

DETECTION OF BLUETONGUE VIRUS VECTOR AND ITS CHARACTERISTICS IN JHARKHAND

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The present surveillance study was conducted to detect the prevalent species of *Culicoides* midges, an important vector of bluetongue virus, in different districts (viz. Hazaribag, Ranchi and Khunti) of Jharkhand state of India covering central and north eastern plateau agro-climatic zone. Habitat of the midges was noted and meteorological parameters were analysed to correlate the breeding and propagation potentiality of the midges in those areas. The principal habitat of *Culicoides* midges were sheds of sheep, goat, cattle or buffalo and decomposed manure. Dirty manger, cracked walls and open drainage system were also noticed to be habitat of midges. The data indicated that conducive environment for multiplication and propagation of *Culicoides* existed in different districts of Jharkhand, at least in studied areas. *Culicoides oxystoma* being observed to be more than 85% in each catch may be considered as the predominant species in these districts of Jharkhand for the first time.

Key words : Bluetongue, *Culicoides* sp., habitat, Jharkhand, midges.

Bluetongue is an infectious, non-contagious, vector-borne viral disease that affects wild and domestic ruminants such as sheep, goats, cattle, buffaloes, deer, antelopes and various other Artiodactyla as vertebrate hosts. The disease is caused by the bluetongue virus (BTV). Cattle and goats are major vertebrate hosts of the virus, but sheep and deer usually exhibit clinical disease characterized by fever, depression, nasal discharge, drooling of saliva, oral lesion,

facial oedema, hyperaemia of coronary bands and muscle weakness (Afshar, 1994). The infection in goat, cattle and buffalo is generally sub-clinical (Luedke and Jones, 1984), while acute and sub-acute infection develops in mule-deer and elk, respectively. In a highly susceptible sheep population it can cause up to 70% mortality (Parker *et al.*, 1975). BT is a multispecies disease and notifiable to the World Organization for Animal Health (OIE, 2006).

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Taxonomically, BTV is classified as a species or serogroup in the *Orbivirus* genus in the family *Reoviridae* (Borden *et al.*, 1971).

BTV is transmitted by biting of blood-feeding insect vectors of the genus *Culicoides* spp. (Diptera: Ceratopogonidae) (Mellor *et al.*, 2000). Midges of just a few species in the genus *Culicoides* (the insect host) transmit BTV among susceptible ruminants, having become infected by feeding on viraemic animals (the vertebrate host) (Standfast *et al.*, 1985). These midges are proven or suspected vectors of the causative agents for various economically important diseases, viz. bluetongue, African horse sickness, epizootic haemorrhagic disease, bovine ephemeral fever, etc.

The family Ceratopogonidae (Order Diptera) currently includes 6056 species; of which 1322 species belong to the genus *Culicoides* (Borkent, 2012). About 60 species of *Culicoides* have been reported to occur in India (Maheshwari, 2012). Very few species of *Culicoides* have been demonstrated to be vectors for BTV, with the principal vectors varying geographically.

Jharkhand state, geographically located at 22°28' N - 25°30' N latitude and 83°22' E–87°40' E longitude with an altitude up to 1142 m shows humid to sub-humid tropical monsoon type of climate. No epidemic of *Culicoides* borne disease has been reported from Jharkhand till date. However, in our previous study, we could detect seroprevalence of bluetongue in ruminants of these districts of Jharkhand (Tigga *et al.*,

2015). Hence, in the next step, it is quite expedient to identify vectors of bluetongue virus that might play a role to carry and circulate the virus in the state. In this background, the present study was conducted to identify prevalent species of *Culicoides* midges and its nature of habitat in Jharkhand. The meteorological parameters of different districts of Jharkhand were also assessed to know whether conducive environment of breeding and propagation of *Culicoides* midges exist during breeding season of *Culicoides* (May to September) or not in the studied areas.

MATERIALS AND METHODS

Collection of *Culicoides* midges : *Culicoides* were collected from different districts of Central and North Eastern Plateau agro-climatic zones of Jharkhand during the months of September to November, 2013 and February, 2014 for species identification. *Culicoides* midges from most of the places were collected (three catches/ place) at the evening hours approximately from 4.30 pm to 6.00 pm. Insects were trapped from near the animal shed, mangers, water trough and mostly from surroundings of manure pit, water channels, irrigated pastures. For species identification, midges were stored in two forms, one in dry form and another in wet form by immersing in 70% glycerol alcohol (10% glycerol in 70% alcohol) (Reddy *et al.*, 2008).

Identification of *Culicoides* midges : The trapped insects were sorted under a light microscope. The number of *Culicoides*

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midges was counted and percentage of midges/ catch was determined in all the cases after screening minimum 200 insects under microscope. The identification of species of *Culicoides* was made based on the morphological characteristics (Sen and Dasgupta, 1959 and Dasgupta, 1995).

Habitat of midges : During collection of *Culicoides* midges from different districts of Jharkhand, their habitat was noted.

Collection of meteorological data : Meteorological parameters (average rainfall, maximum and minimum temperature and relative humidity) of different districts of Jharkhand under different agro-climatic zones have been collected from the Department of Agricultural Physics and Meteorology, Birsa Agriculture University, Kanke, Ranchi, Jharkhand and Jharkhand Meteorological Department during May to September, 2013.

RESULTS

Identification of *Culicoides* midges : After shorting under microscope, *Culicoides* midges were found to be more than 85% in all the catches. The midges were medium sized flies with moderately hairy wings with numerous distinct pale spots including a pale spot over r-m crossvein almost on the center of the vein. Radial cells were absent; anterior border of wing with two dark spots, one at the tip of radial vein forming the stigma, other at about the middle of cell R5. Aedeagus was prominent with saddle- shaped stem. Paramere was broad at the base, curved

and tapered towards the tip with apical hairs indicating midges were of *Culicoides oxystoma*. *Culicoides* samples collected from Hazaribag, Ranchi and Khunti were identified as *Culicoides oxystoma* as shown in Fig.1.

Habitat of midges : The principal habitat of *Culicoides* midges were sheds of sheep, goat, cattle or buffalo and decomposed manure. The detailed observation of the surroundings was done for better understanding about the habitat of midges. Dirty manger, cracked walls and open drainage system were also noticed. The habitat characteristic of *Culicoides* are summarized in Table 1.

Meteorological data : It was observed that average rainfall varied from 71 to 182 mm/yr; maximum temperature varied from 41 to 43°C and that of minimum temperature from 25 to 27°C. The relative humidity varied between 80 to 97% (Table 2).

DISSCUSSION

With the aim of assessing the conducive environment for propagation and breeding of *Culocoides* midges along with habitat of prevalent midges of Jharkhand, studies were conducted on vector biology in different districts belonging to Central and North Eastern Plateau Zone of the state. Identification up to species level was carried out.

The samples of collected midges were mostly found to be *Culicoides* (more than 85%) under microscope and further identified up

Table 1. Habitat of *Culicoides oxystoma* as observed in different districts of Jharkhand

Sl. No.	Collection area	Habitat
1.	Khirgoan District : Hazaribag	Walls of cattle shed, open drain, and water logged ditches, manure, cracked walls, and mangers.
2.	Matwari District : Hazaribag	Shed of cattle and buffalo, surrounded by manure. Open drain by the side of animal shed.
3.	Korra District : Hazaribag	Congested sheds of sheep and goat by the side of roadways and covered by bushes. Cracked walls of shed.
4.	Patratu District : Hazaribag	Goat shed, surrounded by bush and trees, water logged muddy area.
5.	Morhabadi District : Ranchi	Cattle shed. Manure pit, water logged area, open drain.
6.	Dhurwa District : Ranchi	Cattle shed. Muddy and sewage disposal drain is situated just by the side of the shed.
7.	Kanke District : Ranchi	Sheep and goat shed, situated by the side of river. Dirty manger, cracked walls and open drainage system.
8.	Karra District : Khunti	Manure pit and open drain surrounding to cattle and goat shed.
9.	Mahil District : Khunti	Muddy areas beside cattle shed, manure pit, open drain.

to species level by morphological characteristics. *Culicoides* samples collected from Hazaribag, Ranchi and Khunti (Fig. 2) were identified as *Culicoides oxystoma*. It shows the most prevalent insects in all the catches was *C. oxystoma* (Diptera: Ceratopogonidae). It is an important vector species, reported mainly from Asia, with high potential to transmit viral diseases affecting livestock. It is especially distributed in Mediterranean climate regions. It is less common or even absent in arid regions. The

genome based detection of *C. oxystoma* in wide geographical regions, together with its high proportion in the general *Culicoides* population and its vectoring potential, imply that it may be an important vector species in the Middle East (Morag *et al.*, 2012). In 1997, *C. oxystoma* was identified as the species occurring in Haryana, India which was recorded from Central Sheep Breeding Farm and Government Livestock Farm, Hisar. The same species was also identified from Government Cattle Breeding Farm,

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Table 2. Meteorological data* of Central and North Eastern Plateau related to areas of collection of *Culicoides* midges of Jharkhand during monsoon (June to September, 2013)

Sl. No.	Area of Collection and Districts	Mean maximum Temp. (°C)	Mean minimum Temp. (°C)	Relative humidity (%)	Rainfall (mm)/yr
1	Kanke, District : Ranchi	41	25	97	91.90
2	Morahabadi, District : Ranchi	41	25	97	91.90
3	Karra, District : Khunti	41	25	97	182.25
4	Khirgoan, District : Hazaribag	43	27	80	70.95
5	Matwari, District : Hazaribag	43	27	80	70.95
6	Mahil, District : Khunti	41	25	97	182.25
7	Dhurwa, District : Ranchi	41	25	97	91.90
8	Patratu, District : Hazaribag	43	27	80	70.95
9	Korra, District : Hazaribag	43	27	80	70.95

* Data shown are average of 4 months (June to September, 2013)

Patiala, from Bilaspur and from Large Scale Sheep Breeding Farm, Sikar (Bhatnagar *et al.*, 1997). From Tamil Nadu *C. imicola* and *C. oxystoma* were found to be prevalent (Sreenivasulu *et al.*, 2004). Among the 6 spp. identified, *C. oxystoma* was the predominant species in number and the least was *C. perigrinus* (Reddy *et al.*, 2008). From Western State of India (Gujarat) BTV-1 was isolated from *C. oxystoma* (Dadawala *et al.*, 2011).

In the present study, habitat of collected midges was studied. The principal habitat of different *Culicoides* species were sheep, goat, cattle or buffalo shed and decomposed manure. Similar type of habitat was reported

by earlier workers for *C. schulzei* (Halder *et al.*, 2013). In general, developmental sites of *Culicoides* species are poorly known. They can breed a wide range of soils, if they provide enough moisture and organic matter to allow the development of the larvae (Kettle, 1962). The large range of breeding sites can be divided into three principal categories (Meiswinkel *et al.*, 2004): (i) the water saturated soil ecotone between aquatic and terrestrial habitats, (ii) during pats (fresh dung) and (iii) moist, decaying organic matter (including manure). The immature stages of *Culicoides* spp. usually live in surface and layers (0-5cm depth) and rarely are found deeper than 8 cm (Uslu and Dik, 2006).

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Fig. 1. *Culicoides oxystoma*



Fig. 2. Collection Area of culicoides from Central and North Eastern plateau Zones of Jharkhand

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As it has been observed that in India, the breeding season of *Culicoides* spp. ranges from onset of monsoon to latter part of monsoon (viz June to September), the meteorological parameters of different districts of Jharkhand were collected during this period. It was done to ascertain whether conducive environment of breeding and propagation of *Culicoides* midges exist during this period (June to September) or not. It was observed that average (of June to September data) rainfall varied from 71 to 182 mm/yr; maximum temperature varied from 41 to 43°C and that of minimum temperature from 25 to 27°C and relative humidity from 80 to 97% from areas to areas. The spread of BTV is likely to be closely linked to the air temperature and that temperature in the range of 27 to 32°C is optimal for BTV transmission, whereas temperature below 9-15°C is predicted to inhibit virus replication, depending on the strain of BTV concerned (Witmann *et al.*, 2002 and Purse *et al.*, 2005). The data indicated that conducive environment for multiplication and propagation of *Culicoides* existed in different districts of Jharkhand, at least in studied areas.

In our earlier study, we reported substantial seroprevalence (41-51%) of bluetongue in ruminants of these districts of Jharkhand (Tigga *et al.*, 2015). Out of total 480 animal serum samples (sheep-190, goats-210 and

cattle-80) screened, 83 (43.68%) of sheep, 91(43.33%) of goat and 46 (57.50%) of cattle sera were found positive. Seroprevalence followed by identification of competent vector (*Culicoides oxystoma*) further strengthens the anticipation that BTV circulates in the state of Jharkhand that may pose threat to economy in animal husbandry in case of future outbreak, if any.

In short, the present study shows prevalence of *Culicoides oxystoma*, one of the competent vectors of bluetongue, in the state of Jharkhand, for the first time and predicts the existnace of bluetongue and its virus, although not yet recorded officially.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding the present research work.

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REFERENCES

- Afshar A, 1994. Bluetongue: laboratory diagnosis. *Comp Immunol Microbiol Infect Dis*, 17: 221–242
- Bhatnagar P, Prasad G, Kakker NK and Dasgupta SK, 1997. A potential vector of bluetongue virus in north-western India. *Indian J Ani Sci*, 67: 486-488
- Borden EC, Shope RE and Murphy FA, 1971. Physical and morphological relationship of some arthropod borne viruses to bluetongue virus. *J Gen Virol*, 13: 262-271
- Borkent A, 2012. World Species of Biting Midges (Diptera: Ceratopogonidae). <http://www.inhs.uiuc.edu/research/FLYTREE/CeratopogonidaeCatalog.pdf>
- Dadawala AI, Biswas SK, Rehman W, Chand K, De A, Mathapati BS, Kumar P, Cauhan HC, Chandel BS and Mondal B, 2012. Isolation of bluetongue virus serotype 1 from *Culicoides* vector captured in livestock farms and sequence analysis of the viral genome segment-2. *Trans Emerg Dis*, 59: 361–368
- Dasgupta SK, 1995. Morphotaxonomic features and species of Indian *Culicoides*. (Diptera: Ceratopogonidae). In: *Bluetongue: Indian Perspective* (Prasad G and Srivastava RN, eds), HAU Press, Hisar, India, pp 115–188
- Halder A, Joardar SN, Parui P, Banerjee D, Samanta I and Lodh C, 2013. Prevalence of midges: potent vectors for bluetongue virus infection in West Bengal. *Adv Ani Vet Sci*, 1(4S): 5-50
- Kettle DS, 1962. The bionomics and control of *Culicoides* and *Leptocoonops* (Diptera, Ceratopogonidae-Heleidae). *Am Rev Entomol*, 7: 401-411
- Luedke AJ and Jones RH, 1984. Bluetongue diagnosis and significance in the bovine animal. *Bov Pract*, 15: 70-86
- Maheshwari G, 2012. Current Status of Bluetongue Disease, Its Vector and Pathogenesis in India. *Proc Nat Acad Sci, India Sec B: Biol Sci*, 82: 463-475
- Meiswinkel R, Venter GJ, Nevill EM, 2004. Vectors : *Culicoides* spp. In : *Infectious diseases of Livestock* (Coetzar JAW and Tustin R eds.), Oxford University Press, Cape Town, pp 93-136
- Mellor PS, Boorman J and Baylis M, 2000. *Culicoides* biting midges: Their role as arbovirus vectors. *An Rev Entomol*, 45: 307–340
- Morag N, Saroya Y, Braverman Y, Klement E and Gottlieb Y, 2012. Molecular identification, phylogenetic status, and geo-

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- graphic distribution of *Culicoides oxystoma* (Diptera: Ceratopogonidae) in Israel. PLoS One, 7(3): e33610
- OIE, 2006. The process of eliminating List A and B designations in favor of a single notifiable disease list categorized by reporting urgency and other factors http://www.oie.int/eng/edito/en_lastedito.htm
- Parker J, Herniaman KAG, Gibbs EPJ and Sellers RF, 1975. An experimental inactivated vaccine against bluetongue. Vet Rec, 96: 284-287
- Purse BV, Mellor PS, Roggers DJ, Samuel AR, Mertens PC and Baylis P, 2005. Climate change and the recent emergence of bluetongue in Europe. Nat Rev Microbiol, 3: 17-18
- Sen P and Dasgupta SK, 1959. Studies on *Indian Culicoides*. Ann Entomol Soc Am, 52: 617-643
- Reddy C V and Hafeez Md, 2008. Studies on certain aspects of prevalence of *Culicoides* species. Indian J Ani Sci, 78: 138-142
- Sreenivasulu D, Rao MVS and Gard GP, 1995. Bluetongue viruses in India : a review. In: Bluetongue disease in South-east Asia and the Pacific. (St George TD and Peng Kegao, eds). Proceedings of the First Southeast Asia and the Pacific Regional bluetongue Symposium, Greenlake Hotel, Kunming, P.R.China, pp 22-24
- Sreenivasulu D, Subba Rao MV, Reddy YN and Gard GP, 2004. Overview of bluetongue disease, viruses, vectors, surveillance and unique features: The Indian sub-continent and adjacent regions. Vet Ital, 40(3): 73- 77
- Standfast HA, Dyce AL and Muller MJ, 1985. Vectors of BT in Australia. In: Bluetongue and Related Orbiviruses (Barber TL and Jochim MM, eds.), New York, USA, pp 177-186
- Tigga P, Joardar SN, Halder A, Lodh C, Samanta I, Isore DP, Batabyal K and Dey S, 2015. Seroprevalence of bluetongue in ruminants of Jharkhand. Vet World, 8 (3): 346-349
- Uslu U and Dik B, 2006. Vertical distribution of *Culicoides* larvae and pupae. Med Vet Microbiol, 20: 350-352
- Wittman EJ, Mellor PS and Baylis M, 2002. Effect of temperature on the transmission of arboviruses by biting midges, *C. sonorensis*. Med Vet Entomol, 16: 147-156