INCIDENCE OF NEUTRALIZING ANTIBODIES TO CHANDIPURA VIRUS IN DOMESTIC ANIMALS FROM KARIMNAGAR AND WARANGAL DISTRICTS OF ANDHRA PRADESH, INDIA

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Summary. – In order to determine the possible role of domestic animals in the outbreak of acute encephalitis associated with Chandipura virus (CPV) among children in Andhra Pradesh in 2003, a serological survey of domestic animals was carried out during the epidemic in July 2003. Out of 180 animal sera from highly affected areas of the Karimnagar and Warangal districts of Andhra Pradesh 33 (18.3%) had virus neutralizing (VN) antibodies to CPV. The positive animals consisted of pigs (30.6%), buffalos (17.9%), cattle (14.3%), goats (9.3%) and sheep (7.7%). Isolation of CPV and detection of CPV antibodies in patients with encephalitis reported earlier and the evidence of antibodies to CPV in domestic animals shown here suggest that CPV circulates in this region and should be considered an emerging virus of public health importance.

Key words: Chandipura virus; virus neutralizing antibodies; domestic animals; India

Introduction

An outbreak of acute encephalitis with high fatality rate has been reported in children from Andhra Pradesh between June and September 2003 (Rao *et al.,* 2004). This outbreak was associated with CPV (Rao *et al.*, 2004) belonging to the *Chandipura virus* species, the *Vesiculovirus* genus, the *Rhabdoviridae* family.

In India, CPV has been first isolated from a febrile patient from Nagpur, Maharashtra (Rodrigues *et al*., 1972). Later, it was also isolated from sandflies at Aurangabad, Maharashtra (Dhanda *et al*., 1970). In Nigeria, the virus has been isolated from hedgehog *Atelerix spiculis* (Traore-Lamizana *et al*., 2001). The virus is transmissible through sandfly (Tesh and Modi, 1993) and *Aedes aegypti* mosquitoes in the laboratory (Rao, 1967; Ilkal *et al*., 1991). In India, a limited seroprevalence of CPV antibodies in some domestic animals has been reported (Banerjee, 1984).

As a part of integrated investigation of the outbreak in 2003, we carried out a serological survey of domestic animals in highly affected areas of Karimnagar and Warangal districts of Andhra Pradesh during mid July 2003. Different species of domestic animals, namely cattle, buffalos, sheep, goats, pigs and dogs were tested for the presence of VN antibodies to CPV to assess their possible role in the epidemiology of CPV.

Materials and Methods

Study area. The geography of the affected areas has been described earlier (Rao *et al*., 2004). The Karimnagar and Warangal districts have large populations of domestic animals, namely cattle, buffalos, sheep, goats, pigs and dogs.

Collection, storage and transport of serum samples. Altogether 180 animal serum samples were collected from 5 villages in the Karimnagar District (92) and from 7 villages in the Warangal District (88) of Andhra Pradesh. All the animals showed no symptoms of overt illness at the time of collection of serum samples. The animals had been routinely vaccinated earlier against foot-

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Abbreviations: CPV = Chandipura virus; DMEM = Dulbecco's Minimum Essential Medium; FBS = fetal bovine serum; NT = neutralization test; VN = virus neutralizing; VP = virus neutralizing.

and-mouth disease and rinderpest. The animals were bled by venipuncture, the sera separated and transported on ice to the National Institute of Virology, Pune, where they were stored at -20°C until tested.

VN test. All the sera were assayed for VN antibody titers to CPV in Vero cells (Gore *et al*., 1985). Briefly, Vero cells were seeded in 96-well microtitre plates (3 x 10^4 cells/well). Aliquots of serum samples, diluted 1:10 in Dulbecco's Minimum Essential Medium (DMEM) with 2% fetal bovine serum (FBS), were inactivated at 60°C for 20 mins. Further serial three fold dilutions starting from 1:30 to 1:1620 were carried out. The serum dilutions were mixed with 100 $TCID_{50}$ of CPV 1:1. The serum-virus mixtures (100 µl) were incubated at 37°C for 1 hr and added to Vero cell monolayers in microplates. CPV immune mouse serum served as positive control and normal mouse serum as negative control. The antibody titer was expressed as reciprocal value of the highest serum dilution capable of neutralizing 100 $TCID_{50}$ of the virus.

Statistical analysis. The significance of differences in titers was evaluated by the χ-square test, while the significance of differences in geometric mean (GM) titers was calculated by the *t*-test.

Results and Discussion

The VN test revealed that out of 180 animal sera from highly affected areas of the Karimnagar and Warangal Districts of Andhra Pradesh 33 (18.3%) had neutralizing antibodies to CPV. Twenty-three of the 33 positive sera were from the Warangal District and 10 from the Karimnagar. The highest prevalence was found in pigs (30.6%), followed by buffalos (17.9%), cattle (14.3%), goats (9.3%) and sheep (7.7%). Three of 6 dog sera were positive for the antibodies to CPV. However, this result was not included in the comparison, because the number of these sera was very small (Table 1).

The analysis of CPV antibodies-positive sera according to the titer (Table 2) indicated that the seropositivity for CPV among pigs was significantly higher as compared with that for goats and sheep. No significant differences were observed for the other animal species tested. The GM titers of antibodies did not vary significantly among different animal species. Also no significant differences were found in the seropositivity among different animal species from the Karimnagar and Warangal Districts.

The villagers in the two districts surveyed in this study usually rear their animals in large numbers and use to live in close proximity to animal sheds and pig sties. Evidence of VN antibodies to CPV in pigs, demonstrated in this report represents a priority. Interestingly, 4 of the 33 positive animal sera were collected from the animals belonging to the farms from where the cases of encephalitis had been reported during the outbreak in 2003 in the villages of the Karimnagar and Warangal Districts. Apparently, as the animals do not exhibit any symptoms of CPV infection during viremia its clinical diagnosis is difficult.

Summing up, the isolation of CPV from humans, detection of IgG and IgM antibodies in cases of encephalitis, detection of CPV by PCR in sandflies collected from the house of a patient during the outbreak in 2003 (Rao *et al*., 2004) and the evidence of VN antibodies to CPV in the animal populations in the Karimnagar and Warangal Districts of Andhra Pradesh suggest a possible zoonotic nature of the virus. In view of these findings CPV may be considered an emerging virus of public health importance.

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Table 1. Incidence of CPV antibodies in individual animal species

Species	Karimnagar District	Warnagal District	Karimnagar + Warnagal Districts (%)
Pig	2/12	13/37	15/49 (30.6)
Goat	3/26	1/17	4/43 (9.3)
Cattle	2/14	2/14	4/28 (14.3)
Buffalo	2/18	3/10	5/28 (17.9)
Sheep	1/20	1/6	2/26 (7.7)
Dog	0/2	3/4	3/6
Total	10/92	23/88	33/180 (18.3)

Table 2. Analysis of CPV-positive sera according to antibody titers

Species	Titer					GM titer
	10	30	90	270	≥ 540	
Pig	2	3	5	2	3	12.08
Goat	1	2	1	0	0	5.91
Cattle	0	1	2	0	1	7.75
Buffalo	1	2	0	2	0	7.75
Sheep	0	0	1	0	1	6.69
Dog	0	0	1	1	1	34.34

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