The risks of disease transmission by embryo transfer in cattle

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Summary
Guidelines for the safe international movement of livestock embryos are provided in the International Animal Health Code of the Office International des Epizooties, and recommendations for embryo processing, based on numerous research papers on embryo-pathogen interaction studies, are given in the Manual of the International Embryo Transfer Society. Risk assessment is the logical extension of these approaches, since it provides veterinary authorities with a complete package of information on which to base their import/export decisions. Risk assessment includes evaluation of disease prevalence, effectiveness of Veterinary Services and competence of the embryo collection team. It also takes account of the epidemiology and pathogenesis of the disease concerned.

The application of risk assessment for embryo movement is illustrated in this paper by comparisons of the probabilities of transmitting foot and mouth disease, bluetongue and vesicular stomatitis by bovine embryos. The risk scenario pathway was divided into three phases for analysis. The first phase deals with the potential for embryo contamination, which depends on the disease situation in the exporting region, the health status of donor herds and donor cows, and on the pathogenetic properties of the disease agent. The second phase covers risk mitigation by use of the internationally accepted standards for embryo processing, and the third phase considers the risk reductions resulting from post-collection surveillance of donors and donor herds, and also from testing of embryo-collection (flushing) fluids for the disease agent.

It was evident from this assessment that low risks of transmitting disease by international movement of bovine embryos depend initially on a low disease incidence in the exporting region and on easily recognisable disease signs. Competent embryo processing was also of great importance, and in the case of bluetongue, vector ecology had a major influence.

In addition to providing a logical basis for import/export decisions, risk assessment is useful for evaluating the potential outcome of new research and for assessing the safety of the movement of embryos of other species for which little or no research information is available on embryo-pathogen interactions.

Keywords

Introduction

The range of infections which conceivably might be transmitted by embryo transfer is vast, but it appears that the risks are far lower than those associated with movement of postnatal animals, semen and most other animal products. This conclusion is based not only on a large amount of research, but also on field experience. For example, more than 1,000 bovine embryos were imported into France from the United States of America (USA) between 1983 and 1987 without any prior testing of the donors or even washing of the embryos, yet no evidence of disease transmission was detected (37). The recent importation of over 8,000 goat embryos into
Canada from South Africa provides another example: whilst some pre-export testing of the donors was performed in this instance, there has been no evidence of embryo-associated disease transmission (9). Surveys conducted for the International Embryo Transfer Society (IETS) over the past few years (36) indicate that numbers of bovine embryos transferred amount to more than 350,000 per annum, of which about 10% are moved internationally. Numbers of transferred sheep, goat and swine embryos are much lower than those of cattle, but are not insignificant. A high proportion of the transfers, particularly within countries, are made without accurate knowledge of the health status of the donors, so it is noteworthy that there have been no substantiated reports anywhere in the world of disease transmission to recipients by transfer of an embryo. Despite this outstanding safety record, there are still good reasons to be cautious, because there are no non-destructive methods available to test embryos for freedom from pathogens. There is also the possibility of transmitting pathogens via contaminated media, or items of equipment used to collect, wash, freeze and thaw embryos. Biological constituents of media (e.g. serum or serum albumen), and also hormones and enzymes such as trypsin, give rise to special concerns about their inherent disease status (1, 4, 13). Searches for safer alternatives are progressing (20, 25), but meanwhile any materials of animal origin which are used must originate from safe sources and they must be properly processed to remove potential pathogens and contaminants.

**Bovine embryos for international trade**

Most bovine embryos which are traded internationally are obtained from donor cows which have been superovulated using hormone treatments and then artificially inseminated (AI). Such in vivo derived embryos are recovered non-surgically seven days after AI by flushing the uterus with collection medium. The embryos and uterine debris are separated from the rest of the medium either by sedimentation or filtration. The sediment or filtrate is examined systematically at low magnification in order to locate the embryos. The embryos are then transferred to small dishes with fresh medium and evaluated according to the IETS Manual (32).

The zona pellucida (ZP) is an important barrier against pathogens, so only ZP-intact viable bovine embryos, including ZP-intact expanding blastocysts should be exported (Fig. 1).

Bovine embryos considered non-exportable in terms of their disease transmission risk are those with a broken or otherwise defective ZP or with extraneous material adhering to the ZP, as well as blastocysts which have hatched and which therefore do not have a ZP (32). Embryos destined for international movement must be ‘washed’ at least ten times. For this procedure, they are transferred through a series of ten dishes or wells, each containing approximately 2 ml of medium. Each ‘wash’ must constitute at least a one hundred-fold dilution of the medium in the pipette with which the embryos are moved from one dish to the next, and a new, sterile pipette must be used for each of the washes. The embryos are kept under low power microscopic observation and gently agitated during the washings. They are finally observed at higher magnification to ensure that the ZP is still intact and free from extraneous cellular debris. This procedure is described in detail in the IETS Manual (32). Embryos are normally kept frozen from the time of collection until exportation and use.

**Disease concerns**

Time spent by in vivo derived embryos in the oviduct or uterus before collection is only a few days. There is a

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**Fig. 1**

**Bovine embryos at different stages of development**

Embryos D, E and F are non-exportable in terms of disease transmission

*Source: Manual of the International Embryo Transfer Society (IETS) (32)*
possibility that embryos from infected donors may have encountered infectious agents whilst in these sites, but this depends to a large extent on the pathogenesis of the agent. Predilection for the genital tract is of special relevance with respect to transmission by embryo transfer. Among the many known genital diseases of livestock are brucellosis, bovine genital campylobacteriosis and trichomoniasis. It is customary to include infectious bovine rhinotracheitis (IBR) and other infections such as *Haemophilus somnus*, *Leptospira*, *Chlamydia* and genital *Mycoplasma* in this list. Pantrropic and blood-borne agents (of which there are many) may readily gain access to the genital tract and also to the embryos. With other diseases, the risk of embryos having contact with the infectious agent under normal circumstances is remote, and here those which tend to solely infect skin might be included (e.g. poxviruses and dermatomycoses), or gut (e.g. rotaviruses and enterobacteria). However, such agents do occasionally produce generalised infections, and even when very localised there is always a possibility of the embryo collection media and equipment becoming contaminated.

The nature of a disease, especially its epidemiology and potential economic impact on livestock populations, is a factor of paramount importance to veterinary authorities when gauging threats to importing countries (10). For example, agents such as foot and mouth disease (FMD) and rinderpest can pose enormous threats, and measures to avoid them must be commensurate. On the other hand, there might seem little point in imposing strict measures to avoid introduction of diseases which are already endemic in the importing country, especially if no national control policy exists. The exclusion of new strains of endemic disease agents does warrant some attention, however, because these could be more virulent than the indigenous agents, not covered by vaccines, and their spread might cause major losses.

Certain diseases are particularly worrying to veterinary authorities because of the special diagnostic problems encountered when certifying that embryos are specific-pathogen free. The so-called 'slow virus' infections, such as bovine spongiform encephalopathy (BSE) in cattle, are listed in this category because there are no serological or other tests to identify subclinically infected and incubating animals (40). Finally there are common commensal bacteria and the ubiquitous contaminants which may be picked up during embryo collection and processing procedures: while perhaps not posing a direct threat, their identification in samples of flushing and washing media can be a useful indicator of poor sanitary procedures (38).

**Animal health protocols for international movement of embryos**

Early contacts (21) between the IETS and the Office International des Epizooties (OIE) led to collaboration in producing guidelines for the safe international movement of bovine, ovine/caprine and porcine embryos, which were subsequently published by the OIE as Appendices in the *International Animal Health Code* (22). The OIE *International Animal Health Code*, together with the OIE *Manual of Standards for Diagnostic Tests and Vaccines* (23), contains recommended rules and test procedures for the international movement of animals and animal products, and these are recognised by the veterinary authorities of most governments.

A fundamental concept enshrined in the relevant Appendices of the OIE *International Animal Health Code* (22) is the accountability of the 'Officially Approved Embryo Collection Team' which operates under the supervision of the 'Team Veterinarian'. Approved embryo collection teams in exporting countries are held responsible for adhering to all official sanitary protocols for embryos, including collection, processing, identification, grading and certification before export, details of which are covered in the IETS Manual (32). Major benefits of the joint OIE/IETS approach are that when the recommended protocols are followed, not only can the imported embryos be considered safe, and certified as such, but the costly multiple testing regimes which were formerly stipulated for donors and herds of origin in the exporting country, and the quarantine systems sometimes used for recipients and embryo transfer (ET) offspring in the importing country can usually be dispensed with.

The Import/Export Committee of the IETS has categorised several disease agents as 'agents for which sufficient research evidence has accrued to show that the risk of disease transmission by embryo transfer is negligible'. Provided that between collection and transfer the embryos are handled as recommended in the IETS Manual' (5). While this information can be helpful to veterinary officials who make decisions on import risks, the system does have weaknesses. For example, FMD and IBR are both listed in category 1, but for veterinary officials 'negligible' applied to a catastrophic disease like FMD has a very different meaning from when it refers to a commonly endemic disease such as IBR. Obviously, the socio-economic consequences of inadvertent introduction of FMD are far greater than those for IBR, and officials must take account of not only the level of risk of disease introduction, but also the potential impact of the disease on national livestock populations. To expect regulatory officials to allow unregulated international movement of embryos merely because certain diseases are listed in category 1 ('negligible risk of transmission') would obviously be naive. In fact, until recently many countries have applied a 'zero risk' policy with respect to importation from regions where severe epidemic diseases exist, and it is unlikely that this ultra-safe policy will be abandoned without extreme caution. Another possible reason for the apparent reluctance of some national veterinary authorities to relax requirements for importation of embryos may have been that the IETS Import/Export Committee did not define precisely what was meant by the term 'negligible risk'.
Risk assessment of disease transmission by embryos

'Res', in relation to importation of embryos, is a measure of the probability of introduction of an exotic disease and the seriousness of such an outcome. 'Risk assessment' is the process of estimating, as objectively as possible, the probability that an importation would result in the entry of an exotic disease agent, and that indigenous livestock in the importing country would be exposed. 'Risk management' is the process whereby risk is reduced to an acceptable level by appropriate strategies (2).

Consistent, reliable and scientifically based risk assessment is an essential ingredient for effective risk management, and the discipline of 'quantitative risk assessment' (QRA) is increasingly being applied. QRA has been used for many years in engineering and economics, but it is rapidly gaining acceptance in the veterinary profession as a basis for regulatory actions, including those connected with importation of animals and animal products, (7, 11, 15, 16, 18, 19, 24). QRA involves the use of epidemiology, microbiology, pathology, economics and statistics, in addition to common sense, to give precise mathematical estimates of risk, thereby enabling importation conditions that are acceptable and justified. As the chances of transmitting diseases by embryo transfer are so low and because research results (especially those from in vivo experiments) have usually been negative, QRAs are, of necessity, based on statistical probabilities.

'Scenario pathway analysis' (12) is a methodology which is especially appropriate for quantitative evaluation of the risks associated with importation of animals or animal products. For example, in the case of embryo imports, every risk-associated event in the pathway is itemised from the point of origin to final destination of the embryos. The probability or frequency with which such events might occur is evaluated. Accumulation of the numerical answers to these evaluations enables a value to be assigned to the risk associated with the entire pathway. The end result is a quantitative measure of risk that can be clearly visualised and for which the evidence is fully documented.

Recent risk studies applicable to embryo transfer

Sutmoller (33) discussed some of the disease transmission risk factors related to embryo transfer, and Sutmoller and Wrathall (34) attempted to quantify levels of FMD risk reduction which will be achieved by adhering to the IETS Manual recommendations (32). An important conclusion emerging from those studies was that there are three main lines or levels of defence against the introduction of diseases via embryo transfer. The first defence line encompasses an evaluation of the disease risk situation in the exporting country and/or region, the health status of the farms and donor cows from which the embryos are collected, and the pathogenic characteristics of the specified disease agent. The second line depends on the use of accepted standards of handling and processing of the embryos (32). The third line of defence includes any post-collection surveillance of the donors and donor farms, and also the possible testing of embryo collection (flushing) fluids for presence of the disease agent.

Scenario pathway analyses have recently also been used to quantify and compare the risks of transmission of FMD, bluetongue (BT) and vesicular stomatitis (VS) by bovine embryos imported from a region where these three diseases exist (35). Account was taken of the very different characteristics of those diseases. FMD, with its catastrophic effects, was an obvious choice for study; its pathogenicity is well understood, clinical signs are pronounced and, in the region selected to collect the embryos, prevalence is regularly monitored. BT was selected mainly because the possibility of importing infected embryos from BT-endemic regions, such as that under study, gives rise to genuine concerns among veterinary authorities in countries which are free of BT, especially those with large sheep populations. Moreover, the asymptomatic nature of BT in cattle means that a diagnosis on clinical grounds alone is usually impossible. VS is another disease of great concern to veterinary authorities because its clinical signs are easily confused with those of FMD, but, in contrast to FMD and BT, the epidemiology and pathogenesis of VS are poorly understood. Also, VS virus adheres rather firmly to the bovine ZP and cannot be removed very effectively by washing.

In the study by Sutmoller and Wrathall (35), the embryos were assumed to have originated from a sub-tropical region in South America where extensive cattle raising, mainly for beef production, is practised. The region selected for the study is characterised by a rapid turnover of the cattle population with large influxes of young cattle for fattening from adjacent regions. In that region, FMD affects about one herd per thousand herds a year, while BT and VS viruses appear to circulate permanently in the livestock population. Clinical cases of BT are not observed and VS affects cattle and members of the equine species only during sporadic outbreaks (26).

Methodology

Scenario pathway

Figure 2 shows the risk-associated events that were considered for the scenario pathway: if the answer to the first question is 'No', then there is no risk (no infected donor herd included). However, if the answer is 'Yes', then the next
question is 'will the surveillance system and/or the embryo collection team detect the disease?' If the answer is affirmative then there is no risk, but if negative, then a risk does exist. The next consideration relates to whether the pathogen reaches the embryonic environment. If the answer is 'Yes', there is a probability that embryos are contaminated. At this point in the pathway, embryos are divided in two fractions: $f_e$, which is the fraction of viable exportable embryos, and $1 - f_e$, which is the fraction of non-exportable viable embryos (Fig. 1). For fraction $1 - f_e$, the following question is asked: 'Are non-exportable embryos removed?' For fraction $f_e$, two questions are to be answered: 'Are the embryos properly washed?' and 'Does the agent adhere or stick to the ZP?' Finally, there are the post-collection questions related to disease observation in the herd of origin and laboratory tests to detect the presence of the pathogen. A schematic listing of the above risk-related events is presented in Table I.

In Table I three phases of risk mitigation are presented according to the three broad lines of defence against the introduction of diseases by the importation of embryos. The first phase covers the potential for production of contaminated embryos and encompasses the disease situation in the exporting country and/or region of origin, the health status of the herds and the donor cows from which the embryos are collected, and the pathogenic characteristics of the specified disease agent. The second phase covers the risk mitigation measures which depend on the use of internationally accepted standards for processing of embryos. The third phase is the risk reduction resulting from post-collection surveillance of the donors and donor herds, and also the possible testing of embryo collection (flushing) fluids for presence of the disease agent.

**Probability of adverse events occurring**

For each of these risk-associated events (1, 2, 3, … n), rather than a simple 'Yes' or 'No' answer as implied in Figure 2, there is a probability $P(1,2,3, \ldots n)$ of conditions that cause the persistence of the disease agent in the chain of events. The overall risk that the batch of embryos would contain one or more contaminated embryos is the product of the probabilities for each of the events. The evidence on which the probability estimates were based are all fully documented in the paper by Sutmoller and Wrathall (35), but some of the important points are reiterated here.

<table>
<thead>
<tr>
<th>Event no.</th>
<th>Event</th>
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<tbody>
<tr>
<td>1.</td>
<td>Inclusion of one or more infected donor herds</td>
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<td></td>
<td><strong>First line of defence</strong></td>
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<tr>
<td>2.</td>
<td>Detection of the disease in the donor herd by the animal health surveillance system</td>
</tr>
<tr>
<td>3.</td>
<td>Detection of the disease in the donor herd by the embryo collection team</td>
</tr>
<tr>
<td>4.</td>
<td>Lack of contact between embryos and the disease agent in the genital tract of a donor</td>
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<td></td>
<td><strong>Second line of defence</strong></td>
</tr>
<tr>
<td>5.</td>
<td>Removal of the contamination during embryo processing by:</td>
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<td></td>
<td>5.1. Detection and removal of embryos which, although viable, are non-exportable from a disease hazard standpoint</td>
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<tr>
<td></td>
<td>5.2. Proper washing of exportable embryos</td>
</tr>
<tr>
<td></td>
<td>5.3. Removal of the disease agent adhering to the zona pellucida</td>
</tr>
<tr>
<td></td>
<td><strong>Third line of defence</strong></td>
</tr>
<tr>
<td>6.</td>
<td>Detection of the disease agent adhering to the zona pellucida</td>
</tr>
<tr>
<td></td>
<td>7. Detection of the disease agent by diagnostic tests on collection fluid or other samples</td>
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</tbody>
</table>

![Fig. 2](image-url)

**Fig. 2**

**Risk scenario pathway for possible disease transmission by bovine embryos destined for international movement**

Table I

<table>
<thead>
<tr>
<th>List of risk-associated events for disease transmission by bovine embryo transfer</th>
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<tbody>
<tr>
<td>Event no.</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>1.</td>
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<td>2.</td>
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<td>3.</td>
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<td>4.</td>
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<td></td>
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<td>5.</td>
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<td></td>
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<tr>
<td>6.</td>
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</tbody>
</table>
General probability estimates

Certain values used in the simulations are independent of the disease under consideration while others are not. Examples of factors which are independent of disease include the number of donor herds, the fraction of exportable embryos, the probability of non-exportable embryos remaining in the exported batch, and the probability of exportable embryos not being adequately washed. The quantitative estimates for these values were based on the opinions of several commercial and non-commercial ET practitioners and were intended to reflect variable degrees of competence and integrity of the embryo collection team.

Disease-specific probability estimates

Other values used in the study are disease-specific, for example: disease incidence, the duration of the disease episode in the herd and the probability of failure of the animal surveillance system or the embryo collection team to recognise the disease before or at the time of embryo collection.

Prevalence and recognition of clinical signs

The values for the calculated prevalence (18) of FMD and VS in the area were based on the annual incidence of these diseases as reported by the Continental Vesicular Disease Surveillance and Information System (6). Based on those records, for FMD a yearly incidence rate of one infected herd in 1,000 herds was assumed. Since VS occurred, only occasionally in the region, an outbreak affecting 19 herds out of a total of 3,900 was taken as the basis for the study. BT infection occurs in nearly all of the cattle herds in the area, and viraemias were assumed to last between 7 and 60 days. During the low-vector season, the likelihood of active viraemia, which in turn depends on the immune status of the donor, is remote and the most likely value for false negative test results was assumed to be about 5%. Also, if tests for BT were to be used to indicate presence of viraemia, then testing of blood taken from the donor at the time of embryo transfer would be unlikely that FMD or VS outbreaks would go undetected, particularly since embryos for export would usually be collected in well-managed herds with high animal health standards. However, BT in cattle is usually an inapparent infection, and clinical expression is rare (17, 27). Thus, the probability that infection in the donor herd would go unobserved by the surveillance system or the embryo transfer team would be close to 100%.

Disease pathogenicity

In the diseases considered, viraemia is a prerequisite for the contamination of the embryo in the genital tract of the donor cow. Thus the frequency with which the pathogen would reach the embryonic environment depends on the likelihood of viraemia, which in turn depends on the immune status of the donor. Most donor farms are likely to maintain a good FMD vaccination programme and are unlikely to have recorded this disease in recent years.
generally possible for the parties concerned to agree on minimum and maximum probabilities that are reasonably accurate, and on the most-likely value within this range. The information is then used to simulate a so-called probability distribution function (PDF) for each risk event along the scenario pathway (3, 12). In these studies a computer program (@RISK®) was used to simulate PDFs for each of the P values along the pathway (3, 12) and a total risk PDF to illustrate the import risk resulting from the complete scenario pathway, as shown in Figure 3.

A PDF is simulated by carrying out a very large number of recalculations (iterations), each of which is based on different random numbers generated by the computer and on the given distribution parameters such as the minimum, maximum, and the most-likely probabilities. The results of the simulations are presented graphically or as statistical reports. The expected or mean risk is the most-likely outcome of the simulation and is represented by the midpoint of a PDF. The other important statistic is the risk value at which 95% of the iteration results are equal to or below that value.

Simulation results

The simulation results for the contribution made by each line of defence towards the safety of embryo transfer are shown in Table II. This Table, together with Figure 3, illustrates the final import risk of bovine embryos for the three diseases under consideration. In Figure 3 the graphs in the top row are the PDFs for FMD, for BT during the high-vector and low-vector seasons and for VS. The cumulative graphs in the bottom row show the same data presented differently, indicating not only the total range of risk, but also the probability that a risk value will fall within a certain range.

For FMD, under the specified conditions (35), i.e. one FMD infected herd per 1,000 herds per year, the final (mean) import risk of a batch of 300 embryos containing one or more FMD contaminated embryos is one in 1,000 billion \((10^{-12})\), and that risk would be unlikely to exceed one in 100 billion. At the end of phase 1 the most-likely (mean) risk of a batch of 300 embryos containing at least one FMD-contaminated embryo is slightly under one in 10 million, and there is only a 5% chance that this will be greater than one in a million. The second phase would reduce that risk by a factor of 30-60, and the third phase by a factor of 400-1,000.

For BT, during the vector-season, as many as 10% of potential donors were assumed to be viraemic, and, because it is difficult to recover embryos without at least some endometrial trauma, with consequent blood contamination (8) it was also assumed that most of those would yield BT-contaminated embryos. According to the simulation results there would be a chance of 1/10 to 1/20 that the batch of 300 embryos would contain at least one BT-contaminated embryo by the end of the first phase. However, in the low-vector season, with much less BT infection of herds and individual donors, the probability of BT-contaminated embryos would be 1/5,000 and this risk is not likely to exceed 1/1,000. The second and third phases would reduce the risk by factors of 30-60 and 20-30 respectively, and those risk reductions apply to both the low-vector and high-vector seasons.

During the high-vector season the most-likely total risk (i.e. inclusive of phases 1, 2 and 3) that a batch of 300 embryos would contain contaminated embryos would be 1/50,000, but this risk would be unlikely to exceed 1/15,000. The most likely total risk for the low-vector season would be one in 4 million, but would be unlikely to exceed one in a million.

For VS, if embryo collection occurred during a worst case scenario period (i.e. during an epidemic with 19 of 3,900 herds affected by the disease), the probability that a batch of 300 embryos would contain one or more VS contaminated embryos would not exceed one in a million by the end of phase 1. The second and third phases are likely to reduce the risk by factors of 2-3 and 30-60, respectively. However, given

<table>
<thead>
<tr>
<th>Phase</th>
<th>Foot and mouth disease</th>
<th>Bluetongue</th>
<th>Vesicular stomatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Probability of including contaminated embryos</td>
<td>1/million</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>Probability of failure to remove contamination during embryo processing</td>
<td>1/60</td>
<td>1/60</td>
</tr>
<tr>
<td>2.</td>
<td>Probability of failure to detect infected herds post-collection</td>
<td>1/100 billion</td>
<td>1/15,000</td>
</tr>
<tr>
<td>3.</td>
<td>Probability of failure to detect infected herds post-collection</td>
<td>1/1,000</td>
<td>1/30</td>
</tr>
<tr>
<td>Final</td>
<td>Probability of failure to detect infected herds post-collection</td>
<td>1/100 billion</td>
<td>1/50,000</td>
</tr>
</tbody>
</table>

a) 95% of the simulated results for the variable are equal or below the value and 5% are higher
b) Mean risk or most-likely risk
Probability density functions for the risk of foot and mouth disease, bluetongue and vesicular stomatitis by bovine embryo transfer.
the low probability of contamination of embryos with VS during the first phase, the risk of one or more embryos contaminated with VS being present in the imported batch of 300 would be unlikely to exceed one in 100 million.

Defence lines and strategic implications

These QRA studies have confirmed that the risks of disease transmission by bovine embryo transfer are generally very low. However, the reasons can vary for different diseases, and the comparison of FMD, BT and VS serves to illustrate this.

First line of defence

The obvious clinical signs of FMD and VS make it unlikely that embryos would be collected on an infected farm or from a diseased donor. However, in the absence of scientific information that VS viraemia never occurs under natural conditions, a rather high value had to be assigned to the probability that VS virus would reach the embryonic environment. Nevertheless, the first line of defence is almost as efficient in risk reduction for VS as it is for FMD. This is not the case with the first line of defence for BT, and especially not during the vector season when the infection would occur on nearly all farms in the selected region and a rather high percentage of animals could be expected to have viraemia. In addition, due to the asymptomatic nature of BT, there is a high probability of failure to detect this infection. Of course, embryo collection during the low-vector season would greatly reduce the risk of BT transmission.

In these studies the authors did not consider the potential risks arising from an embryo collection team moving from one farm to another, or using contaminated media for embryo collection and washing. Concerns about these are justified, but an assumption was made that the sanitary conditions demanded by the importing country would prevent these risks from occurring.

Second line of defence

Risk-associated factors to be addressed at the second line of defence include detection and removal of non-exportable embryos and adequate washing of exportable embryos. Human failure in these respects is the predominant risk here, and an effective system for accreditation of embryo collection teams will greatly help to ensure their integrity and technical ability. The second line of defence is basically the same for FMD and BT, since both agents can be efficiently removed from the ZP by washing, but it is less effective for VS which has a tendency to adhere to the ZP. The risk that disease transmission might occur as a result of using contaminated media for the collection, processing or storing of embryos is very important and should not be overlooked. Some importing countries stipulate that media must be obtained from a specified source to avoid this risk. In this study it was assumed that the media were properly sterilised, and this risk was not included in the scenario pathway.

Third line of defence

The effectiveness of the third line of defence depends initially on the ease of recognising the disease in the herd of origin while the embryos are in post-collection storage. Clinical signs are not missed easily in cases of FMD and VS, but this is not the case for BT. Laboratory tests could be employed to detect FMD virus in embryo collection fluid, but this would require special bio-containment laboratory facilities. For BT the testing for viraemia is more likely to be effective than the testing of collection fluid. Laboratory tests for VS were not considered effective.

Risk of disease establishment in the importing country

For catastrophic contagious diseases, most importing countries will wish to take account only of the import risk. However, for other diseases, such as those which are transmitted exclusively by vectors, further scenarios might also be considered, including the minimum infective dose of the pathogen and the availability of a competent vector in the importing country. With regard to FMD, import authorities tend to be interested primarily in preventing the importation risk and less in the risk of disease establishment, whereas for diseases such as BT, the risk of establishment may also be considered. BT infection is seasonal, non-contagious and vector-borne, and disease spread seems to be limited by ecological boundaries. The regional nature of BT is evident from studies conducted in the Okanagan Valley of British Columbia (Canada), and in parts of the USA and Australia (17, 30). If the vector does not occur in the receiving country the recipient may still become infected, but since the disease is not contagious it will not become established. Such ecological factors can be taken advantage of to give further reduction in risk. For example:

- embryos can be collected during the period when the lowest number of viraemic cattle would be expected, for instance, from 100 days after the end of the vector season until the beginning of the next season (30).

- embryos can be transferred in the receiving region at the beginning of the period with the lowest vector activity, thus reducing the risk of establishment of BT in the receiving region.

While the pathogenesis of VS is not well understood, geographical distribution of the disease suggests that the risk of establishment outside certain ecological boundaries is very low. Ecological factors seem to be related to types of pastures,
ambient temperatures and amounts of rainfall. For reasons unknown, certain areas and farms are prone to experience VS at frequent intervals. Under those circumstances it appears that the probability of the establishment of VS outside such areas is virtually zero.

Conclusions

Risk assessment is a valid approach for estimating and managing the risks of embryo importation and provides veterinary authorities with an alternative methodology to the disease categorisation system of the IETS (5) on which to base their import/export decisions. The IETS categorisation system gives no quantitative guidance on the meaning of the term 'negligible risk' and is based solely on research data from studies on embryo-pathogen interactions in vitro and in vivo. For a QRA, however, in addition to research data on embryo-pathogen interactions, one can consider disease prevalence, effectiveness of Veterinary Services and competence of the embryo collection team, as well as the epidemiology and pathogenesis of the diseases concerned. The QRA results of this study show that the latter factors play a major role in determining the risk of disease transmission by embryos. For instance, although FMD and BT were both placed by the IETS in Category 1 (negligible risk) because research had shown convincingly that the agents of both diseases can be removed effectively from the ZP by washing, the estimate of risk for BT made here is larger than for FMD because of the asymptomatic nature of BT in cattle. The fact that VS is listed in IETS Category 4 (Diseases or disease agents on which preliminary work has been conducted or is in progress) may lead to undue concerns among regulatory officials, and unnecessary trade restrictions. According to estimates made here, VS has an exceedingly small probability of being transmitted by international embryo movement because its presence, like that of FMD, is easily detected on the farm by clinical surveillance.

Acceptably low risks of transmitting disease through the international movement of bovine embryos depend initially on a low disease incidence in the exporting region and on disease signs which can easily be recognised by those involved in disease surveillance. Nevertheless, embryo transfer practitioners must be aware that estimates of their competence (e.g. in embryo washing and in excluding embryos with non-intact ZPs) and their integrity (e.g. in embryo certification) also have substantial effects on embryo import risks, and estimates of these are incorporated into mathematical models and equations from which the risks are quantified. Thus, in commending the QRA approach to veterinary authorities for the evaluation of embryo transfer risk, the importance of both a reliable disease surveillance system in the exporting region and the integrity and competence of embryo collection teams is emphasised.

The use of the QRA methodology is basically simple and when new data become available the results can be easily re-worked. A QRA can also indicate the areas in which additional research information would be most useful. For instance, if new research on embryo-pathogen interaction in vitro is proposed in the hope of enabling a more precise appraisal of IETS washing procedures, such studies would only be justified if the QRA completed beforehand indicated that the results might significantly reduce the total import risk. In addition, it should be noted that QRA is a valuable tool for assessing the safety of the movement of embryos of species for which little or no research data are available on embryo-pathogen interactions.

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Risques de transmission de maladies lors des transferts d'embryons bovins

P. Sutmoller & A.E. Wrathall

Résumé
Les principes directeurs garantissant l'innocuité des transferts internationaux d'embryons bovins sont énoncés dans le Code zoosanitaire international de l'Office international des épizooties ; par ailleurs, les recommandations relatives à la manipulation des embryons sont décrites dans le Manuel de la Société internationale de transfert d'embryons, en tenant compte des nombreux travaux sur l'interaction embryon-agent pathogène. L'évaluation des risques s'inscrit dans la logique de ces textes, dans la mesure où elle fournit aux Administrations vétérinaires un ensemble complet d'informations sur lesquelles elles peuvent fonder leurs décisions d'importation/exportation. L'évaluation des risques inclut l'estimation de la prévalence des maladies, de l'efficacité des Services vétérinaires et de la compétence de l'équipe de collecte des embryons. Elle prend également en compte l'épidémiologie et la pathogénie de la maladie considérée.

Les auteurs illustrent l'application de l'évaluation des risques lors des transferts d'embryons par une étude comparative des probabilités de transmission de la fièvre aphteuse, de la fièvre catarrhale du mouton et de la stomatite vésiculeuse par les embryons bovins. L'évolution du scénario du risque a été divisée en trois phases. La première concerne la probabilité d'infection des embryons, laquelle dépend de la situation sanitaire de la région exportatrice, du statut sanitaire des femelles donneuses et des cheptels d'origine et, enfin, des caractéristiques pathogènes de l'agent causal. La seconde phase a trait aux méthodes d'atténuation du risque, notamment au respect des normes, agréées à l'échelle internationale, pour la manipulation des embryons. Quant à la troisième phase, elle analyse les moyens de réduction du risque, à savoir la surveillance, après collecte, des femelles donneuses et des cheptels d'origine et la recherche de l'agent pathogène dans les liquides de collecte (ou de lavage) des embryons.

Il ressort de cette évaluation que le risque de transmission de maladies lors de transfert international d'embryons bovins peut être considéré comme faible lorsque l'incidence de la maladie dans la région exportatrice est elle-même faible et lorsque les symptômes de l'infection sont aisément identifiables. La compétence de l'équipe chargée de manipuler les embryons revêt également la plus haute importance ; dans le cas de la fièvre catarrhale du mouton, l'écologie du vecteur joue un rôle majeur.

L'évaluation des risques offre non seulement une base rationnelle aux décisions d'importation/exportation, mais elle sert également à évaluer les résultats de nouvelles recherches et à apprécier les garanties sanitaires entourant les transferts d'embryons d'autres espèces, pour lesquelles aucun travail de recherche ou presque n'a été réalisé en matière d'interactions embryon-agent pathogène.

Mots-clés
Riesgos de transmisión de enfermedades mediante transferencia de embriones bovinos

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Resumen
El Código zoosanitario internacional de la Oficina Internacional de Epizootias contiene las directrices que deben aplicarse para garantizar la inocuidad de los intercambios internacionales de embriones de animales de granja. El Manual de la Sociedad Internacional de Transferencia de Embriones, por otra parte, ofrece recomendaciones acerca del procesamiento de los embriones, basadas en numerosas investigaciones sobre la interacción entre los embriones y los agentes patógenos. La evaluación de riesgos constituye la continuación lógica de estos textos, dado que proporciona a las Administraciones veterinarias un conjunto completo de información sobre la cual basar sus decisiones en materia de importación/exportación. Evaluar los riesgos significa evaluar la prevalencia de la enfermedad, la efectividad de los Servicios Veterinarios y la aptitud del equipo encargado de la recolección de los embriones. El proceso de evaluación debe tomar asimismo en cuenta la epidemiología y patogenia de la enfermedad en cuestión.

Los autores ilustran la aplicación de la evaluación de riesgos al movimiento de embriones mediante la comparación de las probabilidades de que embriones bovinos transmitan la fiebre aftosa, la lengua azul y la estomatitis vesicular. El procedimiento de análisis de las diferentes situaciones de riesgo fue dividido en tres etapas. La primera fase considera la probabilidad de que el embrión contraiga la infección, factor que depende tanto de la situación de la enfermedad en la región exportadora como del estado sanitario de las hembras donantes y rebaños de origen y de las propiedades patógenas del agente infeccioso. La segunda fase estima la atenuación del riesgo que se sigue de la aplicación de normas internacionalmente aceptadas para el procesamiento de los embriones. La tercera, por último, evalúa la reducción del riesgo resultante de la vigilancia de las hembras donantes y rebaños de origen después de la recolección y de las pruebas de detección de agentes patógenos aplicadas a los líquidos de recolección (y lavado).

Esta evaluación puso de manifiesto que la consecución de niveles bajos de riesgo de transmisión de enfermedades debido a los intercambios internacionales de embriones bovinos depende, en primera instancia, de una baja tasa de incidencia de la enfermedad en la región exportadora y de la existencia de síntomas de enfermedad fácilmente reconocibles. Un procesamiento adecuado del embrión es asimismo de la mayor importancia, al igual que, en el caso de la lengua azul, las características ecológicas del vector.

Además de proporcionar una base lógica para la toma de decisiones en materia de importación/exportación, la evaluación de riesgos permite valorar el posible resultado de nuevas investigaciones y estimar el nivel de seguridad que ofrece el movimiento de embriones de especies sobre las cuales existe poca o ninguna información relativa a las interacciones entre embriones y patógenos.

Palabras clave
References


