SEROLOGICAL EVIDENCE FOR JAPANESE ENCEPHALITIS VIRUS AND WEST NILE VIRUS INFECTIONS IN WATER FREQUENTING AND TERRESTRIAL WILD BIRDS IN KOLAR DISTRICT, KARNATAKA STATE, INDIA. A RETROSPECTIVE STUDY

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Received May 22, 2003; accepted September 9, 2003

Summary. – In a serological survey of birds in a Japanese encephalitis (JE) endemic area of Kolar District, Karnataka State, India, 859 bird sera were tested by hemagglutination-inhibition test (HIT) for JE encephalitis and West Nile encephalitis (WNE) viruses. Only 2 (0.002%) and 178 (20.72%) sera were positive for JE virus (JEV) and WNE virus (WNV), respectively. Only 160 (18.63%) of 859 sera could be subjected to neutralizing test (NT). Of these, 20 (12.50%) and 62 (38.75%) were positive for JEV and WNV antibodies, respectively. These findings indicate that bird species such as *Pond Herons* and *Little Egrets* among ardeid birds and *Grey Partridges* and *Quails* among terrestrial birds are infected with JEV and WNV and play probably a role in the maintenance of these viruses in the abovementioned part of India.

Key words: ardeid birds; terrestrial wild birds; West Nile virus; Japanese encephalitis virus; hemaglutination-inhibition antibodies; hemagglutination-inhibition test; neutralizing antibodies; neutralizing test

Introduction

The activity of JEV and WNV has been well documented in South East Asian countries including India (Smithburn *et al*., 1954; Carey *et al*., 1968; Rodrigues *et al*., 1981). Both these viruses are maintained in the nature through a bird-mosquito-bird cycle. Several strains of these viruses have been isolated from humans and mosquitoes, and antibodies to JEV and WNV (JEV and WNV antibodies) have been demonstrated in birds and domestic pigs (Rodrigues, 1984; George *et al*., 1987; Geevarghese *et al*., 1987; Buescher *et al*., 1959). Though the role of birds in the epidemiology of JEV has been worked out on virological and serological grounds extensively, the same is not known with regard to WNV. Recent reports on the WNV activity in birds from the USA (Edison *et al*., 2001) prompted us to take a retrospective study on the prevalence of JEV and WNV antibodies in bird sera, collected during a serological survey in avian fauna of Kolar District, Karnataka State, India between December 1991 and June 1992. This serological survey was performed in connection with investigation of a JE outbreak. This communication summarizes results of the survey.

Materials and Methods

Study area, i.e. Kolar District is situated between 12° 46' and 13° 56' of the north latitude and between 72° 21' and 78° 35' of the east longitude. The district has a maximum north to south and east to west width of approx. 136 km and a total area of 8215.2 square km. Ecological features of Kolar District along with its bird fauna has been reported earlier (Jamgaonkar *et al.,* 1993).

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Abbreviations: HIT = hemagglutination-inhibition test; i.c. = intracerebral; i.p. = intraperitoneal; JE = Japanese encephalitis; JEV = JE virus; NT = neutralization test; WNE = West Nile encephalitis; WNV = WNE virus

Bird trapping. A permission to trap wild birds and to collect blood samples from them was obtained from the Government of Karnataka State. The adult birds were obtained from local professional trappers. Nestling and fledgelings were removed from the nests or caught by hand from trees. They were returned to nets or trees after being bled. The trapped birds were identified on the basis of morphology as described in standard reference books (Ali, 1964; Ali and Ripley, 1983).

Collection of blood samples. Blood samples from birds were collected by venepuncture either from the wing or external jugular vein. The blood samples were held on wet ice for 24 hrs and then the sera were separated and stored on wet ice in an ice box until they were transported to National Institute of Virology Field Station at Bangalore, Karnataka State, India or to National Institute of Virology, Pune, Maharashtra State, India.

Virus strains. The following virus isolates were employed in HIT and NT:JEV human isolate P 20788 and WNV mosquito iso-

Table 1. Hemagglutination-inhibition antibodies to JEV and WNV between water frequenting and terrestrial wild birds in Kolar District, Karnataka State, India

Ser. No.	Species	No. of sera tested	No. of sera positive for antibodies to		
			JEV	WNV	JEV+WNV
	Water frequenting wild birds				
1	Large Egret (*Ardea alba*)	7	–	–	–
2	Pond Heron (*Ardeola grayii*)	260	1	89	7
3	Little Egret (*Egretta garzetta*)	45	–	13	2
4	Reef Heron (*Egreta gularis*)	1	–	1	–
5	Painted Stork (*Mycteria leucocephala*)	2	–	–	–
6	Whitebreasted Waterhen (*Amaurornis phoenicurus)*	1	–	–	–
	Subtotal	**316**	**1**	**103**	**9**
	Terrestrial wild birds				
1	Blackwinged Kite (*Elanus caeruleus*)	1	–	–	1
2	Shikra *(Accipiter badius*)	2	–	1	–
3	Grey Partridge (*Francolinus pondicerianus*)	95		28	38
4	Grey Quail *(Coturnix coturnix*)	95		2	1
5	Rain Quail (*Coturnix coromandelica*)	58		3	1
6	Jungle Bush Quail (*Perdicula asiatica*)	53		14	3
7	Rock Bush Quail (*Perdicula argoondah*)	13	–	6	1
8	Common Bustard-Quail (*Turnix suscitator*)	5	–	2	–
9	Redwattled Lapwing (*Vanellus indicus*)	1	–	–	–
10	Yellow-wattled Lapwing(*Vanellus malabaricus*)	2	–	–	1
11	Indian Stone Curlew (*Burhinus oedicnemus*)	1	–	–	–
12	Spotted Dove (*Streptopelia chinensis*)	1	–	–	–
13	Roseringed Parakeet (*Psittacula krameri*)	2	–	–	1
14	Koel (*Eudynamys scolopacea*)	3	–	1	–
15	Collard Scops Owl (*Otus bakkamoena*)	3	–	3	–
16	Spotted Owlet (*Athene brama*)	2	–	–	–
17	Hoopoe (*Upupa epops*)	6	–	1	–
18	Coppersmith (*Megalaima haemocephala*)	1	–	–	–
19	Common Wood Shrike (*Tephrodornis pondicerianus*)	2	1	–	–
20	Black Drongo (*Dicrurus adsimilis*)	8	–	–	–
21	Brahminy Myna (*Sturnus pagodarum*)	2	–	1	–
22	Common Myna (*Acridotheres tristis*)	7	–	1	–
23	Tree Pie (*Dendrocitta vegabunda*)	1	–	–	–
24	House Crow (*Corvus splendens*)	2	–	1	–
25	Jungle Crow (*Corvus macrorhynchos*)	10	–	–	1
26	Common Babbler (*Turdoides caudatus*)	19	–	5	6
27	Jungle Babbler (*Turdoides striatus*)	8	–	3	2
28	Pied Bush Chat (*Saxicola caprata*)	1	–	1	–
29	House Sparrow *(Passer domesticus*)	14	–	1	–
30	Common Weaver Bird (*Ploceus philippinus*)	10	–	–	–
31	Whitethroated Munia (*Lonchura malabarica)*	80	–	–	1
32	Spotted Munia (*Lonchura punctulata*)	35	–	1	1
	Subtotal	**543**	**1**	**75**	**58**
	Grand total	**859**	**2**	**178**	**67**

late G 22886. Both these virus isolates have been isolated in North Arcot District, Tamil Nadu State, India.

Serological tests. HIT was performed in a standard way (Clarke and Casals, 1958; Sever, 1962). NT with JEV was performed on Swiss albino mice using either infant animals and intraperitoneal (i.p.) route or on young animals and intracerebral (i.c.) route. The virus dose ranged between 145 and 213 LD_{50} (Haldane, 1960). NT with WNV was performed in young adult mice by i.c. route. The virus dose ranged between 178 and 195 LD_{50}. The mice were observed for 21 days. The NT was considered positive or partially positive, based on average survival time (AST) (Smith and Westgarth, 1957).

Results and Discussion

Hemagglutination-inhibition antibodies to JEV and WNV

Out of 859 bird sera (543 sera from 32 species of terrestrial birds and 316 sera from 6 species of water frequenting birds) 178 were positive for WNV and 2 for JEV (Table 1). The sera showing antibody titers ≥1:10 were considered positive. Sixty-seven sera reacted positively with both the virus antigens. Of the 178 sera positive for WNV 103 (57.9%) originated from 3 species of water frequenting (ardeid) birds and 75 (42.1%) from 18 species of terrestrial birds. Among the water frequenting birds, higher WNV positives were from the species Pond Heron (86.4%) followed by Little Egret (12.7%) and Reef Heron (0.9%). Among the terrestrial birds, higher WNV positives were from the species Grey Partridge (37.3%) followed by Jungle Bush Quail (18.7%), Rock Bush Quail (8.0%) and Common Babbler (6.7%). Sixty-seven sera (58 from terrestrial and 9 from water frequenting birds) gave protection against both JEV and WNV. It has been indicated that HIT-determined JEV antibodies in birds, which are either naturally or experimentally infected, disappear within two to three months (Rodrigues *et al.,* 1981).

Neutralizing antibodies to JEV and WNV

Although all the collected 859 sera underwent the antibody HIT, only 160 of them could be subjected to the antibody NT due to insufficient quantity of sera. The NT results showed that 62 (38.8 %) sera were positive for WNV and 20 (12.5%) sera were positive for JEV. Twenty-five (15.6%) sera were clearly positive for both the viruses, while 20 (12.5%) and 18 (11.3%) sera were partially positive for JEV and WNV, respectively (Table 2). The WNV-positive sera belonged mainly to the species *Pond Heron* (45) followed by *Grey Partridge* (12), Jungle Bush Quail (2), Grey Quail (1), Rain Quail (1), and Rock Bush Quail (1). Similarly, JEV-positive sera concerned mainly Pond Heron (14, 15.9%), Grey Partridge (3, 15.0%), Grey Quail (2, 20.0%), and Rain Quail (1, 7.7%). It is probable that the neutralizing antibodies detected in most of the fledglings of ardeid birds was maternal in origin. It is nevertheless an indication of the extent of prevalence of JEV and WNV infections in birds. In Japan, it has been demonstrated that 147 (24.54%) of 599 nestling herons and egrets had maternal neutralizing antibodies to JEV (Buescher *et al.,* 1959). It

Table 2. Neutralizing antibodies to JEV and WNV among water frequenting and terrestrial wild birds from Kolar District, Karnataka State, India

Ser. No.	Species	No. of sera tested	No. of sera positive for antibodies to				
			JEV		WNV		JEV+WNV
			P*	PP*	P	PP	P
	Water frequenting wild birds						
1	Pond Heron (*Ardeola grayii*)	88	14	7	45	6	16
2	Little Egret (*Egretta garzetta)*	16	–	1	–	1	1
	Subtotal	**104**	**14**	**8**	**45**	**7**	**17**
	Terrestrial wild birds						
1	Grey Partridge (*Francolinus pondicerianus)*	20	3	1	12	1	3
2	Grey Quail (*Coturnix coturnix*)	10	2	1	1	1	2
3	Rain Quail (*Coturnix coromandelica*)	13	1	1	1	1	2
4	Jungle Bush Quail (*Perdicula asiatica*)	7	–	–	2	–	1
5	Rock Bush Quail (*Perdicula argoondah*)	5	–	1	1	1	–
6	Common Bustard Quail (*Turnix suscitator*)	1	–	–	–	1	–
	Subtotal	**56**	**6**	**4**	**17**	**5**	**8**
	Total	**160**	**20**	**20**	**62**	**18**	**25**

P* = positive; mortality rate 30% or less.

PP* = partially positive; mortality rate 31–74%.

has been reported that maternal antibodies disappear during the first 50 days of life of the birds (Rodrigues *et al.,* 1981).

Based on virological and serological studies, Kolar District has been considered an endemic area for both JEV and WNV since 1979 (George *et al.,* 1987; Prasad *et al.,* 1982; Geevarghese *et al.,* 1987). Though ardeid birds have been implicated long back in the natural cycle of JEV in many countries including India, the role of terrestrial birds has not been studied in detail with respect to JE and WNE. The findings presented here clearly indicated that also terrestrial birds got infected with both these viruses in the nature. In the past, two major serological surveys of birds have been carried out in JEV endemic areas of South India. During the first survey conducted in North Arcot District, Tamil Nadu State, between 1962 and 1966, only one (the species *Whiteheaded Babbler*) of 410 bird sera was found positive for JEV antibodies in NT All the sera were negative for WNV antibodies (Carey *et al.,* 1968). In the second survey carried out in 1974–1976 in the Krishna-Godavari delta region of Andhra Pradesh State, out of 866 sera from water frequenting birds 188 (21.71%) were positive for JEV antibodies in NT; 111 (59.04%) sera were positive for both JEV and WNV antibodies (Rodrigues *et al.,* 1981). The higher percentage of positive sera concerned the species Pond Heron (35%) and Cattle Egret (34.5%). Thus this study indicates that infections of wild birds with JEV/WNV varies in different geographical areas in India and perhaps the rate of infections increased from the 1960s to the 1970s in this part of India.

In the recent outbreak of WNE in the USA, 17339 bird deaths, including those of 5697 crows have been reported in 1999 (Edison *et al*., 2001). Ebel *et al*. (2002) have reported the presence of WNV antibodies in 23 bird species from New York City using an indirect immunoglobulin G enzyme-linked immunosorbent assay. In our study, only one out of 12 crow sera was found positive for WNV antibodies while only one serum contained both JEV and WNV antibodies. So far, no bird deaths due to WNV have been reported from India. Studies on strain variations in WNV in different geographical regions are likely to throw light on the behavior of this virus.

Acknowledgement. The authors thank Mr P.G. Jacob and the technical staff of the Bangalore Field Station for their assistance. Thanks are also due to Dr. A.B. Sudeep for editorial assistance and Ms S.P. Bohodkar for typing the manuscript.

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