Chapter 14
Viral Hemorrhagic Fevers of Animals Caused by Positive-Stranded RNA Viruses

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Core Message Here we outline serious diseases of wildlife, food and fiber animals, and non-human primates that cause damaging economic effects on producers all over the world. While some zoonotic viruses that occasionally cause serious disease and death in humans are mentioned, the positive sense RNA viruses generally cause economic damage that can have serious societal implications for humans. Finally, honorable mention is given to yellow fever virus, a success of vaccine development efforts. This virus once caused similar serious effects (in humans) during the construction of the Panama Canal, but has been relegated to a footnote in textbooks because of a cheap and effective vaccine.

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1 Bovine Viral Diarrhea Virus

1.1 Etiologic Agent and Natural History

1.1.1 Definition

Bovine viral diarrhea (BVD) was first described as a distinct acute febrile disease of cattle in 1946 [1]. The presence of blood in diarrheic feces and nasal exudate was noted in these early reports along with a fever (40–42 °C) and leukopenia, followed by abortions and the birth of congenitally deformed and weak, nonviable calves. The disease was associated with erosive, ulcerative lesions of the gastrointestinal, oral, and pharyngeal mucosae, and hemorrhages in lymph nodes, gastrointestinal mucosa, subcutaneous tissues, pericardium, and vaginal mucosa. This early association of BVD with bleeding manifestations was overshadowed by the discovery of fetal infections, and the role of immune tolerance in the generation of persistently infected (PI) animals. Cases of hemorrhagic syndrome (HS) associated with BVD likely occurred in the interim, but were not reported again until 1989 and in 1993–1995 [2, 3]. As a result of these outbreaks of severe hemorrhagic disease with significant lethality, interest in BVD HS was reinvigorated [3, 4]. Sequencing of the viruses isolated from HS cases revealed a distinct genotype of BVD viruses, BVDV-2, associated with this syndrome. HS cases are typically caused by BVDV-2 [5].

1.1.2 Etiology and Evolution

BVDV-1 and BVDV-2 are positive-sense, single-stranded RNA viruses with genomes approximately 12.3 kb in length contained within enveloped icosahedral capsids. BVD viruses are members of the genus *Pestivirus* within the family

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Flaviviridae, but are classified in two distinct species. BVDV-1 and BVDV-2 are further classified into several subtypes and multiple strains thereof [6]. Each pestivirus appears to have evolved with its primary ruminant host, but is capable of infecting additional ungulate species. Both viruses occur as two distinct biotypes, noncytopathic (ncp) and cytopathic (cp). Most isolates are ncp viruses (i.e., they do not cause morphologic changes or cell death in infected cell cultures). In animals that are persistently infected with ncp viruses, various spontaneous mutations render the ncp viruses cytopathic in cell culture. Isolation of pairs of cp and ncp viruses is associated with the development of mucosal disease (MD) in PI animals [6]. The BVDV-2 isolates from HS cases are ncp.

1.1.3 Geographic Distribution and Economic Effects

BVD viruses are distributed in cattle worldwide with the exception of countries from which the viruses have been eradicated [7, 8]. On an individual herd basis, monetary losses range widely according to the specific circumstances. The financial losses due to BVD in an individual dairy herd were calculated to be £2,295 and £4,115 in 1982 [9]. Economic losses specifically due to BVDV HS cases have not been calculated. Estimates for the economic impact of BVD at the national level vary from $20 million per million calvings in Danish cattle to a conservative estimate of greater than $400 million for US cattle based on approximately 34 million calves born in 2012 and 2013 [10, 11].

1.1.4 Natural History, Transmission, and Host Range

The persistence of BVDVs in bovine populations is due to the ncp replication and immunosuppressive qualities of the viruses and their ability to be transmitted from susceptible female hosts to developing fetuses with great efficiency. The consequences of fetal BVDV infection include infertility, abortion, stillbirth, weak calves, congenital defects, and growth retardation in calves [12]. Most importantly, infection of the fetus during the first 125 days of gestation results in the generation of PI calves. The survival of PI animals into the breeding season is the key to the maintenance of virus in cattle populations. PI animals shed large quantities of BVDVs in secretions throughout life, and are the source of the infection for susceptible cattle. In nature, infection of cattle occurs via the inhalation of BVDVs in aerosolized respiratory secretions, or ingestion of urine, feces, or saliva from PI-animals, or from contact with aborted infected fetuses and fetal fluids. Inadvertent infection can occur through the administration of live vaccines, contaminated biological products, or through contaminated insemination equipment or semen. BVDVs infect cattle, other domestic and wild ruminants, camelids, pigs, and wallabies [13]. Rabbits can be infected experimentally, but do not develop disease.

1.2 *Pathogenesis and Clinical Features*

1.2.1 Pathogenesis and Immunology

In acute BVDV infections, virus is inhaled or ingested and replicates in the lymphoid tissues of the nasopharynx. Virus-infected lymphocytes and macrophages spread the virus to other lymphoid tissues via the blood, including the bone marrow. Specifically regarding the mechanisms of the hemostatic disorders involved in BVD HS, evidence exists for: (1) direct infection and damage to blood vessels by the virus, and (2) virally induced thrombocytopenia with alteration of platelet function. Evidence for the first mechanism is the localization of BVDV-2 antigen in endothelial cells and smooth muscle cells in blood vessels that may be accompanied by necrotizing vasculitis [14, 15]. Infection and damage to the endothelium triggers thrombus formation and consumption of platelets and other clotting factors. Indirectly, cytokines, such as interleukin-1 and tumor necrosis factor α, produced by monocytes during infection [16] alter the expression of endothelial cell surface proteins, favoring development of disseminated intravascular coagulation (DIC) and vascular leakage.

In naturally occurring BVD HS, the number of platelets is severely diminished (2,000–33,000/μl) with little effect on clotting times (PT, aPTT) and fibrinogen plasma concentrations [4]. In experimental studies, platelets decreased 4–8 days after intravenous inoculation, reaching a nadir at 14–16 days post-inoculation. Hemorrhage was noted when platelet numbers were <5,000/μl [17]. Potential mechanisms for BVDV-2-induced thrombocytopenia include: (1) viral infection of megakaryocytes resulting in decreased production and function of platelets, (2) DIC with consumption of platelets and clotting factors, and (3) immune-mediated destruction or sequestration of platelets. The first mechanism is supported by the identification of viral antigen on platelets by immunofluorescent antibody (IFA) and on megakaryocytes by immunohistochemical (IHC) staining [17–20], and by the observation of decreased numbers of megakaryocytes with degenerative, necrotic changes in the bone marrow [4, 20, 21]. Infection and necrosis of megakaryocytes, however, have not been observed in all studies [18]. Megakaryocyte hyperplasia, possibly compensatory due to platelet consumption and without evidence of virus infection, has been reported by other investigators. These contrasting results may reflect various factors, such as a difference between virus strains in their tropism for megakaryocytes [20, 22, 23], the time post-inoculation at which bone marrow was examined, and/or the age and breed of calves examined [22, 24]. In addition to direct effects of BVDVs on megakaryocytes, platelets from infected calves have altered morphology [20] and a decrease in the aggregation response compared to controls [25]. Recreating BVD HS experimentally is difficult, evidenced by the fact that the hemorrhagic lesions are inconsistently produced even with viruses isolated from HS cases [23]. Clearly, additional factors such as nutritional management and environmental conditions may influence the development of hemorrhagic lesions and the outcome of infection in nature.

The impact of BVDVs on the bovine immune system was initially observed by noting the decrease in leukocyte counts and depletion of lymphoid tissues in the first

reports of the disease [26]. Later, the systemic nature of infections and the viral tropism for T- and B-lymphocytes and macrophages was determined [27, 28]. Acutely infected cattle respond with specific T helper cell, CTLs, and B cell responses. However, the detection of BVDV-specific antibodies in serum may be delayed for 24–49 days in acute infections with HS strains [18]. Once the virus is cleared, surviving cattle maintain strain-specific immunity for years.

In general, viruses that infect these key cells of the immune system significantly reduce the host's defense against a variety of infectious organisms. Consequently, multiple concurrent bacterial, parasitic, and/or viral infections occur in BVDV-infected animals that are not responsive to treatment. The isolation and culture of mixed populations of microbes from cases of pneumonia and enteritis in cattle should trigger suspicion of BVDV infection [29].

The consequences of fetal infection depend on the specific pathogenicity to the fetus of the virus strain, and on the gestational and developmental age of the fetus [30]. Pathogenicity in the fetus is not necessarily similar to infection with the same BVDV strain in adult cattle, and can be unrelated to the biotype of the viral strain as well. Early in gestation, the fetus responds to infection with elements of both the innate and adaptive immune responses; however, these immune functions are not sufficient to clear the BVDV infection [31]. Importantly, the lack of viral clearance results in a PI fetus. When the infection occurs after approximately 150 days of gestation, the fetus is able to develop virus-specific antibodies, mount a virus-specific response, and clear the infection. These are present at birth virus-specific antibodies.

1.2.2 Incubation Period, Clinical Signs, and Gross Lesions

The majority of BVD HS cases occur as a result of acute, postnatal infections following an incubation period of 2–12 days, and should be considered when cattle present with a high fever (40–42 °C), depression, and death within 24–48 h after the onset of signs [15, 18, 23]. Affected animals may also exhibit dyspnea and diarrhea with or without blood in the feces. A complete blood count (CBC) may reveal lymphopenia within 3–18 days [18] and thrombocytopenia within 14 days post exposure [17].

At necropsy, petechial and ecchymotic hemorrhages may be noted on oral or vaginal mucosae, in subcutaneous tissues, and on serosal surfaces of multiple organs [4, 32, 33]. The grossly visible hemorrhages of HS may be accompanied by thymic atrophy in young animals; diffusely reddened, fluid filled lungs reflective of an interstitial pneumonia; and diffusely red segments of the small intestines. The wall of the intestine is thickened by edema and the intestinal contents are scant, blood-tinged, and mucoid in consistency. Peyer's patches are translucent reflecting depletion of lymphoid populations accompanied by small hemorrhages. Mesenteric lymph nodes may be enlarged and edematous. None of these lesions are pathognomonic for BVDV infections and may be mistaken for a number of diseases caused by bacterial and toxic agents. The primary alternative (differential) diagnosis of extensive hemorrhagic lesions in aborted fetuses and neonatal calves is dicoumarol toxicosis

following ingestion of moldy sweet clover hay by the dams [34]. In adults and older calves, clostridial diseases, salmonellosis, leptospirosis, anthrax, and other bacterial septicemias should be considered.

In general, the clinical signs of acute BVD are dependent on the strain of virus and the epizootiological status of the herd. Distinguishing between acute, chronic, and fetal infections, based on clinical signs and gross or histopathologic lesions, is often difficult [17, 32]. For each clinical outcome (e.g., abortion), the list of differential diagnoses is lengthy, and lesions (if present) are not pathognomonic. BVD should be suspected when multiple diseases occur within a herd, such as infertility, abortion, or weak calves with diarrhea and pneumonia. Clinical disease that is refractory to treatment should raise suspicion of underlying immunosuppression and, therefore, BVDV infection.

1.2.3 Morbidity and/or Case-Fatality Rates and Prognostic Factors

As BVD is not a reportable disease in the USA, information on prognosis, morbidity, and lethality of BVD cases with HS is scant. A 1977–1987 retrospective case review of 146 cattle with acute BVDV infection at New York State College of Veterinary Medicine revealed that 15 (10 %) had BVD associated with thrombocytopenia and bleeding and that 9 of the 15 died or were euthanized [4]. Another study demonstrated that an ncp-BVDV could induce thrombocytopenia that was severe enough to observe a morbidity rate of 50 % and case-fatality rate of 20 % [18].

1.3 Diagnosis and Control

1.3.1 Diagnosis and Epidemiology

An array of laboratory diagnostic tests for detecting BVDV proteins (IFA, IHC, antigen-capture enzyme-linked immunosorbent assay [ELISA]), viral RNA (reverse transcriptase-polymerase chain reaction [RT-PCR], real-time RT-PCR) or infectious virus (virus isolation) from individual animal serum, whole blood, ear notch samples, and/or pooled milk is available, as well as serologic tests (serum neutralization, ELISA) [35]. The appropriate choice of samples and tests and the selection of animals to be tested are dependent on the peculiarities of each case. Individual case factors that affect the choice of tests and testing strategies include the management system (dairy, feedlot, beef cow-calf) of the herd, the manifestation of disease (enteritis, bovine respiratory disease, abortions), and whether one is attempting to detect BVDVs in PI or acutely infected animals. In HS cases resulting from acute infection with BVDV-2, whole blood samples from multiple live animals during the febrile phase of disease, or lung and lymphoid tissues obtained from necropsy, should be tested for the presence of infectious virus, viral proteins or viral RNA. BVDV may be detected in the buffy coat of whole blood or tissue samples by virus isolation,

RT-PCR, or real-time RT-PCR in as few as one of ten acutely infected cattle in a HS outbreak, whereas antigen may not be detected by ELISA, IFA or IHC staining in this same scenario. Further evidence of infection may be demonstrated by a fourfold or greater increase in BVDV-specific antibody titers (by serum neutralization or ELISA) in surviving animals. However, interpretation of serologic data is frequently complicated by the prior or concurrent use of BVDV-containing vaccines in cattle.

The epidemiology of BVDV centers on the PI animal, which sheds BVDV in oral and respiratory secretions, feces, and placental fluids and milk, all in high concentrations. PI animals are generated through the infection of susceptible pregnant cows during in the first 125 days of gestation. For this to occur, PI cattle must survive into the breeding season of the herd. PI animals may be inadvertently purchased or be introduced into a herd through the purchase of previously infected female cattle that later give birth to PI calves. Cows may also become infected while pregnant when they encounter PI animals from other herds in shared pasture.

In herds in which breeding is synchronous (as in the US beef industry), one PI animal can infect a large number of cows. The results of this first event can be catastrophic, with losses due to abortions, stillbirths, and weak, non-viable calves. The high proportion of BVDV-associated disease is characteristic of the epidemic phase of herd infection. Any PI calves that are born in this event cause additional morbidity and mortality due to acute BVDV-induced diarrhea and pneumonia within their cohort. Infection with BVDV renders the cow herd largely immune to the specific strain of virus. Births of female offspring by immune dams in subsequent years provide a new generation of cows susceptible to infection. This susceptible cohort serves as the continual source of PI calves in the endemic phase of the herd infection as these heifers are bred and become infected with BVDV.

In herds with year round breeding (e.g., dairy herds), cows are in different stages of gestation. The introduction of PI animals into the herd will result in fewer losses due to abortion, but with the occurrence of characteristic and more easily recognized congenital defects. The reproductive losses may be preceded by a spike in cases of pneumonia and diarrhea in adults and young stock 4–7 months before the birth of defective and PI calves. The epidemiology of BVDV is reviewed by Van Campen in [36].

1.3.2 Vaccination, Control, and Eradication

Ultimately, BVD control is achieved by the identification and removal of PI animals, thereby preventing further infection of pregnant cattle and the perpetuation of PIs. Where instituted, BVD control programs have successfully eradicated BVDVs from domestic cattle [7, 8]. In North America, BVDV-containing vaccines are commonly used to prevent BVD [37–39], but control programs based on PI identification and removal are voluntary. However, under field conditions, BVDV vaccines do not provide complete fetal protection in all vaccinated cows; therefore, PI cattle continue to be generated and perpetuate the infection. Despite a large array of BVDV-containing inactivated or modified live vaccines, the viruses continue to infect cattle and cause disease [36].

1.3.3 Public Health/One Health Crossover

Pestiviruses are uniquely adapted to ruminants of various species, and to pigs. Although humans have been exposed to pestiviruses via viral vaccines contaminated with BVDV through fetal bovine serum, there is no evidence that humans can become infected by them [40].

2 Classical Swine Fever Virus

2.1 Etiologic Agent and Natural History

2.1.1 Definition

Classical swine fever, also known as hog cholera, is caused by classical swine fever virus (CSFV), a virus of great economic and health importance to the global swine industry. CSFV, discovered in 1903, is enzootic in much of the world, persists in wild swine populations, but has been eradicated from the USA and several other countries.

2.1.2 Etiology and Evolution

CSFV is a member of the genus *Pestivirus*, family *Flaviviridae* [41] along with BVDV-1, BVDV-2, and border disease virus (BDV) [42].

As is the case for all pestiviruses, the spherical CSFV particle (40–60 nm diameter) is comprised of an icosahedral nucleocapsid enclosed by a lipid membrane that contains three structural glycoproteins [43, 44]. The approximately 12.5 kb CSFV genome is a single-stranded RNA of positive polarity. The genome consists of a single large open reading frame that encodes four structural and eight nonstructural proteins [45]. Structural proteins, encoded in the 5′ third of the genome, include capsid protein C and envelope (E) glycoproteins E^{rns}, E1, E2. Nonstructural (NS) proteins include N^{pro}, p7, NS2, NS3, NS4A-B, NS5A-B [45]. E2 and E^{rns} play important roles in the attachment of the virion to the cell surface [46, 47], and each protein interacts with a different cell receptor involved in virion attachment and cell-to-cell spread [46].

2.1.3 Geographic Distribution and Economic Effects

CSF was first reported in 1833 from Ohio in the USA [48]. By 1889, 36 US states had reported a disease in pigs with clinical presentation similar to CSF. In the same time frame, outbreaks of a disease resembling CSF were affecting pig herds in Great Britain and across continental Europe.

CSF is classified as a reportable disease to the OIE (World Organisation for Animal Health). CSF is a global concern due to the effect of the disease on pig breeding activities in enzootic areas and as a threat to the pork industry and international pork trading in countries free of the disease.

CSF has been eradicated from Australia, Canada, the USA, and almost all member states of the European Union (EU). Routine vaccination of pig herds has been banned in these countries. However, outbreaks of CSF still occur intermittently in European domestic pigs, leading to significant economic losses. In the EU, from 1992 to 2008, close to 20 million pigs were euthanized due to control measures imposed to combat CSF epizootics, causing total costs of about 5 billion euros. In many countries, CSF is still a major problem [49, 50], and vaccination of pig herds is applied to control spread. Currently, CSFV is considered enzootic in domestic herds in Eastern European countries and Russia, Asia (predominantly in Southeast Asia), in some countries of South and Central America, southern Mexico, and in the Caribbean.

2.1.4 Natural History, Transmission, and Host Range

The only known natural hosts for CSFV are all members of the Suidae [51]. While transmission in domestic pigs is relatively well studied, the epidemiological role of European wild boar is the best understood among wild suids. Wild boars are a source of direct or indirect infection for domestic pigs [52–54]. CSFV infections of wild boar populations can be self-limiting or persistent, with virus circulating within infected populations for years [55]. In wild boars, CSF has a similar clinical presentation as in domestic pigs, including transplacental transmission of CSFV followed by the birth of persistently infected piglets [56, 57]. The most frequent cause of spread of CSFV into a population is probably due to swill feeding [58].

Direct transmission (either horizontal or vertical) is the primary mode of CSFV spread. Horizontal transmission occurs via pig-to-pig by direct contact. In both acute and chronic forms of the disease, virus is constantly shed from infected animals via secretions and excretions even before the onset of clinical signs. Additionally, in adult boars, infections with CSFV lead to excretion of the virus in semen [59]. Subsequently CSFV can be transmitted via artificial insemination. Inseminated sows seroconvert, and virus can be detected in both sows and fetuses [59]. Vertical transmission of CSFV is also common and may occur at any time during gestation when pregnant sows become infected. During an outbreak of CSF in the Netherlands (1997–1998), it was estimated that 17 % of CSFV spread between an infected herd and neighboring herds during the high-risk period (i.e., before the detection of the first infected herd) was due to direct contact. After the high risk period, direct contact transmission decreased to about 1 %, mainly due to the implementation of control measures [60]. Regardless of the source of the virus, it has been observed that during a natural CSF outbreak, the probability of secondary outbreaks of the disease decreases with increased distance from an infected herd [61–63].

Transmission rates for CSFV can be quantified by estimating the reproductive ratio (R_0), described as the average number of secondary cases caused by one infected animal in a fully susceptible population. Experimentally, R_0 values ranging from 81.3 to 100 were estimated for weaned pigs [64, 65]; 13.7 to 15.5 for slaughter pigs [64, 65]; and 13.0 for gilts [66]. From interpen transmission rates of CSFV in weaned and slaughter pigs, R_0 values were estimated to range from 3.39–7.77 [64].

Indirect transmission of CSFV from wild boars to domestic pig herds is a common event [67, 68]. Common sources of infection for domestic pigs are: feed containing meat from wild pigs, silage originating from areas frequented by wild pigs, contact with hunters, and contaminated vehicles. In addition, other indirect modes of CSFV transmission include artificial insemination, swill feed, livestock trucks, personnel, and pig slurry from infected farms. Indirect transmission mediated by animals other than suids, such as ruminants, rodents, birds, or insects has not been documented.

Experimentally, airborne transmission of CSFV over short distances is possible [69–71]. The precise distance that the virus can spread via air is still unknown.

2.2 *Pathogenesis and Clinical Features*

2.2.1 Pathogenesis and Immunology

Historically, CSF has been characterized by sudden death of pigs or by CSF's most common appearance of red to purple discoloration (hemorrhage) of the skin covering the nose, abdomen, inside of limbs, ears, and pubic regions (Fig. 14.1).

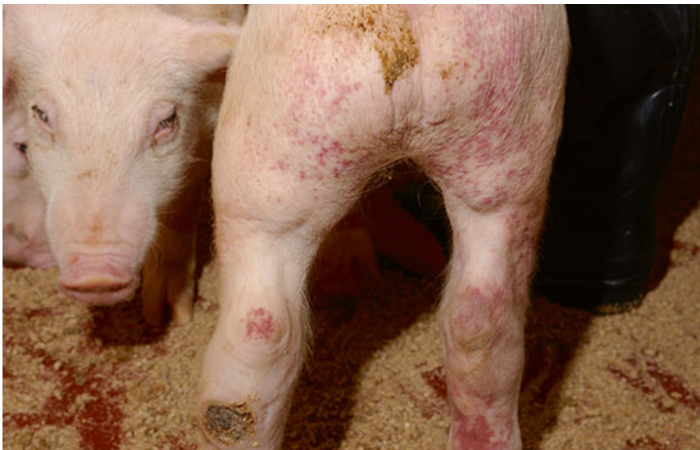

Fig. 14.1 Skin: numerous petechial and ecchymotic hemorrhages along the caudal aspect of the hind limbs in a pig with classical swine fever. Conjunctivitis is present in the second pig pictured (*left pig*). *Source*: Plum Island Animal Disease Center

Postmortem findings are characterized by hemorrhage and congestion in the spleen (Fig. 14.2), lymph nodes (Fig. 14.3), cecum, colon (Fig. 14.4), and lungs.

CSFV primarily causes a hemorrhagic fever characterized by vascular lesions, including splenic infarcts (Fig. 14.2), hemorrhages in lymph nodes (Fig. 14.3) and the urinary system, and disseminated microthrombosis with necrosis of lymphocytes, particularly in the B-cell areas of the lymphoid organs.

The clinical presentation of CSF varies depending mainly on the virulence of the infecting virus. After infection via the oronasal route, CSFV actively replicates in tonsils [72]. Within 2–6 days post exposure, CSFV antigen can be detected in tonsilar crypt epithelium, lymphoid follicles, and para-follicular regions. The virus spreads through the lymphatic vessels into regional lymph nodes, and from there into the bloodstream. Viremia is readily detected between 4 and 6 days post exposure. A second wave of virus replication takes place in several organs, particularly

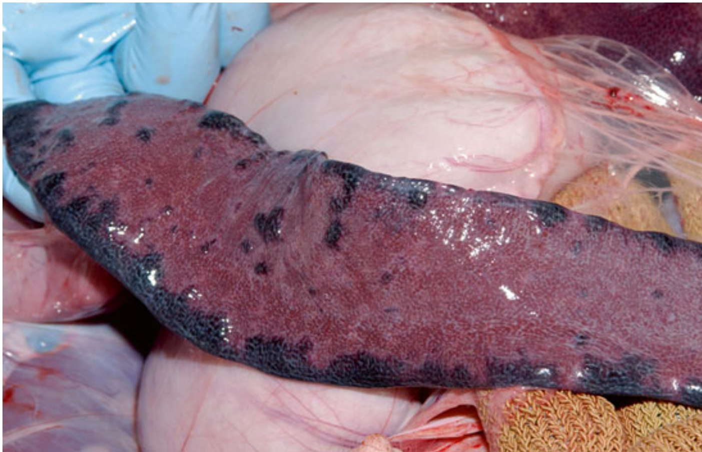

Fig. 14.2 Spleen: severe multifocal splenic infarcts in a pig with classical swine fever. *Source*: Plum Island Animal Disease Center

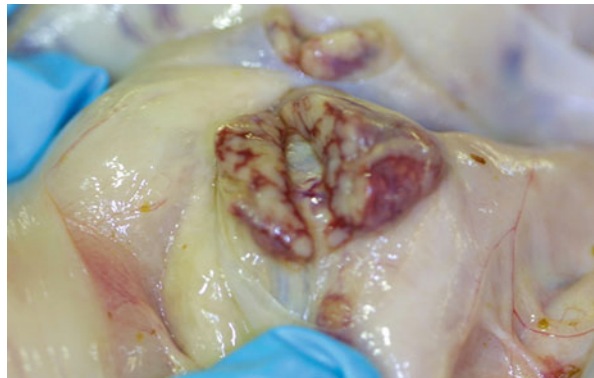

Fig. 14.3 Lymph node, mesenteric: swollen edematous cut surface with typical cortical congestion in a pig with classical swine fever. *Source*: Plum Island Animal Disease Center

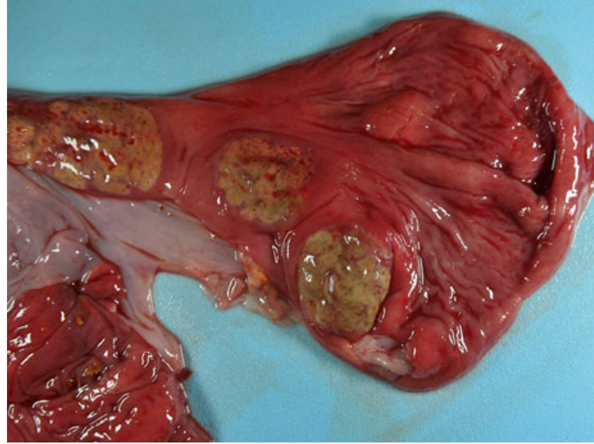

Fig. 14.4 Colon: severe multifocal ulcerative colitis ("button ulcers") in a pig with classical swine fever. *Source*: Plum Island Animal Disease Center

in spleen, bone marrow, and visceral lymph nodes. Lymphoid depletion in the spleen is generally observed with poorly defined peri-arteriolar lymphoid sheaths, and abundant viral antigen accumulation in peri-arteriolar regions and follicles. At this stage of the infection, CSFV is excreted into the environment via nasal, conjunctival, and oral (saliva) routes, as well as via urine and feces.

A hallmark of the infection is the progressive lymphopenia and thrombocytopenia observed during the course of the disease [73]. During the infection, there are profound changes in the bone marrow that may account for decreased white blood cell (WBC) counts [74–78]. The decrease in WBCs also may be mediated by active replication of CSFV in mononuclear cells [79–81], as virus replication leads to changes in monocyte/macrophage gene expression that results in the release of cytokines, some of which can be immunomodulatory or immunosuppressive. At later stages of the disease, infected lymphocytes (as well as granulocytes) are observed. Infected monocytes and macrophages release factors that coincide with the observed onset of fever and coagulation disorders in infected pigs [74]. Observed hemorrhages are the result of vascular changes that affect arterioles, postvenules, and capillaries [82], including a direct effect of CSFV on endothelial cells [83], on platelets leading to thrombocytopenia [73], or by inducing DIC [83, 84] and microthrombosis leading to endothelial damage [84]. Although the mechanisms of CSFV pathogenesis are not well understood, the development of infectious full-length copies of CSFV genomes have led to a better understanding of the role of viral proteins in mechanisms of attenuation and virulence [85–90], and will provide insights into mechanisms of disease progression.

General immunosuppression occurs early after infection with CSFV as indicated by dramatic decrease of peripheral B- and T-cells. Knowledge about the innate immune response triggered by CSFV infection is limited. As with other viral infections, pigs infected with virulent CSFV isolates react with a significant increase in serum concentrations of interferon-α (IFN-α) (500–4,500 U/ml) that is detectable

days 2–5 post-exposure [91, 92]. A viral protein (N^{pro}) has a well described role in inhibiting the type I IFN response [93–95]. Transcriptional analyses of tonsil, retropharyngeal lymph node, and spleen tissues obtained from pigs infected with CSFV strains of different virulence revealed differential expression of 44 host genes by day 3 post exposure [96]. Gene expression changes included those involved in mechanisms of innate and adaptive immune response (including specific antiviral genes), regulation of IFN, apoptosis, ubiquitin-mediated proteolysis, oxidative phosphorylation, and cytoskeleton formation.

Three CSFV proteins are the main targets of antibody response elicited after exposure: envelope glycoproteins, E^{rns} and E2, and a nonstructural protein, NS3. The envelope E2 glycoprotein is the most immunogenic viral protein and induces a strong viral neutralizing antibody response. The neutralizing antibodies induced by envelope glycoprotein E^{rns} are more limited in their avidity than E2 glycoprotein, whereas NS3 protein induces non-neutralizing antibodies. The antibody response is usually detected 2 to 3 weeks post exposure/vaccination, increasing until 4–12 weeks post exposure [97–99]. The critical role of glycoproteins E2 and E^{rns} in the induction of a protective immune response has been elucidated using recombinant vaccinia viruses expressing envelope protein E2 and/or E^{rns} [100]. Based on these findings, subunit vaccines have been developed by expressing the E2 envelope protein in baculovirus/insect cells systems [51].

In contrast to observations of antibody-based protective immunity, pigs vaccinated with live attenuated vaccines (LAV), such CSFV C-strains, mount an early protective cellular immunity (3 days post vaccination) in the absence of circulating neutralizing antibodies. Inoculation of pigs with CSFV C-strain induces virus-specific T-cell responses [101, 102] targeting the E2 and NS3 proteins [103–107]. Furthermore, a close temporal correlation between T-cell responses and the rapid protection induced by a CSFV C-strain inoculation has been observed as early as 3 days post vaccination [108, 109].

2.2.2 Incubation Period, Clinical Signs, and Gross Lesions

The incubation period in individual animals is usually 3–10 days [72]. The course of a CSFV infection varies depending on host characteristics and the virulence of the infecting virus [72]. Acute, chronic, or prenatal forms of the disease are recognized in pigs. At early stages of the acute infection animals show nonspecific signs of disease, such as anorexia, lethargy, increased body temperature, huddling, conjunctivitis, respiratory distress, vomiting and diarrhea. After few days, reddened and purple skin discoloration (hemorrhage) manifest (Fig. 14.1). Some animals may develop signs of neurological disorders evidenced by staggering gait, weakness of hind legs, incoordination, and convulsions.

Chronic forms of CSF are often fatal and usually develop in a low proportion of infected animals [72]. Animals harbor the infection for up to 2–4 months before death. Initially, infected animals develop signs similar to those observed in acute forms of the disease. Skin hemorrhage(s) are manifested as purple discoloration on the ears, tail,

abdomen, and the lower parts of the limbs, usually during the second or third week post exposure. Later, generalized signs of disease may be seen sporadically, but chronic diarrhea and progressive wasting of the animals is consistently observed through to the terminal stage. The severe leukopenia and immunosuppression caused by CSFV infection often leads to secondary enteric or respiratory infections.

In acute cases of CSF, hemorrhage is the predominant manifestation of vascular compromise. Pathological changes can be readily visible in tonsils, lymph nodes, spleen, and kidneys (Figs. 14.2, 14.3, 14.5, and 14.6). Petechial hemorrhages, and even necrotic foci, may be detected on the palatine tonsils (Fig. 14.6). Lymph nodes

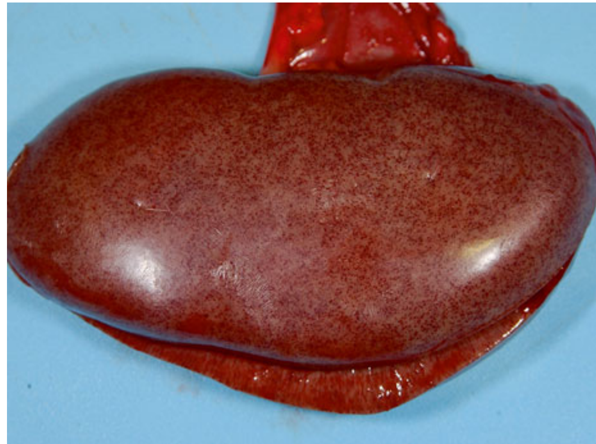

Fig. 14.5 Kidney: multifocal cortical petechial hemorrhages referred to as "turkey egg kidney" in a pig infected with classical swine fever. *Source*: Plum Island Animal Disease Center

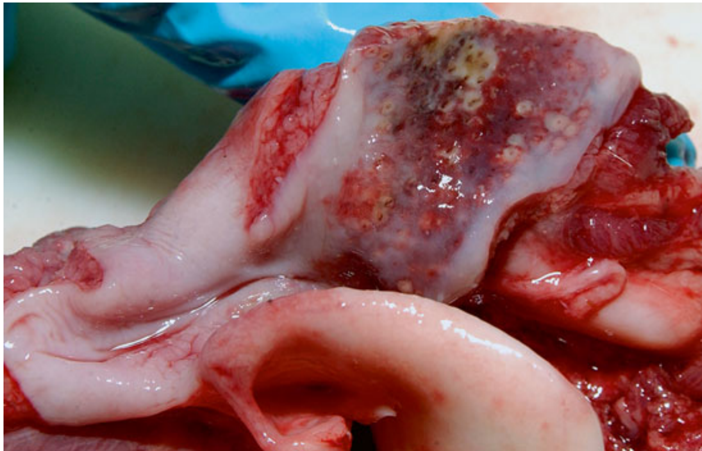

Fig. 14.6 Tonsil: multifocal crypt necrosis and diffuse congestion in a pig with classical swine fever. *Source*: Plum Island Animal Disease Center

are usually enlarged, edematous, and hemorrhagic; the mandibular, retropharyngeal, gastro-hepatic, and mesenteric lymph nodes (Fig. 14.3) are usually the most affected. Enlargement of the spleen and the presence of infarcts in the periphery of the organ are frequently observed (Fig. 14.2). The parenchyma of kidneys may display a yellowish-brown discoloration and petechial hemorrhages (Fig. 14.5) might be observed. Petechial hemorrhages or ecchymoses are also often present in the urinary bladder, heart, and serosae of the abdominal and thoracic cavities. Gross lesions associated with encephalitis include hyperemic and congested blood vessels of the brain. Non-suppurative encephalitis might be observed microscopically in brains of animals showing signs of neurological disorders.

In chronic cases of CSF, gross pathological changes tend to be less accentuated than in acute cases of the disease. Purple skin discoloration might be observed due to hemorrhages in subcutaneous tissues. At this stage, inflammation in the respiratory, gastrointestinal, and urinary tract are often seen as the consequences of secondary infections. Animals usually display chronic diarrhea as a consequence of necrotic and ulcerative lesions on the ileum, the ileocecal valve, and the rectum. “Button” ulcers in the large intestine and colon are considered typical (Fig. 14.4) [72].

In cases of congenital infection, a proportion of piglets may show incomplete development of the cerebellum or other developmental abnormalities, such as atrophy of the thymus [72]. Fetal mummifications, malformations, and stillbirth are a consequence of transplacental infections [110].

2.2.3 Morbidity and/or Case-Fatality Rates and Prognostic Factors

The severity of pathological lesions caused by CSFV depends on time of infection, age of the animal, and virulence of the infecting virus [111]. Case-fatality rates in acute cases of CSF tend to be high particularly among young animals. Acute forms of CSF are associated with virulent isolates of the virus; however, the chronic form of CSF is associated with virus isolates of moderate-to-low virulence. Congenital forms of CSF caused by transplacental infections may occur at any stage of the pregnancy. The course of CSFV infections in sows is mainly subclinical, with animals having transient anorexia and reproductive failures. Infections during the first trimester of gestation often lead to abortions, and repeat breeding could lead to subsequent abortions. Persistently viremic piglets are born to sows that became exposed to CSFV, usually during the second trimester of gestation. These piglets are clinically normal although they may show poor growth, wasting, and tremors. These animals survive for a long period of time (30 days or more) while constantly shedding virus [112]. The outcome of this type of infection, known as “late onset of CSF,” is always fatal. Pregnant sows or “carrier sows” usually do not manifest the disease, although they shed virus, particularly at farrowing. Infections that occur in the third trimester of gestation result in abortion, fetal malformation, or birth of weak or dead litters.

2.3 Diagnosis and Control

2.3.1 Diagnosis and Epidemiology

CSF is diagnosed tentatively on clinical grounds. However, this approach is rather limited since CSF clinically resembles other diseases of pigs such as African swine fever, erysipela, porcine reproductive and respiratory syndrome, coumarin poisoning, purpura hemorrhagica, postweaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, *Salmonella* or *Pasteurella* infections, or any enteric or respiratory syndrome with fever not responding to antibiotic treatment.

Techniques for the detection of CSFV and virus-specific antibodies are established and described in the OIE's Manual of Diagnostic Tests and Vaccine for Terrestrial Animals [113]. Traditionally, virus isolation and detection of viral antigens in frozen tissue sections using antibodies have been used to diagnose CSF. Currently, antibody-detection ELISA, targeted to anti-E2 antibodies, is commonly used for CSF surveillance and for diagnostic purposes. ELISAs based on the detection of antibodies against E^{rns} have also been developed, mainly for distinguishing vaccinated animals from naturally infected animals when subunit vaccines are used to immunize pigs. Antigen detection ELISAs are also available for detecting CSFV antigens in tissue samples. The advent of nucleic acid-based tests, such as RT-PCR and qRT-PCR, has significantly improved the simplicity and sensitivity of tests for detecting CSFV. These techniques are rapid and highly sensitive, capable of detecting low amounts of virus even before the onset of clinical signs.

Genetic typing of CSFV [49, 114, 115] targeting the 5′ untranslated genomic region [49] or E2 and NS5B genes [116, 117] has been widely used for identifying and comparing isolates. Phylogenetic analysis grouped CSFV isolates of different chronological and geographical origins into three distinctive groups, with three or four subgroupings: groups 1.1, 1.2, and 1.3; groups 2.1, 2.2, and 2.3; and groups 3.1, 3.2, 3.3, and 3.4 [72]. Group 1 includes historic CSFV isolates and vaccine strains. Most of these viruses were isolated from around the world in the period dating from 1920s to 1990s, although CSF outbreaks caused by these isolates have been reported in Cuba and Colombia in the early 2000s. Group 2 includes all CSFV isolates that have been circulating in the EU in the last 30 years. The earliest appearance of subgroup 2.3 viruses was reported in West Germany in 1982 [49]. Subsequently, isolates with the characteristics of subgroups 2.1 and 2.2 have been detected in domestic and wild pig populations in different EU countries. The 2.1 viruses have been only sporadically reported in Europe. These isolates were first reported in West Germany in 1989, and thereafter in the Netherlands (1992) and Switzerland (1993). During the major CSFV outbreak in the EU in 1997 and 1998, the virus is believed to have been introduced from Germany into the Netherlands, with subsequent spread to Italy, Belgium, and Spain [118, 119]. Isolates from this group have been detected in Africa and Asia. Group 3 isolates are the most diverse CSFV isolates, and seem to be confined to Asia, particularly Southeastern Asia, where all groups and subgroups have been detected at one point in time.

2.3.2 Vaccination, Control, and Eradication

The first attempts to develop a vaccine against CSF in the USA were made in 1907 by the US Department of Agriculture Bureau of Animal Industry. Vaccination consisted of the inoculation of pigs with anti-CSF serum, followed by inoculation with virulent virus. In 1935, Dorset et al. developed a vaccine killed with crystal violet. In 1965, Baker developed a live attenuated vaccine (LAV) that conferred effective protection against the disease [120]. In 1961, the US Congress authorized a CSF eradication program, but no diagnostic test for CSF was readily available. In 1963, Mengeling et al. developed and described a fluorescence-based rapid diagnostic test for CSF that took less than a day to perform.

LAVs were derived from wild-type CSFVs that were attenuated through repeated passages in either cell culture or experimental animals [98, 121, 122]. CSFV subunit marker vaccines have been developed using recombinant E2 envelope protein [86, 123, 124]. A key feature is that these subunit vaccines induce an antibody response that differentiates *i*nfected from *v*accinated *a*nimals (*DIVA* principle). However, these subunit vaccines are not as efficacious as traditional LAVs, particularly when animals are exposed shortly after vaccination [86, 123, 124]. Solid protection is usually observed after 14 days post vaccination, evidenced by the appearance of circulating neutralizing antibodies.

Other experimental vaccines against CSFV have been designed including peptides, DNA vaccines, vectored vaccines, and trans-complemented, deleted CSFV genomes (replicons; for review see ref. [125]. Vaccines based on immunogenic peptides (mostly derived from the E2 glycoprotein) have been used experimentally [126–129]. DNA vaccines based on the E2 protein have been formulated in combination with cytokine genes to enhance their immunogenicity [130–134]. Vaccinia virus vector-based vaccines have been constructed expressing E2 and/or E^{rns} glycoproteins [100]. E2-bearing viral vectors, including pseudorabies virus [135–138], porcine adenovirus [139–142] swinepox virus [143], and parapoxviruses [144], have been used in pigs. In general, viral vector-based vaccines have DIVA capabilities and induce protection against clinical disease. Alternatives to the use of LAVs (e.g., *trans*-complemented viruses) have been constructed due to the hypothetical potential of any live virus to revert back to its virulent parental strain [145–148]. These viruses lack the E^{rns} or E2 proteins and are complemented in *trans* after their RNA is transfected into helper cell lines expressing the lacking viral gene [146]. When inoculated in pigs, these viruses can enter cells, but they are unable to produce viable viral progeny.

CSF control programs in enzootic areas are based on vaccination of domestic pigs. The most currently used vaccines are LAVs. Vaccination with LAV strains C, GPE−, Thiverval, or PAV-250 induces protective immunity in pigs within a few days after vaccination. In general, these LAV vaccines provide lifelong immunity against disease [98]; however, they do not allow serological differentiation of vaccinated from infected animals. This makes eradication efforts and demonstration of freedom of disease difficult, both of which are prerequisites for removing barriers to animal trade.

With the advent of reverse genetics, experimentally improved LAVs with DIVA capabilities have been developed. Chimeric pestivirus vaccines (CP7_E2alf) and genetically modified FlagT4v CSFV vaccines were constructed [145, 149–153] and included some combination of positive antigenic markers and/or negative markers suitable for differentiation from natural infection. These vaccines confer protection against CSF as with conventional LAV while allowing distinction between vaccinated and infected animals.

In countries free of CSFV, control policies involve depopulation (slaughter) of exposed animals [51]. In the EU, outbreaks of CSF lead to depopulation of infected farms and the destruction of cadavers. During outbreaks, protection zones (3 km radius) and surveillance zones (10 km radius) are established in affected areas, restricting pig movements. An epidemiological investigation is established with the purpose of tracing a CSFV isolate to the source of infection. If considered appropriate, emergency vaccination could be an option for controlling an outbreak of the disease in countries previously free of CSFV.

April 1973 was the first month over a period of 100 years without a CSF outbreak in the USA. In January of 1978, the US was declared free of CSF [79]. Similarly, after implementation of strict control measures, several countries, including Australia, Canada, New Zealand, and member states of the EU, were able to eradicate the disease.

2.3.3 Public Health/One Health Crossover

CSFV is not a risk to humans, other than those public-health effects induced by the economic hardship associated with an outbreak response.

3 Rabbit Hemorrhagic Disease Virus and European Brown Hare Syndrome Virus

3.1 Etiologic Agent and Natural History

3.1.1 Definition

Rabbit hemorrhagic disease (RHD) is caused by the calicivirus rabbit hemorrhagic disease virus (RHDV). RHD was first described in 1984 as a disease of European rabbits (*Oryctolagus cuniculus*) imported from Germany into the People's Republic of China [154], but whether these rabbits were carrying the virus or exposed to a local virus is unclear. The case-fatality rate in these rabbits was over 90 %.

European brown hare syndrome (EBHS) is also caused by a calicivirus, European brown hare syndrome virus (EBHSV), and was first reported in Sweden in 1980 [155]. EBHSV infects European brown hares (*Lepus europeus*) and mountain hares (*Lepus timidus*), causing an acute hepatitis with lower case-fatality rate compared to that of RHD in rabbits.

3.1.2 Etiology and Evolution

Both RHDV and EBHSV are single-stranded, positive-sense RNA viruses in the genus *Lagovirus* within the family *Caliciviridae* [156]. The genomes of both viruses are approximately 7.5 kb long and code for nine viral proteins [157–160].

The hepatotropic, lethal RHDV has likely evolved from closely related but non-pathogenic lagoviruses, which are widely distributed in wild and domestic rabbits [161–164]. These viruses replicate in the gut causing little or no pathology [162, 165, 166]. The mutation or mutations that have altered the cellular tropism of the virus and allowed the very rapid replication in the liver have not been defined. Additionally, the timeframe of this evolutionary step is unclear; researchers still debate whether the switch to pathogenic forms happened once or several times during the evolutionary history of the RHDV [167–169]. Recently, moderately pathogenic lagovirus strains have also been described [170–172].

The origins of EBHSV are unclear. However, viral antigen was detected in tissues of healthy European brown hares in Argentina. The absence of notable hare lethality [173] suggests that non-pathogenic lagoviruses may also exist in hares.

3.1.3 Geographic Distribution and Economic Effects

RHDV was first described in farmed European rabbits in the People's Republic of China in 1984. Over the next few years, RHD occurred in South Korea, Europe, the Americas, Northern and Western Africa, Western Asia, Cuba, and Réunion, and was probably spread by trade of rabbits and rabbit products [154]. Coincident with the emergence of RHD in rabbits, EBHS was reported in Northern Europe in 1980 [155].

RHD causes substantial commercial losses in rabbitries due to the direct costs of deaths in outbreaks. Millions of rabbits have died, and when combined with the associated costs of control and eradication, RHDV is a significant economic burden to producers [154, 174, 175]. RHDV has been established in the wild European rabbit populations of Europe where it has been responsible for an ecologically significant decline of free-living wild rabbit populations [154, 176]. This decline has impacted higher order predators, such as the Spanish imperial eagle (*Aquila adalberti*) and Iberian lynx (*Lynx pardinus*), recreational hunting, and maintenance of traditional landscapes through rabbit grazing [177]. In contrast, RHDV was released in Australia and subsequently New Zealand as a biological control for wild European rabbits, which are major introduced vertebrate pests causing serious agricultural and ecological damage [178, 179]. In these countries, RHDV has had a significant positive economic and ecological benefit [180]. In addition, RHDV appears to have maintained high virulence after its introduction, unlike myxoma virus which was deliberately spread in Australia and Europe in the 1950s [181]. However, emerging genetic resistance to RHDV in Australian rabbits has been reported [182, 183].

3.1.4 Natural History, Transmission, and Host Range

The only known host of RHDV is the European rabbit. Other animals of more than 20 species, including many animals native to Australia, were experimentally infected as a prerequisite for the release of RHDV as a biocontrol agent for rabbits, but no productive infection was observed [178]. Lagomorphs other than European rabbits, including American leporids such as the volcano rabbit (*Romerolagus diazi*), black-tailed jackrabbit (*Lepus californicus*), and eastern cottontail (*Sylvilagus floridanus*), also were not susceptible [184]. This observation suggests that RHDV is unlikely to become endemic in the Americas outside of commercial rabbitries or possibly free-living feral European rabbit populations.

EBHSV has been reported to be less species-specific. While the main host of the virus is the European hare, EBHSV can also infect mountain hares where the host ranges of the two leporids overlap [185], as well as eastern cottontails [178]. Recently, a new lagovirus genetically distinct from RHDV and EBHSV has been described in Europe [171, 172] that causes lethal infections in both European rabbits and Cape hares (*Lepus capensis*) [186].

RHDV is infectious orally, nasally, and conjunctively, as well as by injection. Virus is present in most discharges from infected rabbits, including feces and urine, and in the carcasses of infected dead animals. International spread occurs by direct contact between infected and susceptible rabbits, contaminated rabbit products or other fomites, or by virus present in the environment. Mechanical transmission by flies, especially Australian bush flies (*Musca vetustissima*) and blowflies (*Calliphora* spp.), is of particular importance for distance transmission in the field in Australia and also other countries [154, 187, 188]. Virus can adhere to the legs and mouthparts of flies, and also pass through the gut unchanged [187]. Under laboratory conditions, RHDV could also be transmitted by mosquitoes or fleas that fed on infected rabbits [189].

RHDV is highly stable in the environment. Virus in rabbit carcasses can remain infectious for more than 3 months, particularly inside rabbit warrens [190]. As rabbit carcasses with extremely high virus loads effectively transmit RHDV, selective pressure is probably low for attenuation of RHDV in the field. This hypothesis could explain why field isolates continue to be highly virulent. The frequent reemergence of RHD in rabbit populations, together with detection of viral RNA in recovered rabbits, has led to suggestion of persistent infection [191, 192]. However, although viral RNA can be detected in rabbit tissues months after recovery from disease [193], viral antigen or virus transmission could not be demonstrated. Virus could also not be reactivated by immunostimulation or immunosuppression [194]. Results from recent epidemiological studies suggest that viruses initiating natural outbreaks vary genetically between years in the same location, indicating that the virus does not persist on-site between outbreaks [195].

3.2 Pathogenesis and Clinical Features

3.2.1 Pathogenesis and Immunology

The major target organ of RHDV is the liver, but hemorrhage due to DIC and loss of clotting factors is an inconsistent feature of clinical disease seen in the field. RHDV binds in a strain-specific manner to oligosaccharide histo-blood group antigen (HBGA) receptors expressed by epithelial cells of the gut and upper respiratory tract [183, 196, 197]. Researchers have suggested that these receptors provide an initial site of attachment for RHDV. However, the role of these potential receptors has not been clearly defined. These receptors on epithelial cells are not expressed at high levels in young rabbits [196] even though these animals are readily infected [189, 198, 199], and these antigens are also not expressed in the liver.

The sites of initial replication following oral/nasal inoculation have not been identified. RHDV can be detected in hepatocytes as early as 8 h after oral/nasal inoculation [200]. Rapid virus replication to extremely high titers causes massive hepatic necrosis and fulminant liver failure within 36–96 h after infection. Hepatic encephalopathy may occur as a result of the liver failure. Histopathologically, coagulative necrosis of hepatocytes is observed, starting at the periphery of the lobule and moving inward as the disease progresses. Over 60 % of hepatocytes may be positive for viral antigen [201], and viral RNA can be detected by PCR in the liver [194].

DIC has been observed with thrombus formation in the blood vessels of the liver, kidneys, and lungs, as well as widespread ecchymotic and petechial hemorrhages. Hemorrhage and edema of the alveoli may also be present in the lung [202, 203]. Virus antigen can be detected in alveolar macrophages by in situ hybridization, and viral RNA can be detected by PCR in the lungs [194, 204].

Nephrosis with impaired renal function (elevated BUN and serum creatinine concentrations) occurs late in infection, characterized by congestion of glomerular tufts and the renal medulla, hemorrhages within the renal corpuscles and the interstitium of the cortex, hyaline thrombi within small blood vessels, and hydropic degeneration of the tubules [202, 205]. Depletion of lymphocytes from the white pulp of the spleen is characteristic of RHD. Virus antigen also can be detected in macrophages and lymphocytes in the spleen, blood monocytes, and glomerular mesangial cells by immunohistochemistry or in situ hybridization [201, 204] and in the feces and bile by hemagglutination [194]. Viral RNA can be detected by PCR in bile, spleen, lymphoid tissues, kidneys, WBCs, urine, and feces [194].

3.2.2 Incubation Period, Clinical Signs, and Gross Lesions

The incubation period can be as short as 12–24 h following injection of RHDV. Clinical presentation can be peracute, expressed as sudden death with no premonitory signs: rabbits may be observed grazing then squeal, convulse, and die. In the acute

form of the disease, the infected rabbit may appear depressed and reluctant to move around. These animals will show elevated temperature (up to 42 °C, 107.6 °F), and heart and respiratory rate may be increased. Ataxia, lateral recumbency, convulsions, and coma may occur prior to death and 2–5 days after exposure. Blood-stained discharge from the nose and bloody diarrhea or hematuria may be seen.

Infected rabbits that survive longer than 4–5 days are considered to express a subacute form of the disease during which obvious icterus may be present. Depending on the degree of hepatic damage in these rabbits, death may occur days to weeks later; hepatic cirrhosis has been described in subacutely affected rabbits [202, 206]. Subclinical infection may occur in a small proportion of infected adult rabbits and in young rabbits under 10 weeks of age. These rabbits are infected and seroconvert, but clear the virus with few or no clinical signs of disease. An age-related resistance to disease occurs; infected kits less than 4–5 weeks old rarely show clinical disease although they shed virus [189, 198, 199]. Mild hepatic pathology is present with elevated liver enzyme serum concentrations, and occasional deaths do occur [199]. This resistance is gradually lost, and by 10 weeks of age, rabbits are fully susceptible [207].

The most consistent finding at autopsy is a pale swollen liver, usually with a strong lobular pattern. The spleen is enlarged and black, and the kidneys may be dark. Lungs may be congested and hemorrhagic, with fluid or froth in the trachea and bronchi, and the walls of the trachea are commonly hyperemic due to dilation of the blood vessels. Ecchymotic and petechial hemorrhages may be scattered over all the internal organs, mucosal surfaces, subcutaneous tissues, and muscle, but are not a consistent finding [189]. In subacutely infected rabbits that have survived for some days, the subcutaneous tissue may be yellowish.

Clinical hematology and hemostasis findings include lymphopenia, neutropenia, decreased thrombocytes, increased prothrombin time, and decreased factor V and Factor VII. Clinical chemistry findings include extremely elevated liver transaminase concentrations in serum (aspartate amino transferase >100 times normal; alanine amino transferase >10 times normal), marked elevation of total bilirubin concentrations, elevated BUN and serum creatinine concentrations, elevated serum Na^+ and K^+ concentrations, hypoglycemia, and hyperlipidemia [199, 203, 205, 208].

3.2.3 Morbidity and/or Case-Fatality Rates and Prognostic Factors

Historically, case-fatality rates in RHD-infected adult rabbits were generally 90–95 %. Recently, a complex interaction between clinical outcome, virus genotype, and HBGA receptor type has been demonstrated. Rabbits of particular HBGA types are more likely to survive infection at low doses of particular RHDV strains, although this resistance can be overcome at higher virus doses [183]. In addition, evidence from France and Australia indicates that this natural resistance may be leading to selection in wild populations of rabbits that are negative for the HBGA receptor type that facilitates infection of the predominant virus strain in a specific

geographic region [182, 183]. Emerging genetic resistance to RHDV has been described in Australian wild rabbits, which manifested as resistance to infection rather than resistance to disease [182].

3.3 Diagnosis and Control

3.3.1 Diagnosis and Epidemiology

In commercial rabbitries, RHDV exposure typically leads to a history of rapid onset of high fatality in unvaccinated adult rabbits and survival of younger animals. Gross autopsy findings are largely confirmatory. RHDV does not replicate in cell cultures tested to date [113], so laboratory diagnosis is based on detection of virus, virus antigen, or viral nucleic acid. Virus can be detected in virtually all tissues and secretions using RT-PCR. Liver is the most useful tissue for diagnosis because of the extremely high titers of RHDV in this tissue. Virus can also be demonstrated in liver extracts using antigen-capture ELISA, western blot or by hemagglutination using human erythrocytes. However, some RHDV strains have been reported not to hemagglutinate possibly due to variations in HGBA binding [113, 183]. RHDV RNA can be detected by RT-PCR for prolonged periods in recovered animals, and cross-reaction can also occur with related rabbit lagoviruses. Indirect or competition ELISA can demonstrate antibody to RHDV. Isotype ELISAs are used for detection of IgG, IgM and IgA but are less specific and can cross-react with other rabbit lagoviruses. IgM can be detected as early as 3–4 days after infection. Hemagglutination inhibition can also be used for antibody detection [113].

In Europe, Australia, and New Zealand, RHD is enzootic in wild rabbit populations [178]. The timing of outbreaks is closely linked to the immune status of a population, juvenile resistance, and rabbit breeding patterns, which in turn strongly depend on climatic factors [154]. Young rabbits are resistant to lethal RHDV infection but can become infected and acquire lifelong immunity. Therefore, natural outbreaks of RHD are usually observed towards the end of the breeding season, when a sufficient density of seronegative rabbits of a susceptible age is available [154]. Maternal antibodies can prevent infection [207]. Such antibodies also help to delay exposure of rabbits until they have reached a susceptible age, thereby delaying, but not preventing, outbreaks. Acquired immunity to RHDV is life-long, but boosts in antibody titers have been observed, indicating that rabbits probably get reinfected and mount an anamnestic immune response [209].

Avirulent rabbit lagoviruses related to RHDV occur in both wild and domestic rabbit populations in Australia and Europe. Some avirulent strains may provide a degree of cross-protection against RHD, thus further modifying the epidemiology of the disease. This protection is seen in areas where these avirulent relatives of RHDV are present, which in Australia are predominantly the cooler temperate climatic zones [161–164]. Serological studies are often confounded by the presence of these nonpathogenic lagoviruses, such as rabbit calicivirus (RCV)-A1 in Australia [164].

These nonpathogenic lagoviruses cross react in serological assays for RHDV [209, 210], so specific serological assays have been developed for studying the epidemiology of RHDV in areas where these viruses circulate [211, 212].

3.3.2 Vaccination, Control, and Eradication

In countries where European rabbits are uncommon in the wild, such as the USA, eradication following entry of RHDV has been successful, although further incursions have occurred [174]. In Europe, where wild European rabbits provide a reservoir of infection, eradication is not feasible. Control in commercial rabbitries relies largely on quarantine and vaccination. For meat breeding, control of infection may mean vaccinating the breeders and relying on maternal antibody to protect the meat rabbits prior to slaughter. Inactivated, adjuvanted whole virus vaccines produced from infected rabbit livers are commercially available and provide good protection following a single injection at 8–12 weeks of age, followed by an annual booster. It is not clear if maternal antibody can interfere with vaccination, so delaying vaccination until 12 weeks of age or boosting vaccination at 10–12 weeks may be sensible depending on the situation. A combination of a live myxoma virus vaccine with inactivated RHDV is available commercially (Dercunimix, Merial), and a recombinant attenuated live myxoma virus expressing the RHDV capsid protein was released in 2013 (Nobivac Myxo-RHD, MSD Animal Health). Vaccination in the face of an outbreak is considered effective [113] but should be combined with isolation/culling of affected rabbits, strenuous hygiene, and other biosecurity measures. As noted above, kits may not show clinical signs but will shed virus.

An antigenic variant of RHDV termed RHDVa appears to be replacing RHDV throughout most of its range [167, 213]. Conventional RHDV vaccines are still protective against this variant, although it can overcome suboptimal vaccine doses [214]. However, vaccines appear largely ineffective against the recently described variant strain RHDV2 [172], and immune responses to natural infection with this strain are only partially protective against challenge with classical RHDV [215]. Antigenic variants that overcome vaccination against RHDVa are present in China [216].

Vaccination to protect wild rabbit populations is logistically and economically infeasible although recombinant myxoma viruses expressing RHDV capsid protein have been tested in the field [217]. Nonpathogenic rabbit caliciviruses that provide some cross-protection against RHDV and spread naturally could provide a possible means of immunization of wild rabbits [218, 219].

3.3.3 Public Health/One Health Crossover

The deliberate release of RHDV in Australia as a biological control agent generated controversy because of the broad host range of some caliciviruses and the perception (at the time) that the virus had possibly jumped species into rabbits [174, 220, 221].

However, specific testing and nearly 30 years of experience have demonstrated that RHDV is highly specific for European rabbits, and that it is closely related to (and probably evolved from) a nonpathogenic rabbit calicivirus. The only exception is a recent report describing RHDV2 infection of European rabbits and Cape hares, but not other hares [186]. Despite this, the emergence of a highly virulent virus from an apparently avirulent progenitor provides a warning of how easily new viruses may emerge.

4 Simian Hemorrhagic Fever Virus

4.1 Etiologic Agent and Natural History

4.1.1 Definition

Simian hemorrhagic fever virus (SHFV) is classified as an arterivirus in the family *Arteriviridae*, order *Nidovirales*. Like other arteriviruses, SHFV has a positive-sense RNA genome approximately 15 kb in length, and differs from other arteriviruses by having three additional open reading frames [222].

4.1.2 Etiology and Evolution

SHFV is a relatively uncharacterized virus that causes simian hemorrhagic fever (SHF), a severe disease of Asian macaques (*Macaca* spp.) characterized by fever, facial edema, anorexia, adipsia, petechiae, diarrhea, hemorrhages, and up to 100 % lethality. All outbreaks of SHF have occurred at primate research facilities, but the means of introduction into primate colonies remains undefined. Dependent on the strain, the virus causes subclinical, persistent infection in Kibale red colobus (*Piliocolobus rufomitratus*), red-tailed monkeys (*Cercopithecus ascanius*), and possibly patas monkeys (*Erythrocebus patas*), grivets (*Chlorocebus aethiops*), and Guinea baboons (*Papio papio* spp.), which serve as natural hosts in Africa [223–227].

4.1.3 Geographic Distribution and Economic Effects

Asian macaques, which probably are not exposed in nature to SHFV, are highly susceptible to SHFV infection and disease, whereas African monkeys may carry the virus persistently without clinical signs. The overall distribution of SHFV in wild primates remains unclear. SHFV was a major concern for primate research centers in the past, but physical separation of Asian from African monkeys and improved screening methods have been effective control measures. The last outbreak of SHF was recorded in 1996.

4.1.4 Natural History, Transmission, and Host Range

Much of what is known about SHFV is derived from experiments with the prototype variants LVR 42-0/M6941 and Sukhumi-64. These prototypes were first identified in 1964 during almost simultaneous outbreaks of febrile hemorrhagic disease in Asian macaques that occurred at the Sukhumi Institute of Experimental Pathology and Therapy in the Georgian Soviet Socialist Republic and at the National Institutes of Health (NIH) in Bethesda, MD, USA [224, 228, 229]. Macaques in both institutes were housed in close proximity to African primates, including patas monkeys, baboons, and grivets [226, 229, 230]. During the Sukhumi outbreak, animals presented clinically with a hemorrhagic diathesis and encephalomyelitis. Lethality reached 100 % over 2 months [228, 229]. Conversely, the macaques housed at NIH also presented with hemorrhagic diathesis, but had high fevers in the absence of encephalomyelitis. Transmission was thought to have occurred as a result of reusing needles while tattooing and/or tuberculosis testing the African-origin primates and macaques [224–226]. The lethality during the NIH outbreak did not reach quite the extent seen in the USSR, as there were a few animals that survived infection. Blood and tissue samples from a such a survivor successfully induced hemorrhagic fever in macaques not associated with the initial outbreak, satisfying Koch's postulates [226].

Sporadic SHFV outbreaks of iatrogenic origin have occurred semi-regularly since 1964 [226, 230–232]. During SHFV outbreaks in 1972 and 1989, the virus was thought to be spread by both direct and indirect contact between macaques [232, 233]. Curiously, there seems to be a connection between SHFV and ebolaviruses, as SHFV was found together with Reston virus (RESTV) in macaques with viral hemorrhagic fever in four out of five RESTV emergences [234].

4.2 Pathogenesis and Clinical Features

4.2.1 Pathogenesis and Immunology

Initial exposure of a nonhuman primate colony occurs through infected blood or tissue from carrier animals. The virus is transmitted through direct contact and fomites. Specific subsets of macrophages are the principal target cell for viral infection.

Very little is published characterizing the pathogenesis or immune response to SHFV in English [222, 235, 236]. Similar to other arteriviruses, SHFV principally replicates in macrophages, although there is considerable variation in the cellular tropism, virulence, and immunogenicity of individual strains of SHFV in African monkeys.

Infected monkeys produce complement-fixing and neutralizing antibodies. The humoral immune response of persistently infected patas monkeys varies with the infecting strain of SHFV. Patas monkeys infected with low virulence variants (P-248 and P-741) had minimal or no antibody response and persistently low viremia, whereas a more virulent variant (LVR 42-0/M6941) induced antibodies within

7 days after infection [237, 238]. Additionally, the appearance of these antibodies was associated with clearance of the virus from blood circulation 21 days post inoculation. Neutralizing antibodies against one variant of SHFV do not completely neutralize other variants, suggesting that variation in the neutralization determinants of individual variants of SHFV exists. However, these determinants have yet to be characterized.

4.2.2 Incubation Period, Clinical Signs, and Gross Lesions

The incubation period of SHFV is approximately 3 days, and most animals succumb to the infection within 10–15 days post exposure. Clinical signs begin with depression, ataxia, anorexia, dehydration, edema, cyanosis, and petechial rash. Hemorrhage in the form of epistaxis, hematemesis, ecchymosis, retrobulbar hemorrhage, and melena is common. Hematologic signs of coagulopathy including abnormal coagulation factors with fibrin degradation products are typical. Concentrations of liver enzymes, including LDH, GGT, AST, may be elevated. Elevations of BUN and creatinine concentrations may indicate kidney involvement, but the elevations are variable. Typical gross lesions include random hemorrhage and congestion throughout the gastrointestinal tract, liver, renal capsule, retrobulbar tissue, subcutis, and lung. The proximal duodenum may contain focally extensive congestion, hemorrhage, and necrosis with sharp demarcation at the pylorus. Splenic infarction with significant absence of white pulp may be present. Microscopically, extensive lymphoid necrosis in the spleen is observed, with perifollicular hemorrhage and fibrinous exudate. A unique feature of SHFV is cortical thymic necrosis of the spleen, with sparing of the medulla. Systemic lesions consistent with DIC, including necrosis, fibrin thrombi in glomeruli, hepatic sinusoids, and lung are also present in severe cases.

4.2.3 Morbidity and/or Case-Fatality Rates and Prognostic Factors

Asian macaques are very susceptible to even small doses of SHFV, and usually uniformly develop disease with lethality usually reaching close to 100 %. Other nonhuman primates (NHPs) either do not get infected at all or develop subclinical infections.

4.3 Diagnosis and Control

4.3.1 Diagnosis and Epidemiology

Serologic tests cannot distinguish carriers from previously infected animals, and virus isolation is generally unreliable for diagnosis. Molecular detection by RT-PCR has become the method of choice for detection of SHFV.

4.3.2 Public Health/One Health Crossover

SHFV has not been linked to human disease. SHFV and RESTV were isolated concurrently and repeatedly in crab-eating macaques (*Macaca fascicularis*) maintained in Philippine and US quarantine facilities. SHFV has caused sporadic outbreaks of hemorrhagic fevers in macaques at primate research facilities [234, 239]. Any severe disease of closely related animals such as NHPs must be viewed with caution, as the potential for adaptation of the virus to humans should always be a concern given the common phenotypic and genotypic features of both animals.

5 Other Positive-Stranded RNA Viruses Causing Viral Hemorrhagic Fever-Like Diseases

5.1 *Feline Calicivirus*

A strain of feline calicivirus (FCV-Ari, named for the second observed case) was observed by Pederson et al., to cause disease in domestic cats with hemorrhagic features and this disease could be induced in experimental infections [240]. While vasculitis was an inconsistent finding, severe edema and local necrosis of skin and subcutaneous tissues were present. "Loss of vascular integrity" was considered to be the best explanation for the observed gross and biochemical findings in both the naturally and experimentally infected animals. This loss was supported by finding viral antigen in endothelial cells [241] and identifying a cellular junctional adhesion molecule as a putative cellular receptor [242]. However, it was unclear whether the vascular disruption was due to direct cytopathology by live virions, disruption of tight junctions, or simply antigen uptake and cellular damage in the context of significant epithelial damage. Persistence of viremia appeared to be a prognostic factor. The genetic sequence of FCV-Ari was shown to be within the predicted range of variability when compared to either vaccine or field strains of FCV, but commercial vaccines against FCV were shown to be only partially protective. In an outbreak of FCV-Ari, the virus was suspected to have been transmitted from a shelter cat to the cats of employees and a client of a veterinary practice.

5.2 *Venezuelan Equine Encephalitis Virus*

Venezuelan equine encephalitis virus (VEEV) is an arthropod-borne zoonotic virus affecting both horses and humans [243]. Vectors are typically mosquitoes of the genus *Culex*, but during epidemics members of the genera *Aedes*, *Anopheles*, *Deinocerites*, *Mansonia*, and *Psorophora* may also transmit the virus. The disease in equids can be severe, resulting in a fulminant fatal disease before encephalitic signs have time to develop. In less severe cases, encephalitis with neurological signs

develops during disease progression. In horses that express neurological signs, CNS lesions can be extensive, with necrosis and hemorrhage. Hemorrhage is likely secondary to endothelial destruction and not vascular leakage, as necrotic lesions have been seen to involve the walls of small-to-medium vessels. In fulminant and less severe cases, the viremias in horses are very high, and these horses act as critical amplifying hosts in epizootics. In humans, influenza-like signs and symptoms with a fever and severe headache are seen in the proportion of cases that show any clinical signs (attack rates can be as high as 0.02 %). The virus is normally maintained in a sylvatic (enzootic) cycle, with few differences seen between enzootic viruses and the epidemic (epizootic) viruses recognized in horses or humans.

5.3 Yellow Fever Virus

The disease caused by yellow fever virus (YFV) in nonhuman primates (NHPs) is essentially indistinguishable from the disease in humans, including the hemorrhagic manifestations observed [244]. The main difference is that the disease in NHPs appears to have a more rapid course, with death occurring at 7 days post exposure, instead of the typically longer recurrent fever course seen in fatal human cases. Due to both hepatic and immunologic consequences of infection, yellow fever appears to resemble an inappropriate and overwhelming immune response, similar to that seen in severe sepsis. Conversely, experimental YFV infection of hamsters more closely resembles the hepatic impairment of yellow fever in humans, during which the degree of liver insult is prognostic for lethal disease.

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