Review Article

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Ganjam virus

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Ganjam virus (GANV), a member of genus *Nairovirus* of family *Bunyavirdae* is of considerable veterinary importance in India. Though, predominantly tick borne, GANV was also isolated from mosquitoes, man and sheep. Neutralizing and complement fixing antibodies to GANV have been detected in animal and human sera collected from different parts of the country. Thirty three strains of GANV have been isolated from India, mainly from *Haemaphysalis* ticks. The virus replicated in certain vertebrate and mosquito cell lines and found pathogenic to laboratory animals. One natural infection and five laboratory-acquired infections in men were also reported. GANV is antigenically related to Nairobi sheep disease virus (NSDV) of Africa, which is highly pathogenic for sheep and goats causing 70-90 per cent mortality among the susceptible population. Recent molecular studies have demonstrated that GANV is an Asian variant of NSDV and both these viruses are related to the dreaded Crimean Congo haemorrhagic fever (CCHF) group viruses. The versatility of the virus to replicate in different arthropod species, its ability to infect sheep, goat and man makes it an important zoonotic agent.

Key words Ganjam virus - *Haemaphysalis intermedia* - Nairobi sheep disease - *Nairovirus* - tick

Introduction

Ganjam virus (GANV), a tick-borne arbovirus of veterinary importance causing high morbidity and mortality to exotic and crossbred sheep and goats, is widely prevalent in India[1]. GANV belongs to the genus *Nairovirus* of the family *Bunyaviridae* and is closely associated antigenically with Nairobi Sheep Disease virus (NSDV), the most pathogenic virus known to sheep and goats in Africa[2]. However, despite serologic cross-reactivity, the two viruses are considered distinct due to their occurrence on two different continents and association with different ticks. Studies at the genetic and serologic levels have demonstrated GANV as an Asian variant of NSDV as the two viruses differed only by 10 and 3 per cent at nucleotide and amino acid levels respectively[2]. Hybridization studies using RNA probes demonstrated that both GANV and NSDV are more closely related to Hazara virus (HAZV), a member of the Crimean Congo haemorrhagic fever virus (CCHFV) group than to Nairoviruses[3]. CCHFV is one of the most pathogenic human viruses among *Nairoviruses*, which has a wide geographic distribution in Africa, Europe and Asia. A few fatal cases due to CCHFV have been reported from Pakistan[4]. The wide prevalence of GANV in different parts of India, its association with these important viruses and its versatility to infect sheep, goat and man makes it an important zoonotic agent. This review aims to highlight its potentiality as an emerging virus of veterinary and human importance,

which warrants the need for a surveillance system to monitor its activity in India.

Discovery of the virus: GANV was first isolated from *Haemaphysalis intermedia* ticks collected from goats, suffering from lumbar paralysis from Orissa, India, during 1954-55 and named after the place of isolation[5]. Subsequent studies have yielded several isolations mainly from *Haemaphysalis* ticks and a few from mosquitoes, sheep and man (Table). Recently, for the first time, the virus was isolated from *Rhipicephalus hemaphysaloids* ticks[6]. The areas from where the isolations were made are depicted in Fig.

Disease, public health and animal health: In India, disease associated with GANV in humans has never been reported at an epidemic level. However, a case of natural infection in a 12 yr old European boy in Vellore, Tamil Nadu, and a few laboratory acquired cases have been reported[7,12,13]. All the human cases recovered after a brief illness. The virus, however, was found highly pathogenic to exotic and crossbred sheep and goats causing high morbidity and mortality. Ghalsasi *et al*[8] isolated GANV from exotic rams of the Suffolk and the Dorset Horn breed (sheep) during an epidemic investigation in a sheep farm in Andhra Pradesh. The clinical symptoms included high temperature (107°F), dullness, depression and inappetance. Breathing was rapid and painful and there was muco-purulent nasal discharge. Diarrhoea with mucus or blood in the stools was present. Stiffness of knee joints was also noted. The severity of infection as well as mortality was more in exotic sheep, than in crossbred sheep. Joshi *et al*[14] investigated a disease outbreak in a sheep farm in Veerapuram village of Chengai-MGR district of Tamil Nadu where high morbidity and mortality in sheep was reported. The disease was characterized by high morbidity, mortality and abortion in pregnant ewes in Madras Red breed of sheep. GANV has not been a serious problem to the local breeds so far[8,14].

Table. Isolation of Ganjam virus in India

Source of isolation	No. of isolates	Locality	Year of collection
Haemaphysalis intermedia[5]	1	Ganjam District, Orissa	1954-55
H. intermedia[11]	18	Hubli, Barur and Sagar, Karnataka	1961-62
H. wellingtoni[9]	2	Mysore, Karnataka	1970
H. intermedia[8]	2	Chittoor district, Andhra Pradesh	1981
H. intermedia[6]	5	Pune city	2004-05
Rhipicephalus haemaphysaloids[6]	1	Pune city	2004-05
Culex vishnui complex[10]	1	North Arcot district, Andhra Pradesh	1955-57
Sheep[8]	2	Chittoor district, Andhra Pradesh	1981
Humans[7]	1	Vellore, Tamil Nadu	1969

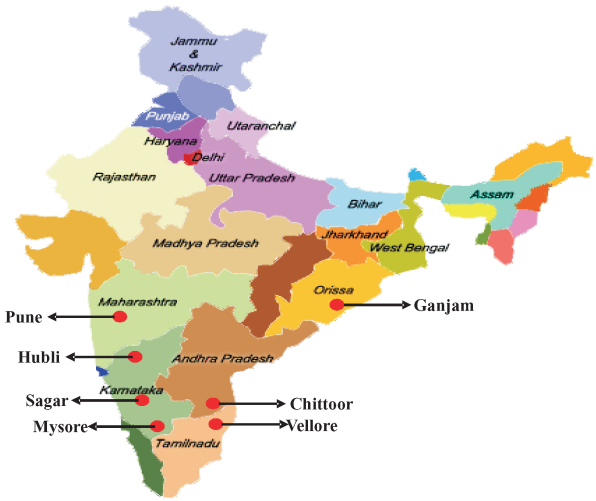

Fig. Map showing places of Ganjam virus isolation in India.

Antigenic relationships with other important members of genus Nairovirus: The family *Bunyavirus* comprises five genera, *i.e*. *Orthobunyavirus*, *Nairovirus*, *Phlebovirus*, *Hantavirus* and *Tospovirus* in addition to several unassigned viruses. The genus *Nairovirus* includes seven serogroups consisting of 34 predominantly tick-borne viruses and majority are associated with severe human and livestock diseases[2]. Their genomes consist of three segments of single-stranded RNA, namely small (S), medium (M) and large (L) segments. The S segment encodes the viral nucleocapsid (N) protein; the M segment encodes the glycoproteins G1 and G2, and the viral polymerase L is encoded in the L segment[2,3]. Cross-immune precipitation analyses have confirmed that viruses in the *Nairovirus* genus share antigenic determinants and are antigenically distinct from representative members of the family *Bunyaviridae*[15]. The important members of the genus *Nairovirus* include CCHFV, NSDV, in addition to GANV, HAZV and Dugbe virus (DUGV). NSDV, GANV and DUGV together constitute the NSDV serogroup due to their close antigenic relationship[3].

Crimean Congo haemorrhagic fever (CCHF) virus: CCHFV is the most important human pathogen among *Nairoviruses*, with a widespread geographical distribution covering Africa, Asia, the Middle East and

Eastern Europe with a case fatality rate ranging from 15 to 100 per cent[4]. Man acquires infection from direct contact with blood or other infected tissues from livestock or through the bite of infected tick. It is a disease found in people who work in close association with livestock such as agricultural workers, slaughterhouse workers and veterinarians. High mortality usually result when the transmission is through blood or respiratory secretions which are most often intrafamilial or nosocomial in nature[4,16]. CCHFV also infects a wide range of domestic and wild animals.

Nairobi sheep disease virus (NSDV): NSDV is the prototype virus of the genus *Nairovirus* and causes acute haemorrhagic gastroenteritis in sheep and goats with high mortality in susceptible populations (70-90%)[2]. The virus is viscerotropic in sheep and produces ecchymosis in intestines and hyperplasia of mesenteric nodes[17]. Virus transmission is mainly through the bite of infected *Rhipicephalus appendiculatus* ticks though a large number of other Ixodid ticks are involved in the maintenance of the virus in nature. NSDV was first isolated in 1910 from Nairobi, Kenya, and further disseminated to other neighbouring countries of East and Central Africa. It is not a major health problem to humans though infections are reported in sheep handlers.

Dugbe virus: DUGV is of veterinary importance in Africa and is the first member of the genus *Nairovirus* to be characterized fully. Unlike NSDV and CCHF, DUGV is relatively apathogenic, however, it can induce thrombocytopenia in man[16]. It has been demonstrated experimentally that the virus is neuro-invasive in mice.

Natural isolations of GANV from India

From ticks: GANV has been isolated on 26 occasions from *H. intermedia,* the incriminated vector, from different parts of the country (Table). GANV was also isolated twice from the bird tick *H. wellingtoni* from the Kyasanur forest area, Karnataka[9]. Recently, one isolation of GANV was made for the first time from *R. hemaphysaloids* collected from domestic animals from Pune district, Maharashtra during routine arboviral investigation studies[6].

From man: GANV was isolated from the acute phase serum of a 12 yr old European boy who was suffering from febrile illness in Vellore, Tamil Nadu[7]. This is the only natural infection reported so far from a human being though several laboratory infections in personnel working on this virus were reported. The first report of a laboratory-associated infection was from Africa where a 16 yr old native male contracted the infection while working[17]. Infection to five laboratory personnel was also reported from India during the investigations of this virus in the laboratory[12,13]. Subclinical infections might be occurring as evidenced by the presence of antibodies in human sera collected from different parts of India as well as in laboratory personnel[17].

From sheep: Two isolations of GANV were reported from exotic rams of the Suffolk and the Dorset Horn breed sheep suffering from febrile illness with mortality in a sheep farm at Palamner, Chittoor district, Andhra Pradesh in 1977[8].

From mosquitoes: GANV was isolated from a pool of 100 mosquitoes belonging to the *Culex vishnui* complex collected from North Arcot district in Tamil Nadu and Chittoor district in Andhra Pradesh during entomologic investigations[10].

Seropositivity in humans: The results of the extensive serological studies carried out in different parts of India indicated the presence of neutralizing (N) antibodies to GANV in man from geographically distant areas *viz*. Jammu & Kashmir, Arunachal Pradesh and Tamil Nadu[7,17].

Seropositivity in animals: Presence of N-antibodies to GANV was detected in sheep, goat and cattle sera collected from Orissa, Gujarat, Karnataka, Punjab and Jammu & Kashmir[1,17]. Joshi *et al*[14] also reported the presence of N-antibodies to GANV in sheep, goat and human sera collected during an epidemic investigation in Tamil Nadu.

Experimental studies with GANV

(i) Molecular studies and phylogenetic analysis: Studies conducted with 34 strains of GANV isolated from different host systems in India demonstrated significant diversity among the isolates when amplification and nucleotide sequence analysis of the PCR products (N-394bp) were carried out (Yadav P, personal communication, 2005). The isolates differed by up to 17 per cent at the nucleotide level for the N gene fragments. The study demonstrated considerable mixing and movement of GANV strains within India, with no clear relationship between genetic lineages, geographic origin or year of isolation. Also NSDV did not seem to represent a distinct lineage among the Indian isolates, but appeared as a minor variant within the GANV monophyletic group (Yadav P, personal communication, 2005).

(ii) Studies on laboratory animals: Infant and adult mouse developed viraemia of varying degree (3-5.5 dex) from 3-5 days post infection (PI) that persisted till their death on 4th to 7th day[18]. The maximum virus yield was observed on the 5th day PI. Hamsters when infected experimentally did not circulate the virus, but developed N and CF antibodies. In sheep, dose dependant viraemia was observed. When infected with 3-4 dex virus, viraemia was observed between 2-5 days PI while a higher dose (4.4 dex) did not induce viraemia. Though bonnet monkeys (*Macaca radiata*) did not develop viraemia, langur monkey (*Presbytis entellus*) infected with GANV, developed viraemia on the 8th day PI. Seven day old chick embryos succumbed to infection when inoculated through the yolk sac route. The embryos died after an average survival time of 3.5 days (NIV unpublished data).

(iii) Susceptibility of mice at different age groups: Both infant and adult mice inoculated intracerebrally (ic), became sick on the 3rd day PI and died either on 4th or 5th day PI. In adult mice, however, death was observed only when inoculated through the ic route[17]. The pathological changes observed at autopsy in different organs were similar in infant and adult mice.

(iv) Histopathological studies in mice: Major histopathological changes were observed in liver, brain, lungs and kidneys of infected mice while heart and intestines remained unaffected. Liver became pale and soft in comparison to the control animals. Patchy areas of necrosis with inflammatory cells consisting of polymorphs and mononuclear cells were observed. Small patchy areas of interstitial pneumonitis were detected in the lungs while dilated sinusoids and reactive follicles with areas of phagocytosis were observed in the spleen cells. The brain appeared congested with patchy areas of haemorrhage and perivascular cuffing with round cells in the cerebrum and cerebellum. However, kidneys showed minimal patchy round cell infiltration of interstitial cells (NIV unpublished data).

(v) Cell culture studies: A number of vertebrate cell lines supported the replication of the virus and induced distinct cytopathic effects (CPE). Paul and Dandawate[19] demonstrated the replication of GANV in Vero cells with distinct CPE. The virus induced polykaryocytosis, which is characterized by the formation of large syncytia with nuclear and cytoplasmic vacuolation, a phenomenon that is not reported in mammalian cells infected with other arboviruses. The virus also replicated in BHK-21 cell line and exhibited distinct CPE. Well-defined plaques were observed in the cell line under agar overlay (NIV unpublished data). Replication of the virus in PS and rhabdomyosarcoma cell lines was also observed recently (unpublished data).

Among the invertebrates, GANV replication was observed in a tick cell line (*H. spinigera*) and in two mosquito cell lines (*Aedes albopictus* and *Ae. novalbopictus*)[20-22]. None of the cell lines exhibited CPE at any period of study. Virus replication in *H. spinigera* cell line was observed after a short incubation period and the cell line yielded 6log $TCID_{50}$ virus/ml on 3rd day PI. The *Ae. albopictus* and *Ae. novalbopictus* cell lines yielded 6 and 4log $TCID_{50}$ virus/ml on 10th day PI respectively. However, GANV did not replicate in *Ae. aegypti*, *Ae. w-albus*, *Ae. vittatus*, *Ae. krombeini* and *Anopheles stephensi* cell lines[19,23-25]. Attempts to propagate the virus in newly established *Ae. aegypti* and *Cx. tritaeniorhynchus* cell lines were also not successful (unpublished data).

(vi) Experimental infection in mosquitoes and ticks: Dandawate *et al*[26] reported the replication of GANV in certain experimentally infected mosquitoes and ticks by parenteral inoculation. *Culex fatigans* and *Cx. tritaeniorhynchus* mosquitoes showed the presence of virus immediately after inoculation with virus titres ranging from 1.8 to 3.2 dex/0.02 ml. However, virus growth was not detected in both the mosquito species though one pool of the latter showed traces of virus on 5th day PI (0.5 dex/0.02 ml). In infected *Ae. aegypti* mosquitoes, the virus was maintained in very low titres (0.7 dex/0.02ml) up to 21st day PI. *Ae. albopictus* mosquitoes on the contrary, did not show even traces of virus on subsequent days PI. However, both the *Aedes* mosquitoes replicated the virus to high titres when inoculated with *Ae. albopictus* cell line-adapted strain of GANV[26]. In both the species, virus was detected in traces immediately after inoculation but the titres increased on subsequent days PI (on days 5 and 12 PI). Two tick species, *i.e*. *H. spinigera* and *H. turturis*, when inoculated parenterally with GANV, showed virus replication with progressively rising titres[26]. Virus was detected up to 15 and 35 day PI in the two species respectively.

Summary & future studies

Though GANV was first reported only in 1969[5], virus isolation and presence of N-antibodies in human and animal sera collected during the early 1950s is suggestive of its prevalence at least since 1954 in India[17]. The virus caused disease in goats and sheep and

was maintained in nature by ticks belonging to the genus *Haemaphaysalis*, mainly *H. intermedia*. The virus still continues to circulate in the country as demonstrated by the antibody prevalence in man and domestic animals and the occasional virus isolations. In India, GANV cause damage mainly to the exotic and crossbred varieties of sheep and goat while the local breeds generally remained either unaffected or mildly affected.

The isolation of GANV from *Cx. vishnui* complex[10] mosquitoes needs further investigation, as no data are available to substantiate this finding. Experimental studies to propagate the virus in this group of mosquitoes did not succeed[26]. Cell cultures derived from *Culex* mosquitoes also did not support the replication of the virus (unpublished data). The probable explanation could be the undigested blood, which the mosquitoes might have had from a viraemic animal.

Impact in veterinary medicine and need for surveillance

The virus isolations from Pune and the presence of antibodies in sheep, goat and human sera in Tamil Nadu are suggestive of the circulation of the virus in the country. In India, GANV affected only the exotic and crossbred sheep causing high morbidity and mortality resulting in the economic damage as observed in Chennai and Andhra Pradesh sheep farms[8,14]. The clinical symptoms observed at both the places matched with that of NSDV infection. The recent isolation of GANV from *R. hemaphysaloids* is a significant observation as the tick mainly infests dogs, which are closely associated with man. Does it point towards a shift in the vector and what will be the difference in pathogenicity in animals and man if GANV adapts to *R. hemaphysaloids*? This area needs urgent attention.

Need for laboratory capability for diagnosis

Diagnosis of GANV was carried out earlier using serologic techniques, which are time consuming and labour intensive. The development of RT-PCR has been a major advancement in the recent years, which has come handy to rapid diagnosis[6]. However, efforts should be made to develop diagnostics against other members of genus *Nairovirus* especially against CCHFV to meet any future eventualities.

The revelation regarding the close relatedness of these viruses to HAZV of the CCHFV group has significance, as CCHFV is the most pathogenic virus of the genus *Nairovirus* to humans causing fatal infections. CCHFV has a wide distribution in Africa, Asia and Europe and the virus has shown its presence in the neighbouring countries like Pakistan with many fatal cases[4]. The conditions in India and Pakistan are almost similar and the virus may make its entry at any time and necessary measures are to be taken. The versatility of the virus, which replicates in different arthropod species, sheep, goats and man makes it an important zoonotic agent, which has the potentiality of an emerging virus of importance in India.

References

1. Banerjee K. Emerging viral infections with special reference to India. *Indian J Med Res*1996; *103* : 177-200.
2. Marczinke BI, Nichol ST. Nairobi sheep disease virus, an important tick-borne pathogen of sheep and goats in Africa, is also present in Asia. *Virology* 2002; *303* : 146-51.
3. Marriott AC, Ward VK, Higgs S, Nuttall PA. RNA probes detect nucleotide sequence homology between members of two different n*airovirus* serogroups. *Virus Res* 1990; *16* : 77-81.
4. Athar MN, Baqai HZ, Ahmad M, Khalid MA, Bashir N, Ahmad AM, *et al*. Short report: Crimean Congo hemorrhagic fever outbreak in Rawalpindi, Pakistan, February 2002. *Am J Trop Med Hyg* 2003; *69* : 284-7.
5. Dandawate CN, Shah KV. Ganjam virus: a new arbovirus isolated from ticks *Haemaphysalis intermedia* Warburton and Nuttall, 1909 in Orissa, India. *Indian J Med Res* 1969; *57* : 799-804.
6. Joshi MV, Geevarghese G, Joshi GD, Ghodke YS, Mourya DT, Mishra AC. Isolation of Ganjam virus from ticks collected off domestic animals around Pune, Maharashtra, India. *J Med Entomol* 2005; *42* : 204-6.
7. Dandawate CN, Work TH, Webb JK, Shah KV. Isolation of Ganjam virus from a human case of febrile illness: a report of a laboratory infection and serological survey of human sera from three different states of India. *Indian J Med Res* 1969; *57* : 975-82.
8. Ghalsasi GR, Rodrigues FM, Dandawate CN, Gupta NP, Khasnis CG, Pinto BD, *et al*. Investigation of febrile illness in exotic and cros-sbred sheep from sheep farm, Palamner in Andhra Pradesh. *Indian J Med Res* 1981; *74* : 325-31.
9. Rajagopalan PK, Sreenivasan MA, Paul SD. Isolation of Ganjam virus from the bird tick *Haemaphysalis wellingtoni,* Nuttall and Warburton, 1907. *Indian J Med Res* 1970; *58* : 1195-6.
10. Dandawate CN, Rajagopalan PK, Pavri KM, Work TH. Virus isolations from mosquitoes collected in North Arcot district, Madras state, and Chittoor district, Andhra Pradesh between November 1955 and October 1957. *Indian J Med Res* 1969; *57* : 1420-6.
11. Boshell J, Desai PK, Dandawate CN, Goverdhan MK. Isolation of Ganjam virus from ticks *Haemaphysalis intermedia*. *Indian J Med Res* 1970; *58* : 561-2.
12. Banerjee K, Gupta NP, Goverdhan MK. Viral infections in laboratory personnel. *Indian J Med Res* 1979; *69* : 363-73.

13. Mohan Rao CV, Dandawate CN, Rodrigues JJ, Prasada Rao GL, Mandke VB, Ghalsasi GR, *et al*. Laboratory infections with Ganjam virus. *Indian J Med Res* 1981; *74* : 319-24.
14. Joshi MV, Elankumaran S, Joshi GD, Albert A, Padbidri VS, Murali Manohar B, *et al*. Post-epizootic survey of rift valley fever-like illness among sheep at Veerapuram, Chennai, Tamil Nadu. *Indian J Virol* 1998; *14* : 155-7.
15. Clerx JP, Casals J, Bishop DH. Structural characteristics of nairoviruses (genus *Nairovirus*, *Bunyaviridae*. *J Gen Virol* 1981; *55* : 165-78.
16. Boyd A, Fazakerley JK, Bridgen A. Pathogenesis of Dugbe virus infection in wild- type and interferon-deficient mice. *J Gen Virol* 2006; *87* : 2006-9.
17. Banerjee K. Emerging arboviruses of zoonotic and human importance in India. In: Misra A, Polasa H, editors. *Virus Ecology.* New Delhi: South Asian Publishers; 1984. p. 109-21.
18. Dandawate CN. Studies on Ganjam virus with reference to viremia in vertebrate hosts & development of antibodies. *Indian J Exp Biol* 1977; *15* : 1058-9.
19. Paul SD, Dandawate CN. Polykaryocytosis in Vero cell cultures with Ganjam virus: an arbovirus. *Indian J Med Res* 1970; *58* : 556-60.
20. Banerjee K, Guru PY, Dhanda V. Growth of arboviruses in cell cultures derived from the tick *Haemaphysalis spinigera*. *Indian J Med Res* 1977; *66* : 530-6.
21. Singh KR, Paul SD. Susceptibility of *Aedes albopictus* and *Aedes aegypti* cell lines to infection by Arbo and other viruses. *Indian J Med Res* 1968; *56* : 815-20.
22. Bhat UK, Guru PY. Susceptibility of *Aedes novalbopictus* cell line to infection with some arboviruses. *Curr Sci* 1974; *43* : 300-2.
23. Bhat UK, Goverdhan MK. Susceptibility of *Aedes vittatus* cell line to infection with some arboviruses. *Curr Sci* 1972; *41* : 480-2.
24. Singh KR, Goverdhan MK, Bhat UK. Susceptibility of *Aedes W-albus* and *Anopheles stephensi* cell lines to infection with some arboviruses. *Indian J Med Res* 1973; *61* : 1134-7.
25. Sudeep AB, Pant U, Dhanda V, Banerjee K. A new *Aedes krombeini* cell line and its susceptibility to some arboviruses. In: Maramorosch K, Mitsuhashi J, editors. *Invertebrate cell culture*: *novel directions and biotechnology applications*. USA: Science Publishers Inc; 1997. p. 11-7.
26. Dandawate CN, Singh KR, Dhanda V. Experimental infection of causes mosquitoes & ticks with Ganjam virus. *Indian J Exp Biol* 1981; *19* : 1185-6.

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