1- COWPOX AND BUFFALOPOX

ETIOLOGY

Cowpox virus (CPXV) and buffalopox virus (BPXV) are members of the genus *Orthopox* virus in the family Poxviridae. Other *orthopox* viruses infecting agricultural animals include horsepox, Uasin, and camelpox. All orthopoxviruses are antigenically extremely similar, but they can be identified by a combination of phenotypic and genetic tests. CPXV received its name as a result of the association of this agent with skin lesions on the teat and udder skin of dairy cattle.

Notwithstanding, it is probably a misnomer because infection of cattle is rare, whereas infection is widespread among rodents in Europe and western Asia.

EPIDEMIOLOGY

Occurrence Infection with CPXV is endemic in wild rodents such as voles (Microtus spp.) in Great Britain, Europe, and western Asia, with infection in different rodent species acting as the reservoir host in different geographic areas. Domestic cats are commonly infected from hunting rodents, but CPXV infection can occur in a number of different mammalian species, one of which is cattle.

The clinical syndrome of cowpox in cattle is now extremely rare, but it occurs sporadically in Europe. In recent decades, reemergence of CPXV infections in cats, zoo animals, and humans has been reported. BPXV was first isolated in India in the early 1930s, and disease outbreaks affecting buffaloes, cattle, and humans have been reported in India, Nepal, Pakistan, Egypt, and Indonesia since then. BPXV is considered an important emerging or reemerging zoonotic viral infection in regions with a large buffalo population.

A similar but distinct vaccinia-like virus has been associated with disease outbreaks among cattle and humans in Brazil.

Origin of Infection and Transmission The origin of CPXV infection is most probably from infected farm cats or humans.

Transmission from cow to cow within a herd is effected by milkers' hands or teat cups. Spread from herd to herd is probably effected by the introduction of infected animals, by carriage on milkers' hands, and in the absence of either of these methods, transport by biting insects is possible. In a herd in which the disease is enzootic, only heifers and new introductions develop lesions. Milkers recently vaccinated against smallpox may serve as a source of infection for cattle, although the vaccinia virus, the smallpox vaccine virus, is a different virus.

BPXV is most commonly isolated from buffaloes, cattle, and people having direct and frequent contact with these animals. Although a primary host species functioning as virus reservoir has not yet been identified for BPXV, peridomestic rodents have been incriminated as potential vectors. Because disease outbreaks in buffalo herds are often associated with high disease occurrence among animal handlers and caretakers, transmission from animal to animal by means of people as vectors is considered to play an important role. It is generally assumed that the virus gains access to tissues through injuries to teat skin, and extensive outbreaks are likely to occur when the environment is conducive to teat injuries. Spread is rapid within a herd and immunity is solid, so that the disease tends to occur in sharp outbreaks of several months in duration, with subsequent immunity protecting the cattle for at least several years.

Economic Importance Losses are a result of inconvenience at milking time because of the soreness of the teats and from occasional cases of mastitis, which develop when lesions involve teat sphincters and decreased milk production.

Zoonotic Implications Human cowpox is not common, although the disease incidence has increased over the past decades, an observation that has been explained by increasing susceptibility of the human population to poxvirus infection following discontinuation of smallpox vaccination in most parts of the world.

Clinical cases in humans usually consist of one or a few lesions on the hand and face with minimal systemic reaction and are most commonly traced back to infected cats or occasionally rats rather than cattle. An increasing incidence of clinical cases of BPXV and Brazilian vaccinia-like virus infection has been reported in humans, particularly among animal caretakers and animal handlers in India but also in Brazil, and has become a serious public health concern in some countries.

Consumption of unpasteurized milk of affected animals has been incriminated as potential route of virus transmission from animal to human.

PATHOGENESIS

Five stages of a typical pox eruption can be observed. After an incubation period of 3 to 6 days, a roseolar erythema is followed by firm, raised papules light in color but with a zone of hyperemia around the base. Vesiculation, a yellow blister with a pitted center, follows. The subsequent pustular stage is followed by the development of a thick, red, tenacious scab. In experimentally produced vaccinia virus mammillitis (produced by inoculation of smallpox vaccine), the lesions have three zones: a central **brown crusty area** of necrosis, surrounded by a **gray–white**

zone of microvesicle formation, again surrounded by a **red border** as a result of congestion. The lesions are essentially hyperplastic.

CLINICAL FINDINGS

Typical lesions are similar for CPXV and BPXV infection and may be seen at any stage of development, but they are mostly observed during the scab stage, with the vesicle commonly having been ruptured during milking. True cowpox scabs are 1 to 2 cm in diameter and are thick, tenacious, and yellow–brown to red in color.

In cows being milked, scab formation is uncommon, with the scab being replaced by a deep ulceration. Distribution of the lesions is usually confined to the teats and lower part of the udder. Soreness of the teats develops, and milk letdown may be interfered with; the cow usually resents being milked. Secondary mastitis occurs in a few cases. Individual lesions heal within 2 weeks, but in some animals fresh crops of lesions may cause the disease to persist for a month or more. In severe cases, lesions may spread to the insides of the thighs, and rarely to the perineum, vulva, and mouth. Sucking calves may develop lesions around the mouth. In bulls, lesions usually appear on the scrotum. Ulcerative skin lesions with raised edges frequently affected by secondary bacterial or fungal infection are commonly observed on the ears of nonlactating cattle and buffaloes infected with BPXV.

CLINICAL PATHOLOGY

The virus can be propagated in tissue culture, and differentiation is possible by electron microscopy. The presence of virus-related DNA sequences can be identified by means of PCR.

DIFFERENTIAL DIAGNOSIS

A number of skin diseases may be accompanied by lesions on the udder and can easily be confused with cowpox if the lesions are advanced in age. Most outbreaks of teat skin disease that clinically resemble classical cowpox are associated with vaccinia virus from contact with a recently vaccinated person. Pseudocowpox Bovine ulcerative mammillitis associated with bovine herpesvirus-2 and bovine herpesvirus-4 Vesicular stomatitis and foot-and-mouth disease Udder impetigo Teat chaps and frostbite Black spot

CONTROL

Prevention of spread is difficult because the virus responsible for the disease is readily transmitted by direct or indirect contact. Udder cloths, milking machines, and hands should be disinfected after contact with infected animals. Dipping of the teats in an alcoholic tincture of a suitable disinfectant, such as quaternary ammonium compounds, is usually satisfactory in preventing immediate spread. Although the prevalence and significance of CPXV infection in cattle is too low to warrant the development of vaccines, the emergence of buffalopox in buffalo and cattle herds and the ensuing zoonotic risk in some parts of the world may warrant

considering the development of vaccines against BPXV for certain regions of the world.

2- PSEUDOCOWPOX (MILKERS' NODULE)

ETIOLOGY

Pseudocowpox virus is a member of the genus Parapoxvirus, with close similarity to the viruses of infectious papular stomatitis of cattle and contagious ecthyma of sheep and goats. It is possible that pseudocowpox virus (PCPV) might be identical to bovine papular stomatitis virus (BPSV). The pseudocowpox virus was previously known as parapoxvirus bovis 2.

EPIDEMIOLOGY

Occurrence Pseudocowpox is reported in most countries. In an affected herd the rate of spread is relatively slow and may result in the disease being present in the herd for up to a year. The morbidity rate approximates 100%, but at any given time it varies between 5% and 10%, and occasionally up to 50%.

Origin of Infection and Transmission The source of infection is infected cattle. The method of transmission includes physical transport by means of contaminated milkers' hands, washcloths, and teat cups. The virus cannot penetrate mucosa, and a preexisting discontinuity of it is necessary for the virus to gain entry.

Transmission by biting insects seems likely. The virus can be isolated from the mouths of calves sucking affected calves, and from the semen of bulls.

Animal Risk Factors Freshly calved and recently introduced cattle are most susceptible, but all adult cattle in a herd, including dry cows, are likely to be affected. The disease does not appear to occur in animals less than 2 years of age unless they have calved.

There is no seasonal variation in incidence. Little immunity develops, and the disease is likely to recur in the herd within a short time.

Economic Importance Pseudocowpox is relatively benign, with most losses occurring as a result of difficulty in milking and an increase in the incidence of mastitis. Zoonotic Implications The disease is transmissible to humans, with infection usually resulting in the development of milkers' nodule on the hand.

PATHOGENESIS

Transmission most commonly occurs at milking time and is mechanical, with the potential for transmission from cow to calf by suckling. The disease can be reproduced by the introduction of the virus onto scarified areas of skin. The lesions are characterized by hyperplasia of squamous epithelium.

CLINICAL FINDINGS

Acute and chronic lesions occur, and there may be up to 10 lesions on one teat (the udder is very rarely infected). Acute lesions commence as erythema

followed by the development of a vesicle or pustule, which ruptures after about 48 hours, resulting in the formation of a thick scab. Pain is moderate and present only in the prescab stage. The scab, varying in size from 0.5 to 25 mm in diameter, becomes markedly elevated by developing granulating tissue beneath it; the scabs drop off 7 to 10 days after lesions appear, leaving a horseshoe-shaped ring of small scabs surrounding a small, wart-like granuloma, which may persist for months. The disease tends to disappear from a herd after 18 to 21 days but may recur cyclically about 1 month later. There are reports of lesions occurring occasionally in cows' mouthshronic lesions also commence as erythema, but progress to a stage in which yellow–gray, soft, scurfy scabs develop.

The scabs are readily rubbed off at milking, leaving the skin corrugated and prone to chapping. There is no pain, and the lesions may persist for months. Milkers' nodules are clinically indistinguishable from human lesions associated with ecthyma virus. The lesions vary from multiple vesicles to a single, indurated nodule. An outbreak of pseduocowpox infection occurred in Brazil, characterized by the presence of severe vesicular, papulopustular, and proliferative scabby lesions on the muzzle of 14 crossbred calves that did not have contact with dairy cattle.

The lesions started as macules and papules on the muzzle that progressed to vesicles, pustules, and scabs with a clinical course of 10 to 15 days, at which time the lesions spontaneously resolved. Nucleotide sequencing of the virus isolated from the lesions revealed 97% homology with pseudocowpox virus and only 84% homology with bovine popular stomatitis virus.

CLINICAL PATHOLOGY AND NECROPSY FINDINGS

Material for examination by tissue culture or electron microscopic examination, the latter being highly recommended as a diagnostic procedure, should include fluid from a vesicle.

DIFFERENTIAL DIAGNOSIS

Differentiation of those diseases in which lesions of the teat are prominent is dealt with in the preceding section on cowpox

TREATMENT

Cattle from affected areas. Locally applied ointments of various kinds appear to have little effect on the lesions. The recommended treatment includes the removal of the scabs, which should be burned to avoid contaminating the environment, application of an astringent preparation, such as triple dye, after milking and an emollient ointment just before.

CONTROL

Recommended measures, such as treatment and isolation of affected cows or milking them last, the use of disposable paper towels for udder washing, and disinfection of teat cups, appear to have little effect on the spread of the disease. An iodophor teat dip is recommended as the most effective control measure because it appears to exert some antiviral effect. An effort should be made to reduce teat trauma because infection is facilitated by discontinuity of the skin.

3- LUMPY SKIN DISEASE (KNOPVELSIEKTE)

ETIOLOGY

Lumpy skin disease (LSD) is a severe systemic disease of cattle associated with the Neethling poxvirus, a Capripoxvirus. It has close antigenic relationship to sheeppox and goatpox viruses, which are in the same genus. There appears to be a difference in virulence between strains.

EPIDEMIOLOGY

Occurrence The disease used to be confined to subSaharan Africa, but spread to many other African countries in the 1970s, then Egypt (outbreaks occurred in 1988 and 2006; the disease is now enzootic) and Israel (outbreaks in 1989, 2006-2007, and 2012). In Israel it was initially eradicated by slaughter of infected and in-contact animals, but vaccination using Sheeppox, and more recently Neethling strain vaccine, has since been used. The virus is actively spreading within and from the Middle East, with cases confirmed in Kuwait (1991), Lebanon (1993), the United Arab Emirates (2000), Bahrain (2003), Oman (2010), Turkey and Syria (2013), Jordan (2013), Iran and Iraq (2013), Azerbaijan and North Cyprus.

There is a risk it could be introduced into European countries, mainly through the illegal movement of animals but also within vectors. Some outbreaks are associated with severe and generalized infections and a high mortality rate, whereas others have few obviously affected animals and no deaths. In general, outbreaks are more severe following introduction of the infection into a region and then abate, probably associated with the development of widespread immunity. Morbidity rates can reach 80% during epizootics, but typically range from 10% to

30% in enzootic areas. In Kenya, the disease is milder, with a lower morbidity rate and an average case fatality of 2%. Outbreaks in Israel produced no direct mortality from the disease. A resurgence of the disease in South Africa was associated with higher rainfall and a decrease in the use of vaccination.

Origin of Infection and Transmission The virus is present in the nasal and lacrimal secretions, semen, and milk of infected animals. However, direct contact is not thought to be the major source of transmission, with most cases associated with transmission by an arthropod vector. LSD virus has been isolated from S. calcitrans and M. confiscata and transmitted experimentally using S. calcitrans and Ablyomma and Rhipicephalus ticks, with evidence that the virus may be transmitted vertically and overwinter in these tick species.

Other vectors are suspected, including Biomyia, Culicoides, Glossina, and Musca spp. However, although the virus was detected in mosquitoes (Anopheles stephensi, Culex quinquefascuatus), stable flies, and biting midges (Culicoides nebeculosis) after feeding on cattle with lumpy skin disease, infection did not transmit to susceptible cattle when these arthropods were subsequently allowed to feed on them. Transmission via infected semen used in artificial breeding has been demonstrated experimentally.

Risk Factors Animal Risk Factors All ages and types of cattle are susceptible, although very young calves and lactating and malnourished cattle develop more severe clinical disease. Recently recovered animals are immune for about 3 months. British breeds, particularly Channel Island breeds, are much more susceptible than zebu types, both in numbers affected and the severity of the disease. Wildlife species are not affected in natural outbreaks, although there is

concern that they might be reservoir hosts in interepidemic periods, such as African buffalo (Syncerus caffer) in the Kruger National Park in South Africa.

Typical skin lesions, without systemic disease, have been produced experimentally with Neethling virus in sheep, goats, giraffes, impalas, and Grant's gazelles, but wildebeests were resistant. Natural cases of lumpy skin disease were recorded in water buffalo (Bubalis bubalis) during an outbreak in Egypt in 1988, but morbidity was much lower than for cattle (1.6% vs. 30.8%).

Environmental Risk Factors Outbreaks tend to follow waterways. Extensive epizootics are associated with high rainfall and high levels of insect activity, with peaks in the late summer and early autumn. Introduction of new animals and communal grazing have been identified as risk factors for LSD infection in Ethiopia.

Pathogen Risk Factors Capripoxviruses are resistant to drying and able to survive freezing and thawing, but most are inactivated by temperatures above 60°C (140F).
Experimental Transmission Experimental transmission can be achieved with ground-up nodular tissue, blood, or virus grown in tissue culture given by intranasal, ID, or IV routes. Although lumpy skin disease is characterized by generalized nodular skin lesions, less than 50% of natural or experimental infections develop generalized skin nodules. The length of viremia is not correlated with the severity of clinical disease.

Economic Importance The mortality rate is usually low (although it can be 10% or more), but economic losses are high. There is reduced feed intake, a reduction in milk production, and occurrence of secondary mastitis associated with lesions on the teats. Losses also accrue from hide damage, reduced body condition, decreased fertility in bulls, and abortion in cows. There has always been a high risk of LSD

spreading out of Africa, and it is now actively spreading in the Middle East. It is also a potential agent for agricultural bioterrorism.

PATHOGENESIS

In the generalized disease there is viremia and fever, followed by localization in the skin and development of inflammatory nodules. Following ID inoculation, local lesions develop at the challenge site but without viremia and systemic infection.

CLINICAL FINDINGS

The incubation period is typically 2 to 4 weeks in field outbreaks and 7 to 14 days following experimental challenge. In severe cases there is an initial rise of temperature, which lasts for over a week, occasionally accompanied by lacrimation, nasal discharge, salivation, and lameness. Multiple intradermal nodules appear suddenly about a week later, often initially on the perineum. They are round and firm, 1 to 4 cm in diameter, and flattened, and the hair on them stands on end. They vary from a few to hundreds and, in most cases, are confined to the skin. However, lesions can occur elsewhere, such as in the nostrils and on the turbinates, causing mucopurulent nasal discharge, respiratory obstruction, and snoring; in the mouth, as plaques and then ulcers, causing salivation; on the conjunctiva, causing severe lacrimation; and on the prepuce or vulva, spreading to nearby mucosal surfaces. In most cases the nodules disappear rapidly, but they may persist as hard lumps or become moist and necrotic, then slough. Lymph nodes draining the affected area become enlarged, and local edema can occur, particularly of the limbs. When the yellow center of nodules slough, this can expose underlying tissues, including testicles or tendons. Lesions where skin is lost may

remain visible for long periods. When lesions coalesce, large areas of raw tissue can be exposed, and these are susceptible to invasion with screwworm fly larvae. Lesions in the respiratory tract are often followed by pneumonia. Convalescence usually takes 4 to 12 weeks, and pregnant cows may abort.

CLINICAL PATHOLOGY

The virus can be cultivated from lesions, and the viral antigen can be detected by a variety of PCR tests. Viral DNA can be detected in the skin up to 90 days after infection using PCR, which is much longer than the virus can be isolated. An antigen ELISA has also been used with samples collected early in the course of the disease, before the development of neutralizing antibodies. Electron microscopy will identify capripox virions in skin biopsies or scabs. This must be used in combination with the history of generalized nodular skin disease; capripox can be distinguished from parapoxvirus (the agent of bovine papular stomatitis) and pseudocowpox, but it is morphologically similar to cowpox and vaccinia viruses. **Histopathology** of lesions reveals a granulomatous reaction in the dermis and hypodermis, with intracellular, eosinophilic inclusion bodies in early lesions. Virus neutralization is the most specific serologic test, but immunity is predominantly cell mediated, and thus it may fail to detect low concentrations of antibodies in many exposed cattle. The agar gel immunodiffusion (AGID) and indirect fluorescent antibody tests are less specific, producing false positives as a result of cross-reaction with bovine papular stomatitis and pseudocowpox viruses.

NECROPSY FINDINGS

The skin lesions are described under “Clinical Findings.” Similar lesions are present in the mouth, pharynx, trachea, skeletal muscle, bronchi, and stomachs, and there may be accompanying pneumonia. The superficial lymph nodes are usually enlarged. Respiratory distress and death are often the result of respiratory obstruction by the necrotic ulcers and surrounding inflammation in the upper respiratory tract, often with concurrent aspiration pneumonia. Histologically, a widespread vasculitis reflects the viral tropism for endothelial cells. Intracytoplasmic viral inclusion bodies may be seen in a variety of cells types. Samples for Confirmation of Diagnosis

- Histology—formalin-fixed lesions from skin, alimentary and respiratory tissue, lymph node (LM)
- Virology—lymph node, skin lesion (ISO, EM)
- Antigen detection—affected tissue, blood, semen (PCR, antigen ELISA).

DIFFERENTIAL DIAGNOSIS

The rapid spread of the disease and the sudden appearance of lumps in the skin after an initial fever make this disease quite unlike any other disease of cattle. Pseudolumpy skin disease (also known as Allerton virus infection and general infection of cattle with bovine herpesvirus-2), is associated with bovine herpesvirus-2, the agent of bovine mammillitis. It occurs primarily in southern Africa, although occasional cases occur in the United States, Australia, and the United Kingdom. Multifocal lesions are distributed over the body, are circular and

up to 2 cm in diameter, and have an intact central area and raised edges, accompanied by loss of hair. Some lesions show a circular ring of necrosis around a central scab. The scabs fall off, leaving discrete hairless lesions that may be depigmented.

The disease runs a course of approximately 2 weeks, and there is no mortality. Only the superficial layers of skin are involved. This is in contrast to the lesions of lumpy skin disease, which are often deep enough to expose underlying tissues. Herpesvirus can be isolated from the periphery of the lesions. Diagnosis can be made by polymerase chain reaction (PCR) on fullthickness skin biopsy.

TREATMENT

No specific treatment is available, but prevention of secondary infection with antibiotics or sulfonamides is recommended.

CONTROL

Lumpy skin disease moves into new territory principally by movement of infected cattle, and possibly by wind-borne vectors. Once in a new area, further spread probably occurs via insect vectors, and ticks have been implicated in maintaining the virus in between epidemics. Control of cattle movement from uninfected to infected areas is an important measure to prevent the introduction of the virus. Once in an area, control is by vaccination.

Vaccination Freeze-dried, live attenuated vaccines are commercially available and the most commonly used. There is antigenic homology between the Capripoxviruses, and thus vaccination of cattle with attenuated sheeppox virus has

been used to protect against infection with LSD virus. This was used in countries previously free of LSD virus because it eliminated any risk of escape of attenuated live vaccine virus from vaccinated herds. However, incomplete protection with a vaccine based upon what was thought to be a Kenyan sheep and goatpox occurred during a 2006 outbreak of LSD in Egypt, and following the use of a sheeppox vaccine in Israel in 2006-2007 in which 11% of vaccinated cattle developed skin lesions.

Consequently, the attenuated Neethling strain vaccine of LSD virus was used in response to a serious outbreak of the disease in Israel during 2012. A battery of three molecular tests was able to differentiate infection with the vaccine strain, and a virulent virus was developed; no spread from vaccinated to nonvaccinated cattle was recorded. A small percentage of cattle vaccinated with the sheeppox virus do develop local granulomatous reactions, but there is no spread of the virus to sheep running with the cattle. However, the commonly used Kenyan sheeppox and goatpox vaccine virus (designated O-240) has been identified as an LSD virus; the low attenuation of this virus probably makes it unsafe for cattle because of its potential to cause clinical disease in vaccinated animals.

Other viruses capable of infecting sheep, goats, and cattle have been identified as potential candidates for vaccines against all capripox diseases. Vaccination of a herd at the start of an outbreak is of limited use. Most animals will already be incubating the disease, and poor needle hygiene in these circumstances may spread the disease. Slaughter of affected and in-contact animals, destruction of contaminated hides, and vaccination of at-risk animals is a common approach when the disease is introduced to a previously free country.