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Nairobi sheep disease virus/Ganjam virus

M.D. Baron* & B. Holzer

The Pirbright Institute, Ash Road, Pirbright, Surrey GU24 ONF, United Kingdom
*Corresponding author: michael.baron@pirbright.ac.uk

Summary
Nairobi sheep disease virus (NSDV) is a tick-borne virus which causes a severe disease in sheep and goats, and has been responsible for several outbreaks of disease in East Africa. The virus is also found in the Indian subcontinent, where it is known as Ganjam virus. The virus only spreads through the feeding of competent infected ticks, and is therefore limited in its geographic distribution by the distribution of those ticks, *Rhipicephalus appendiculata* in Africa and *Haemaphysalis intermedia* in India. Animals bred in endemic areas do not normally develop disease, and the impact is therefore primarily on animals being moved for trade or breeding purposes.
The disease caused by NSDV has similarities to several other ruminant diseases, and laboratory diagnosis is necessary for confirmation. There are published methods for diagnosis based on polymerase chain reaction, for virus growth in cell culture and for other simple diagnostic tests, though none has been commercialised. There is no established vaccine against NSDV, although cell-culture attenuated strains have been developed which show promise and could be put into field trials if it were deemed necessary. The virus is closely related to Crimean-Congo haemorrhagic fever virus, and studies on NSDV may therefore be useful in understanding this important human pathogen.

Keywords
Crimean-Congo haemorrhagic fever – Goat – Nairobi sheep disease virus – Nairovirus – Sheep – Small ruminant – Tick-borne.

Introduction

Nairobi sheep disease virus (NSDV) was first recognised in 1917 as the causative agent of a disease that was affecting sheep brought up from the Masai region of Kenya for sale in the Nairobi livestock markets (1). Montgomery's seminal work established that the disease occurred only in sheep and, to a lesser extent, goats, and was spread only via Ixodid ticks, not directly from sheep to sheep. The disease he observed was particularly severe, with a case fatality rate of around 70% in local breeds of sheep, though somewhat lower mortality in European breeds (1), an observation confirmed by others (2). The virus, or antibodies to the virus, was subsequently found in several parts of East Africa, from Tanzania to Ethiopia (3). Both by comparison of the distribution of antibodies to the virus with the known distribution of different tick species (1, 4) as well as direct studies on the transmission of the virus (2, 5, 6), the dominant vector for the virus was identified as the brown tick *Rhipicephalus appendiculatus*. The virus has also been isolated from pools of *Amblyomma variegatum* ticks collected in the region (7), and this species has been shown to be competent for transmission of the virus (8). No native mammalian reservoir of the virus (i.e. one in which disease does not occur) has been identified in wild ruminants or rodents (4, 9).

Small ruminants bred in areas where the virus is circulating do not appear to get disease, possibly owing to the presence of maternal antibodies in young animals at the time they are first infected, which provides sufficient protection until the animal's own immunity is established. Although NSDV causes very high levels of livestock loss when animals are moved from areas that do not have the disease to areas where it is endemic, the geographical restriction of the virus by the distribution of the relevant tick host has meant that the disease has never had the impact of, for example, peste des petits ruminants (PPR). However, use of more modern molecular techniques has shown that the virus has a much wider distribution than previously suspected,

and may therefore pose a threat to trade and/or breeding programmes aimed at improving the stock of sheep and goats in developing countries. In addition, it has been shown that NSDV is very closely related to Crimean-Congo haemorrhagic fever virus (CCHFV), an important human pathogen, also spread by Ixodid ticks. The global impact of CCHFV is increasing, possibly due to the increased spread of its tick vector, or to increased overlap of human and tick territories as land use patterns change, leading to increased numbers of CCHFV infections of people and consequent mortality. Nairobi sheep disease virus may prove to be a useful model for studying this group of viruses, since the natural hosts for the virus are known and available and, unlike CCHFV, studies on the virus do not require biosafety level 4 containment: only one naturally occurring case of NSDV-caused disease has ever been reported in humans, and that was mild (10), while the extensive studies carried out on the virus in East Africa were never associated with disease in humans. Although the virus requires high levels of containment to prevent possible escape into local tick populations and consequent harm to livestock, the risks to humans are only at the level of, for example, vaccinia virus (biosafety level 2).

Geographic distribution

Perhaps the most surprising discovery about NSDV in recent years was that it was present also in Asia. In 1969, virus isolation studies on ticks in India discovered a novel virus in samples of ticks that had been collected from goats in 1954, in Ganjam province of Orissa state, India (11). Since this original isolation, Ganjam virus (GANV), which is primarily isolated from ticks of the species *Haemaphysalis intermedia* (see review [12]), has been found in many parts of India, and also in Sri Lanka (13) (Fig. 1). Interestingly, it has rarely been isolated from sheep or goats, and there is little recorded disease in small ruminants that can be ascribed to GANV, apart from one recorded outbreak in imported European sheep (14). The different aetiology, and the association with a different tick host, led to GANV for some time being considered as a quite distinct virus. Using defined antibody panels, Davies *et al.* (15) showed that NSDV and GANV were antigenically very closely related, so much so that they suggested that they were the same virus. More recently, sequencing of isolates of the two viruses has confirmed this identity. NSDV/GANV is a bunyavirus of

Fig. 1

Distribution of Nairobi sheep disease virus/Ganjam virus in Africa and Asia

The approximate locations where the virus has been identified, either in infected sheep/goats or in ticks, are indicated by the blue markers

the genus *Nairovirus*, and is thus an enveloped virus with a genome consisting of three segments of negative-sense RNA, S (small), M (medium) and L (large); these segments encode, respectively, the nucleocapsid (N) protein, the surface glycoproteins (Gn and Gc) and the viral RNA polymerase (L). Sequencing showed that the N and L proteins of NSDV and GANV are almost identical, with only around 10% or 2% (respectively) difference at the nucleotide level, leading to the conclusion that they represent different isolates of the same virus (16, 17). Notably, the sequences of the viral surface glycoproteins differed more between GANV and NSDV isolates than did the core proteins (16), possibly reflecting the use of different tick hosts by viruses from different continents (18). The relationships of NSDV and GANV to each other and to other viruses of the same genus, based on the sequence of the viral genomes, are illustrated in Figure 2. It is notable that the Indian and African isolates do not appear to be distinct lineages (16), but are located in the phylogenetic tree as members of a single clade.

NSDV/GANV therefore represents a virus that is widespread in India and in East Africa, and may also be found in other parts of the Indian subcontinent if/when it is looked for. The virus appears to exist without causing notable disease in native sheep/goats in India, yet causes a severe disease in imported European animals in India, and in local breeds of sheep (often derived from Persian fat-tailed sheep) in East Africa. This suggests that the virus is well adapted to its mammalian hosts in India, but is not (yet) adapted to other breeds. Coupled with the fact that East Africa lacks native wild sheep/goat species which might have been the original hosts, these observations suggest that it is possible that the virus originated in India and was brought into Africa more recently, either via a bird-borne tick or on animals shipped from India, that being the main livestock trading direction during the 18th and19th centuries; this was, for example, the route by which rinderpest virus was originally brought into Africa.

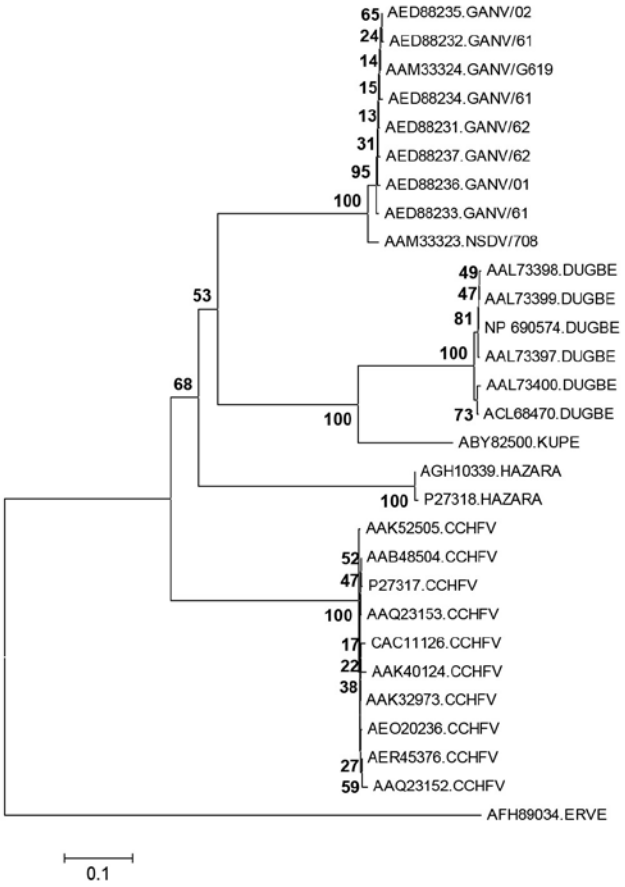

Fig. 2
Evolutionary relationships of nairoviruses
The evolutionary history of the viruses was inferred from the known full-length N protein sequences of the indicated viruses using the neighbour-joining method (19). Sequences were aligned using ClustalW (20). The tree is drawn to scale, with branch lengths in the units of the number of amino acid substitutions per site, which were computed using the James–Taylor–Thornton matrix-based method (21). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches (22). Evolutionary analyses were conducted in MEGA6 (23). For each sequence, the accession number is given in the figure

Diagnosis

In susceptible animals, the virus causes a fever which lasts for three to seven days. Diarrhoea, frequently containing blood, is seen one to three days after the fever starts. Animals rapidly become lethargic and unresponsive and die about a week after the fever starts. Goats show a similar pattern to sheep, just with milder signs and much lower mortality (1, 2). Haemorrhagic petechiae can be seen in the nasal mucosa and in the coronary band above the hoofs. Post-mortem examination shows typical signs of haemorrhage in the longitudinal folds of the lower gastric tract, most severe in the caecum and colon, and sometimes in the respiratory mucosa. The mesenteric lymph nodes and spleen can be swollen. The clinical picture of NSD is usually distinguishable from PPR by the absence of mouth lesions in the former, and the later onset of diarrhoea in the latter, often after the febrile period (authors' own observations). Peste des petits ruminants virus will have higher morbidity, unless the tick infestation is unusually high. Paratyphoid dysentery can have a similar clinical picture to NSD, as can Rift Valley fever, if there is gastrointestinal involvement.

The easiest and most sensitive method of laboratory confirmation of NSDV/GANV infection is reverse-transcription polymerase chain reaction (RT-PCR). The virus can be detected by isolation from blood only during the febrile period (1), but, using PCR, the authors have found virus genome in blood even after temperatures have returned to normal (24). Another advantage of RT-PCR

is that it is quicker and does not require cell culture. The primer pair the authors developed and tested recognises Indian and African isolates and is specific for NSDV/GANV (does not detect the related Dugbe virus or other nairoviruses); the primers can be used either for real-time analysis or conventional gel-based PCR.

Virus antigen can also be assayed by agar gel immunodiffusion (AGID); no commercial test for antigen exists, but full instructions for setting up an AGID test, either for virus antigen or antibody, can be found elsewhere (2). Development of a simple enzyme-linked immunosorbent assay for antibodies to NSDV/GANV has also been described (25).

The original method for virus isolation was by passage in sheep (1); it was later shown that the virus could be passaged in neo-natal mouse brain (26). Few places now have facilities for such procedures, and it is difficult to justify ethically, given the identification of the baby hamster kidney-derived cell line BHK21 clone 13 cells (BHK21/13) as a reproducible and sensitive cell culture system for this virus (27). The authors have observed that another BHK21 clone, BSR cells (28), is also good for virus isolation and growth, with more easily identifiable cytopathic effect than seen in BHK21/13 cells. NSDV/GANV also grew well in Vero cells and a fetal bovine endothelial cell line (24), but showed little in the way of cytopathic effect in these cells, making them less useful than BHK21/13s or BSRs for virus isolation. Whichever cell line is chosen for isolating the virus, the identity of the virus still needs to be confirmed subsequently using PCR or by staining with a virus-specific antiserum (29).

Anti-viral antibodies can be assayed either by AGID or by virus neutralisation assay in BHK cells using standard techniques.

Recent developments

The impact of the virus on livestock has historically been limited to those areas where *R. appendiculatus* is common, and to the occasions when naive animals are introduced from elsewhere. Basic tick control measures will prevent the introduction of the virus with animals brought from an endemic area. Perhaps because of this, there has been little effort to develop a vaccine. Weinbren (30) in the 1950s and Terpstra in the 1960s (2) tested isolates of NSDV that had been passaged >100 times in mouse brain or up to 71 times in tissue culture for their ability to act as live attenuated vaccines. Virus passaged 136 or 141 times in mouse brain still elicited a mild fever in most vaccinated sheep, but no other clinical signs. The animals all developed anti-NSDV antibodies and were protected from lethal challenge (2). A similar picture was found with the cell-culture-adapted virus, where there was mild pyrexia in most animals on vaccination. In addition, some of the animals given cell-culture attenuated virus also showed pyrexia on challenge, although no other clinical signs were seen (2). For both systems of passage, it was observed that, as the virus became fully attenuated, it also lost the ability to elicit a detectable antibody response, and stopped being fully protective.

An alternative to the repeated passage of the virus for the random generation of attenuating mutations would be to deliberately introduce mutations in specific parts of the virus in order to create an immunostimulatory but no longer pathogenic virus. For example, the authors have shown that the amino terminus of the NSDV L (RNA polymerase) protein contains a small motif which is responsible for the ability of the virus to inhibit the host's innate immune response to infection (31); if this motif were removed, the virus would be much more easily controlled by the host defences. For RNA viruses, creation of such targeted mutants requires the development of a system to recover virus from DNA copies of the viral genome, a process referred to as reverse genetics. While systems for the reverse genetics of several genera of bunyaviruses have been developed, and have been used to create vaccine candidates for important pathogens such as Rift Valley fever (32, 33), so far no system for recovering nairoviruses from DNA copies of their genome has been published, although replication of individual genome segments has been achieved for CCHFV (34) and for NSDV (Holzer and Baron, unpublished), so it is only a matter of time before a full reverse genetics system becomes available and an attenuated virus can be developed which is strongly immunostimulatory while properly apathogenic.

Control of NSDV, and other tick-borne pathogens of livestock, may also be achieved in the future by immunisation against the ticks themselves. It has been shown that a vaccine containing the tick cement protein 64P inhibited the transmission of virus between tick and host and protected animals against lethal virus challenge (35, 36). This protein is highly conserved, and the anti-tick vaccine was cross-reactive against several genera of ticks, offering the hope of a vaccine that simultaneously protects animals against NSD and other tick-borne diseases such as heartwater, theileriosis and babesiosis (reviewed in [37]).

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Virus de la maladie du mouton de Nairobi ou virus Ganjam

M.D. Baron & B. Holzer

Résumé
Le virus de la maladie du mouton de Nairobi (MMN) est transmis par les tiques aux moutons et aux chèvres chez qui il provoque une maladie grave, avec de nombreux foyers enregistrés en Afrique de l'Est. Le virus est également présent dans le sous-continent indien où il est désigné sous le nom de virus Ganjam. Le virus ne se propageant qu'à l'occasion des repas de sang des tiques compétentes infectées, sa distribution géographique se limite à celle de ces tiques, à savoir *Rhipicephalus appendiculata* en Afrique et *Haemaphysalis intermedia* en Inde. Dans les zones endémiques, les animaux élevés localement ne contractent généralement pas la maladie ; celle-ci affecte donc essentiellement les animaux déplacés à des fins commerciales ou de reproduction.
Le tableau clinique de la maladie due au virus de la MMN étant très semblable à celui d'autres maladies des ruminants, le diagnostic doit être confirmé au laboratoire. L'amplification en chaîne par polymérase (PCR), la mise en évidence de la multiplication virale en culture cellulaire ou d'autres tests diagnostiques simples sont décrits dans la littérature mais aucune de ces méthodes n'est commercialisée pour l'instant. De même, aucun vaccin contre le virus de la MMN n'est encore disponible ; néanmoins, des souches atténuées en culture cellulaire s'avèrent prometteuses et pourraient faire l'objet d'essais de vaccination sur le terrain, si besoin. Le virus de la MMN étant très proche de celui de la fièvre hémorragique de Crimée-Congo, des études approfondies sur ce premier virus pourraient apporter de précieux éclaircissements sur le deuxième, qui est un agent pathogène majeur pour la santé publique.

Mots-clés
Caprin – Fièvre hémorragique de Crimée-Congo – Maladie transmise par les tiques – Nairovirus – Ovin – Petit ruminant – Virus de la maladie du mouton de Nairobi.

El virus de la enfermedad ovina de Nairobi, o virus Ganjam

M.D. Baron & B. Holzer

Resumen
El virus de la enfermedad ovina de Nairobi es un patógeno transmitido por garrapatas que provoca una grave dolencia en ovinos y caprinos y ha dado lugar a varios brotes en África Oriental. También está presente en el subcontinente indio, donde recibe la denominación de virus Ganjam. Dado que solo se propaga cuando una garrapata competente infectada se fija a un anfitrión para alimentarse, su distribución geográfica se circunscribe a la de esas garrapatas, que son *Rhipicephalus appendiculata* en África y *Haemaphysalis intermedia* en la India. Normalmente los animales criados en zonas de endemismo no contraen la enfermedad, por lo que los efectos de esta se dejan sentir sobre todo en animales desplazados con fines comerciales o reproductivos.

La enfermedad causada por este virus presenta similitudes con otras varias patologías de los rumiantes, por lo que el diagnóstico de confirmación requiere análisis de laboratorio. Se han descrito y publicado métodos de diagnóstico por PCR, por crecimiento del virus en cultivo celular y por otras pruebas de diagnóstico sencillas, aunque ninguno de ellos existe en forma comercial. Tampoco existen vacunas contrastadas contra el virus, aunque se han obtenido cepas atenuadas en cultivo celular que parecen prometedoras y podrían utilizarse en ensayos sobre el terreno si se juzgara necesario. Puesto que el virus de la enfermedad ovina de Nairobi guarda estrecho parentesco con el de la fiebre hemorrágica de Crimea-Congo, los estudios sobre el primero pueden ser útiles para entender el segundo, que es un importante patógeno del ser humano.

Palabras clave
Cabra – Fiebre hemorrágica de Crimea-Congo – Nairovirus – Oveja – Pequeño rumiante – Transmisión por garrapatas – Virus de la enfermedad ovina de Nairobi.

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