Prevention of disease transmission by semen in cattle

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Abstract

To test the safety of semen two approaches can be applied: checking the end product, or continuous surveillance of the bulls before and after semen production. The first method is examination of semen for the presence of infectious agents. This method depends completely on a single investigation and therefore relies only on the sensitivity of the test method. The second method is testing the bulls for diseases before and after semen collection, based on sequential investigations for the absence of either pathogens or antibodies against infectious agents. The EU-Directive 88/407 prescribes that bulls in AI stations must be monitored for the absence of diseases, but only at 12-monthly intervals, which is a severe disadvantage. Furthermore, the directive is specific neither in the tests to be carried out nor in the specification of some pathogens (e.g. Campylobacter foetus).

A programme is presented based on monthly testing of a limited number of bulls for the absence of endemic diseases only, on the basis of Hazard Analysis of Critical Control Points (HACCP). This method only applies to diseases with high transmission rates. Testing some 20\% of the animals on a monthly basis can monitor these highly contagious diseases (e.g. IBR). To monitor BVDv infections, however, monthly testing of all negative animals and semen culture or semen PCR of animals that have seroconverted for this virus seems necessary. Endemic diseases with slow transmission rates in bulls do not suit such a system and can only be monitored on an individual basis. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The main goal for artificial insemination (AI) in cattle is to achieve genetic improvement. However, transmission of infectious diseases by semen constitutes a risk which must be avoided. Semen used for AI must therefore be free of infectious agents.

The EU-Directive 88/407 EEC (EC, 1993) prescribes the health status for bulls in AI stations and the tests to be applied to evaluate this status. The Directive prescribes as a minimum requirement the testing of the health of bulls in AI centres at intervals of 12 months. Free semen trade is allowed for the next 12 months when the bull herd is found to be negative. If, in the next investigation, some animals or the whole herd are found positive, semen of the positive animals collected since the last clear test must be destroyed: most semen will have been used by that time and as a consequence infections might have been transmitted by semen.

Furthermore, the Directive is insufficiently specific with regard to the definition of the pathogens and
prescription of the diagnostic tests to be applied. The sensitivity (and specificity) of diagnostic tests greatly influence the outcome of a screening (positive or negative) and thus also affect the safety of semen released for trade.

Disease agents differ in the number of new cases per primary case per infectious period. The transmission rate depends on the number of infectious animals, the length of the incubation period, the length of the infectious period, and the number of contacts with susceptible animals. The risk of transmission by semen also differs between agents.

Therefore, uniform treatment of all diseases in the Directive includes risks of spreading a disease on the one hand, and may restrict semen trade unnecessarily on the other.

In this article we give concise descriptions of diseases mentioned as having potential venereal transmission in the literature and categorise them according to their potential risk for transmission by semen. For AI centres we propose monitoring for the presence of diseases with high transmission rates frequently by random testing. Diseases with low transmission rates and limited risk for transmission by semen should be monitored annually. Diseases that have been (officially) eradicated should not be investigated.

2. Concise description of diseases in OIE lists A and B and other diseases of relevance to artificial insemination

A concise description is given below of relevant infectious diseases. A summary is given in Table 1 (Horzinek, 1990; OIE, 1996; Eaglesome and Garcia, 1997).

2.1. Viral diseases

2.1.1. Foot-and-mouth disease

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals characterised by high fever followed by vesicles on the mucosa of mouth and tongue, the feet and the udder. FMD is caused by a Picornavirus comprising seven serotypes; infection with one serotype does not induce immunity to other serotypes.

In order to prevent spread of the disease in a country, immediate recognition of the clinical picture is essential. For a positive diagnosis the demonstration of FMD viral antigen by indirect sandwich ELISA techniques preferably in the epithelium of unruptured or freshly ruptured vesicles is sufficient.

The agent is very resistant to environmental factors and may be spread over long distances by air and/or vehicles and/or man. FMD virus is easily transmitted by semen.

2.1.2. Enzootic bovine leukemia

Enzootic bovine leukemia (EBL) is found clinically by enlarged lymph nodes or other lymphosarcomas, or by lymphocytosis in the peripheral blood in the minority (some 30%) of infected animals. Most infections pass completely unnoticed.

The disease is caused by the retrovirus Bovine leukaemia virus (BLV), which may be demonstrated in the blood by PCR-techniques. Routine diagnosis is performed by serology (ELISA, AGID).

The agent does not survive in the environment. Only intact lymphocytes are infective: the infection is transmitted by blood. Transmission by semen is very unlikely (Monke, 1986; Straub, 1988).

2.1.3. Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) is a disease of the upper respiratory tract, or of the genital tract, respectively. IBR is characterised by clinical signs such as high fever, nasal discharge, ocular discharges, and abortion in up to 30% of pregnant animals. The disease has a high morbidity but a low mortality. However, the disease might follow a more severe course if complicated by bacterial infections. IPV is characterised by erosions in the vagina or on the penis leading to adhesions.

The disease is caused by Bovine Herpes Virus type 1 (BHV1) which can be subdivided into strains with a predilection for the respiratory or genital tracts, respectively. Diagnosis is made by demonstration of the virus in secreta from respiratory or genital tracts during the 14 days following infection, and by serology.

BHV1 infections lead to latency. Latently infected animals are the source of new epidemics. The agent may survive in favourable conditions (high relative
Table 1
The main characteristics of infectious diseases that (possibly) could be transmitted by semen or artificial insemination

<table>
<thead>
<tr>
<th>Disease</th>
<th>List OIE</th>
<th>Incubation period</th>
<th>Reservoir</th>
<th>Excretion mainly by</th>
<th>Period of transmission/viraemia</th>
<th>Transmission by semen/Art.</th>
<th>Test sensitivity (serol.)</th>
<th>$R_o$ in bull stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD</td>
<td>A</td>
<td>2–8 days</td>
<td>Animals in the acute phase</td>
<td>Saliva</td>
<td>&lt;14 days</td>
<td>++</td>
<td>&gt;99%</td>
<td>&gt;3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chronic carriers (cattle)</td>
<td>Other secreta / carriers for up to 2 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukosis</td>
<td>B</td>
<td>Up to 35 days</td>
<td>Chronically infected cattle</td>
<td>Intact blood</td>
<td>For life after infection</td>
<td>0</td>
<td>AGID 90%</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Latently infected animals</td>
<td>Nasal discharge</td>
<td>2–20 days</td>
<td>+</td>
<td>ELISA 95%</td>
<td></td>
</tr>
<tr>
<td>Rinderpest</td>
<td>B</td>
<td>2–5 days</td>
<td>Cattle in the acute phase</td>
<td>All se- and excretions</td>
<td>21 days or longer</td>
<td>+</td>
<td>99.6%</td>
<td>&gt;3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue tongue</td>
<td>A</td>
<td>3–6 days</td>
<td>Biting insects</td>
<td>Viraeemia</td>
<td>Viraeemia mostly &lt;14 days</td>
<td>0</td>
<td>?</td>
<td>n.a.</td>
</tr>
<tr>
<td>BVD</td>
<td>–</td>
<td>2–15 days</td>
<td>Persistently infected (pi) cattle</td>
<td>Saliva</td>
<td>2–15 (56) days</td>
<td>+</td>
<td>ELISA 97.8%</td>
<td>&gt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carriers for up to 2 years</td>
<td>All se- and excretions of pi animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant catarhal fever</td>
<td>–</td>
<td>From a few days to year(s)</td>
<td>Sheep during lambing period</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0</td>
<td>n.a.</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Akabane virus</td>
<td>–</td>
<td>??</td>
<td>Biting insects</td>
<td>Viraemia</td>
<td>Viraemia during prolonged periods</td>
<td>0</td>
<td>?</td>
<td>n.a.</td>
</tr>
<tr>
<td>Mycoplasma mycoides</td>
<td>–</td>
<td>2–6 weeks</td>
<td>Chronically infected cattle</td>
<td>Nasal discharge</td>
<td>For life after infection</td>
<td>+</td>
<td>?</td>
<td>&gt;2</td>
</tr>
<tr>
<td>(CBPP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine brucellosis</td>
<td>B</td>
<td>14–120 days</td>
<td>Chronically (latently) infected animals</td>
<td>Vaginal discharge</td>
<td>Prolonged periods</td>
<td>+</td>
<td>Max. 90%</td>
<td>&lt;1 in bull stations</td>
</tr>
<tr>
<td>Bovine tuberculosis</td>
<td>B</td>
<td>&gt;3 weeks</td>
<td>Chronically infected cattle</td>
<td>Nasal discharge</td>
<td>For life after open tuberculosis</td>
<td>+</td>
<td>90%</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Johne’s disease</td>
<td>B</td>
<td>&gt;(12) 24 months</td>
<td>Chronically infected cattle</td>
<td>Faeces</td>
<td>From 18 months before overt clinical disease onwards</td>
<td>0</td>
<td>ELISA 40%</td>
<td>&lt;1 in bull stations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFT 35%</td>
<td></td>
</tr>
<tr>
<td>Leptospira hardjo</td>
<td>B</td>
<td>&lt;7 days</td>
<td>Chronically infected cattle</td>
<td>Urine</td>
<td>Prolonged periods after infection</td>
<td>+</td>
<td>99.8%</td>
<td>&lt;1 in bull stations</td>
</tr>
<tr>
<td>Genital campylobacteriosis</td>
<td>B</td>
<td>&lt;3 days</td>
<td>Chronically infected cattle: bulls without symptoms</td>
<td>Copulation</td>
<td>For life after infection</td>
<td>+</td>
<td>90%</td>
<td>&lt;1 in bull stations</td>
</tr>
<tr>
<td>Haemophilus somnis</td>
<td>–</td>
<td>??</td>
<td>Harbours genital and respiratory tracts in healthy animals</td>
<td>Nasal discharge</td>
<td>For life after infection</td>
<td>+</td>
<td>95%</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Query-fever</td>
<td>–</td>
<td>??</td>
<td>Harbouring (serol.)</td>
<td>Effluents from genital tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Q-fever)</td>
<td></td>
<td></td>
<td></td>
<td>Fetal membranes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genital trichomonos</td>
<td>B</td>
<td>&lt;3 days</td>
<td>Chronically infected cattle: bulls without symptoms</td>
<td>Vaginal discharge</td>
<td>Prolonged periods</td>
<td>0</td>
<td>65%</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Bovine spongiform encephalopathy (BSE)</td>
<td>B</td>
<td>4 years</td>
<td>CNS of contaminated cattle</td>
<td>Contaminated food</td>
<td>No transmission reported</td>
<td>0</td>
<td>No test</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* 0, unlikely; +, possible; ++, easily.
* a Based on information from the Animal Health service, Deventer, the Netherlands, and ID/DLO, Lelystad, the Netherlands.
* b Best estimations based on literature cited.
* c ?, no experience and no information found in the literature.
* d n.a., not applicable.
humidity, low temperature) for 30 days in the environment. BHV1 is easily transmitted by semen (Van Oirschot, 1995).

2.1.4. Rinderpest

Rinderpest is a very contagious disease, mainly of cattle and buffaloes. The disease is characterised by high fever, erosions on the gums, tongue and palate, together with nasal and ocular discharge and diarrhoea. Rinderpest is caused by a Morbillivirus that can be demonstrated in blood or lymph nodes and spleen, mainly by culture.

Infected animals are the main means of transmission. The virus survives in the environment for limited periods. Transmission by semen is possible.

2.1.5. Blue tongue

Blue tongue (BT) occurs mainly in small ruminants but may also occur in cattle. If clinically overt (many infections pass unnoticed in cattle) the disease is characterised by fever, facial oedema, haemorrhages and ulceration of the mucous membranes.

An Orbivirus consisting of 24 serogroups causes the disease. The virus is identified by culture and by PCR techniques on blood samples from febrile animals.

The agent is transmitted by insects of the genus Culicoides; transmission from cow to cow has not been reported. Transmission by semen is very unlikely.

2.1.6. Bovine virus diarrhoea

Bovine virus diarrhoea (BVD) is normally observed clinically as transient diarrhoea in only 5% of infected animals. In exceptional cases severe diarrhoea, fever, ulceration of the buccal mucosa, haemorrhages and death can occur. The remaining 95% of infections pass unnoticed. When infected in the first 4 months of pregnancy, cows may deliver persistently infected (pi) calves that shed the virus throughout life.

The pestivirus bovine virus diarrhoea virus (BVDv) causes the disease. Diagnosis is by antigen ELISA or culture of the virus from the peripheral blood, and by serology.

Persistently infected animals transmit the virus throughout life and infect sentinel animals easily ($R_0 > 2$), but primarily infected animals transmit the virus for a limited period (the virus can be demonstrated in broncho-alveolar washings up to 56 days after infection). The transmission rate by primarily infected animals is below 2. However, one exceptional bull has been described that shed BVDv in the semen over prolonged periods (11 months) though the animal was serologically positive for BVDv. It may be speculated that a primary infection during puberty or an intrauterine infection around the time of maturation of the immune system could have caused this uncommon pattern.

The agent survives for some days in the environment. Transmission by semen is easy (Meyling and Jensen, 1988; Kirkland et al., 1991; Kommisrud et al., 1996; Kirkland et al., 1997).

2.1.7. Malignant catharral fever

Malignant catharral fever is a sporadic, almost invariably fatal disease of cattle of all ages. The disease is characterised by high fever, bright red coloration of all mucous membranes, enlarged superficial lymph nodes, and very often diarrhoea.

A Herpes virus (BHV3) causes the disease. Diagnosis in the live animal is done on clinical signs, and by PCR on heparinized blood. Culture of the virus is not possible.

Information on the resistance of the virus against environmental influences is not, therefore, available. Cow to cow transmission has never been reported. Transmission by semen is very unlikely.

2.1.8. Akabane virus

Akabane virus infections were shown to cause sporadic epizootics of premature births and developmental deformities in the newborn (arthrogryposishydramnioncephaly) in cows, sheep and goats. The disease is reported from Southeast Asia, the Arabian Peninsula, the Middle East and African countries.

Diagnosis is done retrospectively by serology in adult animals and by culture techniques in abnormal foetuses and calves.

Biting insects transmit the agent. After experimental infection in bulls Akabane virus was not demonstrated in the semen; transmission by this route is therefore unlikely (Parsonson et al., 1981).
2.2. Bacterial diseases

2.2.1. Contagious bovine pleuropneumonia

Contagious bovine pleuropneumonia (CBPP) is characterised by fever, dyspnoea, coughing, nasal discharges and anorexia. CBPP is caused by Mycoplasma mycoides ssp. mycoides, which can be demonstrated in nasal secreta and bronchoalveolar washings, or in pleural fluids collected after puncture by culture in appropriate media.

The agent is vulnerable to environmental influences and is transmitted by infective animals over limited distances. Transmission by semen is possible.

2.2.2. Brucellosis

Brucellosis is manifested by abortions in the last third of pregnancy. The causative organisms are excreted in abundance with uterine discharges and with milk (Berchovich, 1998).

The disease is caused by Brucella (B.) abortus, occasionally by B. melitensis (lower numbers of aborting cows in the herd), and exceptionally by B. suis. The disease is diagnosed by culture of the causative organism from uterine discharges and milk, by serology (CFT, BUA), or by a specific skin test (Berchovich et al., 1989).

The organisms survive in the environment in favourable circumstances for prolonged periods. Brucellosis is a zoonosis. Transmission by semen is possible.

2.2.3. Bovine tuberculosis

Bovine tuberculosis passes unnoticed in the early phases after infection, but in advanced cases emaciation, coughing and enlargement of the lymph nodes develop.

The disease is caused by Mycobacterium (M.) bovis and occasionally by M. tuberculosis (the human tuberculosis strain) when a human infective source is present.

Tuberculosis in live animals is diagnosed by a specific skin test, gamma-interferon assay using peripheral blood lymphocytes, serology (ELISA), and by culture from nasal secretions.

The organism is resistant to environmental influences and may survive for several months. Tuberculosis is a zoonosis. Transmission by semen is possible.

2.2.4. Johne’s disease

Johne’s disease (paratuberculosis) is chronic enteritis of ruminants characterised in cattle by severe diarrhoea, emaciation and submandibular oedema.

The disease is caused by Mycobacterium (M.) paratuberculosis, which only leads to clinical disease and excretion after infection at a very young age. Infection leads to progressive thickening of the gut wall causing a protein loss enteropathy with diarrhoea in the final stages after 24 months or longer. M. paratuberculosis was found in the semen of bulls suffering from severe clinical disease.

In the clinical stages, the agent can be demonstrated in faeces, and, exceptionally, in milk and the foetus by culture and/or PCR techniques. The latter method lacks sensitivity when applied to faeces.

For the detection of subclinically infected animals a skin test, ELISA and CFT are applied. Each method has the disadvantages of limited specificity and sensitivity.

Johne’s disease might be a zoonosis (Crohn’s disease in man) (Chiodini, 1989). Transmission by contaminated semen or semen from contaminated bulls has never been demonstrated (Ovdienko, 1974; Larsen et al., 1981; Merkal et al., 1982).

2.2.5. Leptospirosis

Leptospirosis is a contagious disease of animals and humans. Clinically, infections are characterised by fever, icterus, haemorrhages, uraemia, and blood tinged urine.

Cattle are carriers for Leptospira hardjo, but end hosts for other leptospira species. The agent is excreted over long periods in the urine of carrier animals and can be demonstrated by PCR techniques or culture.

Leptospira may survive in the environment in humid and warm condition (summer) for longer periods. L. hardjo is a zoonosis and causes milker’s fever in man. Transmission by semen is possible.

2.2.6. Bovine genital campylobacteriosis

Bovine genital campylobacteriosis is a venereal disease characterised by infertility, early embryonic death and abortion. In bulls, infections are not apparent.

The disease is caused by Campylobacter foetus ssp. venerealis, which in bulls can be cultured from
preputial washings. Serological methods in bulls are unreliable.

*Campylobacter foetus* ssp. *foetus* is a gastrointestinal inhabitant of healthy cattle and may be isolated from the prepuce of bulls. This agent may, in rare cases, cause abortions only after bacteraemia following oral infections.

The organisms survive in the environment for limited periods (hours). Mating and/or semen are the main causes of transmission.

2.2.7. **Query fever**

Query fever (Q-fever) in cattle is characterised by abortions, retained placenta, metritis and infertility.

The disease is caused by *Coxiella (C.) burnetti*. Diagnosis is established by microscopy of foetal membranes or aborted foetuses, or by culture of the agent by inoculation of embryonated eggs with uterine effluents. Routine diagnostic tests are done by serology (CFT, ELISA).

The agent is extremely resistant to environmental influences. Transmission is by biting insects or by inhalation of contaminated dust. Q-fever is a zoonosis. Transmission by semen is limited or unlikely (Kruszewska and Tylewska-Wierzbanowska, 1997).

2.2.8. **Haemorrhagic septicaemia**

Haemorrhagic septicaemia (HS) is a highly fatal disease characterised by initial fever, respiratory signs, and terminal septicaemia leading to recumbency. It is reported from Asia and Africa.

HS is caused by certain strains of *Pasteurella multocida*. The agent can be cultured from blood and from internal organs.

*P. multocida* is moderately resistant to environmental influences. Transmission by semen is very unlikely.

2.3. **Protozoan diseases**

2.3.1. **Bovine genital trichomoniasis**

Bovine genital trichomoniasis is characterised by infertility and abortion. Bulls may be infected without clinical signs.

The disease is caused by the protozoan *Trichomonas foetus*, which comprises three serotypes of equal pathogenicity. The agent can be cultured from uterine discharges and preputial washings.

Trichomonas is rather resistant to environmental influences. Mating and/or semen are the main causes of transmission.

2.4. **Other diseases**

2.4.1. **Bovine spongiform encephalopathy**

Bovine spongiform encephalopathy (BSE) is a fatal neurologic disease of cattle with a long incubation period. Clinically behavioural changes and locomotion disturbances such as ataxia manifest the disease. Feed is generally regarded as the most likely source of infection. After (oral) uptake of abnormal protein the bodily protein is stereometrically altered making the protein invulnerable to proteases, leading to intracellular accumulation of prions. This stereometrical change causes death of brain cells resulting in the neurological signs.

There are no diagnostic tests for live animals available, though promising results have been reported (Korth et al., 1997).

The prions are extremely resistant to environmental factors. There is not any evidence that prions might be present in the semen (Fraser and Foster, 1994). BSE is considered to be a zoonosis (nvCJD).

3. **Additional remarks on diseases in AI stations**

Many parts of the world are free of some of the diseases mentioned above (OIE, 1996). This disease-free status in itself should guarantee bulls’ freedom from these diseases and thus the safety of semen collected from bulls born and raised in that area and kept in local AI stations.

However, if diseases are endemic, several hazards exist for the introduction of disease agents to AI studs. Preventive measures should concentrate on the admission of only disease free animals, on optimal hygiene for humans entering the premises (showering and changing clothes), on 48-h storage periods (including forage) or disinfection of materials before admission into the station, the use of piped water only, and the eradication of vermin. One risk factor, however, remains: infectious particles transported by air and/or birds. Only large distances (more than 1 km) between the AI station and other cattle in the area will suffice in this case.
In this section we concentrate on diseases that are endemic in the northern part of Europe: IBR, BVD, Johne’s disease, *Leptospira hardjo*, and BSE. Furthermore we will pay attention to *Campylobacter* infections because of recent isolates of *Campylobacter foetus* ssp. *foetus* in the Netherlands and Belgium.

At admission, bull calves must be free of all the diseases mentioned above. However, reliable tests must be performed with negative results before admission to an AI station.

Latent infections with BHV1 are a real threat. Therefore only animals which are serologically negative should be accepted. Latently infected animals are normally serologically positive for BHV1, but some seronegative latently infected cows have been reported (Straub, 1988; see Horzinek, 1990). However, the sensitivities of the tests applied in these animals are doubtful. Straub described seronegative latently infected animals after the application of a 1-h incubation Serum Neutralisation test with a limited sensitivity: the animals probably would have been positive if more sensitive tests had been applied.

Seronegative latently infected animals are believed to develop during the period of maternal immunity after an infection with very low doses of BHV1 (Hage, Thierry, personal communications 1996 and 1998).

In the past, in The Netherlands and in the United States bull centres accepted calves with maternal antibodies, which possibly could have suffered a low dose infection before admission. In recent years only calves negative for IBR at the age of 3 weeks have been accepted. The applied test methods are those found to be the most sensitive in the testing evaluation in 1995 (Kramps et al., 1996; de Wit et al., 1998). Occasionally, single serologically positive animals over 6 months of age were found in monthly routine tests. These single reactors occurred both in animals completely negative for IBR since birth (0.002%), as well as in animals that were introduced into the bull studs in the presence of maternal antibodies (0.003%). Reactivation is not applicable in the group of animals negative since birth, so airborne infections might play a role (Mars et al., 1998; Bitsch, personal communication). The animals that seroconverted did not spread the disease.

AI stations in the USA traditionally admitted bulls at the age of 6 months: in the last decade only animals serologically negative at the age of 6 months were admitted. However, as a large percentage of dairy cows in the USA were serologically positive for IBR, a large percentage of these bulls will have been serologically positive due to maternal antibodies. In a limited number of farms virus might have spread and infected some bull calves. In a limited number of animals the infection dose could have been low enough to establish latent infections without the development of a detectable level of active antibodies, producing latently infected serologically negative animals. In these bull stations, however, no IBR infection was observed (apart from some single reactor) during the last decade, making a BHV1 infection-free period of more than 3000 bull years. Therefore, it must be concluded that the number of latently infected serologically negative animals is very low or they do not exist at all.

Bovine virus diarrhoea is endemic worldwide. Persistently infected (pi) animals born after an early intrauterine infection transmit the disease easily. Transiently viraemic animals however transmit the disease to a limited extent (Wentink et al., 1989). The sensitivity for detection of these pi animals is almost 100% (Zimmer, 1999, personal communication).

However, as some routes of infection for the introduction of BVDv are not completely understood, in endemic areas BVDv might infect bulls in an AI station. Primary infections pass clinically unnoticed in more than 95% of cases (Wentink et al., 1989; Brownlie, 1990) and therefore these transient infections are a real threat for the dissemination of BVDv by semen. Primary infections might lead to prolonged periods of viraemia (up to 40 days) (Bruschke, 1998), the presence of virus in bronchoalveolar washings (Brownlie, 1990) (up to 56 days after infection), and in semen (Kirkland et al., 1997) even after seroconversion had taken place. Therefore, the risk for transmission of BVDv by semen is substantial.

Johne’s disease is endemic in most countries of the world. The prolonged incubation period and test systems with low sensitivity lead inevitably to the introduction of infected calves. However, excretion in semen occurs only after prolonged excretion in the faeces (Larsen et al., 1981) and shedders can be traced by applying annual faecal culture tests from
the age of 2 years. Furthermore, there is no evidence that contaminated semen infects cows after insemination (Ovdienko, 1974; Larsen et al., 1981).

With regard to Campylobacter infections, Campylobacter foetus ssp. venerealis causes abortion and infections of the genital tract of cows. Actually, this agent was the impetus for the world wide application of artificial insemination techniques and for the obligatory addition of antibiotics to the semen (Directive 88/407; Chen et al., 1990).

A similar agent to this Campylobacter, Campylo-
bacter foetus ssp. foetus is associated with abortion storms in sheep. In cattle, this agent infects the gastrointestinal tract after oral uptake and is shed in the faeces. In exceptional cases, oral infections in adult pregnant cattle may lead to bacteremia and occasionally to infection and expulsion of the foetus (OIE, 1996). Giacoboni et al. (1993) isolated Campylobacter foetus ssp. foetus from the faeces of 26.5% of young calves, and 15% of older cattle. Excretion in the faeces leads inevitably to contamination of the environment and thus to the risk of contamination of the prepuce. We isolated Campylo-
bacter foetus ssp. foetus from the prepuce of bulls ranging in age from 13 months to 8 years. The latter agent is not considered to be a venereally transmissible agent and should therefore not influence semen trade. Specification of the agent causing genital Campylobacteriosis in cattle is needed in the legislation.

Many Leptospira species exist, each with their specific carrier host (reservoir) in which infection leads to diseases of limited severity and prolonged excretion. Cattle are the carrier host for L. hardjo and transmission by semen is possible.

Infection in other animal species (end hosts) leads to very severe life threatening disease as mentioned above. After recovery, the Leptospira are completely eliminated from the end host’s body, and transmission by semen is very unlikely. Attention in AI stations should be concentrated on Leptospira hardjo infections.

BSE prions have never been demonstrated in the genital organs of diseased bulls or in the semen and experimental insemination of susceptible cows with semen from BSE contaminated bulls did not induce BSE in the cows or progeny. The risk of transmission of BSE by semen is negligible and therefore there should be no restrictions on semen trade imposed by BSE (Fraser and Foster, 1994).

4. Quantification of detection of the presence of diseases

The next step is to minimise the consequences of the unintentional introduction of agents into AI-stations. Regular checks must be performed for the presence of disease agents that might be transmitted by semen: animals found positive must be separated immediately.

The rates at which diseases are transmitted to other animals depend on a number of characteristics of the pathogen, the host and of the contact structure between animals. An important parameter related to transmission is the basic reproduction ratio, i.e. the number of animals that are infected by one typical infectious animal during its entire infectious period. This number depends highly upon the number of effective contacts between animals. A contact is effective when the pathogen is transmitted in the case where one of the animals is infectious (excretion titre above the minimal effective dose). These contacts might be either direct (animal–animal) or indirect (by air, people, equipment etc). High transmission rates in AI stations will be achieved when the number of effective contacts is high, and the length of the incubation period is short. BHV-1 for example has an incubation period of 2 days, the length of the infectious period is about 10 days and the reproduction ratio in a bull station is estimated to be larger than 3 (Table 1). This means that, initially, each infected bull generates more than three other infected bulls within 12 days.

Using a classical Reed–Frost model (well described by Thrusfield, 1995), implemented in WinEpiscope (Frankena et al., 1990), the number of susceptible (S), infectious (I), and recovered (R) animals can be assessed for each time-unit. The time unit is about equal to the length of the incubation period + half the length of the infectious period assuming that effective contacts take, at average, place half way through the infectious period. Thus, for BHV-1 this time unit is 2 + 5/2 = 7 days. Using sensitive tests, BHV-1 seroconversion can be measured at 7 days after infection, thus, coincidentally,
Table 2: Probability of detecting an outbreak of disease in a population of 100 animals, depending on the day, number of seroconversions and the sample size (n) assuming a SE of 100% and p = \frac{3}{100}, I_0 = 1, S_0 = 99 (for explanation see text)

<table>
<thead>
<tr>
<th>Days post introduction</th>
<th>No. converted</th>
<th>Probability of detection using n=20</th>
<th>Probability of detection using n=10</th>
<th>Probability of detection using n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1</td>
<td>0.20</td>
<td>0.10</td>
<td>0.005</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>0.60</td>
<td>0.35</td>
<td>0.19</td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>0.94</td>
<td>0.74</td>
<td>0.48</td>
</tr>
<tr>
<td>28</td>
<td>31</td>
<td>0.99</td>
<td>0.98</td>
<td>0.85</td>
</tr>
<tr>
<td>35</td>
<td>61</td>
<td>1.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
</tbody>
</table>

at the start of the next time unit.

The cumulative number of susceptible, infectious and recovered animals for the situation in which the BHV-1 infection starts with a single animal (I_0 = 1, S_0 = 99, thus N = 100) is shown in Table 2, assuming that each infectious animal makes three effective contacts. Table 2 also presents the probability of detecting the infection.

Subsequently, the number of infectious animals on each day was assessed by linear interpolation within a time-unit. Based on the number of seroconversions, the population size, and the sample size, the probability of detecting the epidemic can be calculated for each day. For this probability calculation, the formula of Cannon and Roe (1982), implemented in WinEpiscope, was used. Results are shown in Table 2 and Figs. 1 and 2. The epidemic can be detected with a probability of 95% at day 22, 26 and 32 post-introduction using a random sample of 20, 10 and five animals, respectively (n = 20, 10 and 5).

In fact, it is not the number of seroconversions that determines the detection probability but the number of seroconversions that can be detected. This number is equal to the number of conversions times the sensitivity (SE) of the test (assuming that false positive test results are ruled out by other test procedures). From Table 1 it can be seen that the SE for BHV1 is over 99% and thus the number of detectable seroconversions is almost identical to the number of actual conversions and the sensitivity is of minor importance. If the model is calculated with an SE of 0.90 (or 90%) then the column under n = 20 in Table 2 would read: 0.18, 0.56, 0.92, 0.9993, 1.00.

In Fig. 2, the probabilities are shown for a population of N = 100, but an epidemic starting with three animals (I_0 = 3, S_0 = 97, R_0 = 0). This epidemic can be detected with a 95% probability at 15, 19 and 25 days post introduction for n = 20, 10 and 5, respectively.

5. Discussion

For safety testing of semen, two approaches can be applied: examination of the end product, or continuous surveillance of the bulls before and after semen production. The tests for the presence of
infectious agents in the semen depend entirely on a single investigation and therefore rely on the sensitivity of the test methods only. Continuous surveillance of the semen donors for infectious diseases before and after semen collection is based on sequential investigations, increasing the reliability by the application of multiple tests. For health security of semen, testing for the freedom of infectious diseases of the donors 28 days after semen collection is beyond doubt the best method.

The methodology used in this paper is mainly for illustrative purposes. The Reed–Frost model is deterministic and chance processes do not play a role. In reality chance does play a role and stochastic models would reflect reality better. For example, in reality an initial infection in one animal has a probability not to be transmitted to other animals and in that case the infection is restricted to this single animal (single reactor). Also, all infectious animals are considered identical with regard to lengths of incubation and infectious periods, the amount of virus excreted and the number of contacts they make. Another constraint of the Reed–Frost model is that contacts between animals occur at random, which is not the case when animals are housed individually or in small groups. With some effort more refined models can be developed but it is not expected that the outcomes will differ greatly.

For a specific infection, whether or not the epidemic will be detected at a sampling point depends mainly on the sample size and the number of seroconversions. For the example of BHV-1, the worst case scenario is that an infection is introduced just before a sampling point. Few animals will then be infected before that sampling and none will have shown seroconversion. If the next sampling is 4 weeks later at semen release from quarantine, the infection will be detected with a probability of 0.99 (day 35 post-introduction, \( n = 10 \)). In the best case scenario, an infection is introduced just after a sampling point. Then the probability of detection equals 0.98 (day 28, \( n = 10 \)). Thus with a sampling interval of 4 weeks and a sample size of 10 bulls, an epidemic of a disease with a high transmission rate (e.g. IBR), present for more than 4 weeks, will be detected with a high probability. If one wants to increase the probability to detect a younger epidemic then the sample size should be increased; e.g. a sample size of 20 bulls will detect a 3-week-old epidemic with a probability of 0.94.

What is the effect of the number of infected animals at introduction? If a single infectious animal is brought in then this number will be 1. However in an AI station this is not very likely to occur because the animals are kept in quarantine for 4 weeks and they are serologically tested before entering the stud. If the introduction is due to aerogenic transmission, e.g. from a neighbouring farm, then several bulls might be infected at the start. In this case, the probability that the initial infection will not be passed on to others by chance is lower and more animals will have shown seroconversion at the next

Fig. 2. Probability of detecting an infection based on days post-introduction and using several sample sizes (\( S_0 = 97 \), \( I_0 = 3 \)).
sampling, increasing the probability of detection (Fig. 2).

The outcomes rely heavily on seroconversion. In many epidemics clinical signs will be apparent and the infection will be noted in an earlier phase and semen will be withheld from trading.

In Table 3 the diseases are subdivided into six categories based on their estimated risk for transmission by semen, and by their likely transmission rate within a bull station. Furthermore, the endemic situation should be taken into consideration when evaluating the safety of semen to be imported.

Diseases with both a high transmission rate within the bull herd and a high risk for transmission by semen (I: FMD, Rinderpest, IBR, BVD) should be tested very frequently to guarantee safe products. For this category the safest method would be to test the animals before release of semen 28 days after production. When working according to this method, clinical and serological checks of the health status can be performed before release. AI stations situated in areas officially free of particular diseases should be allowed to release semen without testing the bulls.

The countries in the EU and North America are free from FMD and Rinderpest. For both diseases the clinical phenomena are so clear that the presence of the diseases will never pass unnoticed during a period of 28 days for semen quarantine. A periodic testing programme of a representative number of animals in the area for the absence of both diseases will guarantee reliable certification. However, when the diseases are endemic, investigations for the freedom of the animals from these diseases 1 month after semen collection seems to be unavoidable. The use of fresh semen presents a high risk and should be prohibited.

IBR, however, is endemic in most countries of Europe and North America. The disease can be either clinically very overt, or pass completely unnoticed (Hage et al., 1996). The only safe method is to test the bulls for the presence of antibodies for BHV1 1 month after collection. BHV1 has a basic reproduction ratio greater than 3 (Hage et al., 1996; Bosch, 1997). From the graphs in Figs. 1 and 2 it can be concluded that 1 month after infection an at-random sample of 20% of the animals detects an infection with 99.99% certainty (de Wit et al., 1998). If a random sample is negative, the whole herd can be considered to be negative until 7 days before the moment of sampling. If later random samples are found positive, the herd was infected after at least 7 days before the last sampling day. A monthly random sampling procedure of a limited number of animals (20%) can be performed providing more than 99% certainty of semen safety before release. Single reactors will probably be missed in a random

<table>
<thead>
<tr>
<th>Risk of transmission by semen</th>
<th>High</th>
<th>Moderate</th>
<th>Unlikely</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 secondary cases per month</td>
<td>I FMD, IBR, BVDV (persistently infected animals), Rinderpest</td>
<td>III Mycopl. mycoides ssp. mycoides</td>
<td>V</td>
</tr>
<tr>
<td>≤2 secondary cases per month</td>
<td>II Camp. foetus ssp. venerealis, Trichomonosis, BVDV (transient infections)</td>
<td>IV Brucellosis, Leptospirosa hardjo, Tuberculosis</td>
<td>VI BSE, Other Leptospira spp.</td>
</tr>
</tbody>
</table>

Table 3
Ranking of the diseases transmissible by semen according to their risk for transmission in semen and by their rate of spread in a bull station according to data from the literature
sample. These single reactors obviously did not infect other animals in the herd: they obviously did not excrete sufficient virus or maybe no virus at all. The semen of such animals will very likely be free of BHV1. In conclusion: random samples collected 1 month apart are a safe surveillance system to monitor BHV1 infections in bull studs.

Bovine virus diarrhoea virus infections are endemic worldwide. Infections will be detected in the case of pi animals, but might be missed when transient infections occur in an AI station. As this disease is easily transmitted in semen, both from pi animals (Meyling and Jensen, 1988) and from transiently infected bulls (Kirkland et al., 1991, 1997), great attention must be paid to ensuring the absence of this agent. Furthermore, one exceptional case has been published in which a bull excreted BVDv in its semen over prolonged periods (11 months) in the presence of active antibodies (Voges et al., 1998). There is information that this phenomenon might occur more frequently (Bruschke, 1999, personal communication). Even applying monthly whole herd sampling for BVDv antibodies, virus excretion might be missed.

In this situation, monthly tests for serologically negative donor bulls and, in the case of seroconversions, checking the end product for the presence of BVDv might be the only solution.

Initially, AI techniques were developed to avoid transmission of the venereal diseases Trichomoniasis and genital Campylobacteriosis. The optimal preventive measure is to admit only virgin bulls at an age younger than 3 months to AI stations: such bulls will be free of venereal transmissible diseases. Annual tests for both diseases will suffice.

Campylobacteriosis was the original reason for the addition of antibiotics to the semen. If this procedure is properly executed, semen is free from this bacterial agent (Chen et al., 1990). Bull stations should be free of this agent, but the low transmission rate makes more frequent testing than once annually useless. When, however, a positive result is obtained, all semen batches after the last negative result should be checked for the presence of the agent and positive ejaculates destroyed.

Genital Trichomoniasis is also only contracted after mating with infected females. However, if an infection with Trichomonas is diagnosed, no treatment is available and the bull and its semen must be destroyed.

The diseases mentioned in categories III to VI are of limited risk. Bulls must be negative for these diseases, but an annual check for their absence will suffice.

6. Conclusions

The disease-free status of areas for particular diseases 1 month after semen collection is sufficient guarantee for semen safety, e.g. in Europe for FMD, Rinderpest, Akabane virus, enzootic bovine leukosis, brucellosis, tuberculosis, blue tongue, Mycoplasma mycoides ssp. mycoides. Regular testing of bulls on AI stations does not improve the safety of the semen produced by bulls born and raised in that area.

For endemic diseases with a high transmission rate a random sampling procedure of 20% of the animals 1 month after semen collection is sufficient to guarantee freedom from infectious agents. This is applicable to IBR in endemic areas.

For BVD there now exist several problems with the application of this procedure, and therefore regular serological control of negative animals should be done. In animals that seroconvert, semen should be checked for the absence of the virus, i.e. end product control.

Tests for genital Campylobacteriosis should concentrate on Campylobacter foetus ssp. venerealis only, because only this type causes genital infections. Admission of only virgin bulls to an AI station in itself guarantees safe semen.

For the diseases with a slow spreading pattern, particularly in areas or regions in which those diseases are eradicated, investigations of individual bulls once a year are sufficient for optimal semen safety.

Ubiquitous agents that might be or are present in semen, e.g. E. coli, Proteus spp., Pseudomonas spp., Haemophilus spp., Campylobacter spp. except C. foetus ssp. venerealis, Mycoplasma spp., Ureaplasma spp. should not affect semen trade: the addition of antibiotics is a sufficient guarantee of safety.
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References


