp. depends on habitat availability as well as the physicochemical factors of habitats, i.e., moisture, dissolved oxygen, pH, salinity, organic contents and conductivity[15,16]. Thus, the occurrence of species richness and distribution of *Culicoides* spp. determined by the interactions of the physicochemical parameters. Worldwide *Culicoides* species showed a widespread habitat distribution from freshwater to coastal areas such as *Culicoides crepuscularis* Malloch, and *Culicoides bermudensis*Williams found in freshwater in the United States, but in Bermudas, these species are associated with a salinity range of 1.2 to 36.2 parts per thousand (ppt)[24]. The *Culicoides furens* (Poey) were also reported from salt marsh and freshwater while *Culicoides mississippiensis* Hoffman from salt marsh areas of Florida in low abundance[25]. *Culicoides melleus* (Coquillet), *Culicoides hollensis* (Melander and Brues), and *C. furens* were isolated from the salt marshes of Georgia[26]. The levels of dissolved salts influence the suitability of aquatic habitats for immature populations of the *C. variipennis* complex[15]. However, the highest abundance of the larval habitat of *Culicoides distinctipennis* Austen was in the freshwater lake edges[27]. *Culicoides furens* showed a wider habitat range in both salt marsh and freshwater habitats[25]. The soil chemistry characteristics substrate was examined for*Culicoides peliliouensis* Tokunaga, of Hooghly estuary[28], while a similar investigation was done for *C. variipennis*, *C. sonorensis* and *Culicoides occidentalis* Wirth and Jones throughout their geographic ranges[29] and various *Culicoides* spp.[16]. Habitat parameters show variations within seasons and for species[29,16].

DISCUSSION

Laboratory-based studies were also reported for the oviposition site preference and the emergence of *Culicoides* are stimulated by different salinity of habitat i.e., in *C. imicola*[30], *C. obsoletus*[31] and *Culicoides impunctatus* Goetghebuer[32]. Salt concentration below 0.06 g 10 ml^{-1} was selected in *C. imicola* as the preferable oviposition substrate[30] whereas *Culicoides variipennissonorensis* (Coquillett) females also chose to oviposit in 0‰ rather than 19‰. Still, it was noticed that no eggs were laid on 34‰[33]. Oviposition and survivability of *C. peregrinus* were gradually affected when exposed to high salinity; 30-40 ppt[34]. Many oviposited eggs were deposited in 0 ppt but reduced sharply in 5 ppt and then continuously decreased to 20 ppt. Oviposited eggs were not observed in 30 ppt to 40 ppt salinity. Egg retention was observed in stressful high saline concentrations. The hatching rate of eggs was highest at 0 ppt (without salt) and gradually decreased with increasing salinity. The hatching duration has not differed with salinity. The larval survivability and pupation recorded maximum in 0 ppt and significantly reduced in 25 ppt[34]. The developmental period from egg to adult was about 18-23 days in the control condition (without salt)[3], but it became delayed in high salinity with increasing mortality.Similarly, all the immatures of *Culicoides molestus* (Skuse) were died at three to four times the salinity of seawater[35]. They opined that seawater salinity could be an essential factor of habitat suitability for *C. molestus* immatures. The higher saline concentrations of seawater inhibit the survivability and maturation of immatures of *C. molestus*, whereas lower concentrations of natural seawater are more suitable for survivability. A correlation between larval habitat and salinity has been established for several *Culicoides* species[36,37]. *Culicoides circumscriptus* and *C. furens* could survive in hypersaline water, i.e., 1.5 times and three times that of seawater, respectively[38]. The increased larval mortality in higher salinity indicates that salt concentration may be responsible for disrupting the larval osmotic homoeostasis by gain of ions and loss of water.

CONCLUSION

Substrate parameters and environmental conditions play a vital role in habitat selection and survivability of *Culicoides* spp. The survival of *Culicoides* spp. was maximum during rearing, with temperatures ranging from 20-26°C. During inconducive conditions, the mortality rate increased with an altered sex ratio. In the laboratory alterations in sex ratios affect mating experiments. Likewise, temperature ranges affect the vector species' distribution, ultimately linked to the outbreak of diseases worldwide. Most species adapted to low salinity with increased salinity but very few species tolerated >2 ppt salinity. As larval mortality increased, their survivability was significantly affected in the higher salinity range. Our understanding of these parameters will help establish a laboratory colony, ultimately generating information about disease epidemiology and the vectorial capacity of these vectors. The emergence of bluetongue virus and other *Culicoides*-borne arboviruses are dependent on environmental drivers[39]. Previous studies reported the pattern of *Culicoides*-borne disease in tropical endemic areas related to climatic factors, primarily temperature and rainfall[40]. Besides several other factors like vegetation, physiochemical parameters of habitat and hosts greatly influence the abundance of vector species which are related to the appearance of disease outbreak and vectorial capacity[39].

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1st June, 2024

Review article

ONE HEALTH GLOBAL KNOWLEDGE PARTNERSHIPS

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Received : 16th March, 2024

Accepted : 30th May, 2024

ABSTRACT

With emerging issues such as climate change, transformations in global agri-food systems, increased migration, rising international trade, and growing pollution, there has been a surge in the outbreaks of new pathogens, leading to novel disease threats and incidences. These challenges are often complex and multifaceted, requiring innovative approaches. There is a renewed interest in strengthening One Health approach, which promote cross-disciplinary collaboration and communication across human, animal, plant, and environmental health sectors. Educational institutions are crucial in studying and addressing health security issues, promoting equity in services, and contributing to human progress and prosperity. Michigan

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Cite this article as:

Ghimire, RP, Maredia, K, One health global knowledge partnerships. *J Med Arthropodol & Public Health*. 2024; 4(1): 63-83.

State University (MSU), a premier land-grant university in the United States, is actively engaged in promoting the One Health approach nationally, regionally, and globally. Through interconnected missions of research, teaching, and extension, MSU strengthens human and institutional capacities in One Health, aiming to improve global health security. These programs are implemented both at MSU and internationally, in close collaboration with national, regional, and international organizations, with the goal of building the next generation of leaders in One Health research, policy, education, and outreach. This paper discusses the global health security scenario and MSU's efforts to enhance health security through the One Health approach.

Keywords: Animal, human and environmental health; capacity building; health security; international collaborations; One Health

INTRODUCTION

We live in a global village and interconnected world. Diseases or disease threats occurring in one corner of the world can easily and quickly transcend the country and continental boundaries and reach other corners of the world. The COVID-19 is a recent example. The COVID-19 which was first reported in Wuhan in China spread across the world in a matter of few weeks and months and affected the entire world. As of March 16, 2024, there were 774.8 million total cases of and 7 million total deaths from COCID-19 globally with largest share coming from the U.S. (cases: 103.4 million, death: 1.2 million)[1].

This case shows that even developed and resource rich countries are unprepared to respond to and contain endemic and pandemic on time before they spread widely inflicting heavy loss to lives, economies, livelihoods, and environments[2]. Furthermore, the average overall Global Health Security Index (GHSI) of 195 countries in 2021 was 38.9 out of a possible score of 100 and no country scored above 75.9[3]. Global average GHSI and as well as GHSI of a few representative countries show that the world is less prepared for preventing global health security threats (Table 1). Global average score of 28.4 for prevention which is the lowest among six categories contributing to GHSI strongly supports this logic. Score for response (37.6), for norms (47.8) and for risk (55.8) infer that countries and the world are becoming reactive when endemic or pandemic occurs[3]. This is contrary to the fact that "primary pandemic prevention actions cost less than

1/20th the value of lives lost each year to emerging viral zoonoses and have substantial cobenefits"[4] and preventing diseases is many times more effective than controlling diseases.

Table 1. Global Health Security Indices (GHSI)

Countries	Overall	Ranking (195)	Prevent	Detect	Respond	Health	Norms	Risk
USA	75.9	1	79.4	80.1	65.7	75.2	81.9	73.3
Lao PDR	34.8	99	18.7	37.9	38.3	22.0	44.1	47.6
Thailand	68.2	5	59.7	91.5	67.3	64.7	68.9	57.2
Vietnam	42.9	65	40.3	55.1	30.6	24.0	53.3	53.9
Cambodia	31.1	126	24.8	37.1	21.3	12.3	52.4	38.4
Myanmar	38.3	85	21.7	46.8	37.8	19.5	63.7	40.4
Philippines	45.7	57	27.7	52.6	38.8	46.5	55.9	52.8
Indonesia	50.4	45	31.8	55.4	50.2	41.2	68.9	55.0
Malaysia	56.4	27	37.7	72.5	61.4	36.6	56.4	73.9
Global Average	**38.9**	**-**	**28.4**	**32.3**	**37.6**	**31.5**	**47.8**	**55.8**

Source: Homepage - GHS Index[3]

There are many wicked and complex diseases, disease threats and health security issues the world has been facing. Both eco- and humancentric changes are on the rise that are threatening the world ecosystems and leading to the emergence of many deadly and contagious pathogens. Changes in lifestyle, changes in food production and supply practices, rampant inequality and persistent poverty, increased travel and trade, both nationally and internationally, increase in use of agrochemicals in agriculture and associated industries are some of the factors contributing to this issue. Similarly, incidences such as climate change, increase in environmental contamination, loss of habitats and biodiversity threatening wildlife and aquatic species are threatening animal, human, and environmental health. These global challenges - environmental degradation, public health issues, and socioeconomic disparities - are interconnected and complex, demanding multifaceted solutions.

Furthermore, lifestyle changes such as the shift towards more sedentary lifestyles and high-consumption habits contribute significantly to health issues and environmental strain. The current trends in food production systems are unsustainable, contributing to deforestation, water scarcity, and biodiversity loss. Inequality and poverty are both causes and consequences of environmental degradation and poor health outcomes. While global connectivity has numerous benefits, it also poses risks such as the spread of diseases and environmental impacts. The heavy reliance on agrochemicals has led to numerous environmental and health problems, including soil degradation, water pollution, and the decline of pollinator populations[5].

Articles titled "Which Is the World's Deadliest Animal: A Lion, Tiger, Shark, Snake or Mosquito?"[6] and "The World Deadliest Animal"[7] demonstrate that mosquitoes are the deadliest among all arthropods worldwide. This is because besides biting human beings and inducing irritation, allergy and pain, mosquitoes serve as the vectors for transmitting many life-threatening diseases such as malaria, yellow fever, dengue, chikungunya, Zika, West Nile, filariasis and Japanese encephalitis[6, 7]. The WHO (2024)[8] reports, globally in 2022, an estimated 249 million malaria cases and 608 000 malaria deaths in 85 countries. The changes in climate and environmental variables such as rise in temperature, pollution, and other humancentric actions such as increase in movement of people and goods are creating favorable environments for these vectors to breed and propagate and move from one place to another, thereby transmitting infectious diseases.

Antimicrobial resistance (AMR) which is also called silent pandemic is affecting and killing several thousands of people, animals and destroying a swath of biodiversity worldwide. As of 2019, antibiotic-resistant bacteria cause 1.3 million direct deaths and five million indirect deaths each year and these numbers have gone up after COVID[9]. Rayan (2023)[10] reports that AMR is the world's most serious public health threat which happens when bacteria, viruses, fungi, and parasites change and stop responding to medication. Elderly people and those with underlying medical conditions such as cardiovascular diseases, diabetes, chronic respiratory diseases, and cancer, are affected by this than other groups (WHO, 2024)[11].

The current global health security scenario warrants us that the world needs a strong and resilient public health system at all levels that can prevent, detect, and respond to infectious disease threats. Global health security can be improved through education, research and training by engaging global health networks across regions to collect and share health surveillance data. Furthermore, low-and middle-income (LMIC) countries represent a disproportionate burden of diseases with higher likelihood of adverse public health outcomes, driven in part by limited access to health care, clean water, and a safer food supply. LMICs need support such as preventing, detecting, and responding to diseases and diseases threats to strengthen their health security systems.

Addressing these issues requires a combination of individual action, community initiatives, and policy reform at both national and international levels. Public awareness and education play crucial roles in driving change, as well as the development and adoption of innovative technologies and best practices that reduce harm and enhance sustainability. Collaboration across sectors and disciplines is also essential to create resilient systems that can withstand and adapt to the challenges of the 21st century and beyond.

To this end, educational institutions such as universities and colleges which provide education to youths play crucial roles in preparing competent human resources who can effectively tackle the above-mentioned global health challenges. The educational institutions need to be mindful of the evolving scenarios – local, regional, global – and develop clear and realistic teaching and learning programs for various stakeholders. Educational institutions that have a strong global presence and have wide national and international outreach programs and alumni networks have even bigger roles to play.

MICHIGAN STATE UNIVERSITY (MSU) AND GLOBAL HEALTH

Michigan State University (MSU) is a premier Land-Grant University that has been embracing three interconnected missions — education, research and extension/outreach. MSU is working in 172 countries around the world on a range of global research endeavours with more than 500 developed partnerships. MSU faculty are actively involved in applied research initiatives and training activities in the areas of vector borne and zoonotic diseases; antimicrobial resistance; food safety/security; and water safety and environmental health through local, national, and global partnerships aimed at growing our ability to improve the health of communities across the world. MSU also has strong teams leading global food security initiatives.

MSU has a long tradition of serving as excellence in One Health education and knowledge. MSU is a leading world institution in the Global Health initiative. MSU recognizes five essential interlinked elements of Global Health: Human Health, Animal Health, Agriculture, Food Safety and Security, and Environmental Health. MSU possesses considerable strengths in veterinary diagnostics as it houses a renown referral diagnostic laboratory. Beyond diagnostics, several faculties at MSU are engaging in cutting edge research on vector borne diseases, antimicrobial resistance, aquatic diseases, avian influenza, and wastewater treatment, among others.

This paper discusses global knowledge partnerships in One Health and shares the experiences of Michigan State University. This paper is the culmination of the discussions on various forums including an international conference held on December 13, 2023, in Calicut, Kerala, India. Michigan State University is always open to exploring mutually beneficial partnerships.

WHAT IS ONE HEALTH?

There are many different definitions of One Health in the literature. The one most widely followed is by the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC). According to WHO (2024)[12], 'One Health' is "an approach to designing and implementing programs, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes". The WHO (2024)[12] further notes that the "One Health' is "an integrated, unifying approach to balance and optimize the health of people, animals and the environment which is important to prevent, predict, detect, and respond to global health threats". While Centers for Disease Control and Prevention (CDC)[13] reports that "One Health recognizes that the health of people is connected to the health of animals and the environment". It is a collaborative, multisectoral, and trans-disciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment[13].

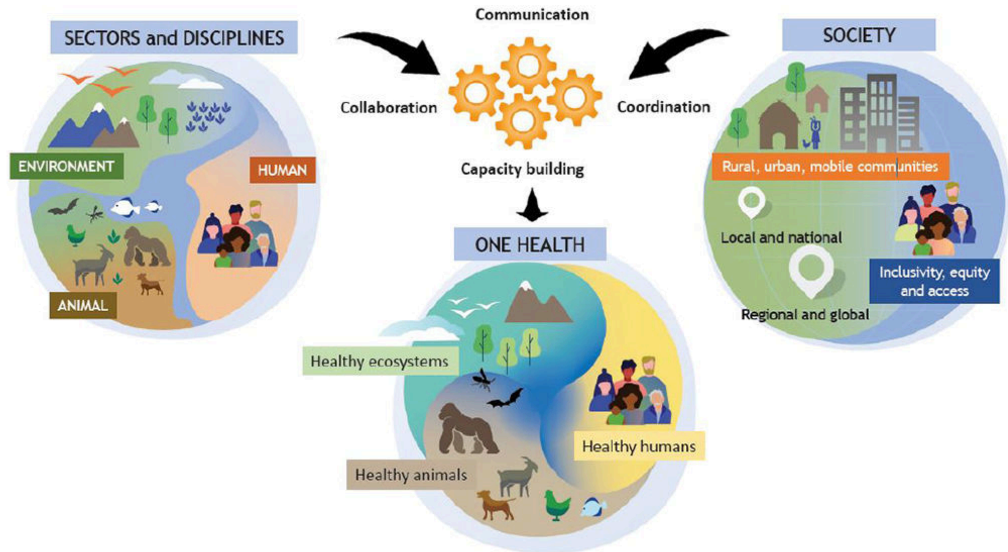


Fig. 1. One Health Definition[12]

The new One Health Framework (Fig. 1) emphasizes that there needs to be effective communication, collaboration and coordination and capacity building efforts not only across sectors and disciplines (human, animal, environment) but also across local, national, regional and global levels. The One Health approach posits that to ensure healthy human beings, there must be healthy animals and healthy ecosystems.

ONE HEALTH AT MSU

At Michigan State University (MSU), College of Agriculture and Natural Resources, College of Veterinary Medicine, Institute of Global Health at the College of Osteopathic Medicine, Department of Biosystems and Agricultural Engineering, Department of Animal Science, and Veterinary Diagnostic Laboratory are engaged in and offering One Health related programs. There are other units and colleges at MSU such as Department of Geography, Asian Studies Center, Asia Hub, and James Madison College also offer some programs that promote the One Health approach. Various One Health programs at MSU are described here.

MEKONG REGION ONE HEALTH INNOVATION PROGRAM (MOHIP)

The MOHIP is a primary One Health focused program that MSU has been implementing in lower Mekong subregion (LMSR) covering Lao PDR, Thailand and Vietnam since the year 2022. The overarching goal of MOHIP (2022-2025) is connecting the U.S. and Mekong One Health researchers and practitioners and encouraging them to share One Health tools (research, education, outreach) to facilitate One Health promotion in the LMSR. Funded by the U.S. Department of State, the MOHIP also aims to establish a transnational network of One Health researchers and practitioners that will help continue and sustain One Health program that MOHIP has initiated. There are three core components in the MOHIP: One Health education, One Health research, and One Health outreach (Fig. 2). These are described as follows.

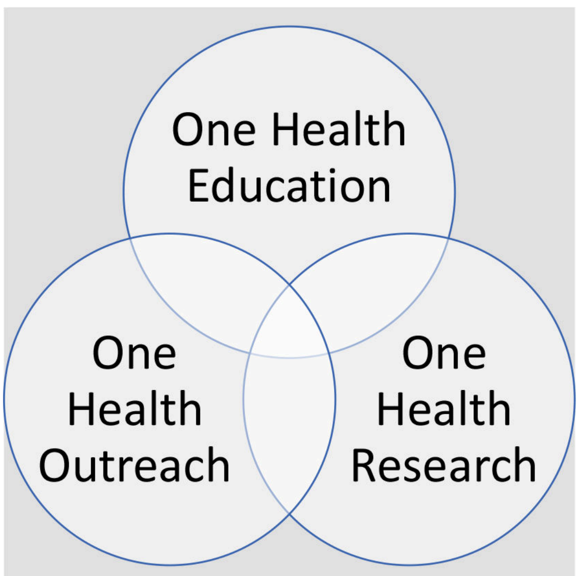


Fig. 2. MOHIP Core Components

The MOHIP has a strong outreach component. MOHIP team connects with beneficiaries and stakeholders in the Mekong through formal and informal connections to network and build relationships and leverage those connections to outreach its programs. Webinars are the core of the outreach for MOHIP. As of

June 23, 2024, 14 webinars have been organized in many different topics related to One Health and inviting speakers from all over the world (Table 2). The flyer (Fig. 3) shows Webinars #12, 13 and 14 with the topics, speakers, program dates and time and links to register. Many of these webinars had two or more speakers, representing both the Mekong and outside Mekong, specifically the U.S. The motivation behind having those combinations of speakers is to encourage those speakers also connect and collaborate and share ideas and tools which is the core of MOHIP.

Fig. 3. MOHIP Webinar #12-14 Flyer

Table 2. MOHIP Webinar Series

Webinar Topics
1. Public Health Approach for Detection and Surveillance of Emerging Zoonotic Diseases
2. One Health Principles and Practices
3. Antimicrobial Resistant Foodborne Pathogens: A One Health Concern
4. Epidemiological Surveillance of Zoonotic Diseases
5. One Health Implementation and Experience in Latin America: South-South Partnership with the Lower Mekong Region
6. Improving Health Security through Environmental Stewardship: The System Integration for Small-scale Wastewater Treatment
7. Health Impacts of Air Pollution: A One Health Perspective Research on Air Pollution in Thailand
8. Soil Health, Animal Health, & Human Health Nexus
9. Household Water Insecurity - Intersections between Humans and Livestock Simple and Feasible Method for Swine waste water Treatment Using Intermittent Aeration
10. Antimicrobial Use and Resistance in Humans and Animals: A Case of Lao PDR
11. SMART Biosensors for One Health Monitoring and Surveillance
12. Biodiversity and One Health
13. The Importance of the One Health Approach for Understanding Infectious Diseases: An Example of a Neglected Tropical Disease Health
14. Land Use, Climate Change and Human Health: A One Health Approach

SHORT COURSE ON HEALTH SECURITY, ONE HEALTH AND ZOONOSES

The MOHIP at MSU offers a short course on Health Security, One Health and Zoonoses. The course on zoonoses that the World Technology Access Program (WorldTAP) launched virtually during the mid-COVID pandemic period in 2021 is the foundation for this course. As the title of the course suggests in the first year of offering in 2021, the course focused on zoonoses. It was important given that the burden of zoonotic diseases is an important global issue affecting human and animal health, food value chains, international trade, and the environment. Twothirds of the infectious diseases affecting human health are of animal origin. Information and knowledge of epidemiology, prevention, control and treatment of zoonotic diseases is critical for managing these diseases. To help address these contexts, the WorldTAP at Michigan State University designed and offered an online international short course on zoonotic diseases in March 2021.

A diverse group of forty-two participants from 15 countries in Africa, Asia, Middle East, and North America participated in this program. Key contents of this comprehensive course included epidemiology of zoonoses, zoonotic diseases of wildlife origin, One Health approach to managing zoonoses, and roles of regional, and international organizations in strengthening zoonotic disease management capacities, lessons-learned from the pandemic on diagnosis, prevention and prediction of zoonotic diseases. The participants appreciated the content of this online short course[14].

Leveraging experiences from the first offering, the MSU team revised the course content and included health security and additional topics on One Health. The MSU offered the updated version titled “Health Security, One Health, and Zoonoses” twice, i.e., in 2023 and 2024. In 2023 this course was limited to the three MOHIP countries and about 500 people applied for it. In 2024, this course was further modified based on the input received from the participants of 2023 cohort. The topics on One Health Economics, the concept and state of One Health research, artificial intelligence and One Health, and antimicrobial resistance and One Health were added. In 2024, about 300 people applied for the course. One hundred and ten of them attended this course. There were 26 resource persons from various disciplines representing Asia, Europe, USA, and Australia sharing their knowledge and experiences through this course – a true global knowledge partnership in One Health!

RESEARCH PROJECTS

The MOHIP at MSU provides funding for qualified projects to be led by researchers from three MOHIP countries, namely Lao PDR, Thailand, and Vietnam. In 2023, the MOHIP selected three proposals. In 2024, other three projects will be funded. The MOHIP has selected three research proposals for 2023 which are: (i) Lao PDR: "Improve the efficiency control and surveillance of brucellosis from animals in Vientiane Capital, Lao PDR"; (ii) Thailand: "Exposures and potential health risk from animals and environmental exposures among the hill tribe and stateless people living in border areas of Thailand-Myanmar-Laos: One Health Approach"; and (iii) Vietnam: "Surveillance of antibiotic contamination, coliform bacteria and resistome in Mekong River Basin environment of Thailand and Vietnam from the One-Health perspective". These three projects represent diverse public health, animal health and environmental health issues ranging from AMR, incidences in Zoonoses and brucellosis. It is envisaged that these research studies will be able to study public health issues facing the region and suggest policies and programmatic measures to address those issues and improve health security.

GLOBAL HEALTH STUDIES

MSU offers the Master of Science in Global Health and the Graduate Certificate in Global Health which are new programs initiated by the Institute for Global Health at Michigan State University (MSU)[15]. This course is uniquely desigend with faculty and courses representing many different colleges and disciplines within the university, such as Veterinary Medicine, Human Medicine, Social Science, Arts and Letters, as well as non-governmental organizations and other agencies with a focus on global health. It has multidisciplinary faculty and student body, focusing on the inter-relatedness and importance of human, animal and environmental health summarized in the concept of One Health.

ONE HEALTH ONE WORLD DAY

To commemorate the start and adoption of One Health approach and to make people aware of this great approach among students, researcher, faculty, and professional societies, the Institute for Global Health (IGH) at MSU organizes the One Health Day annually. It was held on November 3 in 2023 and the theme was "Food Safety, focusing on Pollution, Agriculture, Planetary Health, Eco Health and One Health" (Fig. 4). Besides its own faculty as the speakers, the IGH invited speakers representing plant health and animal health from outside the MSU. This event was well-attended by more than 150 students, faculty, and visiting faculty and scholars, among others.

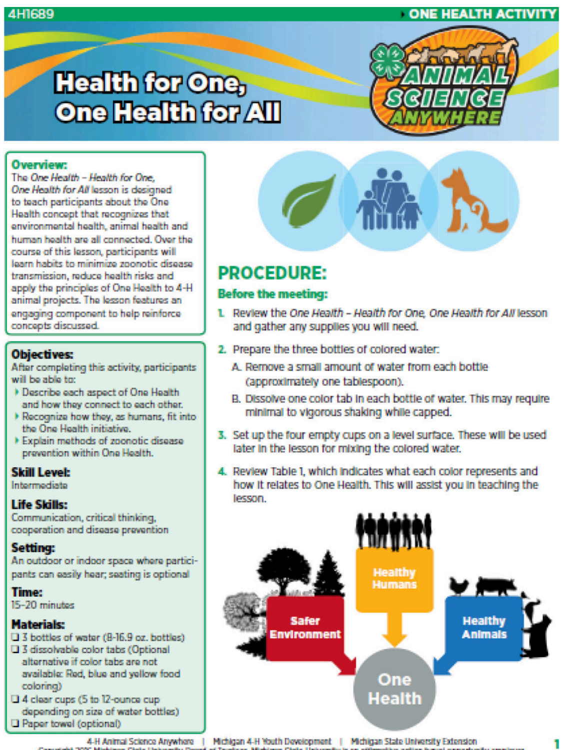
Fig. 4. Flyer for One Health Day 2023

EDUCATION ABROAD PROGRAM ON ONE HEALTH

To give a real-world exposure to students on One Health and facilitate their learning of health and diseases through an interdisciplinary lens, various units of MSU organize education abroad programs in countries outside the US including Nepal. The education abroad programs are also opportunities for both visiting and host country teams to share their issues and experiences and co-learn and pursue collaborations in the future.

4-H ANIMAL SCIENCE ANYWHERE: HEALTH FOR ONE, ONE HEALTH FOR ALL

The Animal Science Anywhere aims to help leaders "engage 4-H youth at club meetings or events in learning more about the science and life skills involved in animal and veterinary science projects"[16] including on One Health[16] (Fig. 5). Youth work in teams or individually in their projects and strive to attain the learning objectives. "Lessons are flexible, providing adaptations for various locations, ages and audiences)[16]. One of the lessons is on One Health and it is entitled "The One Health – Health for One, One Health for All." This is specifically designed to teach students about the One Health approach. In this lesson, "participants learn to minimize zoonotic disease transmission, reduce health risks and apply the principles of One Health to 4-H animal projects"[16]. The lesson includes ample opportunity for hands-on experiences to apply the concepts that they are taught[16].


4H1689 ONE HEALTH ACTIVITY

Health for One, One Health for All

ANIMAL SCIENCE ANYWHERE

Overview:
The *One Health – Health for One, One Health for All* lesson is designed to teach participants about the One Health concept that recognizes that environmental health, animal health and human health are all connected. Over the course of this lesson, participants will learn habits to minimize zoonotic disease transmission, reduce health risks and apply the principles of One Health to 4-H animal projects. The lesson features an engaging component to help reinforce concepts discussed.

Objectives:
After completing this activity, participants will be able to:
- Describe each aspect of One Health and how they connect to each other.
- Recognize how they, as humans, fit into the One Health initiative.
- Explain methods of zoonotic disease prevention within One Health.

Skill Level:
Intermediate

Life Skills:
Communication, critical thinking, cooperation and disease prevention

Setting:
An outdoor or indoor space where participants can easily hear; seating is optional

Time:
15–20 minutes

Materials:
- ❑ 3 bottles of water (8-16.9 oz. bottles)
- ❑ 3 dissolvable color tabs (Optional alternative if color tabs are not available: Red, blue and yellow food coloring)
- ❑ 4 clear cups (5 to 12-ounce cup depending on size of water bottles)
- ❑ Paper towel (optional)

PROCEDURE:

Before the meeting:
1. Review the *One Health – Health for One, One Health for All* lesson and gather any supplies you will need.
2. Prepare the three bottles of colored water:
   A. Remove a small amount of water from each bottle (approximately one tablespoon).
   B. Dissolve one color tab in each bottle of water. This may require minimal to vigorous shaking while capped.
3. Set up the four empty cups on a level surface. These will be used later in the lesson for mixing the colored water.
4. Review Table 1, which indicates what each color represents and how it relates to One Health. This will assist you in teaching the lesson.

4-H Animal Science Anywhere | Michigan 4-H Youth Development | Michigan State University Extension 1
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Fig. 5. One Health as an Integral Part of 4-H in Michigan

ONE HEALTH CLUB

The College of Veterinary Medicine (CVM) at MSU operates a One Health Club that promotes collaboration and communication in all aspects of health care and well-being for humans, animals, and the environment. The Club organizes seminars, community engagement activities, and share updates on global One Health happenings among students and faculty so that they are able to challenge the world view, consider different perspectives, and begin thinking about healthcare with an interdisciplinary mindset[17].

GLOBAL ALLIANCE FOR RAPID DIAGNOSTICS (GARD)

The GARD (2024)[18] is a multidisciplinary and multicultural peer-to-peer network of equals and committed to improving global health through early diagnosis. "Its vision is to save lives and sustain health through rapid diagnostics. GARD has the mission to develop portable, affordable, accessible, and deployable nano-enabled biosensor technologies for rapid and early detection of infectious and antimicrobial-resistant diseases to support sustainable health in populations who need help the most but can afford the least"[18]. As described in the GARD[18] the sustainable health refers to accessible and affordable healthcare, safe and nutritious food, clean water, and clean environment in alignment with sustainable development goals.

MEKONG CULTURE WELL (MCW)

The James Madison College at MSU is implementing the MCW program which undertakes collaborative and innovative projects to understand complex and dynamic ecological, social, and cultural transformations that are taking place. MCW implements and also facilitates diverse projects, activities, and learning opportunities in Southeast Asia, at Michigan State University, and virtually. MCW MSU team has been studying knowledge of human liver fluke among the people of Thailand and Cambodia. Liver fluke is a big problem in Mekong, specifically along the Mekong River and humans get infection after they ingest infected fish.

MSU'S STAKEHOLDERS ON ITS CAMPAIGN ON ONE HEALTH

MSU believes in collaboration, coordination with and empowering local and country partners that could lead to sustainable outcomes. For the MOHIP, MSU has been collaborating with One Health University Networks (OHUN) such as Southeast Asian One Health University Network (SEAOHUN), Laos One Health University Network (LAOHUN), Thailand One Health University Network (THOHUN) and Vietnam One Health University Network (VOHUN).

Established in 2012 with the support from the Emerging Pandemic Threats program funded by USAID to build regional, national and local One Health capacities for early disease detection, prevention, rapid response and containment, and risk reduction, THOHUN has 14 universities and 83 faculties covered under its umbrella (Fig. 6).

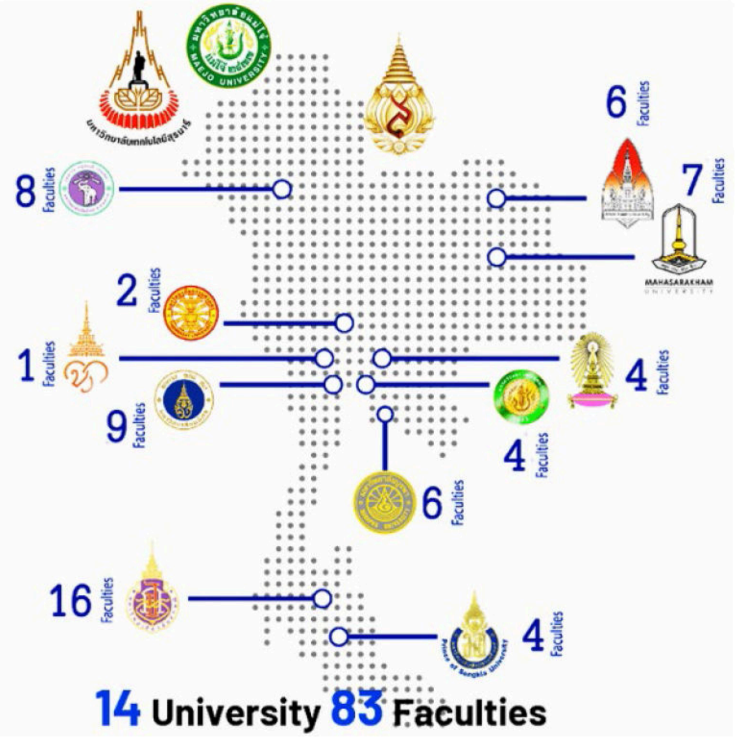

Fig. 6. THOHUN (Thailand One Health University Network) (seaohun.org)[20]

Vietnam One Health University Network (VOHUN) was established in 2011, with the support of USAID, through the RESPOND project[19,20]. There are 20 universities under VOHUN which represent the field of medicine, veterinary science, public health, nursing and food technology to promote the One Health (OH) approach, through university training and research, to equip a new generation of lecturers and researchers with a full range of knowledge and skills to practice the OH approach.

Established in 2018, the Lao One Health University Network (LAOHUN) is the youngest network within SEAOHUN. LAOHUN comprises five universities and 18 faculties. The LAOHUN aims to become a collaborative network of universities fostering multi-sectoral effort on One Health (OH) workforce and policy development.

GOVERNMENT INSTITUTIONS

MSU One Health team collaborates with government ministries, preferably with Ministries of Agriculture, Health, and Environment in many Asian, African and Middle Eastern countries. MSU team also works with the U.S. Government posts and missions in Asia and Africa to implement One Health programs.

DISCUSSION

Michigan State University has a long tradition of excellence in One Health education and knowledge generation and knowledge sharing. MSU is a leading public university in the Global Health initiative. MSU recognizes five essential interlinked elements of Global Health: Human Health, Animal Health, Agriculture, Food Safety and Security, and Environmental Health[21]. MSU Global Health Programs seek to promote, protect, and improve worldwide health by encouraging and enhancing interactions and scientific collaborations in these areas[21].

MSU strives to adopt the One Health approach to prevent, predict, and control diseases results in positive outcomes, such as increased awareness, coordination, and cooperation among stakeholders, and resource sharing for zoonoses management. Inter- and trans-disciplinarity are powerful approaches to co-creating actionable knowledge and increasing the impact of science. MSU will form alliances with like-minded national and global partners to incorporate the One Health model (human-animal-environment nexus) and share resources and tools related to health security with three foci: (i) *Networking*: Strengthen transnational networks of One Health practitioners. (ii) *Co-creation of Research*: Continue to collaborate in identifying gaps and developing training programs for community trade networks, partners and one health researchers, and (iii) *Collaborative Outreach and Research*: Through webinars and virtual symposiums to educate stakeholders globally in emerging diseases and health threats and prepare them to address those threats.

We believe that the proposed approach will be a cornerstone to understanding the complexity of climate and environmental variables on food production and the onset of zoonotic diseases and how they individually and in combination affect food security. It is our hope that these efforts will inform policy and programmatic measures to address these challenges specifically through the One Health approach.

CONCLUSIONS AND WAY FORWARD

This paper discusses global knowledge partnerships in One Health and shares the experiences of Michigan State University in One Health programs. MSU is always open to exploring mutually beneficial partnerships. We live in a global village and inter-connected world. We have to be mindful of and gather intelligence about the new developments and changes that are happening in the world whether they are in our proximity or far way in another continents. Such changes might affect and impact us and our ecosystems.

One Health is a holistic, transdisciplinary, and systems approach and works well to understand and address health issues facing the world. The One Health approach is necessary to address global "wicked problems" like zoonotic diseases. Since One Health approach allows us to think outside of the box and this is helpful to better protect the health of humans, plants, animals, and the environment. However, implementation of One Health approach requires political and sectoral commitment, cooperation, and ownership; resources, skills, capacity, and incentives; international collaborations and partnerships; and continued research, education and outreach using multiple perspectives, stakeholders, and disciplines to generate holistic solutions.

In conclusion, with the emergence of new threats, there are innovations or innovative technologies coming up too. For example, the global community now has smart communication tools which we did not have before to connect, share information and interact globally. These digital tools allow us to easily access and exchange information and be informed of any new developments globally. As has been emphasized in the new model of One Health, there is an urgency to coordinate, collaborate and communicate with people and institutions globally. Partnerships are becoming critical as resources become scarce and competitive. Partnerships bring benefits to everyone. Partnerships also provide opportunities to share resources. Scientific conferences and symposia are opportune platforms to meet and network with professional colleagues and build collaboration to share information and knowledge and explore and nurture and solidify communication, collaboration and coordination with not only with our professionals from our own fields but also from other disciplines.

We conclude the paper with the quote from WHO Director General, Dr. Tedros Adhanom Ghebreyesus[22]. "*Human health does not exist in a vacuum, and nor can our efforts to protect and promote it. The close links between human, animal and environmental health demand close collaboration, communication and coordination between the relevant sectors*." The quote provides insight to the entire world including us at the MSU that the people from human, animal and environmental health realms must work together in a coordinated manner, sharing information on a timely manner and acting proactively during any disease outbreak or even before and after that while also learning from the past experiences.

Author Contributions: RG: Conceptualization; Writing - original draft; review and editing; KM: Conceptualization; review and editing.

Conflict of Interest: The authors declare no conflict of interest.

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1st June, 2024

Scientist's Bio-bibliography

DR. SHRI PRAKASH: LIFE AND WORKS OF AN EXTRAORDINARY DEFENCE ENTOMOLOGIST OF PRACTICE

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Received : 3rd May, 2024
Accepted : 20th May, 2024

ABSTRACTS

Bio-bibliography of Dr Shri Prakash, an eminent entomologist in the Defence Research & Development Organization of the Government of India, is presented highlighting his contributions to facilitate working of Indian armed forces in difficult terrains infested by vicious mosquitoes and other hematophagous flies, mites, ticks etc., besides leeches, and other nuisance organisms. One of his major contributions to the Defence Entomology is playing a

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Cite this article as:
Veer, V, Mendki, MJ, Tyagi BK, Dr Shri Prakash: Life and works of an extraordinary Defence entomologist of practice. *J Med Arthropodol & Public Health*. 2024; 4(1): 85-106.

pivotal role in facilitating industrial production of the indigenously invented mosquito repellent, DEPA (N'N' diethylphenylacetamide).

PART – I: BIOGRAPHY

"If you want to shine like a Sun,
first burn like a Sun."

– Dr. A.P.J. Abdul Kalam

The above metaphor emphasizes the message that achieving a successful life requires a commitment to hard work, dedication, honesty, sincerity, and truthfulness. It suggests that there are no easy shortcuts to success; instead, one must put in consistent effort and uphold certain values to achieve their goals.

Fig. 1. Dr. Shri Prakash Ph.D., Former Associate Director & Head, Entomology Division, Defence Research & Development Establishment (DRDO), Gwalior, MP, India.

Shri Prakash has embodied all these attributes which are reflected in his life through his research and achievements. His life is hugely motivational, and he likely serves as a role model for others by demonstrating how applying these qualities can lead to a successful and fulfilling life. This metaphor also underscores the importance of integrity, diligence, and perseverance in the pursuit of one's aspirations in the realms of science (Fig.1).

Dr. Shri Prakash has earned a great name and fame largely through dint of his hard work reflected by highly prestigious and technology developments. His vision in research mainly focused on translating the classical biology of the organisms to the technologies by an impressive number of original research publications. He always researched objectively with a focus on carrying the output of his work to industry for commercial production so that the benefit could reach end-users. He championed in the field of conducting research that earned him several copyrights, patents, transfer of technologies etc., all for the use in defense services. He achieved phenomenal success in instituting this philosophy of inventive and innovative research in the Division of Entomology headed by him. During headship many technologies were transferred to the industry for wider production and circulation through commercialization. The story of DEPA (N' N' diethyl phenyl acetamide) is not only highly inspiring but also immensely gratifying to witness its use by the Indian defense forces in the frontier areas where blood-sucking mosquitoes, flies, bugs, leeches roaches, etc. make the living full of challenges. During his headship, the Entomology Division (now department is known as Vector Management Division) progressed largely in the 'delivery mode' of products, and in this indispensable activity he was ably supported by a team of a handful of industrious and brilliant scientists like Dr. Vijay Veer, Dr. B.D. Parasher, Dr. M.J. Mendki, Dr. S.N. Tikar, Dr. Kshitij Chandel and others.

Dr. Shri Prakash was born into a middle-class Kayastha family in Lucknow, Uttar Pradesh, on 17th July 1949. His father, Late Shri Shyam Manohar Nath, served as a government official in the industries department of the UP government in Kanpur, while his mother was a homemaker. Among his siblings, he was the third child, with an elder sister, an elder brother, and a younger sister. Both sisters pursued careers in the teaching profession, and his elder brother retired as a branch manager at the State Bank of India in Kanpur. He got married to Rekha Shrivastava in 1976. She was a teacher in a government higher secondary school, Gwalior, Madhya Pradesh. She served for 38 years and was greatly admired for her dedication and honesty. They have two children, a daughter and a son who is elder, both doctors by profession. Their son, Dr. Advait Prakash, a pediatric surgeon, is currently a professor in a medical college. His wife, Dr. Dipti Saxena, is also a professor in Anesthesiology. They have a twelve-year-old daughter, Aditi Prakash. Dr. Shri Prakash's daughter, Dr. Pragya Prakash, is an Ophthalmologist currently working as an Assistant professor in a medical college. She is married to Dr. Anurag Shrivastava who is an ENT Surgeon, currently an Assistant professor. They have a eight-year-old son, Amey Shrivastava. After retirement Dr. Shri Prakash settled down in Indore (MP) along with his family (Fig. 2).

Fig.2. Family members. Dr. Shri Prakash and Mrs. Rekha Shrivastava (Sitting). L to R.(Standing) Ms. Aditi Prakash, Dr. Dipti Saxena, Dr. Advait Prakash, Dr. Pragya Prakash, Dr. Anurag Shrivastava, Master Amey Shrivastava.

Dr. Shri Prakash did his schooling, graduation and post-graduation from Kanpur. He completed his M.Sc. in Zoology in 1969. His professional career started in 1971 when he joined Defence Research Laboratory Materials (DRLM; a laboratory of DRDO and presently known as Defence Material Stores Research and Development Establishment, DMSRDE), Kanpur as Junior Scientific Assistant (JSA). His main area of work there was in storage entomology. After one and half year of service, he was transferred to Gwalior - one of the detachments of DRLM, Kanpur - in 1973.

In the same year Gwalior laboratory was made an independent unit as Defence Research & Development Establishment (D.R.D.E.), with a mandate of research and development in the fields of biology and chemistry. The research divisions that were mainly focused on included Microbiology, Entomology, Pharmacology and Toxicology, Medicinal Chemistry, Synthetic Chemistry, Analytical Chemistry etc. so that most of the research activities can be done under one umbrella on a molecule of interest, to enable a comprehensive understanding of its properties, effects, and potential applications. This collaborative multidisciplinary approach helped to generate necessary data for regulatory purposes/registration and subsequent technology transfer. Later the DRDE laboratory underwent a successful transformation and achieved a higher level of research excellence.

In 1974 Dr. Shri Prakash was transferred to the D.R.D.E, Gwalior which proved a turning point both in his personal and professional life, with the D.R.D.E. becoming his *karmabhoomi* destined to shape his highly checkered and meandering career in defence science. Having joined the Entomology Division, he laid an emphasis on Medical Entomology. To grasp the discipline and update his skills in medical entomology he underwent a streak of training in (i) National Institute of communicable diseases (now known as National Center for Disease Control), Delhi for three months in 1974, (ii) Breeding and maintenance of laboratory mice at National Institute of Virology in 1977, (iii) Safety aspects in the research applications of ionizing radiations at Bhabha Atomic Research Center (1986), (iv) Project management course at Institute of Technology Management, Mussoorie (1991), and (v) Course on Intellectual Property Rights at Institute of Technology Management, Mussoorie (2002). He also did M.Sc. specialization in entomology, in 1976, albeit already a specialization in ichthyology (Fish & fisheries). Later, he was awarded the degree of Ph.D. in 1983 in the field of chemo-sterilants application in genetic control of pest and/or vector insects then an emerging discipline with much promise.

Dr. Shri Prakash's main area of research is development of personal protection measures for defence frontier regions and other difficult terrains. Since these troops were often posted in the dense forest areas the spraying of insecticides was neither practical nor economical. Therefore, alternative methods were to be developed keeping diverse terrains in mind. Personal protective measures such as repellants were an obvious choice. Dr. Shri Prakash is instrumental behind the gift of DEPA (N' N' diethylphenyl acetamide), the indigenously produced repellant functionally paralleled with repellent, DEET, earlier imported to India at a huge cost. Repellent work was begin under the leadership of Dr. R.V. Swamy (then Director of DRDE, Gwalior and a well known chemist) and Mr. K.M. Rao (then head of the Entomology Division). Later Dr. Shri Prakash and his team lead multi-insect repellent research and development work and encounter a long and zigzagged peregrination full of challenges. He, however, finally braced the success and his name will thus be written in the annals of science with great honour and glory. Under his leadership a team of scientists comprising chemists, biochemists, entomologists and toxicologists contributed to develop indigenously available safe, effective and nontoxic product for use by the armed forces. After more than three years of rigorous and hard work, few chemical compounds were shortlisted based on various parameters such as molecular weight, molecular length, hydrophobicity, vapor pressure etc. They were tested for biological efficacy in laboratory and field evaluation was done against pestilent and vector mosquitoes as well as other blood sucking organisms especially those prevalent in the northeastern sector such as Assam and Arunachal Pradesh. After successful completion of field evaluation, the compound selected was tested for safety by doing acute, subacute and long-term studies employing various routes such as oral, dermal and inhalation as per the protocol of the Drug Controller General of India (DCGI), New Delhi. The data collected after extensive studies, was submitted to DCGI for registration for use in human beings as topical application. Subsequent to its approval, the product was up scaled in pilot plant at D.R.D.E., Gwalior and National Chemical Laboratory, Pune. Various formulations such as cream and lotions were developed and distributed in the Army. They instantly became a mainstay for protection against mosquitoes. Patents were obtained and the product was included in Indian Pharmacopeia (IP). The technology was transferred to more than half a dozen entrepreneurs for development of various formulations. ***The fable of DEPA discovery is a classical example of product development from lab-to-land of immense practical utility*****,** reminding us the ageless significance of the famous adage: "*The voyage of discovery is not in seeking new landscapes but in having new eyes.*" (Kandasamy, C., 2021. DEPA (N, N-Diethyl Phenylacetamide): Commercial Journey of India's First Indigenous Mosquito Repellent with Novel Properties. Pages 369-377; In: *Genetically Modified and other Innovative Vector Control Technologies Eco-bio-social Considerations for Safe Application.* Editor: B.K. Tyagi; Springer-Nature, Singapore, xxiv+449 pp.

Dr Shri Prakash has diverse interests in animal science. He was actively associated with the management of rodents in naval ships. His research exploration in rodent control is considered highly useful. Since rodents were difficult to control with available rodenticide baits and mostly died in inaccessible places after consumption of poison, causing stinking odour to emanate from airconditioning systems, he undertook alternate control measures against rodents in ships and succeeded with great aplomb. A rat attractant plus poison bait (Ratox^{TM}) was developed. It was found very effective in rodent control. Now waiting for its commercialization. His grasp of different animal sciences came quite handy to him when he contemplated to find an alternative tool to prevent mosquito bite – the DEPA! The late President of India and Bharat Ratna Dr. A.P.J. Abdul Kalam has also emphasized step-by-step learning just as knowing well one science breeds another science coming easily to you:

"Learning gives creativity
Creativity leads to thinking
Thinking provides knowledge
Knowledge makes you great"

Dr. Shri Prakash knew early in his research career that the real science is in basic science and, therefore, was interested in conducting contemporary basic research. His work included DNA marker for the identification of mosquito vectors and other pests and their microbial diversity in midgut and salivary gland. These studies were carried out in collaboration with the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad and National Centre for the Cell Science (NCCS), Pune. Midgut and salivary gland microflora have been reported to influence the development of parasites/pathogens in mosquitoes. It has been also demonstrated that the genetic profile of insects plays important role in determining the vector competence. The study aimed to understand the microbial diversity in the midgut and salivary gland of field-collected mosquitoes and genetic variability in mosquitoes.

Other significant basic research done by Dr Shri Prakash focuses on the role of human skin emanations in host-seeking behaviour of mosquitoes. Human skin emanations are known to attract host-seeking female *Culex quinquefasciatus*, the vector of human lymphatic filariasis. Various chemical components of human skin emanations (carboxylic acids, alcohols, and aldehydes) were evaluated separately at different doses for electroantennogram (EAG) and for behavioral assay. The studies have very clearly indicated that mosquito use air-borne olfactory cues produced by chemical components found in human skin emanations which are associated with host-seeking behaviour.

Dr Shri Prakash has won many prestigious awards among which the "D.R.D.O. Scientist of the Year Award – 2007", given away to him by Dr. Manmohan Singh, the then Prime Minister of India, is very close to his heart (Fig. 3). Other significant awards won included: Defense Technology Spin-off Award (2001), Laboratory Group Technology Award (2003), Technology Day Commendation Award (2004), and Best Innovations Futuristic Development Award (2008).

Fig. 3. Dr. Shri Prakash, Associate Director & Head of the Entomology Division, D.R.D.E., Gwalior receiving highly coveted award of "The DRDO Scientist of the Year Award' (2007) from the then Prime Minister of India, Dr. Manmohan Singh during the D.R.D.O. Awards Function in Delhi.

Dr. Shri Prakash had a highly prolific and gratifying nearly three-decade- long career in his *alma mater* D.R.D.E., Gwalior and his glittering innings ended in the year 2009 when he retired from the post of scientist 'G' at DRDE, Gwalior. However, absolutely inevitably irrefutably befitting to his glorious contribution toward the D.R.D.O. through his immaculate research findings, the Government of India was pleased to extend his service by two more years as the Emeritus Scientist.

Dr. Shri Prakash is one of those rare defence scientists who by their energetic character not only practically educate the budding researchers in habits of industry, but by the example of diligence and perseverance which they set before them, largely influence the scientific activity in all directions and contribute in a great degree to form the scientific character. The national progress, it is aptly said, is after all the sum of individual industry, energy and uprightness, and Dr. Shri Prakash is a glorious example in our own lifetime. He is very wise, affable and considerate, always ready to help others. He has very many good, close friends and colleagues who still associate with him and seek his advice. Currently he is living a peaceful life with his near and dear ones, fully satisfied what almighty God has bestowed upon him in the service of science and society, like what Louis Pasteur, the well-known scientist who discovered the food preparation process known as pasteurization:

"Let me tell you the secret that has led me to my goal.
My strength lies solely in my tenacity."

PART - II: BIBLIOGRAPHY

I. Products developed by Dr. Shri Prakash

1. **Roachtox**™ and **Roachline**™ – These products are for cockroach control. Product Roachtox is in tablet form while Roachline is chalk stick form. These products are superior to others in having attractant plus insecticide. Both are highly effective against all three common house cockroaches. These are approved by the Central Insecticide Board (CIB), Faridabad under Act 9 (3B). Therefore, licensing is easier.

2. **DEPA** (diethyl phenylacetamide)- a multi-insect repellent. It can be used as cream and spray formulations for protection against mosquitoes and other biting insects. Product is approved by the Drug Controller of India. DEPA spray is regularly used by the Armed Forces for many years now.
3. **Ratox™-**This is for rodents control and highly effective against *Rattus* spp. found in home, railway stations and ships. It contains a rodenticide plus rodent attractant**.** Highly safe to users.
4. **Attracticide™-** This product was for mosquito population control. It contains mosquito oviposition attractant plus a growth regulator. Eggs laying female mosquitoes are attracted to water baits to lay their eggs which are subsequently die due to the presence of growth regulator in bait water. This product is also highly effective in reduction of mosquito population at area.
5. **Insecticidal Paint-** This product is found highly effective against crawling insects such as flies, ants and cockroaches, etc.

   N.B.: *Further information on these products can be obtained from the Director, D.R.D.E., Jhansi Road, Gwalior-47400. One can also contact to Dr. Shri Prakash at his email ID: shripra2004@yahoo.co.in*

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III. Patents acquired by Dr. Shri Prakash (as inventor/ coinventor)

(A) *International Patents:*

1. Composition for use in the control of dengue transmitting mosquito. (European Patent; No: EP 1850666B1)
2. Composition for use in the control of Dengue transmitting mosquito. (US Patent No: 2008/0274,076)
3. Composition for use in the control of dengue transmitting mosquito. (World intellectual property organization, PCT, No: WO 2006/090410 A1)
4. Composicao para atriar matar para uso em atrair o mosquito *Aedes aegypti* processo para a preparacao da composicao. (Brazil No. A01 N27/00.2009.01)
5. A wool care composition. (European patent, No: EP 2382351 B1)
6. A wool care composition. (US Patent No: 2011/ 0305644 A1)
7. A wool care composition. (PCT No. WO2010/086872 A1)
8. N, N Di alkyl morpholine4-4 carboxamide compounds as insect repelling agents and process for their preparation (PCT, No. WO 2012/104865A8)

(B) *National patents:*

1. A process for the preparation of glossy slow-release insecticidal paint for insect control (Patent no. 178869, dt 24.12.2004).

2. An insect repelling compound for repelling disease transmitting insects like mosquito and process thereof. (Indian Patent Application no. 204/DEL/2011)
3. An insect repelling compound and process thereof (Indian Patent Application no. 1676/DEL/2010)
4. A wool care composition (Indian Patent Application no. 545/DEL/2009)
5. Attractant composition for bed bug (Indian Patent Application no. 545/DEL/2009)
6. Composition for use in the control of Dengue transmitting mosquito (Indian Patent Application no. 404/DEL/2005)
7. Improved multi-insect repellant formulation and process thereof (Indian Patent Application no. 1741/DEL/2005).

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Graphical Abstracts

WING GEOMETRIC MORPHOMETRY: A TOOL FOR DISCRIMINATING *CULICOIDES* VECTOR SPECIES OF INDIA

Nabanita Banerjee and Abhijit Mazumdar

Department of Zoology, University of Burdwan, West Bengal

Received : 17th April, 2024

Accepted : 13th May, 2024

ABSTRACT

Culicoides spp. are significant vectors of medically and veterinary important pathogens, like bluetongue virus (BTV), African horse sickness virus (AHSV), and epizootic hemorrhagic disease virus (EHDV). BTV has caused substantial losses in Indian sheep and goat populations, while cattle often show subclinical infections. In India, *Culicoides oxystoma, C. peregrinus, C fulvus, C. imicola, C brevitarsis, C. actoni, and C. orientalis* have already been reported as the putative vectors of BTV. Therefore, the taxonomic sufficiency of these vectors is essential for effective disease control. Traditional morphometric methods rely on morphological characters, wing patterns, linear measurements, and ratios, which complicate differentiating closely related *Culicoides* species, especially cryptic ones. Thereby, these methods often result in misidentifications that impair epidemiological

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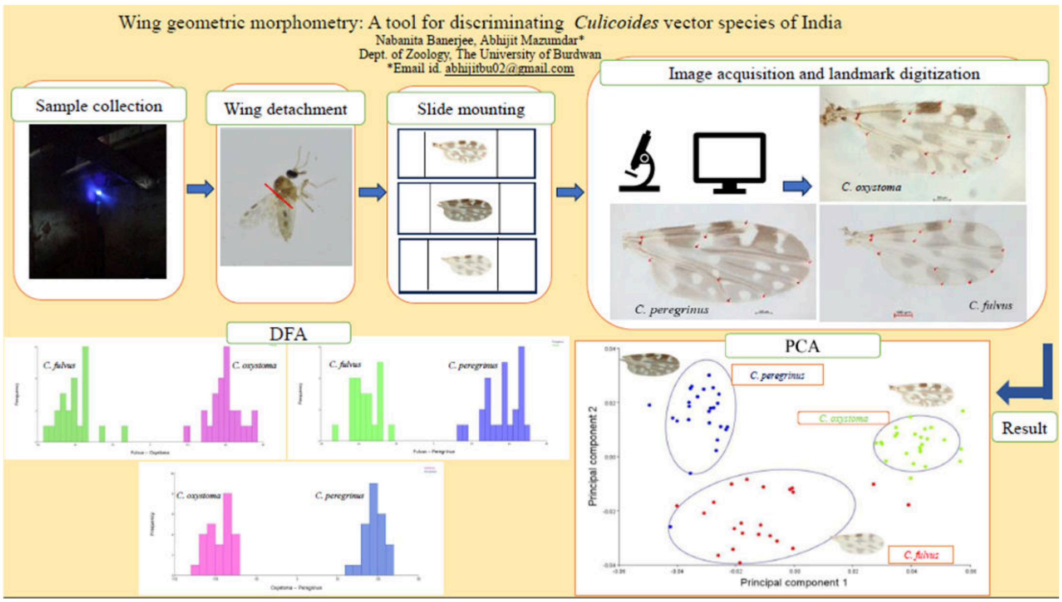
Cite this article as:

Banerjee, N, Mazumdar, A, Wing geometric morphometry: a tool for discriminating Culicoides vector species of India. *J Med Arthropodol & Public Health*. 2024; 4(1): 107-108.

assessment. Molecular techniques, while precise, are costly and need comprehensive reference databases.

Geometric morphometrics offers a promising alternative by analyzing shape variations among species or individuals. This method utilizes anatomical landmarks to capture geometric information, describing shape, size, and their relationships. By harnessing statistical power, in some instances, geometric morphometrics also enables differentiation between cryptic and sibling species. Our study focuses on applying GM to distinguish the three most abundant BTV vector species, viz. *C. oxystoma, C. peregrinus,* and *C. fulvus*. This article presents the species-specific differences to some extent through variability estimated by plotting the shape-based GM.



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1st June, 2024

Graphical Abstracts

POPULATION STRUCTURE OF *CULICOIDES* WITH SPECIAL REFERENCE TO VECTORS OF BLUETONGUE VIRUS (BTV) IN INDIA

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Received : 17th April, 2024

Accepted : 13th May, 2024

ABSTRACTS

Bluetongue virus (BTV) is mainly transmitted by species within the subgenera *Avaritia* Fox, *Hoffmania* Fox and *Remmia* Glukhova in India. Five of the seven reported putative BTV vector species are from subgenus *Avaritia* i.e., *C. actoni, C. brevitarsis, C. orientalis, C. fulvus* and *C. imicola* while *C. peregrinus* belongs to subgenus *Hoffmania* while *C. oxystoma* to *Remmia*. Traditional taxonomy of species in this subgenus is based on wing pattern which often leads to misidentifications due to minute differences in the pattern of pale and dark spots among species. For example, *C. oxystoma* and *C. schultzei*, and *C. fulvus* and *C. orientalis* have similar wing patterns. The presence of intraspecific morphological variations lead to misidentification of species. Other morphological characters like

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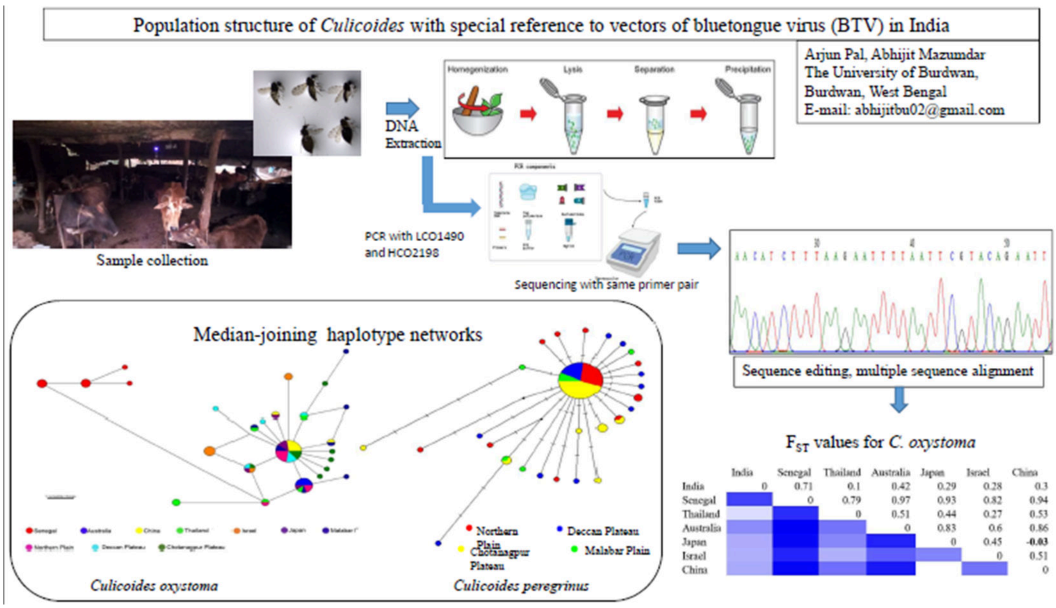
Cite this article as:
Pal, A, Mazumdar, A, Population structure of Culicoides with special reference to vectors of Bluetongue virus (BTV) in India. *J Med Arthropodol & Public Health*. 2024; 4(1): 109-110.

sensilla coeloconica pattern, number of mandibular teeth among others often show overlap among species.

Molecular phylogenetic analyses have sought to address some of the problems faced in morphological taxonomy. However, the presence of cryptic species, hybrid individuals and highly divergent genetic structure from different populations have given rise to newer problems. Recent studies have uncovered genetically divergent lineages within five of the seven Indian BTV vectors, i.e., *C. actoni, C. brevitaris, C. imicola, C. peregrinus* and *C. oxystoma* including the presence of multiple BINs which is indicative of presence of cryptic species or possible misidentification.



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Request for contributing manuscripts for the next issue scheduled to be published on December 1, 2024

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Vol. 4, No. 1, of *Journal of Medical Arthropodology & Public Health* (June 1, 2024) was published on date. We take this opportunity to request the scientific fraternity having research interests in medically important arthropods (e.g., insects, arachnids, centipedes, millipedes, crustaceans etc.) to submit their research manuscripts for consideration of publication in the Vol. 4, No. 2 (December 1, 2024) on or before September 30, 2024. For SUGGESTIONS TO AUTHORS please see either this issue or the SOMA website, www.soma16.org.

Expecting your kind understanding and cooperation in this regard, and looking forward to receiving your manuscripts before very long, we remain, with best wishes,

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