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Risk of disease transmission by llama embryos

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Summary

An assessment was made of the risk of transmission of foot and mouth disease (FMD), vesicular stomatitis, bluetongue, tuberculosis and brucellosis by llama embryos. The study suggests that embryo transfer is a safe method for the international movement of llama embryos despite the special characteristics of these embryos, such as the absence of a *zona pellucida,* and despite the lack of data on pathogen-embryo interactions.

For acute viral diseases such as FMD, vesicular stomatitis or bluetongue, embryo transfer reduces the risk of international embryo movement by a factor of 10^4. Therefore, if favourable epidemiological or ecological conditions exist in the region of origin of the embryos, the risk of contamination of a batch of llama embryos with the above agents is close to zero. The risk of contamination with *Mycobacterium* or *Brucella* depends on the incidence of these diseases, but under the most unfavourable prevalence levels, the risk does not exceed $10^{-3.3}$, given that the results of diagnostic tests of the herd and of donor animals are negative before and after collection of the embryos. This study demonstrates that risk assessment can be a valuable tool to facilitate international movement of embryos, particularly for those species for which little or no data are available regarding embryo-pathogen interactions.

Keywords

Bluetongue – Brucellosis – Embryo transfer – Embryos – Foot and mouth disease – Llamas – Risk assessment – Tuberculosis – Vesicular stomatitis.

Introduction

The *International Animal Health Code* (*Code*) of the Office International des Epizooties (OIE) (6) provides detailed recommendations for the collection, handling and processing of embryos destined for international movement, in order to minimise the risk of disease transmission. The safety of international movement of llama embryos is considered in detail in the Appendix 4.2.3.8 of the *Code*. However, the absence of a *zona pellucida* (ZP) as a pathogen barrier and the lack of research data concerning the interactions between llama embryos and pathogens are factors that negatively impact on the development of import protocols.

Sutmoller and Wrathall performed an assessment of the risk of transmission of various acute viral diseases by bovine embryos from a sub-tropical region in South America (9, 10). The authors demonstrated the feasibility and power of risk assessment methodology to obtain estimates of the level of safety of embryo transfer techniques when following the recommendations of the OIE (6) and the International Embryo Transfer Society (IETS) (8).

The present paper evaluates the levels of risk posed by some acute viral diseases and chronic bacterial diseases in llama embryos. Llamas are induced ovulators and the ovum is released from the ovary within 24 hours following copulation. Llama embryos mature and hatch more quickly than bovine embryos and reach the uterus later. Consequently, llama embryos differ substantially from bovine embryos in that they are hatched blastocysts without a ZP at time of collection. Specifically considered were the risks for importing countries of introducing foot and mouth disease (FMD), vesicular stomatitis (VS), bluetongue (BT), tuberculosis and brucellosis.

Materials and methods

The risks of animal diseases posed by an animal product or commodity depend firstly on livestock management practices and the animal health situation in the region and farm of origin. However, for the purpose of the risk assessment described below, only the additional safety that embryo transfer would provide was considered.

Embryo collection

Superovulation of llamas is usually fairly unsuccessful, therefore single embryos are collected as a continuous process. The embryo donor llamas are bred and flushed after a week. One or two weeks later, depending on follicle size, the llama is bred again (P. Taylor, personal communication).

Embryos are collected using a closed system, with phosphate buffered saline flushing medium flowing from a closed, sterile bag through the attached tubing and into and out of the uterus through a sterile Foley catheter. The medium, with any debris from the uterus, flows through a closed filter and the excess is discharged. When most of the fluid has been recovered, the filter is removed from the system, the contents are poured into a sterile plastic search dish and viewed under a microscope at low power magnification. This last step and any further processing of the embryo are performed in a biosafety cabinet, to avoid contamination of the embryos by micro-organisms.

Llama embryos are 'washed' as described in the IETS *Manual* (8). However, because of the absence of a ZP, research findings obtained for embryos with an intact ZP cannot be applied to the llama embryo (hatched blastocysts). Trypsin cannot be added to the medium since it is likely to damage the unprotected embryo cells.

Following collection, embryos are frozen until export. The frozen embryos are kept in officially sealed containers and remain under official supervision (P. Taylor, personal communication).

Scenario risk pathways

The problem to be modelled is presented schematically in Figure 1. This scenario, similar to the one used by Sutmoller and Wrathall (9, 10), shows the events related to the risk of disease transmission, from the origin of the embryos to the time when the embryos are ready for international movement. For transmission of a disease agent to occur, the agent must be present in the herd of origin. It must then remain undetected by diagnostic tests and clinical observation, the embryos must become contaminated in the genital tract of the donor and, finally, after collection, the infection must again remain undetected while the embryos are in storage. Each of the links in the scenario pathway is associated with a probability that the disease agent persists in the chain of events (11). The accumulation of these probabilities provides the probability that the imported embryos are contaminated.

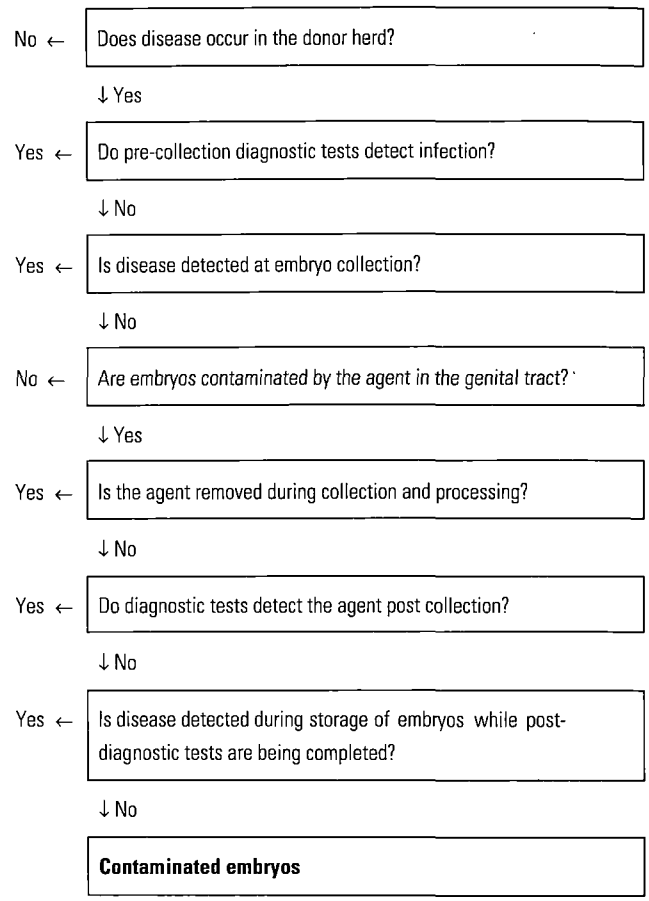

Fig. 1
Risk scenario pathway for disease transmission by the international movement of llama embryos

Estimation of risk and computer simulation

The models for the different scenarios used the Microsoft Excel® worksheet application as the modelling environment and the @RISK© risk analysis add-in to give Excel the ability to generate Monte Carlo sampling from probability distributions (11). The extra functions in Excel provided by @RISK© are characterised by starting with the letters 'Risk' followed by the input parameters in parenthesis, e.g. RiskTriang(10%, 25%, 30%), which are the minimum, most likely and maximum estimated probabilities, respectively.

Triangular distributions were used where the range and the most likely value within that range could be estimated (11). The Pert distribution was used to model expert opinion. This is similar to the Triang distribution, but has the advantage of being more naturally shaped and less sensitive to the estimation of the minimum and maximum values. Pert distributions use similar input parameters as the Triang distribution (11). The probability values for each event in the scenario pathway were based on published data as well as expert opinion.

For each simulation, 10,000 iterations (re-calculations) of the worksheet were performed to establish probability

distributions (11). A probability distribution is an effective way to present all the possible outcomes of a simulation since this expresses the uncertainty of that particular estimation. Simulation outcomes of particular interest are the 'expected' or 'mean results' (the most-likely outcomes) and 'percentile probability values', indicating, for instance, the upper 95% confidence limit on the risk.

Risk assessment

Foot and mouth disease

Probability that foot and mouth disease occurs in the donor herd

The disease prevalence in the area can be calculated as the number of affected herds in the area per year divided by the total number of herds in the area and multiplied by the duration of a disease episode in a herd as a fraction of a year (5).

The probability of including at least one infected donor herd is a function of the number of donor herds required and the calculated disease prevalence in the area. It is calculated as follows:

$$P = 1 - (1 - p_c)^n$$

where:

n is the number of donor herds

p_c is the calculated prevalence (11).

If the incidence of FMD is low, the probability of including an infected donor farm will be very low; in particular on well-managed farms involved in international movement of embryos. However, this important risk factor must be calculated for each particular case and is not included in this risk assessment.

Probability that pre-collection diagnostic tests fail to detect foot and mouth disease in the herd of origin

Serological tests are adequately sensitive to detect llamas infected with FMD (1). However, serological tests may have a fairly high failure rate when FMD is still in the early stages of development. The high probability value for failure of the tests to detect the FMD infection, shown in cell F5 of Figure 2, reflects this reality.

Probability that foot and mouth disease is not detected on the premises of the llama donor herd at embryo collection

The planning and execution of an embryo transfer programme is complex and requires frequent contacts over several weeks between farm personnel and embryo transfer technicians for selection, examination, and insemination of the donors. In addition, management of llamas is very intensive and all animals are checked daily. Therefore, since FMD has a short incubation period, the clinical signs should be evident by the time of the embryo collection, even though the clinical signs of FMD in the llama may be less pronounced than in other livestock species (3).

The estimated probability that the embryo transfer technicians would detect FMD in the donor herd ranges from 90% to 99% with a most likely value of 95% (cell F6 in Figure 2).

Probability that embryos are contaminated with foot and mouth disease virus in the genital tract of the donor

The model assumes that the herd of llamas is not vaccinated against FMD and in the absence of circulating antibodies the virus is likely to reach the genital tract of an infected llama. Therefore, in this case, the probability value for cell F9 is 1.

Probability that foot and mouth disease virus is not removed by processing

No research data are available reagarding the interactions between the embryo and the FMD virus. The llama embryo (hatched blastocyst) lacks a ZP, therefore the information available for other species with a ZP cannot be applied. The virus is likely to be absorbed by embryonic cells of hatched llama blastocyts and, therefore, the washing is expected to be ineffective in removing FMD virus from the embryo. Therefore, the value of cell F10 is 1.

Probability that post-collection diagnostic tests fail to detect foot and mouth disease

During the period between collection of the last embryo and export of the embryos, sufficient time exists for the development of positive serological reactions for FMD. The range for false negative results was estimated to be between 1% and 10%, with 5% as the most likely value (cell F13).

Probability that foot and mouth disease is not detected in livestock while the embryos are in storage and diagnostic tests are being completed

The time-lapse between collection of the embryos and the completion of the post-collection diagnostic tests is at least 30 days. The likelihood that an outbreak of FMD will be detected during that period is not any lower than for cell F5.

Vesicular stomatitis

Probability that vesicular stomatitis occurs in livestock or in the donor herd

In countries eligible for the international movement of llama embryos, suspected vesicular disease cases are investigated within the framework of FMD surveillance. It is therefore unlikely that the herd would be infected, but this important epidemiological factor was not considered in this risk assessment.

	A	B	C	D	E	F
						Result of 1 iteration
1	**Disease situation**					
2	Probability of including a FMD-infected herd					?
3						
4	**Pre-collection testing of donors**					
5	Probability that pre-collection diagnostic tests fail					0.51
6	Probability that FMD is not detected					0.052
7						
8	**FMD virus/embryo interaction**					
9	Probability that embryos are contaminated in genital tract					1
10	Probability that FMD virus is not removed by washing					1
11						
12	**Post-collection observations**					
13	Probability that post-collection tests of donors fail					0.053
14	Probability of failure of post-collection quarantine					0.052
15						
16	Probability that llama embryos are contaminated with FMD					– 4.1

Cell address	Formula
F5	= RiskPert(10%,50%,95%)
F6	= 1–RiskPert(90%,95%,99%)
F13	= RiskTriang(1%,5%,10%)
F14	= 1–RiskPert(90%,95%,99%)
F16	= LOG(PRODUCT(F5:F14))

FMD: foot and mouth disease

Fig. 2
Excel/@RISK© worksheet for the simulation of the risk of foot and mouth disease by the international movement of llama embryos

Probability that pre-collection diagnostic tests fail to detect vesicular stomatitis in the herd of origin

The probabilities of failure to detect VS by diagnostic tests performed prior to collection of embryos are not higher than those estimated for FMD.

Probability that vesicular stomatitis is not detected in the llama donor herd at embryo collection

Clinical signs of FMD and VS are similar and equally detectable for VS as for FMD.

Probability that embryos are contaminated with vesicular stomatitis virus in the genital tract of the donor

Contamination of embryos in the genital tract is less likely for VS as compared to FMD. While for FMD this probability was set to unity, for VS the value is probably close to zero because of the absence of a detectable viraemia.

Probability that vesicular stomatitis virus is not removed by processing

The effect of 'washing' the embryos is likely to be identical to that described for FMD.

Probability that post-collection diagnostic tests fail to detect vesicular stomatitis in the herd of origin

The probabilities of failure to detect VS in diagnostic samples taken post-collection of embryos are not higher than those estimated for FMD.

Probability that vesicular stomatitis is not detected in livestock while the embryos are in storage and diagnostic tests are being completed

The time-lapse between collection of the embryos and the completion of the post-collection diagnostic tests is at least thirty days. The likelihood that an outbreak of VS will be detected during that period is not lower than for cell F5.

Bluetongue

Probability that bluetongue occurs in livestock or in the donor herd

Bluetongue is a vector-borne disease and the ecological conditions of the habitat of llamas do not favour the occurrence of the disease. The probability of BT infection in the herd or donor animal was not considered in this risk assessment.

Probability that pre-collection diagnostic tests fail to detect bluetongue in the herd of origin

The probability of failure of pre-collection diagnostic testing to detect BT may be slightly higher than those estimated for FMD.

Probability that bluetongue is not detected in livestock or in the llama donor herd at embryo collection

No information is available regarding the clinical expression of BT in camelids. This risk factor may be higher than for FMD.

Probability that embryos are contaminated with bluetongue virus in the genital tract of the donor

Contamination of embryos in the genital tract is likely to be similar to that described for FMD. For FMD this probability was set to unity.

Probability that bluetongue virus is not removed by processing

The effect of 'washing' the embryos is probably the same as for FMD.

Probability that post-collection diagnostic tests fail to detect BT in the herd of origin

The probability of failure of post-collection diagnostic testing to detect BT is probably slightly higher than that estimated for FMD.

Probability that bluetongue is not detected in livestock while the embryos are in storage and diagnostic tests are being completed

The time-lapse between collection of the embryos and the completion of the post-collection diagnostic tests is at least thirty days. The likelihood that an outbreak of BT is detected during that period probably will be somewhat lower than for cell F5.

Tuberculosis

Four major species of acid-fast staining mycobacteria (*Mycobacterium avium, M. bovis, M. paratuberculosis* and *M. tuberculosis*) affect livestock, and all four have been reported in camelids, either as an experimental or a natural infection (M.E. Fowler, personal communication). However, camelids are not highly susceptible to *Mycobacterium* infection. Reports of confirmed natural cases of tuberculosis in camelids in South America are rare. In North America, *M. bovis* was isolated from eight llamas during a five-year period at the Veterinary Services Laboratory of the United States Department of Agriculture (2). In a game farm in Iowa, United States of America, diffuse granulomas were found during necropsy of a llama. Tuberculosis was diagnosed in cervids at this same farm. Ten llamas from the game farm were euthanised, but no lesions were found, even though these animals had been in direct contact with the tubercular llama that died. Similar findings were noted in a game farm in Canada where cervids and llamas were kept in close proximity. Many of the cervids and one of the llamas had granulomas; however the remainder of the llamas did not present lesions (2).

Probability that tuberculosis occurs on the donor farm

The lower the disease prevalence, the lower the probability of including an infected llama among the embryo donors. This reduces the risk, but also reduces the probability of test failure. Simulations were performed with five prevalence levels of *Mycobacterium* infection: 10%, 5%, 1%, 0.1% and 0.01% (Figure 3, cell range F2:J2).

The probability of including at least one infected llama in the donor group is a function of the *Mycobacterium* prevalence in the donor herd (cells F2:J2) and the number of donor animals (cell E3) according to the following equation:

$$P = 1 - (1 - p)^n$$

where:

p is the prevalence

n is the number of donors (11).

The number of donors for the batch of embryos is assumed to be 15 (cell E3). The results of the calculations that at least one infected llama is included in the donor group is calculated for each prevalence level in the cell range F4:J4.

Probability that herd tuberculin testing fails

The relatively fibreless area in the axillary space is the preferred site for tuberculin testing and either a single intradermal test or a comparative balanced bovine purified

	A	B	C	D	E	F	G	H	I	J
					Results of one iteration					
1	**Disease situation**									
2	Prevalence of tuberculosis in the herd					10%	5%	1%	0.1%	0.01%
3	Number of donors				15					
4	Probability of including one infected donor					0.79	0.54	0.14	0.01	0.001
5	**Herd testing**									
6	Group size (n)				750					
7	Number of llamas tested (t)				120					
8	Sensitivity of tuberculin test (s)				0.70					
9	Probability that herd testing failed					0.0001	0.01	0.41	0.9	0.99
10	**Pre-collection testing of donors**									
11	Number of potential donors				40					
12	Pre-collection diagnostic tests fail					0.01	0.09	0.62	0.95	1.00
13	**TB/embryo interaction**									
14	Embryos contaminated in genital tract					0.12	0.12	0.12	0.12	0.12
15	TB not removed by embryo washing					0.06	0.06	0.06	0.06	0.06
16	**Post-collection testing of donors**									
17	Probability that post-collection tests fail					0.16	0.40	0.83	0.98	1.00
18	Probability of contaminated embryos					–9	–5.8	–3.7	–4	–5

Cell address	Formula
F4:J4	= 1–(1–F2)^$E3
E8	= RiskTriang(66%,70%,75%)
F9:J9	= (1–$E7*$E8/$E6)^(F2*$E6)
F12:J12	= (1–$E8)^(F2*$E11)
F14:J14	= RiskPert(0%,10%,30%)
F15:J15	= 1–RiskPert(85%,95%,99%)
F17:J17	= (1–$E8)^(F2*$E3)
F18:J18	= LOG(PRODUCT(F4:F17))

TB: tuberculosis

Fig. 3
Excel/@RISK© worksheet for the tuberculosis model to simulate the risk of llama embryos

protein derivative (PPD) and avian PPD test is recommended on this site (M.E. Fowler, personal communication). Comparative axillary intradermal tuberculin tests, based on a 3 mm increase in the double fold skin thickness, showed the sensitivity to be 66% to 75%, with a specificity of 100% (2). An experiment conducted in Mexico on animals that were artificially infected, indicated that the axillary site is sensitive, however, the response may be more diffuse and slightly more difficult to interpret than that at the lateral cervical area (2).

MacDiarmid (personal communication) noted that in New Zealand at least 90 days are required between tuberculin tests due to the possibility of desensitising the animal.

The probability (P) of detecting at least one infected llama in the group depends upon the number of animals tested (t), the size of the group (n), the sensitivity of the test (s) and the disease prevalence (p), according to the equation: $P = (1 - ts/n)^{pn}$ (4). The group size was assumed to be 750 llamas (cell E6), the number of llamas tested as candidate donors was 120 (cell E7). The sensitivity of the test is noted in cell E8. The results of the computations are shown in cells F9:J9.

Probability that pre-collection diagnostic tests for *Mycobacterium* infection fail to detect tuberculosis in the herd of origin

For the collection of a batch of embryos, the model assumes that at least forty potential donor llamas will be tuberculin tested (cell E11), even though a smaller number will ultimately be selected as embryo donors (cell E3). Any positive test result will disqualify the whole group.

Probability that embryos become contaminated with *Mycobacterium* infection in the genital tract of the donor

No information is available concerning the probability that embryos could become contaminated in the genital tract of an infected llama donor. No particular affinity of *Mycobacterium* for the genital tract has been reported. In other species, such as cattle, tuberculosis of the genital tract has been observed in

a small percentage of infected animals. Since one can safely assume that only a small percentage of infected llamas will show pathology of the genital tract, the probability that embryos become contaminated in the genital tract is set tentatively between 0% and 30%, with a most likely value of 10% (cells F14:J14).

Probability that tuberculosis is not removed by processing

Mycobacterium is assumed to have no particular affinity for the cells of the hatched blastocyst, therefore the 'washing' can be expected to act as a simple dilution procedure for the pathogen. In that case, the theoretical dilution factor will be of the order of 10^{-20}. Thus, the 'washing' can be expected to be effective in removing not less than 90%-99% of the *Mycobacterium*. The probability distribution for non-removed *Mycobacterium* is expressed as 1 – RiskPert (85%, 95%, 99%), but this probably underestimates the risk reduction effect of embryo processing (cells F15:J15).

Probability that post-collection diagnostic tests fail to detect *Mycobacterium* infection

The final number of embryo donors is assumed to be 15 (cell E3) and the test sensitivity is given in cell E8. The calculated results are shown in the cell range F17:J17.

Brucellosis

Probability that brucellosis occurs on the donor farm

Brucellosis is not a major disease of camelids, but these animals have been proven to be susceptible to *Brucella melitensis*. *Brucella melitensis* is the primary species found in goats and sheep and the infection of sheep was believed to be the source of infection in a Peruvian herd of llamas (2). Three llamas died at London Zoo a few weeks after contact with newly imported camels. Serum titres for *B. melitensis* were greater than 1:1,000, indicating active infection (2).

Brucella abortus infection (including abortion) has been produced experimentally in llamas, but no natural cases have been reported (M.E. Fowler, personal communication). If brucellosis occurs in a llama herd, infection is likely to spread more widely than tuberculosis.

Probability that herd testing fails

Studies have confirmed the validity of bovine testing procedures in camelids. The sensitivity (90%-95%) and specificity (90%) seem to be similar to other livestock (M.E. Fowler, personal communication). Due to the higher sensitivity of the brucellosis test compared to the tuberculin test, fewer brucellosis-infected llamas will escape detection.

Probability that pre-collection diagnostic tests for brucellosis fail to detect *Brucella abortus* in the herd of origin

Given the high sensitivity of the test for brucellosis and the high prevalence of brucellosis, it is highly unlikely that the test results will not indicate at least one infected llama among the forty potential embryo donors, if brucellosis is present in the herd. One positive result is assumed to lead to a full epidemiological investigation and confirmed disease occurrence would lead to the cancellation of the export of llama embryos.

Probability that embryos are contaminated with *Brucella abortus* in the genital tract of the donor

The affinity of *Brucella* for the genital tract is probably higher than that for *Mycobacterium*. Unlike *Mycobacterium*, *Brucella* probably contaminates the genital tract of the infected llama. In this respect *Brucella* is a slightly higher risk than *Mycobacterium*.

Probability that *Brucella abortus* is not removed by processing

Probability values for the efficiency of the washing of llama embryos are assumed to be similar to those of *Mycobacterium*.

Results

For the acute viral diseases, this risk assessment did not consider the epidemiological situation or risk classification of the country and farm of origin, but only estimated the risk reduction as a result of embryo transfer procedures. A probability distribution was simulated for the risk of FMD, assuming a probability of 1 that the source farm would be infected. In this case, the most likely risk for a batch of llama embryos from that farm would be $10^{-4.2}$ or 1:15,000 with a 95% probability that the risk would not exceed $10^{-3.6}$ or 1:4,000 (Figure 4). A sensitivity test showed that all variable factors contributed approximately equally to the simulation results. Thus, embryo transfer procedures reduce the risk of FMD by a factor of approximately 10^4. If the risk of disease in the country or farm of origin is also low, the total risk of FMD posed by the international movement of llama embryos is negligible.

The risks for VS and BT were compared qualitatively with the FMD risk. The risk for VS is smaller than the risk of FMD,

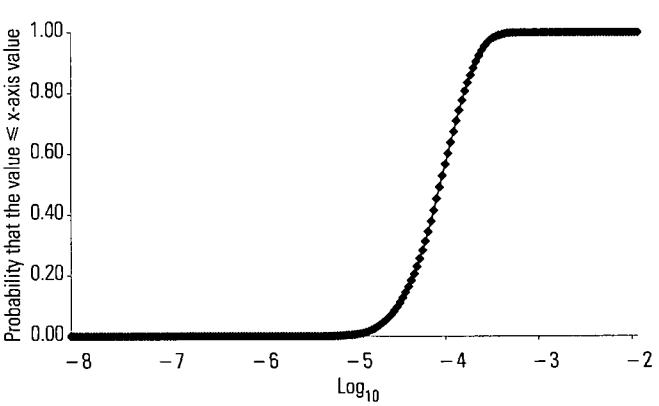

Fig. 4
Probability distribution for the risk of foot and mouth disease from llama embryos

mainly because VS is unlikely to reach the genital tract. The BT risk for a batch of llama embryos is probably somewhat higher than the risk of FMD, depending on ecological conditions.

The probability of not detecting a *Mycobacterium* infection using tuberculin testing depends upon the prevalence of the disease in the herd and the number of animals tested. The lower the prevalence, the higher the probability of failing to find an infected animal in a particular group. However, with a lower prevalence, the chance of including an infected animal among the donor llamas is also reduced. The results of simulations for a batch of embryos using decreasing levels of tuberculosis prevalence are shown in Table I.

Table I
Simulation results of the final risk that a batch of llama embryos is contaminated with *Mycobacterium* at different herd prevalence levels

Herd prevalence (%)	Mean probability or most likely probability	Maximum risk [a]
10	−9.2 [b]	−8.5
5	−5.9	−5.3
1	−3.8	−3.3
0.1	−4.2	−3.7
0.01	−5.1	−4.6

a) 95% confidence that the value will not be exceeded
b) $\log_{10}$

At 5%-10% prevalence levels, the probability of contaminated embryos being included in a batch of embryos to be exported is very low. This is mainly because at these prevalence levels the probability that the tuberculin test (even with a rather low sensitivity) will fail to detect at least one infected llama in the group is very low. At a prevalence level of 1%, the probability of including an infected llama among the donors is still considerable, however, when tuberculin testing is performed, the probability of missing an infected llama approaches 90%. At a still lower prevalence, the probability of failure of the diagnostic test approaches 100%, but the probability of including an infected llama becomes significantly less. Thus, given that 175 tests were performed on 750 animals, the risk of contaminated embryos in the export batch, under the most unfavourable prevalence levels, is very unlikely to exceed $10^{-3.3}$ (1:2,000), but is likely to be several orders of magnitude less.

The number of animals in the herd was taken to be 750 (Fig. 3, cell E6). Simulations with 120 and 1,200 llamas, respectively, did not significantly change the results. A sensitivity test indicated that at the 10% prevalence rate, the sensitivity of the tuberculin test contributed the most to the simulation results. At the lower prevalence levels, the factors that most influenced the results were 'the contamination of embryos in the genital tract' and 'the pathogen not removed by washing the embryos'.

In a qualitative comparison for brucellosis, a higher within-herd infectivity of brucellosis and a higher sensitivity of serological test would result in a lower probability of including a *Brucella*-infected llama donor. Thus, the overall risk of *Brucella* is less than that of tuberculosis.

Discussion

Foot and mouth disease

The risk of FMD is extremely low, but the consequences of the introduction of FMD are disastrous for all sectors of the livestock industry of the importing country.

Vesicular stomatitis

The risk approaches zero and the consequences of VS introduction for the livestock industry of the importing country are probably localised.

Bluetongue

The risk of BT may be slightly higher than the FMD risk, depending on ecological conditions. The consequences of BT introduction by llama embryos for livestock depend upon ecological factors in the importing region, such as the existence of competent vectors.

Tuberculosis

The risk of importing a batch of llama embryos contaminated with *Mycobacterium* is very low. The consequences of the import of contaminated embryos will mostly affect the owner of recipient llama herds. Introduction may have consequences for the regional tuberculosis status.

Brucellosis

The risk for brucellosis is lower than the risk of tuberculosis. The consequences of importing a batch of contaminated llama embryos will mostly affect the owner of the recipient herd. Introduction may affect the brucellosis-free status of a farm or area.

Conclusions

Llama embryos are hatched blastocysts without a ZP. Research findings regarding embryos of other livestock species with intact *zonae* cannot simply be applied to llama embryos. Washing of llama embryos probably does not significantly reduce the risk of viral diseases such as FMD, VS and BT. However, the risk that a batch of llama embryos is contaminated with the above agents is close to zero when the epidemiological and ecological situation in the region of origin of the embryos is favourable. The risk of contamination with *Mycobacterium* or *Brucella* is low when the results of diagnostic tests of the herd and of donor animals before and after collection of the embryos are negative. Washing of embryos may be a risk reduction factor for bacterial agents.

This study shows that risk assessment can be a valuable tool to facilitate international movement of embryos of those species for which little or no data are available on interactions between the embryo and pathogens.

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Risque de transmission de maladies par des embryons de lama

P. Sutmoller

Résumé

Une évaluation des risques de transmission de la fièvre aphteuse, de la stomatite vésiculeuse, de la fièvre catarrhale du mouton, de la tuberculose et de la brucellose par des embryons de lama a été effectuée. Cette étude montre que malgré les particularités des embryons de lama, notamment l'absence de *zona pellucida*, et en dépit des lacunes qui subsistent concernant les interactions entre ces embryons et les agents pathogènes, le transfert d'embryons de lama en vue des échanges internationaux ne présente pas de danger sanitaire.

Pour les maladies virales aiguës, telles que la fièvre aphteuse, la stomatite vésiculeuse ou la fièvre catarrhale du mouton, le transfert d'embryons représente un facteur de réduction des risques d'environ 10^4. Par conséquent, si les conditions épidémiologiques ou écologiques de la région de collecte des embryons sont favorables, le risque d'infection d'un lot d'embryons de lama par les agents des maladies mentionnées ci-dessus est proche de zéro. Le risque de contamination par *Mycobacterium* ou *Brucella* dépend de l'incidence des infections par ces agents pathogènes : toutefois, aux taux de prévalence les plus défavorables, ce risque n'excède pas $10^{-3,3}$, sachant que l'élevage d'origine et les femelles donneuses sont testés avant et après la collecte des embryons et que les résultats doivent être négatifs. Cette étude montre que l'évaluation des risques facilite les déplacements internationaux d'embryons, en particulier chez les espèces pour lesquelles les interactions entre l'embryon et l'agent pathogène sont peu connues.

Mots-clés

Brucellose – Embryons – Évaluation des risques – Fièvre aphteuse – Fièvre catarrhale du mouton – Lamas – Stomatite vésiculeuse – Transfert d'embryons – Tuberculose.

■

Riesgo de transmisión de enfermedades a través de embriones de llama

P. Sutmoller

Resumen

El autor describe una evaluación del riesgo de transmisión a través de embriones de llama de fiebre aftosa, estomatitis vesicular, lengua azul, tuberculosis y brucelosis. Del estudio se desprende que, pese a las particularidades de los

embriones de llama (por ejemplo la ausencia de zona pelúcida) y a la falta de datos sobre las interacciones entre el embrión y los agentes patógenos, la transferencia de embriones es un procedimiento seguro para los intercambios internacionales de embriones de llama.
En el caso de enfermedades víricas agudas como la fiebre aftosa, la estomatitis vesicular o la lengua azul, la transferencia de embriones representa un factor de reducción del riesgo del orden de 10^4. De ahí que el riesgo de contaminación de un lote de embriones de llama se aproxime a cero cuando la región de origen presenta condiciones epidemiológicas y ecológicas favorables. El riesgo de contaminación por *Mycobacterium* o *Brucella* depende de la incidencia de la infección por estos agentes patógenos. Pero aun con los niveles más desfavorables de prevalencia, cuando las pruebas de diagnóstico realizadas en el rebaño y en los animales donantes, tanto antes como después de la extracción de los embriones, dan resultados negativos, el riesgo no es superior a $10^{-3,3}$. El presente estudio demuestra que la evaluación de riesgos puede constituir un valioso instrumento para facilitar los intercambios internacionales de embriones, sobre todo para especies de las que poco o nada se sabe en cuanto a las interacciones entre el embrión y los agentes patógenos.

Palabras clave
Brucelosis – Embriones – Estomatitis vesicular – Evaluación de riesgos – Fiebre aftosa – Lengua azul – Llamas – Transferencia de embriones – Tuberculosis.

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