Chapter 14

ARTHROPOD-BORNE DISEASES

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14.1 SUMMARY

Rift Valley Fever (RVF), Wesselsbron disease (WSL), Bluetongue (BT) and Akabane disease (AKA) are arthropod-borne viral diseases of sheep and goats, transmitted primarily by mosquitoes and biting midges, that can result in abortion, neonatal death and/or congenital malformations when the pregnant animal is infected. The outcome of infection by RVF, WSL, BT and AKA viruses may differ from inapparent to severe. The main factors responsible for this variation are the stage of gestation at the time of infection, viral characteristics and susceptibility of the ewe.

The unequivocal diagnoses of RVF, WSL, BT or AKA virus as a cause of abortion in sheep and goats is not easy. The climatological conditions and clinical manifestations combined with virus isolation, histopathological changes in specific tissues, viral antigen detection and presence of antibodies in the sera of the dam or precolostral sera from affected young have varying relevance in the diagnosis of involvement of each of the four viruses in abortion.

14.2 INTRODUCTION

Known arthropod-borne viral diseases most likely to result in abortions, neonatal deaths and/or congenital malformations in sheep are Rift Valley Fever (RVF), Wesselsbron disease (WSL), Bluetongue (BT) and Akabane disease (AKA). Strictly speaking BT and AKA are not single disease entities. Bluetongue includes 24 serotypes all of which are interrelated in a complex network of cross-relationships but often with insufficient cross-protection to prevent infections by other serotypes. Akabane virus on the other hand is a single member of the Simbu group. Several members of this group may produce similar defects in the foetus. Akabane virus is nevertheless by far the best studied and probably the most pathogenic member. Other members of the Simbu group which have been associated with natural disease are Aino and Tinaro and the list of viruses involved in this syndrome will probably grow. On rare occasions other Flavi viruses like Banzi and West Nile, members of the Palyam-serogroup, may cause sporadic abortions and congenital defects. It is quite possible that other yet unidentified arthropod-borne viruses may also be responsible for abnormalities in sheep.

Wild-type RVF viruses referred to as hepato-, viscero- or pantropic viruses usually cause a peracute or acute zoonotic disease of domestic ruminants in Africa. In sheep and goats there is a high mortality rate in lambs and kids and affected ewes and nanny goats usually abort. In fully susceptible flocks natural infections cause abortion storms of foetuses at all stages of pregnancy. Livers of infected animals are enlarged, soft and friable with small necrotic foci and are, in lambs and kids, almost invariably accompanied by generalized haemorrhages. Icterus may be evident. The peripheral and visceral lymph nodes are enlarged, oedematus and may show petechia.

The clinical signs of WSL in adult sheep and lambs are very similar to those

of RVF but icterus is more prominent. Subclinical infections in adult sheep are common and WSL normally occurs sporadically with a much lower mortality rate particularly in adult sheep. Congenital malformation accompanied by arthrogryposis has been reported in a few outbreaks but are mostly restricted to pregnant ewes which have been vaccinated with neurotropic attenuated live vaccines. The occurrence of WSL under conditions which favour the occurrence of RVF are a probable reason for the infrequent diagnosis of WSL.

Bluetongue particularly affects sheep and the virus causes haemorrhages, ulceration, cyanosis, coronitis, laminitis, oedema of the head and neck and torticollis. Mortality can vary from 2 to 30 percent. Abortions and congenital deformities in sheep as a result of BT are extremely rare but have been produced under experimental conditions and following vaccination of ewes during pregnancy.

Akabane disease produces clinically inapparent infections in adult sheep. In pregnant ewes, however, marked teratology, particularly of the foetal nervous system may result. The physical effects are usually only observed with the birth of affected lambs. Abortion and stillbirth are other, variable manifestations of the infection.

As these diseases are insect transmitted they have a seasonal occurrence affected by the distribution, seasonal activity and abundance of insect vectors. Mosquitoes are proven vectors of RVF and WSL, and distinct species act as the most important vector in a particular region. *Culicoides* species are the only proven vector of BT and both mosquitoes and biting midges have been incriminated as vectors of AKA. It has also been shown that AKA virus can multiply in *C.brevitarsis* under experimental conditions. The isolation of all four viruses from a wide variety of other arthropods does not indicate their importance as vectors of the virus but provides evidence of the presence of the virus.

Rift Valley Fever and WSL are African diseases. In Eastern and Southern Africa where RVF occurs as epidemics in relatively dry countries, outbreaks have always been associated with above average rainfall and in irregular cycles of 5 - 20 years and even longer. In endemic areas, where insect vectors are present continuously, the disease occurs more regularly and indications are that WSL follows a similar pattern. The occurrence of RVF in Western and Northern Africa is probably the result of the movement of infected persons or the transportation of infected slaughter animals to markets in these areas.

Bluetongue, on the other hand, is present in subtropical and tropical areas of Africa, the Middle East, the Americas and Australia and can occur regularly every year although it has a higher prevalence in wetter years. The continuous presence of BT is most likely due to the involvement of dung breeding Culicoides species which are much less dependent on wet seasons for their presence.

Most of Africa, Asia (excluding Russia) and Australia may be regarded as endemic for AKA virus and, in all probability, many of its endemic relatives. The American continent (except for the presence of Cache Valley virus in Texas), Papua-New Guinea and the island countries of the Pacific are free of infection.

The incidence of RVF, WSL and BT increases in summer and reaches a peak in late summer to early autumn. This pattern correlates closely with the increase and abundance of insect vectors. After the first frost in winter both insect vec-

tors and disease usually disappear. However, abnormal lambs may be born up to 3 months after disappearance of the vectors. In warmer climates where insect vectors occur continuously, seasonality may not be a common feature of these diseases. It is also clear that under normal conditions these diseases cannot occur in the absence of the insect vector. Consequently the introduction of animals from infected countries into vector free areas or in winter, when the vector populations are insignificant, may be a relatively safe procedure. However, global warming may result in the enlargement of regions where vectors can survive and this may expand the areas where arthropod-borne diseases can occur.

Based on the success of diagnosis, arthropod-borne viruses causing abortion or malformations can be divided into two groups. Rift Valley Fever virus usually produces non-characteristic signs in individual animals, but causes a high mortality rate in lambs and is accompanied by abortion in ewes at all stages of pregnancy. The virus can usually be isolated from the blood of the ewe at the time of abortion and almost invariably from tissues of the aborted foetus. On the other hand, isolation of WSL, which rarely, and AKA, which never, produces clinical signs in adult non-pregnant sheep is mostly unsuccessful. It is widely accepted that BT virus does not cause abortions and the isolation of this virus from an aborted foetus is very unlikely.

General recommendations made to farmers concerning the control of arthropod-borne infections of livestock include housing of sheep at night when mosquitoes and midges are most active and moving stock away from low-lying, poorly-drained areas to high, wind-swept grazing where biting arthropods are usually less prevalent. Where *Culicoides* species are involved, the use of insecticides, lavicides and sterilization of males by irradiation can help control vector populations. Keeping cattle in close proximity to sheep has also been reported to be effective, apparently because *C.imicola* (the most prevalent vector in South Africa) has a preference for cattle. Such methods are usually impractical, instituted too late and, at best, palliative in the face of viral epidemics where immunization remains the only effective way to protect livestock.

Two vaccines are currently available for immunization against RVF. A modified live vaccine that uses the Smithburn strain of RVF is available for use in South Africa and Kenya. The vaccine induces durable immunity in sheep six to seven days after a single inoculation. In a proportion of pregnant animals it may cause abortions or teratology of the foetus and hydrops amnii and prolonged gestation in the dam. A formalin-inactivated cell culture vaccine is available in South Africa and Egypt which has proved safe to use even in pregnant animals. The vaccine, however, is expensive to produce and only induces a short-lived immunity so that administration of regular booster doses is necessary to ensure adequate protection. An attenuated live vaccine is available for use against WSL in non-pregnant sheep and goats. The immunity induced following vaccination is life-long so revaccination is not recommended.

Prophylactic immunization of sheep against BT is complicated by the existence of multiple serotypes. Three live attenuated pentavalent vaccines have been developed in South Africa and they are administered to sheep at three-weekly intervals and repeated annually. After two or three annual immunizations most sheep are immune to all serotypes in the vaccine.

There are indications of temporary infertility in both ewes and rams vaccinated for the first time which necessitates immunization well before or after the mating season. Immunization of pregnant ewes can result in brain defects in foetuses ruling out vaccination during pregnancy.

A formalin-inactivated vaccine is available in Japan for use against AKA and a similar vaccine will soon be commercially available for use in Australia.

14.3 SAMPLES

Proper collection and handling of samples is highly important to the successful recovery of any virus which might be present. Samples should be taken with sterile precautions to prevent contamination with bacteria. Post-mortem materials should be taken with care to avoid cross-contamination of tissues and it is recommended that separate sets of sterile instruments are used for recovery of each tissue.

Fresh non-fixed tissue samples should be treated as described in the chapter on Border Disease.

Pre-colostral blood samples from newborn lambs should be collected when possible together with blood samples from aborted ewes for antibody detection. Samples should be taken into sterile, evacuated glass blood (e.g. Vacutainers) and treated as described in the chapter on Border Disease.

14.4 RISKS TO HUMAN HEALTH

In man, both RVF and WSL can cause an influenza-like syndrome, which may be lethal in the case of RVF and is caused by the handling of infected material and through transmission by mosquito vectors. It is advisable to protect people likely to handle RVF infected animals or products by wearing of protective clothing and vaccination where available.

14.5 DIAGNOSIS

The available methods of diagnosis, both direct and indirect, and their applicability for each of the four diseases is summarised in Table 14.1. In the case of RVF, WSL and AKA the history and epidemiology of the disease, including a relatively high rate of abortion, foetal abnormalities and/or mortality in newborn lambs or kids, as well as climatological conditions which favour the occurrence of large populations of suitable vectors, should warn of the possible presence of these viruses. Viral isolation and detection, histopathological changes in specific tissues and detection of viral antigens are the main diagnostic methods but their relevance varies for each of the viruses. A variety of methods are also available to detect antibodies to each of the viruses in sera. Sera collected from aborted ewes or nanny goats has limited diagnostic value: sera that contains no antibodies to a specific virus can establish that the virus was not the cause of abortion, but the presence of antibodies does not confirm viral involvement in the abortion as it cannot be proven that infection occurred during pregnancy. Where lambs and kids are born live, antibody determination in precolostral blood samples can be used, in conjunction with other methods, to help confirm viral infection as the cause.

14.5.1 Rift Valley fever

Histopathological lesions in the liver of aborted lambs and kids leave

little room for doubt about the diagnosis of RVF. The virus can be readily isolated from the aborted placenta and foetal tissues by intracerebral or intraperitoneal injection of infected materials into suckling mice and/or cell culture with detection by immunofluorescence (IF). Viral antigen can frequently be detected rapidly in impression smears of infected tissues by IF, in tissue suspensions by complement fixation (CF) and immunodiffusion and in tissue sections by immunoperoxidase staining. The presence of antibodies to RVF in sera can be determined by a number of techniques (Table 14.1).

Table 14.1 : Summary of diagnostic methods and their applicability for the detection of arthropod-borne diseases as the cause of abortion in small ruminants

	Rift Valley Fever	Wesselsbron Disease [a]	Bluetongue [b]	Akabane Disease [c]
Virus Isolation from:				
Placenta	+	-	+	-
Foetal tissue	+	-	+	+
Virus Isolation by :				
Mice inoculation	+	(+)	+	+
Cell culture	+	(+)	+	+
Embryonated Hens eggs	(+)	-	+	(+)
Histopatholgy	Foetal liver	Foetal liver	-	Foetal brain
Antigen Detection in Fixed Tissues	+	+	+	+
DNA Probes/PCR	+	-	+	-
Antibody Detection by :				
Virus Neutralisation Test	+	+	+	+
Serum Neutralisation Test	+	+	+	-
ELISA	+	+	+	+
Indirect / Direct				
Haemagglutination Test	+	+	+	+
Complement Fixation Test	+	+	+	+
Indirect / Direct Fluorescent				
Antibody Test	+	-	-	-
Gel Diffusion	+	-	+	-

a - Wesselsbron virus has only ever been isolated from experimental infections, never from field samples.

b - Bluetongue virus has only been shown to produce abortion in sheep under experimental conditions or following vaccination of ewes during pregnancy. Tests, however, may be relevant when young are born with congenital defects.

c - Akabane virus has rarely been isolated from aborted foetuses although placental material has occasionally been shown to contain the virus.

14.5.2 Wesselsbron disease

Wesselsbron virus has never been isolated from an aborted foetus in the field although it has been isolated from foetuses aborted from experimentally infected pregnant ewes. The macroscopic and microscopic lesions in the liver of aborted foetuses are often sufficient to make a diagnosis of WSL. The presence of antibodies to WSL in sera can be determined by haemagglutination inhibition (HAI), CF and virus neutralisation test.

14.5.3 Bluetongue

Since infection of pregnant ewes with BT virus produces foetal abnormalities rather than abortion, clinical signs and lesions in the affected animals are significant in diagnosis. Bluetongue virus can be isolated from clarified tissue suspensions of affected young most readily by intravascular inoculation of 10-12 day old embryonated hen's eggs followed by passage in suckling mice brain or cell cultures with detection by CF or IF. An indirect peroxidase-antiperoxidase (PAP) test has been used to demonstrate BT virus in sections of the chorio-allantoic membrane as has an avidin-biotin amplified staining procedure to demonstrate BT virus antigens in tissue samples from infected foetuses. The presence of antibodies to BT in sera can be determined CF, gel immunodiffusion and ELISA. Type specific antibodies are best detected by means of virus neutralisation tests, such as plaque reduction.

14.5.4 Akabane disease

Lambs which develop hydranencephaly (HE) or arthrogryposis (AG) or both as a result of intra-uterine infection with AKA virus may be aborted, stillborn or delivered alive at term. Culture of tissues from affected animals for virus is usually negative although placental material may contain virus which can be isolated in suckling mice and/or cell culture. Diagnosis therefore requires that parts of the brain affected are determined macroscopically and microscopically. Precolostral serum collected from affected animals can be used to detect antibodies to AKA virus by virus neutralisation test, agar-gel diffusion or ELISA and provide strong evidence that the affected animal was infected in utero.

14.6 DIRECT DIAGNOSIS

14.6.1 Virus isolation

Demonstration of virus in placental material or aborted foetuses is the best way of diagnosing RVF, BT and AKA viruses as the cause of abortion or congenital malformation. Virus isolation is best carried out in suckling mice inoculated intracerebally or in a variety of cell cultures including VERO, BHK 21, CER as well as primary foetal calf and lamb kidney and testis cells. Viruses are detected by tests such as immunofluorescence, ELISA, or complement fixation. For isolation of BT increased sensitivity can be achieved by intravenous inoculation of 10-12 day old embryonated hen's eggs with blood or clarified tissue suspensions. After a single passage in eggs the virus can be readily cultivated in cell cultures or in mouse brain. Virus neutralization tests are commonly used to distinguish between serotypes of BT.

N.B. It is essential that laboratories undertaking virus isolation have a guaranteed supply of virus-free susceptible cells and foetal bovine or equivalent serum which contains no antivirus activity and no contaminating virus.

14.6.1.1 Preliminary virus isolation

a / Mouse inoculation

Principle

Sample materials are inoculated intracerebrally into 1-2 day old suckling mice. Any virus present will multiply and cause death of the mice. The brains are removed from dead mice and used to inoculate susceptible cell cultures as described in Section 14.6.1.2.

Materials and reagents

- 1-2 day old suckling mice.
- Maintenance media (p.190)

Procedure

1. Inoculate 20µl volumes of each sample intracerebrally into each of at least 5 suckling mice.
2. Observe the mice at least twice daily for at least 10 days.
3. Aseptically remove the brains from any mice that die.
4. Homogenise brains in maintenance media to give a 10% suspension (w/v).
5. Inoculate cell monolayers with the resultant homogenate or store homogenate at 4°C for up to 2 weeks, at -20°C for between 2 weeks and 3 months and at -70°C for period of longer than 3 months.

Note

• When only one or two mice die or there are no deaths, surviving mice should be killed, their brains removed, homogenised and a second passage undertaken in infant mice.

b / Virus isolation in embryonated hen's eggs

Principle

Samples are inoculated intravenously into embryonated hen's eggs at 10-12 days of age. Any viruses will multiply there and cause the death of the embryo after 2-5 days depending on the number of viral particles inoculated. The embryos in the dead eggs, recovered on the 4th day after inoculation are very rich in virus and are used to inoculate susceptible cell cultures as described in Section 14.6.1.2.

Materials and reagents

- Specific pathogen free embryonated hen's eggs.
- Egg incubator and candler.
- Phosphate buffered saline (PBS).
- Antibiotics: gentamycin (gentalline) or streptomycin and amphotericin B (fungizone).

Procedure

1. Incubate the embryonated hen's eggs at 38°C in a humid atmosphere (50 - 70% humidity) until 10-12 days of age. Candle to verify their vitality which is indicated in each by the air sac and the embryo.
2. Dilute the samples 1 in 10 with PBS containing antibiotics (500µg/ml gentamycin or 200µg/ml streptomycin and 0.5µg/ml amphotericin B).
3. Inject 0.1ml of the neat and 1 in 10 dilution of samples intravenously into each of 3 to 5 eggs.
4. Incubate the eggs at 34°C and candle daily.
5. Aseptically harvest embryos dying from the 2nd day after inoculation.
6. Homogenise each embryo with 5ml of PBS, then centrifuge at 900 xg for 10 minutes.
7. Remove the supernatant and either inoculate cell monolayers or aliquot and store at 4°C for up to 2 weeks, at -20°C for between 2 weeks and 3 months and at -70°C for period of longer than 3 months.

14.6.1.2 Tissue culture isolation and detection

Viral isolation can be undertaken following the method described in the chapter on Border Disease using the relevant reference virus strain, viral antiserum, a rabbit or mouse IgG conjugated with fluorescein isothiocyanate (FITC) and the following cell maintenance media :

Eagles minimum essential medium (MEM)
10% tryptose phosphate broth (TPB)
2% foetal bovine serum (FBS)

Penicillin G	200 IU/ml
Streptomycin sulphate	100 µg/ml
Kanamycin	100 µg/ml
Fungizone	2.5 µg/ml

Reading and interpretation of results

Viral presence is confirmed by the presence of fluorescence characteristic of virus.

14.7 INDIRECT DIAGNOSIS

14.7.1 Histopathology

Principle

A suitable method for histopathological preparation of tissue samples and haematoxylin and eosin (H & E) staining is described in the chapter on Toxoplasmosis. For diagnosis of RVF and WSL virus infections characteristic changes occur in the liver whereas in AKA virus infections changes in the brain and spinal cord are the most useful diagnostic tool. In the latter case specific myelin stains such as Luxol fast blue can be used as described in the chapter on Border Disease.

14.7.1.1 Rift valley fever

Histopathological examination of the liver reveals primary foci of necrosis comprising dense aggregates of cytoplasmic and nuclear debris, some fibrin, and a few neutrophils and macrophages which can be discerned against a background of parenchyma reduced by nuclear pyknosis, karyorrhexis and cytolysis, with only narrow rims of degenerated hepatocytes remaining reasonably intact close to portal triads. Destruction of hepatocytes may be so marked that most of the normal architecture of the organ is lost, giving the liver almost the appearance of lung tissue. Intensely acidophilic cytoplasmic bodies which resemble the Councilman bodies of yellow fever are common, and rod-shaped or oval eosinophilic intranuclear inclusions are found in about 50% of affected livers. Mineralisation of necrotic hepatocytes may be evident as small purplish-blue cytoplasmic granules in H & E stained sections in approximately 60% of lambs while bile casts are present in about 30% of livers.

14.7.1.2 Wesselsbron disease

Histopathological examination of the liver reveals mild to extensive necrosis of the parenchyma; individual or small groups of necrotic hepatocytes are scattered throughout the lobules. Apart from the necrotic hepatocytes, most other liver cells reveal cloudy swelling and hydropic degeneration. Other changes in the liver include mild to moderate Kupffer cell proliferation, cholestasis, mild to moderate proliferation of bile ductules, and infiltration of mononuclear cells, particularly histiocytes, in the portal triads. Intranuclear inclusion bodies, which stain eosinophilically in H & E tissue sections and which are inconsistent in shape and size, are present in some necrotic hepatocytes.

14.7.1.3 Akabane disease

The pathogenic effects of AKA virus on the developing foetus depend on the gestational age at the time of infection and may vary from arthrogryposis (AG) and hydranencephaly (HE) to porencephaly (PE) and micrencephaly (ME). The extent of damage to the brain of infected lambs can vary within and between brains and between regions of the brain.

In lambs exhibiting AG and HE the skull tends to appear normal but most of the forebrain is replaced by membranous sacs of liquid and structures remaining are not recognisable. The midbrain, cerebellum and medulla oblongata may be reduced in size and the meninges may consist of fused membranes with focal areas of necrotic debris and fibrous thickening. In the medulla of the thymus Hassall's corpuscles may be prominent and contain much keratin and eosinophilic hyaline material. The cortex may be narrow and sparsely populated with thymocytes. The spinal cord may be reduced in size with marked reduction in the extent of myelination of the ventral cord and reduction in ventral cord neurons many of which exhibit degenerative changes. Small mineralised plaques may be present beneath the meninges of the spinal cord.

In lambs exhibiting PE there may be large liquid filled cavities in the subcortical areas of the cerebellum and numerous small to large areas of caviation and small malacic foci present in the cerebral cortex. The rest of the brain and spinal cord tend to appear normal.

Lambs with ME have small heads with abnormally thick skull bones and a reduced cranial cavity. The brain is markedly smaller than in similar uninfected lambs due to a reduction in the size of the cerebral hemispheres. Sections of the brain show few changes but there may be decrease in the parenchyma of the cortex of the cerebellum but no degenerative changes.

14.7.2 Detection of viral antigen

Immunohistochemical labeling techniques, such as immunoperoxidase and peroxidase anti-peroxidase (PAP) staining methods, can also be applied to histological tissue sections to detect RVF, WSL and BT antigens. The PAP technique can be carried out as described in the chapter on Toxoplasmosis using suitable primary and binding antibodies.

14.7.3 Detection of viral antibodies

Serological diagnosis is of limited value in the case of RVF, WSL and BT but it is currently the only way to assist in the diagnosis of AKA in which serological and circumstantial evidence and the exclusion of other causes must be employed. The only evidence of specific infection in an abnormal lamb is the presence of specific antibody in serum collected before the lamb has received colostrum. It may also be possible to demonstrate antibodies against the causative virus in the pre-colostral serum of normal newborn lambs born in the same period as abnormal lambs as not all infected lambs will necessarily develop clinical signs.

The presence of IgG antibodies indicates the presence of virus but is poor evidence of recent infection. Where tests for IgM antibodies are available these should be the serological test of choice to establish recent infection. Virus neutralisation tests are the most specific serological test available.

14.7.4 Virus Neutralisation test

The virus neutralisation test described in the chapter on Border Disease can be used to diagnose AKA using a suitable cytopa-

thic AKA virus, suitable control positive and negative sera and the growth media given below with the following changes :

1. Add virus at a concentration of 200 TCID50 per 25µl.

2. Following the addition of cells, incubate plates for up to seven days.

- Growth Media

Eagles minimum essential medium (MEM)

10% tryptose phosphate broth (TPB)

10% foetal bovine serum (FBS)

Reading and interpretation of results

The VN titre for each serum is the dilution at which the virus is neutralised in 50% of the wells. This can be calculated by the Spearman-Karber method. A seronegative animal will show no neutralisation at the lowest dilution.

14.8 FUTURE WORK

In recent years, genomic probes have been developed. The sensitivity of probe assays is generally lower than that of most other methods and their use is limited to well equipped laboratories. However, the advent of the polymerase chain reaction (PCR) and its application to transcription-based in vitro gene amplification has led to the development of highly sensitive assays using non-radioactive probes. This rapid method is relatively expensive and needs further investigation and/or modification before it can be used routinely for diagnostic purposes. Demonstration of viral antigen by PCR in cases of abortion must be evaluated cautiously as this sensitive technique can detect virus antigens produced long before the actual infection that precipitated the abortion and may lead to a false positive result.

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Chapter 15

NAIROBI SHEEP DISEASE

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15.1 SUMMARY

Nairobi sheep disease (NSD) is a tick-transmitted virus infection of sheep and goats. The disease is characterized by a marked febrile response followed by profuse watery diarrhoea that often becomes haemorrhagic and frequent abortion in pregnant animals. The course of disease may be peracute, acute, mild or inapparent, with a case mortality frequently over 80 percent.

As with other arthropod-transmitted viral infections, the unequivocal diagnosis of NSD is not easy. Nairobi sheep disease virus can be isolated from aborted materials and tissues of adult animals by inoculation either into infant mice or into baby hamster kidney cells with identification by immunofluorescence. In recovered animals the detection of serum antibody by indirect immunofluorescence, indirect haemagglutination or enzyme-linked immunoabsorbent tests will indicate previous infection with NSD.

15.2 INTRODUCTION

The virus of NSD belongs to the Nairovirus genus within the family of arthropod-transmitted viruses known as the Bunyaviridae. Nairoviruses have distinct structural and antigenic characteristics and can be subdivided into six serogroups. The serogroup to which NSD virus belongs also contains Dugbe and Ganjam viruses isolated from ticks in Nigeria and India, respectively. The virus measures 90 to 100 nm in diameter and contains RNA, and all isolates appear to be antigenically identical.

Nairobi sheep disease was first described in Kenya in 1910 and it was subsequent established that the disease was caused by a virus transmitted by Rhipicephalus appendiculatus ticks that had previously engorged on sheep reacting to infection. Following the recognition of NSD in the area around Nairobi, its distribution within Kenya was found to correspond generally to that of *R.appendiculatus*. However, other ticks can transmit the virus and it has been isolated from *Amblyomma variegatum* and *R. pulchellus*, suggesting that the epidemiology of NSD may be complex. The disease has now been identified in Uganda, Northern Somalia, and probably Zaire and Ruanda. There is serological evidence that it is present in Botswana, Ethiopia, Tanzania and South Africa.

Natural transmission between sheep occurs only be tick bite and it has been concluded that NSD is maintained in a sheep-tick-sheep cycle. Both transtadial and transovarial transmission have been demonstrated in the tick vector. The virus is shed in the urine and faeces but the disease is not spread by contact. Clinical signs in animals reared in areas infested with infected ticks are usually not observed. In contrast, the response of naive sheep moved to endemic areas is dramatic.

Following exposure to infected ticks,

the incubation period is four to six days. Pyrexia of sudden onset is the first sign, with temperature rising rapidly to 40 to 42°C. Affected animals are dull and anorexic, and there may be a mucous nasal discharge. Shortly thereafter, watery green faeces are voided and may become haemorrhagic. Distress and constant straining become evident, and the nasal discharge often becomes blood-tinged. The external genitalia of ewes swell, and pregnant animals frequently abort. Death may follow within 24 hours of the onset of signs but can be delayed for as long as six days. It is generally considered that the prognosis is poor if diarrhoea develops.

All breeds of sheep and goats are susceptible. The mortality rate of African sheep tends to be high, even up to 80%, whereas that of European breeds is seldom more than 50%. Goats appear to be less susceptible, with a mortality rate of 10%, but losses in the field have been reported to reach 88%.

At postmortem examination the hindquarters are usually soiled with faeces and the nostrils caked with blood-tinged nasal discharge. There is general lymphadenosis and haemorrhages occur throughout the alimentary and respiratory tracts in all sheep except those dying peracutely. The genital tract, particularly of pregnant ewes, is oedematous and hyperemic, and the mucous membranes are catarrhal. Foetuses exhibit dermal haemorrhages. The only histological changes that are consistently present and considered to be of diagnostic value are a severe glomerulotubular nephritis associated with hyaline and epithelial casts and a generalized vascular congestion of the kidney that is most marked around the glomeruli.

Virus serially passaged by intracerebral inoculation of mice or in tissue culture loses pathogenicity for sheep and has been used as attenuated live virus vaccines. However, the degree of attenuation is accompanied by a loss of immunogenicity. Thus, owing to the variability in susceptibility of different breeds of sheep, none of these vaccines has proved generally acceptable. An inactivated vaccine prepared from virus propagated in baby hamster kidney cells and then formalinized and precipitated with methanol produced a high level of resistance to laboratory challenge but has not yet been assessed in the field.

Nairobi sheep disease virus can infect man and is related to a hazardous viral zoonosis of man known as Crimean-Congo haemorrhagic fever.

15.3 SAMPLES AND DIAGNOSIS

The clinical and postmortem picture cannot be relied on to provide a specific diagnosis although the occurrence of the clinical symptoms described above and death of adult animals at the same time as abortion can rule out many other common abortive agents. Confirmation depends on laboratory tests which should be undertaken on both aborted materials and tissues (including spleen , mesenteric lymph nodes and liver) from ewes dying at the time of or soon after abortion. Since the virus is heat sensitive samples must be collected promptly after abortion or death and sent to the laboratory as quickly as possible on ice and also in phosphate buffered glycerol solution at pH 7.2.

In the laboratory virus may be detected in tissue homogenates by intracerebral inoculation of suckling mice or in baby hamster kidney cell line (BHK 21)

cultures in which it produces characteristic perinuclear inclusion bodies. Tissue culture combined with detection by direct or indirect immunofluorescent staining provides the most rapid and certain means of detection [1]. Reliance on viral detection by cytopathic effects in tissue culture is less sensitive as generally isolates must be serially passaged two to five times before cytopathic effects are detected. The presence of antigen in tissue sections or impression smears of material from infected sheep can be carried out by direct or indirect immunofluorescent techniques. In recovered animals the detection of serum antibody by indirect immunofluorescence, indirect haemagglutination or enzyme-linked immunoabsorbent tests will indicate previous infection with NSD. The relevant methodologies are described in full in the chapter on Arthropod-Bourne Diseases.

REFERENCES

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Whether under intensive, semi-extensive or extensive farming system sheep and goat flocks suffer heavy losses from abortive infections throughout the world. Brucellosis due to Brucella melitensis is known to be widespread and well established in most countries of the Mediterranean region and it is also constituting a worisome emerging problem in other geographical regions such as southern Africa, Central Europe or North Asia. However, this infection is not the only important cause of abortions. Indeed, even when the incidence of the disease has been successfully reduced in countries where it occurs, the abortion rate in small ruminants may be only slightly affected.
In these countries, as in others, Chlamydiosis, Toxoplasmosis, Q fever and other viral and bacterial diseases require a better knowledge of their epidemiology and an effort of prevention sustained with a view to maintain their impact at an acceptable level.
This work strives to contribute to fill the gaps with this respect especially in developing countries.

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