

Three-day fever

A.J. Akakpo

B.P. 12 104 Dakar-Yoff, Senegal
E-mail: ayia11612@gmail.com

Summary

Three-day fever is a viral disease caused by an *Ephemerovirus* of the family *Rhabdoviridae*, transmitted by arthropod vectors. It is common in tropical and subtropical regions, where it affects mainly domestic cattle and buffaloes, especially in intensive dairy or fattening production systems. It is of economic importance because it reduces milk production and fertility and causes abortion. The disease is generally benign. It manifests in several susceptible subjects simultaneously, with a sudden episode of fever accompanied by muscle involvement with arthritis, stiffness of the limbs, and lameness, followed by rapid recovery. The presence of a serofibrinous exudate in the joints is indicative of the disease. Clinical diagnosis is often difficult in the absence of pathognomonic signs. Epidemiological factors (proliferation of arthropod vectors), associated with a short-lived fever and the presence of many immature neutrophils, point strongly to three-day fever. In the absence of any specific treatment, the symptoms are treated with antibiotics and anti-inflammatories. Medical prophylaxis currently uses live attenuated vaccines, pending the development of recombinant vaccines, which are giving promising results.

Keywords

Arbovirosis – Buffalo – Cattle – *Ephemerovirus* – *Rhabdoviridae* – Three-day fever – Tropical region.

Introduction

Three-day fever (also known as bovine ephemeral fever, bovine epizootic fever, three-day stiffness, three-day sickness and dengue of cattle) is a virulent, inoculable and non-contagious viral infectious disease of domestic cattle and buffaloes. It is caused by an RNA virus belonging to the family *Rhabdoviridae*, transmitted by arthropod vectors. Clinical signs of the disease include mild fever associated with joint pains, muscular weakness, stiffness of the limbs and lameness, sometimes accompanied by anorexia and depression. Although morbidity may affect 100% of the herd, mortality is generally low (1–2%). In most cases the disease resolves itself suddenly within three days. This disorder is of considerable importance for intensive dairy farms because of its impact on reproduction and because it can lead to a decrease, or even complete cessation, in milk production.

Background and geographical distribution

Three-day fever is most often reported in countries close to the equator and in tropical and subtropical regions of

Africa, Asia and Australia, as well as in a few temperate zones of Asia.

In 1878, Schweinfurth (1) wrote a brief description of the disease in Africa. However, it was Piot (2) who gave us the first scientific description in his excellent report on the Egyptian epidemic of 1895. He referred to the disease as *epizootic dengue fever of cattle* to mark the similarity between the signs of the disease in animals and those of dengue fever in humans. In 1907, Bevan (3) described the clinical signs of the disease in former Northern Rhodesia (now Zambia). In 1910, Freer (4) highlighted two important points:

- the need for intravenous inoculation of a healthy animal with blood taken from a sick animal to reproduce the disease
- the intervention of arthropod vectors in the transmission and spread of the disease.

In 1967, Van der Westhuizen (5) succeeded in isolating and characterising the causal agent as a member of the family *Rhabdoviridae*. In 1974, Davies and Walker (6) demonstrated the possibility of viral replication in biting arthropods, by isolating the virus from a mixture of *Culicoides* during an epizootic outbreak of the disease in cattle in Kenya.

Between 1936 and 1940, the disease spread beyond Africa to other countries. Subsequently, the disease has been reported in the Near and Middle East (Israel, Syria, Iran, Iraq), Central Asia (Pakistan, India, Bangladesh, People's Republic of China), Southeast Asia, Australia and later Japan (1968) (7, 8, 9, 10, 11).

In Senegal, despite the lack of scientific reports, the disease is well known to Fulani herders, who refer to it as 'rainy season fever'. Rural veterinarians are also well acquainted with it.

The disease is unknown in New Zealand, Europe and the Americas.

Affected species

Species belonging to the genus *Bos* (taurine cattle, zebu) and domestic buffaloes are responsive and susceptible to three-day fever. Experimentally infected goats and sheep and many wild herbivores, such as wildebeest (*Connochaetes taurinus*), buffalo (*Syncerus caffer*), hartebeest (*Alcelaphalus buselaphus*), waterbuck (*Kobus ellipsiprymnus*), kudu and giraffes, are responsive but not susceptible because they experience a silent infection with seroconversion (12, 13, 14).

Importance

The importance of three-day fever is largely economic in nature. While it does not cause significant mortality, apart from during the rare epizootics reported in Egypt and South Africa (15, 16), on intensive dairy farms the disease leads to abortion and a very sharp drop in milk production (8, 13, 17, 18).

Aetiology

The three-day fever virus is a cone-shaped capsule with a spiky surface, similar to the rabies virus. The nucleocapsid, 180 nm in length and 73 nm in diameter, is formed of a negative-sense single-stranded RNA and proteins and is helical in structure. The lipoprotein envelope has fine projections on its surface called spicules.

The virus contains five major proteins (19). These are:

- the glycoprotein envelope (G protein), which is immunogenic because it induces neutralising antibodies that aid resistance
- four non-membranous proteins: the N protein, generally associated with the nucleocapsid, the L protein, which is a polymerase, and the matrix proteins M1 and M2.

There are four serotypes of this virus, only one of which is pathogenic. In 1986, a fifth serotype was reported to have been isolated in Japan, but this was unconfirmed (20).

Culture is possible on cell lines (BHK-21 and Vero), as well as on young mouse brains.

The virus is vulnerable under external conditions. It is stable at a pH of between 5 and 10 and inactivated by the meat maturation process, during which the pH falls below 5. The virus is very sensitive to disinfectant chemicals and to lipid solvents.

The pathogenicity of the three-day fever virus is variable; as a general rule it is not particularly pathogenic, making the disease fairly mild, but there are some strains with quite high pathogenicity that cause more serious sickness in lactating females and fattening animals.

The three-day fever virus belongs to the genus *Ephemerovirus*. It belongs to the group *Lyssavirus* in the family *Rhabdoviridae* (21). As it belongs to the group *Lyssavirus*, the three-day fever virus shares antigens with the Adelaide River, Kimberley and Berrimah viruses, as well as with the Puchong and Malakal viruses. This can cause cross-reactions and problems interpreting serological reactions. According to Nandi & Negi (13), a subacute infection with the Kimberley virus induces weak production of antibodies that neutralise the three-day fever virus, but confers no real protection.

The most powerful protection comes from the strong and lasting immunity that develops in animals that recover. This humoral immunity is based on neutralising antibodies directed against the glycoprotein of the envelope. It seems that this confers relative, rather than absolute, immunity because relapses in cured animals have been reported by St Georges in Australia (17). The author also speculates on the possible role of related rhabdoviruses, such as the Kimberley virus, which triggers the initial appearance of non-protecting xenogenic antibodies, leading to possible confusion with specific antibodies during serological testing.

Pathogenesis

After entering the body, the three-day fever virus produces polynuclear neutrophils in the blood 24 hours after infection. Infection of endothelial cells, which is associated with hypocalcaemia, leads to the development of clinical signs and lesions (8). Inflammation and toxæmia in the blood vessels, joints and mucosa are linked with the massive production of interferon in cells infected with the virus, with plasma fibrinogen found in the joints and the peritoneal, pleural and cardiac cavities. A sharp fall in blood

calcium levels is responsible for the signs of nerve paralysis observed.

Clinical signs

The incubation period under normal conditions is very short: 36–48 hours. Under experimental conditions, this may vary from 29 hours to 10 days, but the average is three to four days.

The disease causes a sudden rise in temperature and affects the general state of health. There is a similar sudden fall in temperature, culminating in a general recovery after three to five days without complications. Hyperthermia generally occurs in two phases (22, 23). During the first peak of fever, the clinical signs are discreet and hard to detect. In more severely affected animals there is sudden fever with temperatures reaching 40–41°C, as well as depression, loss of appetite, anorexia, stiff gait, salivation and nasal discharge, inflammation of the joints, rapid pulse and respiration rate, shivering and oedema of the subcutaneous muscles, eye sockets and head. The animal, sometimes lying in sternal recumbency and sometimes on its side, shows some reflexes, but these gradually disappear as the disease progresses. Loss of the swallowing reflex, lack of rumination, constipation and profuse salivation become evident. Total loss of reflexes followed by coma leads to the death of the laterally recumbent animal. It should be noted, however, that these clinical signs may also disappear as suddenly as they appeared. The second peak of fever occurs 12–24 hours after the first, affecting the lungs (tachypnea, rattling) and causing lacrimation. Secondary complications may include signs of pneumonia and pulmonary emphysema, abundant discharge, stiffness of the limbs, arthritis, lameness and lasting paresis, forcing the animal into prolonged sternal recumbency. This phase of hyperthermia may last from two to four days.

In addition to hyperthermia, other clinical signs may appear or persist, such as subclinical mastitis leading to a sharp reduction in milk yield, abortion in 5% of pregnant females and infertility in bulls. Generally speaking, animals in good physical condition (fat, good milkers) show more severe signs than lean animals and non-lactating females.

The disease can lead to death in some individuals following a gradual loss of reflexes, or to cessation of swallowing and rumination. But other individuals recover in five to six days without complications (pulmonary emphysema, locomotor ataxia, persistent stiffness of the limbs). Milk yield in recovered milking cows is always lower than it was prior to the disease.

Lesions

Serofibrinous polyserositis, of varying degrees, in the articular synovial membranes and in the thoracic and peritoneal cavities is characteristic of the disease (23). Serous surfaces may also show signs of bleeding and oedema to varying degrees. The oedema fluid in the thoracic or abdominal cavity contains fibrin. In the joints, this periarticular inflammatory fluid is yellow or brown and gelatinous in appearance.

Other lesions may also occur, such as pulmonary and lymph node oedema, inflammation of the parietal and visceral pleura, pericarditis (especially at the base of the heart), necrosis at certain points of the skeletal muscles, and, sometimes, emphysematous lesions of the lungs, mediastinum and subcutaneous connective tissue.

Epidemiology

Three-day fever is especially common in tropical and subtropical regions of Africa, Asia and Australia, as well as in certain temperate regions of Asia. It affects intensively reared domestic cattle and buffaloes and has a significant economic impact. The disease is seasonal, with increased prevalence in the hot, wet season (rainy season in tropical and subtropical zones, summer and autumn in temperate zones), when conditions favour the proliferation of arthropod vectors.

The main sources of infection are sick live animals and asymptomatic carriers of the virus, as well as arthropod vectors. Owing to its vulnerability (low resistance to desiccation, heat and ultraviolet rays, etc.), the virus is non-persistent in the external environment.

The responsiveness and susceptibility of individuals are influenced by both intrinsic and extrinsic factors. One of the intrinsic factors that has a significant influence is the species of the animal. Three-day fever is pathogenic mainly to domestic cattle and buffaloes. Wild ruminants (eland, buffalo, wildebeest) often show no signs of infection. Heavy animals and dairy cows are more susceptible and succumb to severe forms of the disease. As demonstrated by Momtaz *et al.* in Iran, in a given herd, the prevalence of the disease is higher in females than in males and it also increases with age (14). Extrinsic factors include: a warm, wet season; heavy rain; physical fatigue; low-lying, wet swampy areas; flood plains and irrigation zones.

Direct transmission of three-day fever is unknown; transmission is exclusively indirect via vector-borne hematophagous arthropods belonging to various species

of mosquitoes or *Culicoides* (24), particularly the family Culicidae, which includes the genera *Culex*, *Aedes* and *Anopheles* (*Culex annulirostris* and *Anopheles bancrofti* are the prime suspects), and the family Ceratopogonidae (*Culicoides brevitarsis* and *C. imicola*) (16, 25).

Three-day fever develops in an enzootic or sporadic form in infected countries where the vectors survive throughout the year. Prevalence increases with the proliferation of arthropod vectors in favourable seasons (the rainy season in tropical zones or summer and autumn in temperate zones). Prevalence decreases once again with the arrival of the less conducive dry or cold season. During epizootic outbreaks, morbidity may be high (50–100%), but mortality is always low (2–3%). When the disease appeared in Saudi Arabia, morbidity of 59% and mortality of less than 1% were recorded (26).

Diagnosis

Three-day fever can be diagnosed either in the field or in the laboratory. The first cases are difficult to diagnose in areas previously free from the disease, and diagnosis is not always any easier in areas where the disease is well known, because of its non-characteristic appearance and its discreet early clinical signs.

In the field, diagnosis of the disease relies on epidemiological and clinical elements and lesions. In general, three-day fever may be suspected in an enzootic zone or in the season conducive to the proliferation of arthropod vectors (hot, wet season, low-lying, wet, swampy areas, delta or flood plain). It should also be suspected in an intensive dairy or fattening cattle system with improved breeds if several animals simultaneously present with fever, stiffness of the limbs and lameness, and subsequently recover rapidly. The presence of a serofibrinous exudate in the joints is also highly indicative of the disease.

A differential diagnosis may be necessary, depending on the location, for hyperthermic and nervous or respiratory disorders. Diseases that induce hyperthermia include Rift Valley fever and bluetongue. The ability to induce hyperthermia is not the only similarity between these two diseases: both are caused by viruses transmitted by arthropod vectors (arboviruses), and both occur mainly in the hot, wet season, which is conducive to the proliferation of arthropod vectors. Diseases causing nervous and respiratory symptoms include botulism, ehrlichiosis of ruminants, intoxication by poisonous plants (genera *Crotalaria*, *Diplochia*), hypocalcaemia, pulmonary emphysema and acute pulmonary oedema.

In general, three-day fever evolves benignly towards a cure in three to four days if there are no complications.

In the field, the disease cannot be diagnosed with certainty if there are no pathognomonic signs, making it necessary to resort to laboratory tests to confirm or invalidate a clinical suspicion.

Laboratory diagnosis entails both direct and indirect microbiological testing of blood samples taken from animals in the hyperthermic phase, to identify the virus and specific antibodies.

Direct microbiological diagnosis involves the isolation and detection of the pathogenic agent. Blood collected during the hyperthermic phase in a tube with an anticoagulant can be used for direct examination, as well as to isolate the virus in a culture and identify the viral genome.

– Histological analysis of a blood smear on a slide can be used for a blood cell count, as well as immunofluorescence.

a) A cell count is used to determine the number of formed elements in the blood. The presence of neutrophilia (neutrophils significantly higher than 30%) associated with many immature forms strongly indicates three-day fever. This test can be used for rapid diagnosis in the field.

b) Immunofluorescence can be used to detect and identify the virus or its antigens on the smear using immune serum (the virus-specific antibodies are marked with a fluorochrome).

– The virus is isolated either in a culture of blood leukocytes on cell lines (BHK-21 or Vero) or by intracerebral inoculation of newborn mice. The virus is identified by neutralisation of the viral culture in the presence of the specific antiserum or immunofluorescence on the cell lawn. A result is obtained after three to four days using this costly reaction.

– The viral genome can be identified using the classic reverse transcription polymerase chain reaction (RT-PCR). In 2011, Zheng *et al.* (27) described a real-time loop-mediated isothermal amplification (RT-LAMP) assay used to detect the three-day fever virus. This test is more sensitive than isolation and identification of the virus using traditional RT-PCR and thus allows earlier detection of the virus.

Indirect microbiological diagnosis aims to detect neutralising antibodies using viral neutralisation tests or an enzyme-linked immunosorbent assay (ELISA). The detection of antibodies in the second of two blood samples taken after an interval of two to three weeks demonstrates infection by the three-day fever virus. However, the existence of antigens in common with other lyssaviruses, such as the Kimberley virus, can make it difficult to interpret serological responses because of cross-reactions. The blocking ELISA test can overcome this problem because the reaction is more specific than viral neutralisation. It can also differentiate between infection with the three-day fever virus and infection with other similar viruses (22, 28).

Treatment

There is no specific treatment for infection with the three-day fever virus. Nevertheless, the symptoms can be treated and hygiene measures implemented.

Anti-inflammatories and antibiotics can be used to reduce the severity of the disorder, as was done in India (29). The intravenous administration of calcium, taking care not to overdose, can remedy signs of hypocalcaemia such as constipation, rumen atony, muscle tremors or paresis.

Hygiene measures are implemented to avoid or remedy situations conducive to the disease. It is important to rest sick animals because, if they go in search of water or food in a pastoral system, this could exacerbate the clinical signs and increase mortality. Convalescent animals must also be nursed to enable them to regain their health.

In Senegal, Fulani herders make febrile cattle take cold baths in order to lower their body temperature. Ousseynou Diouf, Doctor of Veterinary Medicine, treats seriously sick animals with antipyretics, anti-inflammatories and antibiotics, in particular tetracyclines, which results in a spectacular improvement in their condition (personal communication).

Prophylaxis

Efforts to prevent infection are based on controlling arthropod vectors and implementing livestock hygiene measures to reduce the impact of favourable conditions. The results are limited, especially in countries where livestock rearing is pastoral and vector control programmes are not entirely satisfactory. ■

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