Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis: BHV-1 infections

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Summary: This short review article summarizes recent information on the molecular biology of bovine herpesvirus 1 (BHV-1) and the IBR-like as well as IPV-like genomes. This may provide a better understanding of BHV-1 latency.

DNA fingerprinting of isolates allows us to trace diseases and gain a picture of the molecular epizootiology. The major immunogenic proteins, especially a 74 Kd glycoprotein which gives rise to neutralizing antibodies, have been defined as those structural components being involved in cross-reactions with the goat herpesvirus (BHV-6) and being of importance for diagnostic assays.

Besides the known clinical appearance of BHV-1 infections, expressing themselves mainly as respiratory or genital infections, the immune response is discussed and its value outlined for diagnostics and epizootiology.

In conclusion, recommendations are given to control or eradicate BHV-1 infections.

KEYWORDS: Bovine herpesvirus - Cattle diseases - Immunity - Latent infection - Viral diseases.

INTRODUCTION

This review gives a synthesis of clinically orientated data with recent information on molecular biological and immunological research in this field. A more precise understanding of the genetics and immunological function of bovine herpesvirus 1 (BHV-1) may have consequences for prophylactic and control measures concerning the corresponding diseases.

Previous reviews on these cattle diseases and the infectious agent, bovine herpesvirus 1, deal in more detail with clinical aspects (24, 13, 21, 43, 29) or the latest biological aspects of the virus (26).

HISTORY

In continental Europe the genital form of BHV-1 infection has been known for more than 100 years. The first reliable report stems from Rychner (39) in Switzerland who mentioned, in 1841, a venereal disease (in a bull and several contact cows) which was subsequently referred to as "Bläschenausschlag" (45) or "Exanthema
vesiculoso/pustulosum coitale" (51, 49). Reisinger and Reimann (37) were the first to show the filterability and transmission of the infectious agent, demonstrating that this disease was not of bacterial origin. It took thirty years for the virus to be isolated and it was then named "infectious pustular vulvovaginitis (IPV)" virus (23, 18).

In contrast to this clinical picture, infectious bovine rhinotracheitis (IBR) was first described in the 1950's in Colorado and California (40) from whence it spread rapidly. The close antigenic relationship between IBR and IPV viruses and the induced clinical pictures were recognized by Gillespie et al. (14). In 1960 the first case of an IBR virus infection was reported on the European continent (19), followed by descriptions of this respiratory tract infection in the United Kingdom and in other countries. Further tracing of these infections throughout the world was (and still is) hampered by the fact that serological surveys performed subsequently in different countries could not distinguish between IBR- and IPV-virus infections because of the antigenic cross-reactivity. No fundamental distinction was made between the genital and respiratory manifestations of the virus, and they have been indiscriminately called IBR-IPV virus infections since that time.

CLASSIFICATION AND BIOLOGY OF THE VIRUSES

Genetic material

The IBR/IPV viruses have been classified as BHV-1. This is based on serology. Their identity was later proved by DNA studies. With certain restriction enzymes such as 'HpaI', however, a clear separation into IBR-like and IPV-like strains is possible, grouping all the foetal isolates, some of the brain isolates, and abortigenic viruses with the IBR-like viruses (26). Different findings with viruses from other countries (34, 7, 32), where the classical picture of European IPV is not known or has become modified, most probably reflect rapid changes of the viruses in cattle populations.

BHV-1 does not cross-react with BHV-2 (bovine herpes mammillitis virus), with the African malignant catarrhal fever virus (tentatively grouped as BHV-3) or with BHV-4 (the Movar-type virus) (26, 46). Caprine herpesvirus (tentatively grouped as BHV-6; 10) is antigenically and serologically closely related to BHV-1, but clearly differs in DNA fingerprinting.

The BHV-1 genome is similar to that of pseudorabies virus, equine herpesvirus types 1 and 3, and varicella-zoster virus, belonging to the class D herpesviruses. The BHV-1 genome has a molecular weight of 135 Kd and is known to be composed of a unique short (Us; 13 Kd) sequence which inverts relatively to the unique long (UL; 100 Kd) sequence, leading to two isomeric forms of DNA which are flanked by internal (11 Kb) and terminal repeats (11 Kb). Recent findings show that the BHV-1 genome has a small unique DNA “tail” on the terminal repeat sequence (Hammerschmidt, personal communication) which might be involved in replication and could turn out to be important for the latency of the virus. Short repeats in BHV-1 DNA terminal fragments which are responsible for the heterogeneity of the genomes appear to play a role in the circularization or concatamerization of the viral genome during DNA replication and subsequent cutting. Although BHV-1 replication is far from being understood, genome structures of this virus have proved to be a good model of the general properties of herpesviruses, e.g. the terminase cleaving the DNA into the right genome length size (20).
Proteins and antigens

BHV-1 induces approximately fifty infected cell proteins, about thirty of which are structural proteins of the virus, and approximately fifteen are non-structural polypeptides. Eight to eleven are glycosylated (33). The glycoproteins appear on the outer envelope of viral particles as well as on the surface of infected cells and represent the main target for the immune system, inducing a response which may neutralize free virus and destroy infected cells.

BHV-1 glycoproteins are divided into three groups, each consisting of two or three polypeptides which appear to be different forms of the same basic molecule, i.e. as a monomer or dimer or as a precursor and its final products (47, 28). It emerged from different studies — and was essentially pointed out for the first time by Gregersen (15) — that a glycoprotein with a molecular weight between 74,000 and 82,000 represents the main immunogenic component, which induces the strongest neutralizing immune response (8, 16, 28). Therefore, we believe that the 74 Kd glycoprotein is the most suitable candidate for a possible future subunit or recombinant vaccine. Antibodies against other glycoproteins seem to neutralize BHV-1 weakly or only in the presence of complement (16, 28).

BHV-1 strains of different origin and pathogenicity exhibit a high degree of uniformity in their protein composition, although strain-specific differences were observed with some minor polypeptides (16, 31). It is of special interest that the goat herpesvirus (BHV-6) shares the major immunogenic glycoproteins with BHV-1, which is the reason for strong immunological cross-reactivity and a marked one-way cross-neutralization of BHV-6 by antibodies directed against BHV-1 (10, 15, 22, 25).

CLINICAL MANIFESTATIONS

BHV-1 infections are known as the classical picture of infectious pustular vulvo-vaginitis (or infectious balanoposthitis in bulls) and typical infectious rhinotracheitis.

IBR is associated with more or less severe affliction of the respiratory tract. The pathological pictures vary from slight inflammation of the mucous membranes to severe alterations in the tissue of the respiratory tract. Simultaneous infection with other viruses (paramyxovirus, rhinovirus) or bacteria may result in bronchopneumonia. In conjunction with respiratory illness, conjunctivitis is a frequent event which can lead to keratoconjunctivitis ('pink eye'). Another rare form of BHV-1 infection is encephalitis in the calf, seldom seen in adult bovines (12). Recently, variant viruses of BHV-1 have also been isolated from a neurological disease of calves in Argentina (32). BHV-1 has also been reported to cause abortions (30). In this context, it should be mentioned that live attenuated virus vaccines led to abortions, especially in the USA. We identified abortigenic viruses to be of the IBR type (16). In contrast, live virus vaccines based on IPV-like viruses were non-abortigenic (26).

The typical clinical entity defined as IPV (or as "Bläschenausschlag" in German-speaking countries) was widespread in small farms in Europe where the bull transmitted the disease. A circumscribed exanthema on the vulva and in the vagina, or balanoposthitis in male bovines, is associated with pustules followed by necrotic erosions of the genital mucosa. Good healing tendency appears to be typical.
The clinical appearance of BHV-1 infections has changed in the past decade. With the introduction of artificial insemination, IPV has become rare whereas IBR is now the prominent clinical entity, distributed world-wide in cattle. Rapid transmission of IBR-like viruses in large herds under crowded conditions may have led to variations in IBR virus isolates, identified by DNA fingerprinting. It is conceivable that viruses able to spread easily, and having a high replication rate, extensively use their genomic ability of variability (20).

IMMUNE RESPONSE

Humoral immunity

This is the most prominent immune response, and measurement of neutralizing antibodies is usually taken as the sole parameter for the immune status. Although neutralizing antibodies against the 74 Kd and a 90 Kd glycoprotein clearly reflect that an infection has taken place in the herd (16, 28), it is conceivable that BHV-1 infections without neutralizing antibodies can be traced by using sensitive ELISA tests. In all assay systems except for the neutralization test, cross-reactions with other bovine herpesviruses have always raised doubts concerning the specificity of the reactions.

More refined methods and further investigations are necessary to definitely clarify this point. In the meantime, the neutralization test positive with 1:2 diluted serum can be regarded as proof of infection in a herd. A cross-reactivity of BHV-1 with BHV-6 might cause diagnostic problems in countries where goats and cattle are tested. Both viruses induce a strong cross-neutralization response, although the response is always stronger in the homologous system (22).

As in other herpesvirus systems, even a strong humoral immune response does not prevent BHV-1 latency. The status of the immune response, however, is believed to control the amount of activated virus. In any case, each recurrence boosts the existing antibodies. In general, one should consider that the humoral immune response only partly reflects the immune status, and that the cellular immune response, which has been less investigated, might play a more important role in defence against the disease.

Cell-mediated immunity

A strong humoral immune response may neutralize free virus, but BHV-1 cannot be eliminated from the body. *In vitro* models where infectious virus spreads from cell to cell in the presence of neutralizing antibodies exemplify the situation in the animal. Although little is known about the cellular immune response against BHV-1, several mechanisms attack infected cells, such as a specific cell-mediated immunity by helper and suppressor T-lymphocytes and cytotoxic T-cells as well as their precursors, and a non-specific response by natural killer cells and antibody-dependent cellular cytotoxicity (ADCC). A balanced activation of such lymphocytic sub-sets — as achieved by replicating virus — is essential for an effective T-cell response. Non-replicating herpesviruses and their glycoproteins are weak antigens for the stimulation of a cellular immune response (38). This may explain why inactivated BHV-1 vaccines were considered to be less effective than live virus vaccines. However, an increased amount of antigen, a better presentation of inactivated antigens, and appropriate adjuvants may lead to sufficiently effective inactivated vaccines.
The fact that newborn calves are more susceptible to BHV-1 infections, and more often show severe clinical signs including encephalitis, may be due to immature cellular immune functions.

Testing for the delayed type of hypersensitivity enables us to detect a T-cell immune response in vivo (9) even if neutralizing antibodies are not traceable (3). Because the required simple and sensitive routine methods for testing cell-mediated immunity are not commercially available, we are still unable to define the real immune status of BHV-1 infected animals.

Local immunity

Humoral and cellular immune mechanisms cooperate in eliminating infectious virus, and are thus of major importance for the epizootiology of BHV-1 infections, whereas local immunity strongly influences the severity of the disease in the infected individual.

Non-specific defence mechanisms (i.e. anatomical structures, mucus, cilia, interferon, macrophages), locally produced antibodies and a local cell-mediated immune response all cooperate at mucous membranes and comprise the local immunity. Among different secretory immunoglobulins IgA predominates (5). It is mainly effective in the upper respiratory tract, whereas cellular mechanisms could play a major role in recovery from severe lung infections. Natural and artificial BHV-1 infections induce the production of interferon on respiratory and genital mucous membranes (4) where it contributes directly, or by the stimulation of other immune mechanisms, to an early immunity (44, 48).

Immunosuppression

Recent immunological studies indicate that the immune response against a BHV-1 infection not only stimulates, but also partly inhibits immunological functions, leading to an increased susceptibility for secondary bacterial infections (50). BHV-1 infections have been reported to suppress the chemotactic response of neutrophils, natural cell-mediated cytotoxicity, and the responses of peripheral blood lymphocytes to mitogens. Decreased immunological functions against secondary respiratory bacterial infections were also observed when no cell damage on respiratory mucous membranes could be noted after intramuscular application of virus (11). Live virus vaccines may have a similar suppressive effect on the immune response in cattle.

EPIZOOTIOLOGY

Infectious pustular vulvovaginitis or balanoposthitis was the only clinical form of BHV-1 infection seen during the century which followed its first description. Transmission of virus occurs from individual to individual by direct genital contact. Therefore this disease spreads relatively slowly. In regions where only small cattle herds are kept and artificial insemination is not exclusively performed, the situation has not changed much. Artificial insemination has drastically reduced the IPV cases.

BHV-1 gained major economic importance when a virus variant, which caused severe respiratory infections, appeared in the late 50's. This virus spread rapidly in feedlots and in cattle populations with high fluctuation. Exports of cattle and
semen from the United States to almost every continent subsequently spread the
new respiratory disease all over the world. Virus latency has certainly contributed
as a co-factor to its efficient distribution. This reconstruction of events can be
deduced from recent molecular epizootiology based on restriction enzyme analysis
of BHV-1 DNA's in selected isolates with known disease history. The results
demonstrate differences between IBR and IPV isolates. With a suitable restriction
enzyme (e.g. Hpal), discrimination between the classical IPV-like and IBR-like
virus strains is possible. IBR-like viruses seem to be more pathogenic since isolates
from abortions and encephalitis are associated with the IBR-like Hpal restriction
pattern (16). It should be noted that IPV viruses can cause mild respiratory diseases
and vice versa, IBR strains have been isolated from the genital tract (14), which fur­
ther complicates the situation. IBR viruses among themselves exhibit a striking
homology, confirming the theory that a single variant was originally responsible for
the sudden appearance of respiratory infections. On the other hand, IPV isolates
show a divergence within their DNA-fingerprints, which indicates that different
virus types may have developed within decades. Although the appearance of new
strains with altered pathogenicity seems to be rather a rare event, BHV-1 is not
totally stable. The recent findings that a specific neuropathogenic strain from calves
has a different genome pattern (7, 32) indicate that another highly pathogenic IBR
virus variant has developed.

Distribution

BHV-1 occurs throughout the world. Infections occur regularly in European,
Northern American and some African countries, as well as in Australia. Although
reports from certain Asian, African and South American countries are not avail­
able, it can be assumed that BHV-1 infections are also present in those regions, but
are simply not diagnosed or not reported because other diseases are more impor­
tant. Apart from cattle, neutralizing antibodies against BHV-1 have also been
found in other animals, partly phylogenetically related, like goats, buffaloes, wilde­
beest, red deer and other free-living wild ungulates. We cannot prove that these
antibodies are induced by BHV-1 infections because they might have been origin­
ated by other cross-reacting herpesviruses.

Latency and reactivation

A general feature of all herpesviruses is their ability to remain latent. This topic
is discussed in detail by Thiry et al. in this issue. Here, the major characteristics of
latent BHV-1 infections and their consequences for efficient disease control are
summarized. Viral latency must be considered as a regular event, and is certainly
not exceptional. It is accepted that the regional ganglia are a major site for latency.
The virus persists there as a DNA copy (1, 2). Other sites for latent virus may exist,
because BHV-1 has been isolated from normal calf foetuses (27).

Reactivation of latent BHV-1 occurs spontaneously under the influence of natu­
ral stress factors (diseases, transportation, parturition), or may be induced artifi­
cially by corticosteroid treatment (41, 36). Reactivated virus may cause recrudes­
cence of the disease and virus is shed from the local mucous membranes as well as
in semen from bulls (17). Problems and principles concerning latency and reactiva­
tion are also valid in cases where live virus vaccines are used. It is well accepted that
antiviral antibodies have no effect on latency and recrudescence. Humoral anti­
obodies do not prevent the establishment of latent infections (35, 42), but may
reduce the amount of excreted virus, and may influence the course of recrudescent disease.
Latency after BHV-1 infections or live virus vaccination is an obstacle for the control of this disease. It reduces the value of diagnostic efforts since successful virus isolation or the presence of antibodies (or a fourfold increase of antibody levels) do not always indicate an external infection. It may also indicate reactivation of a latent infection. Furthermore, an animal once infected must be considered as a virus carrier for years, and thus a potential source of infection for other individuals.

**PROPHYLAXIS AND DISEASE CONTROL**

Sensitive and simple diagnostic methods are a basic requirement for the successful control of IBR/IPV infections. Neutralization tests and virus isolation are used mainly to detect infected animals or virus carriers. Both methods are rather tedious, require the use of cell cultures and thus can only be performed in laboratories with appropriate experimental equipment and highly-trained personnel. Neutralization assays should be done as a plaque reduction assay using not more than 100 plaque-forming units per assay, and should start with at least a 1:2 dilution of inactivated serum to guarantee maximum sensitivity. The detection of chronic virus carriers by virus isolation should be conducted after prior activation of a latent infection by corticosteroid treatment of animals. Virus neutralization tests and virus isolation followed by virus neutralization with a specific antiserum give the highest specificity. However, ELISA or RIA tests are more practical and are used for testing large numbers of blood or milk samples, although their specificity may be somewhat lower. Their advantage is higher sensitivity, which may be useful for detecting low antibody titres in latently infected animals.

The delayed hypersensitivity test may be very useful, too, for detecting latent infections, but this test is still at the experimental stage. It is important for the test not to evoke humoral antibodies which could interfere with serological antibody tests. None of these test procedures differentiates between IBR and IPV virus infections, although, in several countries, the governmental regulations treat venereal (IPV) and respiratory (IBR) diseases differently. Normally, only genitally transmitted BHV-1 infections are subject to regulations. It should be recommended that both diseases be controlled without distinction.

**Successful disease control may be achieved by the following measures:**

1. Elimination of risk factors by artificial insemination, separation of positive from pre-tested negative animals, export restrictions for BHV-1 positive animals, and embryos or semen from positive animals.

2. Establishment of BHV-1 free herds. This includes serological testing twice a year, removal of positive reactors and strictly separate handling of animals from positive and negative herds. Breeding cattle and bulls in artificial insemination centres should be kept apart in units composed of animals free from virus and antibody.

3. Animals in BHV-1 positive herds may be vaccinated, if at all, preferably with inactivated vaccines to avoid the risk of latent infections. Live virus vaccination should be avoided. The only indication for application of live attenuated vaccines is a direct danger of infection in a certain herd.

The aim of successful disease control should be the absence of virus, and not just of disease. Thus, an early decision as to whether to combat disease or to elimi-
nate the infectious agent is required, because the control of overt disease by vacci-
nation interferes with the total elimination of the infectious agent. A complete,
organized strategy is a fundamental condition to achieve control of BHV-1 infec-
tions.

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REFERENCES

Genitaltrakt nach experimenteller Infektion mit Rhinotracheitis (IBR)- und Bläschen-
ruminant herpesviruses including bovine, buffalo and caprine herpesvirus 1 and bovine
8. COLLINS J.K., BUTCHER A.C., RIEGEL C.A., MCGRANE V., BLAIR C.D., TERAMOTO
Y.A. & WINSTON S. (1984). — Neutralizing determinants defined by monoclonal antibo-
9. DARCEL C. LE Q. & DORWARD W.J. (1972). — Skin reactivity and infectious bovine rhino-
bovine herpesvirus type 1 on bovine leucocyte functions. Infect. Immun., 42, 106-112.
12. FRENCH E.L. (1962). — A specific virus encephalitis in calves: Isolation and characteriza-
lar vulvovaginitis virus with infectious bovine rhinotracheitis virus. Cornell Vet., 49,
288-297.


