

CHAPTER 9

VIRAL INFECTIONS AND BOVINE MASTITIS

A review

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Abstract

This review deals with the role of viruses in the aetiology of bovine mastitis. Bovine herpesvirus 1, bovine herpesvirus 4, foot-and-mouth disease virus, and parainfluenza 3 virus have been isolated from milk from cows with clinical mastitis. Intramammary inoculations of bovine herpesvirus 1 or parainfluenza 3 virus induced clinical mastitis, while an intramammary inoculation of foot-and-mouth disease virus resulted in necrosis of the mammary gland. Subclinical mastitis has been induced after a simultaneous intramammary and intranasal inoculation of lactating cows with bovine herpesvirus 4. Bovine leukaemia virus has been detected in mammary tissue of cows with subclinical mastitis, but whether this virus was able to induce bovine mastitis has not been reported.

Bovine herpesvirus 2, vaccinia, cowpox, pseudocowpox, vesicular stomatitis, foot-and-mouth disease viruses, and bovine papillomaviruses can play an indirect role in the aetiology of bovine mastitis. These viruses can induce teat lesions, for instance in the ductus papillaris, which result in a reduction of the natural defence mechanisms of the udder and indirectly in bovine mastitis due to bacterial pathogens. Bovine herpesvirus 1, bovine viral diarrhoea virus, bovine immunodeficiency virus, and bovine leukaemia virus infections may play an indirect role in bovine mastitis, due to their immunosuppressive properties.

We conclude that viral infections can play a direct or indirect role in the aetiology of bovine mastitis; therefore their importance in the aetiology of bovine mastitis and their economical impact needs further attention.

1. Introduction

Bovine mastitis is a highly prevalent disease in dairy cattle, and one of the most important diseases affecting the world's dairy industry; it places a heavy economic burden on milk producers all over the world (Miller and Dorn, 1990; Schakenraad and Dijkhuizen, 1990; Miller et al., 1993; Bennett et al., 1999). Worldwide, annual losses due to mastitis have been estimated to be approximately 35 billion US dollar. In the US, the annual costs of mastitis have been estimated to be 1.5 – 2.0 billion US dollar, while losses of milk productions, due to subclinical mastitis, and higher cow replacements costs associated with high somatic cell counts were estimated at 960 million US dollar (Wells et al., 1998). Each case of clinical mastitis in the US and California costs approximately 107 and 200 US dollars, respectively (Miller et al., 1993). In Scottish dairy herds, facing high bulk-tank somatic cell count, the average annual costs of subclinical mastitis was 100 Pound Sterling/cow (Yalcin et al., 1999), while in the UK and the Netherlands, the annual average revenue losses were calculated to be 42–84 Pound Sterling/cow (Esslemont and Peeler, 1993) and approximately 130 Dutch Guilders/cow (Schakenraad and Dijkhuizen, 1990).

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature (International Dairy Federation, 1987). Mastitis is characterized by physical, chemical and usually bacteriological changes in the milk and by pathological changes in the glandular udder tissue. The diagnosis of mastitis is based on clinical signs, e.g. swelling of the udder, tender to the touch, fever, and depression. In many cases a reduced milk production can be observed. Because of the large number of subclinical mastitis cases, the diagnosis of mastitis can also depend on indirect tests which in turn depends on e.g. the leukocyte numbers in the milk (Radostits et al., 1994).

Bovine mastitis is generally considered to be of infectious nature leading to inflammation of one or more quarters of the mammary gland and it is often affecting not only the individual animal but the whole herd or at least several animals within the herd. If left untreated, the condition can lead to deterioration of animal welfare resulting in culling of affected cows, or even death.

Mastitis-causing pathogens include bacteria and non-bacterial pathogens, like mycoplasmas, fungi, yeasts, and chlamydia (Watts, 1988, Radostits et al., 1994). These pathogens infect the udder generally through the ductus papillaris, which is the only opening of the udder to the outside world.

Despite intensive aetiological research, still around 20-35% of clinical cases of bovine mastitis have an unknown aetiology (Miltenburg et al., 1996; Wedderkopp, 1997). Miltenburg et al. (1996) found a 28% negative rate in 1045 cases of clinical mastitis, and Wedderkopp (1997) did not note pathogens in 35% of 6809 milk quarters in 3783 cows suffering from clinical mastitis. The percentage of culture-

negative samples of both clinical and subclinical mastitis cases in the Netherlands has recently been determined to be approximately 25% (Barkema et al., 1998). An explanation for these high percentages of culture-negative samples might be a low concentration of udder pathogens, e.g. *Escherichia coli*. Other pathogens such as mycoplasma, yeasts and moulds are difficult to cultivate. But these agents cannot be the explanation for all culture negative milk samples from mastitis cows, because these agents are no common udder pathogens (Pfützner, 1994; Wendt, 1994). Due to the high percentages of unknown causes of mastitis, it is obvious to study the role of viruses in the aetiology of bovine mastitis. This in spite of the fact that viruses are generally considered not to play an important role. Watts (1988), for example, identified 137 microbial species as causative agents of bovine mastitis, including agents involved in its pathogenesis. However, viruses were not included.

The reasons for this negligence could be manifold. Historically, mastitis research has concentrated on bacterial pathogens. In case of viral infections, signs of mastitis may not have been recognised because other clinical signs were more prominent. Subclinical mastitis cases are often not diagnosed and consequently their aetiology is not investigated. This may cause an underestimation of virus infections involved in bovine subclinical mastitis. Another reason might be that lactating cows are seldom used in viral pathogenesis studies. In, e.g. most BHV1 pathogenesis studies, young calves are used due to economic aspects. A disadvantages thereof is that it does not yield any indication as to whether BHV1 can be involved in the aetiology of bovine mastitis. In addition, milk samples from mastitis cows are often not properly collected, treated and stored for virological research, as this requires special care. The laboratory diagnosis of viral mastitis is laborious and expensive. Diagnostic tools, e.g. susceptible cells, for the detection of viruses are often not optimally used. These arguments might explain why it is difficult to estimate the importance of viral infection on the aetiology of bovine mastitis and their economical impact. It also explains the low number of viral mastitis reports, and it may explain why the last brief review on viral infections of the bovine mammary gland has been published 30 years ago (Afshar and Bannister, 1970). This review paper aims to make an inventory of the updated evidence that demonstrate whether viral infections are associated in a direct or indirect way with bovine mastitis (Table 1).

Table 1. Viral infections and their association with bovine mastitis

Virus	Natural cases	Experimental reproduction		Indirect by teat lesions	Epidemiological studies
	Virus isolation	IM route ^a	Natural route		
Bovine herpesvirus 1	+	+			D ^b
Bovine herpesvirus 4	+ ^c	+			+
Foot-and-mouth disease virus	+	+	+	+	
Parainfluenza 3 virus	+	+			
Bovine leukaemia virus	+ ^d				+/-
Bovine herpesvirus 2				+	
Cowpox virus				+	
Pseudocowpox virus				+	
Vesicular stomatitis virus			+		
Bovine papillomaviruses				+	
Bovine viral diarrhoea virus					+
Bovine immunodeficiency virus					D ^b
Rinderpest virus					D ^b
Bovine enterovirus		+			

IM^a: intramammary

D^b: data are considered to be insufficient for association

+^c: virus isolation from cases and not from matched controls

+^d: no virus isolated but viral particles detected by electron microscopy

2. Viral infections and bovine clinical mastitis

Bovine herpesvirus (BHV) 1 (Gourlay et al., 1974; Roberts et al., 1974), BHV4 (Wellenberg et al., 2000), foot-and-mouth disease virus (Burrows et al., 1971), and parainfluenza 3 virus (Kawakami et al., 1966a and 1966b) have been detected in milk from cows with clinical mastitis. However, the detection of virus in milk from cows with mastitis obviously does not prove that these agents are the cause of mastitis, or that they are involved in an indirect way.

2.1 Bovine herpesvirus 1

Bovine herpesvirus 1, a member of the *Alphaherpesvirinae* subfamily within the *Herpesviridae* family, causes infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis. In 1974, BHV1 was isolated from a cow with mastitis in the USA. Although, bacterial culture was negative and only BHV1 was isolated from the milk, the evidence that the virus caused the mastitis was, at most, circumstantial as the milk sample was collected three days after vaccination with a live IBR-vaccine (Roberts et al., 1974). In France, BHV1 was isolated from milk samples from cows with mastitis in combination with *Mycoplasma agalactiae* (Espinasse et al., 1974; Gourlay et al., 1974). BHV1 was also isolated from one of the milk samples obtained from one out of 96 cows with mastitis (Bilge, 1998). Besides the isolation of BHV1 in milk, the virus was also isolated from vesicular lesions on the udder and on the teats of a cow. Thus, BHV1 was associated with cutaneous lesions of the bovine udder, however it was difficult to ascertain whether the lesions were primarily caused by the BHV1 infection (Guy et al., 1984).

A possible role of BHV1 in the aetiology of bovine mastitis, without or in combination with bovine viral diarrhoea virus (BVDV), was also suggested by Siegler et al. (1984), who described a high incidence of mastitis cases in a number of herds with BHV1 and BVDV infected cows. Bacteriological examination of bovine milk samples in a number of herds suffering from mastitis revealed no udder pathogens, while others suffered from mastitis induced by e.g. staphylococci and streptococci. Immunisation of cows in the affected herds with IBR/IPV vaccine, without or in combination with mucosal disease/BVD vaccine, resulted in an effective control of their mastitis problems, including the herds suffering from mastitis induced by e.g. staphylococci and streptococci. Any clear evidence that BHV1 or BVDV were involved in these mastitis cases was not presented, as no attempts were made to isolate BHV1 or BVDV from milk of affected cows, and no data were presented about unvaccinated control cows within the same herds.

Bovine herpesvirus 1 has been shown to replicate in the bovine mammary gland and to induce signs of clinical mastitis after an intramammary inoculation (Greig and Bannister, 1965; Straub and Kielwein, 1966; Corner et al., 1967). An intramammary inoculation of one young heifer with a BHV1-IBR or a BHV1-IPV strain induced swollen quarters, hard and tender to the touch (Greig and Bannister, 1965). A strong reduction in milk yield was recorded, and milk samples showed abnormal morphology, with clots and blood, after the intramammary inoculation of cows with the BHV1-IBR strain. Virus was first isolated from infected quarters on day 2 post-inoculation (pi) which continued up to day 10-15 pi. The virus reached titres up to $10^6 - 10^7$ TCID₅₀/ml. No virus was detected in the milk from the two uninoculated control quarters. Clinically, the experimental mastitis produced by the BHV1-IBR strain was similar to that induced by the BHV1-IPV strain. Dilution series further demonstrated that about 10^3 TCID₅₀ BHV1/ml was required to produce infection of the mammary gland by the intramammary route (Greig and Bannister, 1965). In another study, an intramammary inoculation with BHV1-IPV resulted in clinical mastitis as evidenced by an increase in body temperature, decreased appetite, painful and swollen udders, and a strong reduction of the milk yield. It was possible to isolate the virus from the milk of inoculated quarters until day 11 pi (Straub and Kielwein, 1966).

Experimental BHV1 infections of the mammary gland resulted in necrosis of the alveolar epithelial layer, infiltration and accumulation of polymorphic and mononuclear cells, and inclusion bodies in the nuclei of epithelial cells (Corner et al., 1967).

The above mentioned studies demonstrate that BHV1 has been isolated from natural cases of mastitis and that the bovine udder is susceptible to BHV1. However, its impact on bovine mastitis cases in general is unclear. In view of the ubiquitous character of this virus, the number of reported mastitis cases in which BHV1 played a role is probably low. Bovine herpesvirus 1 was not isolated from milk of any of the 58 natural clinical mastitis cases from 10 herds examined virologically by Wellenberg et al. (2000). BHV1 is probably not a major primary udder pathogen.

2.2 Bovine herpesvirus 4

Bovine herpesvirus 4, a rhadinovirus and member of the *Gammaherpesvirinae* subfamily within the *Herpesviridae* family, has been isolated from cows with various clinical signs, including mammary pustular dermatitis (Reed et al., 1977), and chronic ulcerative mammary dermatitis (Cavirani et al., 1990). Recently, BHV4 has been isolated from 3 milk samples of 3 (5%) out of 58 cows with clinical mastitis, and not from the 58 matched control cows. Two of the three cows from which BHV4 was

isolated developed antibodies against BHV4, while no increase in antibodies against BHV4 were detectable in the third cow within 21 days. A possible role of BHV4 in bovine mastitis was further supported by the fact that in 4 of the 10 herds examined there was an ongoing BHV4 infection at the same time as mastitis occurred (Wellenberg et al., 2000). In a second case-control study, a part of the gene coding for BHV4-glycoprotein B was detected by PCR (Wellenberg et al, 2001) in milk samples from 2 (4%) out of 54 mastitis cows. From the same milk samples, BHV4 was isolated on bovine umbilical cord endothelial cells, a cell type highly susceptible to bovine herpesviruses (Wellenberg et al., submitted). A significant increase in BHV4 antibody titres was detected in one of these two mastitis cows at the same time as mastitis occurred. No BHV4 was detected in milk from their matched control cows by gB-PCR or virus isolation. In both case-control studies, the presence of BHV4 was in most cases accompanied by bacterial udder pathogens, e.g. *Staphylococcus aureus* and *Streptococcus uberis*. An experimental study, performed to further investigate the role of BHV4 in bovine mastitis, showed that a simultaneous intramammary and intranasal inoculation of lactating cows with BHV4 did not result in clinical mastitis. However, subclinical mastitis was induced in 2 out of 4 inoculated lactating cows (Wellenberg et al., in press). A significant increase of SCC was recorded in milk from 50% of the BHV4 inoculated quarters on days 8, 9 and 11 pi, compared to the non-inoculated quarters of the same cows (within-cow controls) and the quarters of the mock-inoculated cows. Virus was isolated from milk samples of inoculated quarters only; from day 1 pi to days 9 - 14 pi. A *Streptococcus uberis* infection appeared to trigger BHV4 replication in cows infected 2 weeks before with BHV4. Bovine herpesvirus 4 was isolated from the milk from 2 out of 4 quarters after an intramammary *Streptococcus uberis* inoculation.

During an epidemiological study, a positive association between the presence of BHV4 antibodies in cows and the incidence of bovine mastitis caused by *Staphylococcus aureus* was recorded (Zadoks et al., submitted). This finding suggests that a previous BHV4 infection promotes the development of mastitis especially caused by *Staphylococcus aureus*. BHV4 has also been isolated from the cellular fraction of milk samples from cows with antibodies against BHV4. Unfortunately, no clinical data were reported on mastitis in these cows (Donofrio et al., 2000).

All above mentioned studies strongly suggest a role for BHV4 in bovine mastitis. Although, BHV4 probably does not appear to play an important role as primary udder pathogen in the aetiology of clinical mastitis, it may play a role in subclinical mastitis cases, or in an indirect way. More research is warranted to establish a possible indirect role of BHV4 infections in bovine mastitis, e.g. as a result of immunosuppression. The virus can infect cells involved in the immune system, e.g. mononuclear blood cells (macrophages), and recently, a possible role of BHV4 has been postulated by playing a role in damaging vascular tissues (Lin et al., 2000). In addition, bovine endothelial cell cultures are highly

susceptible to BHV4.

2.3 Foot-and-mouth disease virus

Foot-and-mouth disease (FMD) virus, a member of the *Aphthovirus* genus within the family of the *Picornaviridae*, in general causes an infection whereby the virus is widespread through various tissues and organs of the host. Although, a primary infection of the mammary gland is unlikely to be a common occurrence in the pathogenesis of FMD, the virus can also replicate in the secretory epithelial cells of the mammary gland. Many researchers have isolated FMD virus from milk of FMD affected cows (Burrows, 1968; Ray et al. 1989; Fuchs, 1994), and also teat and udder lesions have been reported in FMD-affected cattle during an outbreak with an Asia-1 serotype (Firoozi et al., 1974).

The results of experimental inoculations of the udder show that it is a highly susceptible organ that is capable of producing large amounts of virus. Evidence for the replication of FMD virus in the mammary glands, as a result of a systemic infection, was found in cattle that were infected by (simulated field-type) contact exposure to FMD virus infected animals (Blackwell et al., 1983). Infection of FMD virus by the oronasal route also resulted in virus replication in secretory epithelial cells of the alveoli of the udder (Blackwell and Yilma, 1981), and in progressive temporal necrosis in the alveoli. Clumps of necrotic secretory epithelial cells and detached membrane-limited structures (cellular debris) were observed within the alveolar lumen and in the milk (Blackwell et al., 1983). During experimental infection, an increase of leukocytes was not recorded up to day 17 pi. This means that FMD virus infections of the bovine udder result in necrosis of the alveolar epithelial cells, but this occurs without a strong increase in leukocytes as observed for most bacterial udder infections. So, FMD virus is not the cause of a viral clinical mastitis as such, but secondary bacterial infections may result in clinical mastitis. The necrosis process is probably responsible for the observed decrease in milk yield (Blackwell and Wool, 1986).

Replication of FMD virus in the mammary gland has also been reported after cows were exposed to the virus either by aerosol, by a combination of intramammary-intravenous inoculation (Blackwell and Yilma, 1981), or after an intramammary inoculation via the ductus papillaris (Burrows et al., 1971). After intramammary inoculation, affected quarters became swollen and tender to the touch. The milk showed abnormal morphology (with clots), and a drop in the milk yield of approximately 60% was recorded. The FMD virus multiplied rapidly and virus titers of $>10^7$ plaque forming units/ml were recorded within 8-32 hours pi. Dissemination of the virus from the mammary gland was recorded by virus isolation from milk within 4-24 hours pi. The ability of FMD virus to persist in the mammary tissue was confirmed by the intermittent recovery of the virus from cows up to day 51 pi, which indicates virus multiplication in the udders of immune cows (Burrows et al., 1971).

Based on reports of natural mastitis cases and experimental infections, we may conclude that the udder is a highly susceptible organ for FMD. Infection of the secretory epithelial cells of the mammary gland will usually be the result of a systemic infection, because a primary infection of the mammary gland by this virus is unlikely to be a common occurrence. Mastitis associated with FMD virus is assumed to be due to secondary bacterial infections.

2.4 Parainfluenza 3 virus

In 1966, parainfluenza 3 (PI3) virus, a member within the *Paramyxoviridae* family, was recovered from Japanese cattle with acute respiratory illness from nasal secretions, and also from milk (Kawakami et al., 1966a). On one of the examined farms, the virus was recovered from milk in 14 of 58 cows (24%). The cows from which PI3 virus was recovered from the milk did not show signs of clinical mastitis, but an increased milk SCC was recorded in many milk samples.

Parainfluenza 3 virus was also isolated from quarter milk from one cow of the same farm with typical aseptic mastitis. An intramammary inoculation of PI3 virus resulted in respiratory signs and other signs e.g. fever, malaise, and losing condition, as observed in calves infected with the same PI3 virus by intravenous or intranasal inoculation. The affected udders developed swelling and induration. The milk showed a color change, an increased pH and increased numbers of glandular epithelial cells, neutrophils, lymphocytes and monocytes. Virus was excreted in high titers (up to 10^7 TCID₅₀/0.1 ml) in milk from inoculated quarters up to day 10 pi. The histological examination revealed that the major change was an interstitial inflammation, consistent of large lymphoid cells (Kawakami et al., 1966b). Both studies indicate that the mammary gland is highly susceptible to PI3 virus, and that in naturally PI3 virus infected cows udder infections may also occur. In some cases the infection may result in overt clinical mastitis. These findings await confirmation.

3. Viral infections and bovine subclinical mastitis

A possible role of viruses in bovine subclinical mastitis has been suggested before (Fuchs, 1994). Subclinical mastitis occurs frequently, and may lead to high economical losses due to reduced milk yields, and to penalties because of too high bulk-tank somatic-cell counts. Losses resulting from both clinical and subclinical mastitis may amount to 20% of the potential production (Beck et al., 1992). In practice, subclinical mastitis cases are often not detected rapidly, or may even not be recognized by the farmer.

3.1 *Bovine leukaemia virus*

Bovine leukaemia virus (BLV), belonging to the *Deltaretrovirus* genus and member of the family of *Retroviridae* (Pringle 1999), causes enzootic bovine leukosis. It preferentially infects lymphocytes of the B-lineage in cattle. Recently, BLV particles have been detected by electron microscopy around lymphocytes in the mammary tissue of BLV antibody positive cows affected by subclinical mastitis (Yoshikawa et al., 1997). No macroscopical lesions were detected in the mammary glands of the 6 cows examined, but histological lesions were found in some lobules of the mammary gland, i.e. an infiltration of lymphocytes, plasma cells, and neutrophils into alveoli and interlobular connective tissue. The alveoli also contained numerous macrophages and desquamated alveolar lining cells. No information was recorded about the milk SCC, the presence of bacterial udder pathogens or milk yields. Consequently, whether this virus was the causative agent in this “subclinical” mastitis case is unknown.

A possible association of BLV infections and mastitis in dairy cows has been investigated on individual and on herd level, however with contradictory results. A positive association has been reported by Milojevic et al. (1991) and Rusov et al. (1994), who reported that the occurrence of mastitis and increased cell counts are more often recorded in cows with enzootic leukosis than in healthy cows. A significant association between BLV seropositivity and higher milk SCC has also been recorded for older cows (Jacobs et al., 1995). According to Emanuelson et al. (1992), the risk for infectious diseases seemed to be greater among BLV-infected herds than among non-BLV infected herds. In this study a positive association between BLV antibody positive bulk milk and bovine mastitis, and also for bulk SCC, was recorded. However, no data were presented on the differences in management on the herd level, which is an important factor in bovine mastitis prevalence.

In the above mentioned studies a possible association of BLV infections and mastitis in dairy cows

is noted, but this is not the case in all studies performed. In one of these studies, 226 adult dairy cows were examined for BLV infection and mastitis, but no statistically significant association was found between the BLV infection and mastitis (Fetrow and Ferrer, 1982). Of the cows positive for BLV-infection, 22.7% had bacteriological cultures positive for mastitis, whereas in the group of BLV-infection negative cows 23.2% suffered from bacteriological mastitis pathogens. Scott et al. (1991) reported that the number of subclinical mastitis cases in BLV positive cows was even lower than that in BLV negative cows. In this study, the number of *Staphylococcus sp.* and *Micrococcus sp.*, isolated from milk from 50 BLV-positive cows, was significantly lower than that in 35 BLV-seronegative cows. In a matched case-control study, to assess the risk of clinical mastitis in BLV-infected cows, the BLV-infected cows did not produce less milk, or did not develop mastitis more often than did noninfected cows ($P > 0.05$) (Huber et al., 1981).

Also studies on milk yields, which also might be an indication for the presence of subclinical mastitis cases within herds, on BLV positive herds showed contradictory results. Heald et al. (1992) could not find a significant association between milk yield, somatic cell counts and BLV-seropositivity. One of the reasons why results on studies on herd level are not in agreement is that many studies did not use a proper study design. None of the studies performed on individual or herd level clarify the role of BLV in the aetiology of bovine subclinical mastitis. No experimental studies have been reported to investigate whether BLV is able to induce subclinical or clinical bovine mastitis. Such experiments are necessary to gain more insight into the role of BLV in the aetiology of bovine mastitis.

4. Viral infections and their indirect role in bovine mastitis

Can viral infections play an indirect role in the pathogenesis of bovine mastitis? Damage of teat and ductus papillaris (as natural barrier) and immunosuppression may lead to a higher susceptibility for bacterial mastitis cases, and bacterial infections may run a more severe course.

4.1 Teat lesions

Bovine herpes mammillitis virus (BHV2), vaccinia, cowpox, pseudocowpox, FMD viruses, and to a lesser extent vesicular stomatitis virus can cause a local dermatitis, often with ulcerations in the ductus papillaris, leading to secondary bacterial infections in the sinus lactiferus and the corresponding mammary gland (Turner et al, 1976; Francis, 1984; Scott and Holliman, 1984).

4.1.1 Bovine herpesvirus 2

Bovine mammillitis is an acute viral disease of cattle caused by BHV2 (Martin et al., 1966), a virus of the genus *Simplexvirus* and member of the *Alphaherpesvirinae* subfamily within the *Herpesviridae* family. Bovine herpesvirus 2 often infects young heifers and young cows at first parity or in the first lactation period. The infection may be subclinical or relatively mild (Turner et al., 1976; Letchworth and LaDue, 1982; Scott and Holliman, 1984), but it can also be very severe causing extensive painful ulcerations on one or more teats and udders (Scott and Holliman, 1984). Lesions can range from vesicles and ulcerations of large (up to 10 cm wide) areas of teat skin to single small (2-3 cm wide) plaques of oedema. Severe BHV2 infections may also result in damage of the ductus papillaris. The functions of the keratin in the ductus papillaris, with its fatty acids and proteins, and the macrophages, lymphocytes and plasma cells in the ductus papillaris and the sinus lactiferus may be impaired due to this BHV2 infection (Paape et al., 1985; Senft and Neudecker, 1991). This may enhance the susceptibility of the mammary gland for bacterial mastitis (Martin et al., 1969; Letchworth and LaDue, 1982; Scott and Holliman, 1984; Gourreau et al., 1989).

BHV2 has also been isolated from milk from cows with ulcers on teats (Martin et al., 1969), but leakage from these lesions was probably the cause of the presence of BHV2 in the examined milk samples. Turner et al. (1976) recorded mastitis cases in cows with BHV2 infection. These cows had ulcers in the ductus papillaris, and therefore its function was impaired. Chronic mastitis was observed in cows with udder ulcers up to the ductus papillaris. These reports suggest that BHV2 may induce mastitis due to damage of the mechanical defence of the udder. Mastitis indirectly due to BHV2

infections (Letchworth and LaDue, 1982; Scott and Holliman, 1984) mostly affect a few cows within a herd, but also percentages of 22% have been recorded for BHV2 affected cows that developed mastitis (Martin et al., 1969). Under experimental conditions, an intradermal and intravenous inoculation of a 30-month-old heifer with BHV2 resulted in several clinical signs e.g. mammilitis. However, in this study mastitis has not been recorded and the mammary gland has not been examined for histopathological lesions (Tabbaa et al., 1987).

In conclusion, BHV2 infections can result in damage of the natural defence mechanisms of the udder, which results in a higher susceptibility to bacterial mastitis.

4.1.2 *Vaccinia virus and cowpox virus*

Infections with vaccinia virus and cowpox virus, both belonging to the genus *Orthopoxvirus* within the subfamily *Chordopoxvirinae* of the *Poxviridae* family, do not occur anymore or are very rare, respectively (Mayr and Czerny, 1990). Clinical signs are comparable to those described for BHV2 infections. As a result of teat lesions, mastitis may occur on the same way as it occurs after a BHV2 infection. After an intramammary inoculation with vaccinia virus, the virus that has been used for smallpox vaccination, an inflammatory reaction in the bovine mammary gland was produced (Easterday et al., 1959). The intramammary inoculation of the mammary glands via the ductus papillaris of 6 cows with vaccinia virus (strain IHD) induced systemic signs e.g. elevated body temperatures, and udder swelling in 5 of the 6 cows inoculated. Lesions appeared on the ends of all vaccinia virus inoculated teats, and progressed from a papule to a vesicle to a scab. The SCC increased up to >500.000/ml, and vaccinia virus was isolated from the milk of 4 out of 4 lactating cows up to 9 days pi (Easterday et al., 1959). Natural cases of bovine mastitis, in which vaccinia virus was involved, are unknown.

Outbreaks of cowpox virus are extremely rare. The virus enters through teat skin injuries and several stages of lesion development can be observed. Erythematous areas appear on the teat and can change into raised papule and ruptures with pitted centers. Lesions spread rapidly throughout the herd. Healing occurs within two to three weeks although secondary bacterial infections may delay resolution (Francis, 1984). During a cowpox virus infection in India of cows and buffalo's some animals suffered from mastitis (Sambyal et al., 1983). Most of the affected cows showed teat lesions, and udder pathogens like *Staphylococcus aureus* and *Klebsiella spp.* were isolated from the milk of affected cows. The role of cowpox virus in this case of mastitis was not clear, but the teat lesions, induced by cowpox virus, might have resulted in secondary bacterial mastitis. The above mentioned studies indicate that cowpox virus may play a role in bovine mastitis, but the incidence is probably very low.

4.1.3 Pseudocowpox virus

The pseudocowpox virus belongs to the genus *Parapoxvirus* within the subfamily *Chordopoxvirinae* of the *Poxviridae* family. Only one report was found concerning the isolation of a poxvirus from milk (Dawson et al., 1968). The virus was isolated from a pooled milk sample and typed as a virus from the paravaccinia subgroup, but no lesions suggestive of pseudocowpox were recorded, neither was clinical or subclinical mastitis. An intramammary inoculation of one lactating cow with this strain did not result in systemic disturbance, swelling or induration of the udder. Only a few small clots were recorded in the milk on days 4 and 5 pi. No lesions developed on teats and on the udder. In milk from one out of two inoculated quarters, the virus was isolated on only 24 hours pi, but clinical mastitis was not noted (Dawson et al., 1968). No further reports on pseudocowpox virus and bovine mastitis were found, despite the fact that this virus is ubiquitous and the infection induces comparable clinical signs as reported for BHV2 infections (Gibbs, 1984). This suggests that, in addition to BHV2, pseudocowpox virus may also induce mastitis due to damage of the mechanical defence mechanism of the udder. The role of pseudocowpox virus in the aetiology of bovine mastitis is still an interesting area for research; this virus was detected in 5 out of 14 cases of bovine teat lesions in Dutch cattle (Wellenberg, 2001, unpublished data).

4.1.4 Foot-and-mouth disease virus

FMD virus can play a secondary role in bovine mastitis in that FMD virus infection may result in ductus papillaris lesions and therefore enhances bacterial infections as reported for an experimental *Arcanobacter pyogenes* (*A. pyogenes*) udder infection (Saini et al., 1992). After an infection of lactating cows with FMD virus, *Arcanobacter pyogenes* had been isolated from 15 quarters showing purulent mastitis (Saini et al., 1992), while an intramammary inoculation of quarters with *A. pyogenes* alone did produce only mild inflammatory reactions (Vecht et al., 1987). This suggests that the teat epithelium of the quarters had already been damaged by FMD virus that supported the involvement of *A. pyogenes* as the causative agent of purulent mastitis. The injury to teat epithelium was essential for the establishment of infection (Seinhorst et al., 1991). Field studies also support a secondary role of FMD in bovine mastitis. An increased incidence of bovine mastitis cases with secondary bacterial pathogens has been reported after an infection with FMD virus (Ray et al., 1989; Seinhorst et al., 1991).

4.1.5 Vesicular stomatitis virus

Mastitis has been associated with some other virus diseases, but it has not been demonstrated that these viruses were the primary invaders of the mammary gland. Strozzi and Ramos-Saco (1953) reported teat lesions and associated mastitis in cases of vesicular stomatitis virus infections; a virus belonging to the genus *Vesiculovirus* within the *Rhabdoviridae* family. An intramammary inoculation of 8 cows with vesicular stomatitis virus (New Jersey) did not induce udder swelling, but it resulted in increased milk SCC of >500.000/ml in all 5 inoculated lactating cows. The virus was isolated from milk from 4 out of 5 lactating cows. In 5 out of 8 cows elevated body temperatures were recorded. No changes were recorded in the bacterial flora of any quarter inoculated with vesicular stomatitis virus during this study (Easterday et al., 1959). Although vesicular stomatitis virus may play a role in bovine mastitis, the incidence is probably very low as the number of reported mastitis cases in which vesicular stomatitis virus has been involved is nil.

4.1.6 Bovine papillomaviruses

The bovine papillomaviruses belong to the genus *Papillomavirus* within the family *Papillomaviridae*. At least six types of bovine papillomavirus (BPV) have been recognised, and certain types can cause fibropapillomas on teats (Olson, 1990). Fibropapillomas in the ductus papillaris due to bovine papillomavirus may result in damage of the natural defence mechanisms of the udder and therefore in a predisposition for mastitis (Francis, 1984). An ascending bacterial infection may result in mastitis (William et al., 1992).

4.2 Immunosuppression

In addition to viruses that cause teat lesions, other viral infections may induce or enhance bovine mastitis due to their immunosuppressive effects. Although, so far there is not any clear evidence for this.

4.2.1 Bovine herpesvirus 1

Bovine herpesvirus 1 infections can impair the bovine immune system (Bielefeldt-Ohmann and Babiuk, 1985; Straub, 1991; Nataraj et al., 1997; Saini et al., 1999; Koppers-Lalic et al., 2001). Based on the immunosuppressive properties of BHV1, it has been proposed that the virus may play a

secondary role in the aetiology of diseases caused by bacteria (Filion et al., 1983; Bielefeldt-Ohmann and Babiuk, 1985; Hutchings et al., 1990), but whether and which secondary role BHV1 plays in the aetiology of bovine mastitis is not clear. Epidemiological studies, to examine whether BHV1 seropositive animals are more prone to bovine mastitis than BHV1 seronegative animals, are unknown. Hage et al. (1998) reported a significant drop in milk production, which might be an indication for subclinical mastitis, during a subclinical BHV1 infection on a dairy herd. However, no association was found between the BHV1 infection and mastitis, since the milk SCC was unaltered and clinical mastitis was not observed.

4.2.2 Bovine viral diarrhoea virus (BVDV)

Another virus that causes immunosuppression is BVDV, a member of the *Pestivirus* genus, within the family of the *Flaviviridae* (Roth et al., 1981; Bolin et al., 1985; Markham and Ramnaraine, 1985; Welsh et al., 1995). Persistently infected animals show chronically impaired immunoresponses (Roth and Bolin, 1986; Brownlie, 1989), and a delay in the onset of BRSV-specific IgG response and reduced antibody titres has been noted in cattle infected concurrently with BVDV (Elvander, 1996). These data indicate that BVDV may play an (indirect) role in the susceptibility of the animal to secondary infections, or may enhance the possibility of secondary infections to run a more severe course (Potgieter et al., 1984).

Studies on the immunosuppressive role of BVDV in relation to bovine mastitis are very scarce. Siegler et al. (1984) reported an increased amount of mastitis cases in BVDV and BHV1 seropositive herds, however which role BVDV played in these mastitis cases is unclear. Furthermore, a positive association between BVDV and bovine mastitis, based on the BVDV antibody titres in bulk milk of 237 herds, has been reported. The number of mastitis cases increased in herds with an increased BVDV antibody milk titre (Niskanen et al., 1995). In a retrospective longitudinal study, which was conducted to examine whether the exposure of dairy herds to BVDV affected udder health, a 7% increase was noted in the incidence rate of clinical mastitis in herds exposed to BVDV as compared with non-BVDV exposed herds (Waage, 2000). A reduction in the milk yield was shown in cows that seroconverted for BVDV antibodies, although no information was presented on mastitis (Moerman et al., 1994). Further studies are warranted to clarify the role of BVDV in bovine mastitis. No intramammary inoculation of cows with BVDV has been reported, and in addition there are no reports on the isolation of BVDV from milk of cows with mastitis. However, BVDV genomic sequences can be detected by PCR in milk and bulk milk samples (Radwan et al., 1995; Drew et al., 1999), but this is likely to be caused by the presence of persistently infected cows in the herd, and consequently does not

mean that the virus is involved in a direct or indirect way in bovine mastitis cases.

4.2.3 Bovine leukaemia virus

An association of a virus infection with a higher susceptibility for bovine mastitis has also been suggested for BLV. The primary target cells for BLV are cells of the B-lymphocyte lineage in cattle. Infection of B-lymphocytes may influence the humoral immune responses, e.g. a reduction in plasma IgM levels, and the cellular responses are very probably as well impaired in BLV-infected cattle (Yamamoto et al., 1984; Meiron et al., 1985). These data suggest that the immune system of BLV infected calves can be affected, although its effect on the incidence and the severity of bovine mastitis cases is unclear.

4.2.4 Bovine immunodeficiency virus

Bovine immunodeficiency virus (BIV), a lentivirus within the *Retroviridae* family, was detected for the first time in 1972. Although most infections run a subclinical course, BIV infections may also result in clinical signs such as lymphadenopathy, lymphocytosis, lesions of the central nervous system, wasting and several secondary bacterial infections (Snider et al., 1996). Lymphoid depletion with a reduction of the follicular development and depletion of B and T-cell compartments in lymph nodes are observed in BIV infected animals. Secondary infections were often multiple such as, e.g. metritis and mastitis (Snider et al., 1996). In this study, 24 (40%) out of 59 cows with a BIV infection showed chronic mastitis. Necrotising udder tissue were recorded in combination with a few udder pathogens like *Escherichia coli*.

The effect of co-infection with BLV and the influence of immunosuppression on the severity of chronic bovine mastitis cases remains to be of interest for future investigations.

5. Other viral infections, including non-bovine related virus infections, of the bovine mammary gland

A few other viral infections have been associated with bovine mastitis. For example, mastitis, which may be secondary, has been attributed to a systemic virus disease such as malignant catarrhal fever (Beckman, et al., 1960). This report suggests that severe lesions in the mammary gland may account for a decline in milk production and cracking of the epithelium of the teats. However, this is the only report on any possible relation between malignant catarrhal fever and mastitis.

5.1 Rinderpest virus

The role of rinderpest virus, a Morbillivirus and one of the members of the *Paramyxoviridae* family, in bovine mastitis has not been examined thoroughly. Infection of two swamp buffaloes with a rinderpest virus, isolated from the spleen of an infected buffalo, resulted in clinical signs as fever, depression and conjunctivitis, and vesicles appeared on lips and mammary gland (Tesprateep et al., 1987). Natural primary or secondary cases of mastitis due to rinderpest virus infections have not been reported.

5.2 Bovine enterovirus

Bovine enteroviruses are members of the genus *Enterovirus* in the *Picornaviridae* family. This virus has been isolated from healthy cattle and cattle with enteric, respiratory and reproductive disease problems (Knowles and Mann, 1990), but its role in mastitis is unknown. After an intramammary inoculation of two cows with bovine enterovirus, an acute catarrhal mastitis with marked increased milk SCC, and only mild clinical symptoms were recorded in both cows. The virus was isolated from milk of inoculated quarters (Straub and Kielwein, 1965).

5.3 Non-bovine related viruses

Also non-bovine related viruses have been shown to replicate in the bovine mammary gland after intramammary inoculation (reviewed by Afshar and Bannister, 1970). An inflammatory reaction in the bovine mammary gland was produced by infusion of the Newcastle disease virus (strain Roakin). The intramammary inoculation of 3 cows, one of which did not lactate, with Newcastle disease virus resulted in udder swelling and an increase in body temperature in one of the inoculated cows. An

increase in milk SCC was recorded in the two inoculated lactating cows, and the milk also contained virus on day 6 after inoculation, but not on day 9 (Easterday et al., 1959).

Mitchell et al. (1956) have demonstrated that influenza and NCD viruses will multiply when inoculated into the mammary gland (Mitchell et al., 1956). No mention was made of any inflammatory processes. These studies show that non-bovine viruses are able to replicate in the bovine mammary gland.

6. Concluding remarks

This review shows that viruses can be involved, in a direct or indirect way in the aetiology of bovine mastitis. In natural cases of mastitis, BHV1, BHV4, FMD and PI3 viruses have been isolated from milk. In addition, experimental infections via the ductus papillaris clearly demonstrated that these viruses replicated in the mammary gland tissue, followed by clinical mastitis in the cases of BHV1, FMD virus and PI3 virus infections, and subclinical mastitis after a BHV4 infection. However, no investigations have been performed to examine whether this route of infection is of importance in the field. Because bacterial pathogens usually infect the udder through the ductus papillaris, it may be expected that also viruses can infect the mammary gland tissue via this route after transmission by, e.g. milking devices. Especially in cases when hygienic measures are not well taken. However, we assume that BHV1, BHV4, FMD and PI3 viruses are mostly transmitted by direct contact and by aerosols. That an experimental infection through the natural route leads to mastitis, has been shown only for FMD (Table 1). However, in the Western world FMD virus induced bovine mastitis is not of practical relevance because cattle infected with FMD virus will be destroyed immediately after the diagnosis has been made. With regard to BHV1, BHV4, PI3 virus and BLV infections, the data in the literature do not convincingly demonstrate that these viruses can play a primary role in causing mastitis in the field.

It is likely that viruses that cause teat lesions (BHV2, cowpox, pseudocowpox, FMD, vesicular stomatitis virus, and papillomaviruses), and thereby damaging the integrity of the bovine udder, indirectly contribute to mastitis. The impact of these virus infections may be great within individual herds but less within regions or districts of countries.

Although it is plausible that virus-induced immunosuppression underlies mastitis, there are no data that underpin this assumption. In addition, only very few well-designed epidemiological studies have been performed to support a causal relationship between virus infections and mastitis.

Further research should be performed to firmly establish the importance of viral infections on bovine mastitis in the field. Such research should certainly take into account that mastitis is a multifactorial disease, consequently such studies are difficult to design. Investigations should deal with well-designed case-control studies, more experimental viral infections whether or not in conjunction with bacterial infections, and various epidemiological studies. Application of new more powerful diagnostic tools, based on the detection of viral genomic sequences e.g. by (multiplex) PCR or micro-array devices, offer new opportunities for a rapid simultaneous detection of most viruses involved in bovine mastitis. In the future, these new screening methods may also provide a better insight in the prevalence of viruses in milk from cows with mastitis.

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