REGRESAR A
MODULO 2

QUANTITATIVE ASSESSMENT OF THE RISK OF DISEASE TRANSMISSION BY BOVINE EMBRYO TRANSFER

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FOREWORD

The Pan American Foot-and-Mouth Disease Center (Pan American Health Organization/World Health Organization) has a long standing commitment to assist countries of the Western Hemisphere in the safe and legal international exchange of livestock and their genetic material. To prevent the spread of foot-and-mouth disease in particular, the Center has cooperated actively over the years with countries of the Region in the development of protocols and testing procedures for the disease.

Over the past decade, it has become apparent that moving embryos is probably the safest method for moving genetic material between countries. Numerous scientific papers have shown the safety of embryo transfer techniques with regard to disease transmission. However, progress has been slow in relaxing the existing regulations to permit legal embryo movements between countries that have a different animal disease status. One reason for this apparent reluctance to change on the part of veterinary authorities has been the inability to provide satisfactory estimations of the risk involved. Moreover, trading policies will, in the future, be largely dependent on risk management, under the terms of NAFTA and GATT.

In this issue of the Centers scientific-technical monograph series, two distinguished scientists n the field of disease transmission and embryo transfer present a quantitative assessment of the risk of disease transmission by bovine embryos. We are hopeful that the application of this methodology will not only result in freer exchanges of embryos, but will also provide fresh insight into how to trade more freely and safely in other products of animal origin.

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INTRODUCTION

Embryo transfer (ET). especially in cattle, ‘snow big business in many parts of the world. Surveys by Thibier *(363?)* showed that in 1991 more than 240.000 bovine ETswere done worldwide and in 1992 at least 280,000. Since these data are incomplete it is estimated that the current level is probably at least 350,000 annually and a conservative estimate of embryos moved between countries is approximately 35,000 annually *(40).*

Until a few years ago, most countries that were free of major epidemic diseases such as rinderpest and foot-and-mouth disease (FMD) officially maintained a zero-risk policy for the importation of embryos from countries where such diseases existed, The drawbacks of such a complete import prohibition are:

- The countries were not legally able to obtain desirable genetic material.
- It stimulated illegal movement of embryos.

Also, this zero-risk policy will be untenable in the near future in view of emerging international trade agreements such as the North American Free Trade Agreement (NAFTA) and the General Agreement on Trade and Tariffs (GATT). Under these, health requirements cannot be used as non-tariff trade barriers. Instead, international trade must be based on risk management, which in turn must rely on risk assessments that are founded on scientific evidence, and are consistent, transparent and well documented.

Because research and field experience have shown that embryos can be moved with little risk of disease transmission, many regulatory officials now favor some form of risk management, particularly when dealing with potentially catastrophic diseases such as FMD and rinderpest. However, the problem is to define what is the risk of a specific import activity and what is an acceptable level of risk relative to the consequences of disease introduction.

The Research Subcommittee of the International Embryo Transfer Society (IETS) Import/Export Committee classified FMD in their 1992 categorization as a disease agent for which sufficient evidence has accrued to show that the risk of transmission is **negligible** provided that the embryos are properly handled between collection and transfer (4,33). However, for veterinary regulatory officials the term **“negligible”** may have a quite different meaning when applied to a catastrophic disease such as FMD from when it is applied to a commonly endemic disease like infectious bovine rhinotrachitis (IBR). This is because the magnitude of the socio-economic consequences of inadvertent introduction of these two pathogens is totally different. Thus, for each disease agent a risk assessment must take account of both the risks and the benefits of embryo importations, including the possible consequences of disease introduction.

Quantitative risk analysis (QRA) is a rapidly developing technique which has been used for many years in engineering and economics (16,21), but which is relatively new to veterinary medicine *(2,13,1417,22,34).* It makes use of various branches of science such as epidemiology, statistics, mathematics, microbiology and pathology, as well as of FT technology.

A QRA starts out with the identification of a hazard which, in the case of international movement of embryos, is the introduction of a specific foreign animal disease by an importation of embryos from a country where that disease exists. Next, a pathway of specific events from the point or origin to the destination must be identified. At each point or event in the pathway the following questions must be asked: What can go wrong; bow often is that likely to happen and what are the consequences? The accumulation of answers to those questions for all of the events will constitute the final risk related to the importation. A QRA must be consistent, well documented, flexible and transparent. It must clearly present the information and conclusions about the risks involved, It will also show which measures contribute best to risk reduction. The results can then be used by decision-makers to help choose a course of action.

The objective of Pad I of this publication is the development of a QRA model (or disease transmission by ET. Bovine ET was chosen for the discussions because bovine embryos are the most commonly used in international trade. In Part I, Section I some of the general aspects of bovine ET procedures are briefly reviewed, while Section 2 contains a general discussion of the risk factors involved, in section 3 the scenario pathway is shown and the general principles of risk quantification are outlined.

Practical application of the QRA model is presented in Part II of the paper. This deals with the construction of a specific QRA model for the risk of transmission of FMD by bovine ET from a FMD infected country. Because of the important potential for export of embryos of Zebu cattle breeds from Brazil, particularly from the area comprising the north-eastern part of the state of Sao Paulo, that region was selected for constructing the model.

Part II Section 1 gives some specific details of the epidemiology and control of FMD in the area concerned. The status of the ET industry of Brazil and in the selected area is also reviewed. In Section 2 a quantification of the risks is proposed. Also, documentation, is provided on the evidence and intermission used for the proposed risk values, followed by the statistical elaboration of the risk estimates, discussion and conclusions.

Obviously, the risk estimates presented here will not be the last word on this subject. However, new data can easily be incorporated and the risks can then be recalculated. This is one of the great strengths of the ORA technique.

PART I

DEVELOPMENT OF A OUANTITATIVE RISK ASSESSMENT MODEL FOR DISEASE TRANSMISSION BY BOVINE EMBRYO TRANSFER

OVERVIEW OF BOVINE EMBRYO TRANSFER PROCEDURES

This brief overview covers general aspects of the bovine ET procedures, particularly those that have a bearing on animal health safety aspects of international bovine embryo movement. Important sources for the information that follows in this section have been the Training Manual for Embryo Transfer in Cattle", FAO Animal Production and Health Paper 77(26), and the Manual of the International Embryo Transfer Society (IETS Manual) (33). For more detailed descriptions of FT methods and procedures the above mentioned publications should be referred to.

Embryo Donor Herd Management

Embryo donors cows should ideally he healthy and fertile. They are likely to be valuable animals and are usually kept under intensive management. Embryo collections can be done at the farm or in an embryo collection center, but moving donor cows to a center may cause management problems particularly with lactating cows. In beef cattle, oestrus detection and superovulation may also be adversely affected by unfamiliar environments. Moreover, the gathering together of animals from different origins into an embryo collection center may create disease problems.

The planning and execution of an ET program is complex and requires frequent contacts. Sometimes over a period of several weeks, between the farm management personnel and tine Embryo Collection Team (ECT). For a successful ET program, both farm personnel and the ECT must pay close attention to herd health aspects during this time.

On the donor farm it is necessary to have suitable facilities, including chutes for handling cattle, a refrigerator for keeping drugs and an appropriate laboratory area for works with the embryos. Accurate oestrus detection is a key factor for the success of the FT program. The observation of behavioral and possibly endocrinological changes requires frequent and close inspection of the animals in the herd.

Superovulation

Collection of embryos by flushing the uterus following natural oestrus and insemination is possible, but usually yields only one embryo. In order to produce multiple embryos the donor cows are given a series of hormone injections over a period of approximately 3 to 5 days in the mid-luteal phase. Several donors can be treated simultaneously so that they come into oestrus about the same time. This facilitates insemination and embryo collection. The hormone treatments are usually administered by the owners' own veterinarian or by farm personnel under the direction of the ECT.
Following their superovulatory treatment donors are observed closely for signs of oestrus and then inseminated. Because the multiple follicles ovulate over a period of hours, and because the

transport of sperm and ova may be altered by superovulatory treatment, donor cows are inseminated more often than normal. The use of high quality semen is recommended.

Recovery of Embryos

Embryos are usually recovered six to eight days after oestrus, but not later than day nine, and the first day of oestrus is counted as day 0. An idea of the number of embryos present can be obtained by palpating the ovaries per rectum to estimate the number of *corpora lutea*. Before the start of the uterine flushIng. it is standard practice to administer an epidural anaesthesic. Recovery procedures are done as aseptically as possible; the perineum and tail area of the cow should be cleaned and the vulvar area disinfected with iodine soap and/or alcohol swabs.

Flushing of the uterus is done by a catheter with a small inflatable cuff near to its end. The operator, with hand in the rectum, manipulates the catheter through the cervix into the uterus. Once it is in position, the cuff is inflated to secure the catheter in place. The uterus anterior to the cuff is then filled and emptied repeatedly with varying amounts of collection medium, depending on the size of the uterus. Alternatively, a continuous flow system may be used by holding a container such as a disposable plastic infusion bag about one meter above the cow. In the latter case the flow of the fluid is controlled by clamps on the tubing. The fluid is collected either in a graduated cylinder or it is allowed to flow through a filter that retains the embryos.

When cylinders are used, the collection fluid is allowed to sediment for several minutes, whereupon most normal embryos settle at the bottom. The top fluid is siphoned off and the remainder is poured into flat-bottomed recovery dishes. The cylinder is rinsed at least twice with further medium to ensure recovery of any retained embryos.

When filters are used to obtain the embryos, the embryos, uterine debris and mucus are rinsed from the filter membrane with medium and placed in a recovery dish. The whole surface area of the recovery dishes is examined systematically at about 0-20 times magnification to locate embryos. The embryos are then transferred to smaller dishes with fresh medium and evaluated for their stage and grade, and the integrity of the *zona pellucida* (ZP) is checked using a stereomicroscope at e.g., 50X magnification.

Embryos destined for international movement must be washed an additional 10 times, according to the method described in the IETS Manual *(33).* This involves transferring embryos, in groups of 10 or fewer, through 10 changes of medium. The washing can be done conveniently in disposable plastic multi-well cell culture plates. At least 2 ml volumes of fluid are used in each well and the group of embryos is transferred between each well with a fresh, sterile micropipette. Each “wash” must constitute at least a one-hundred-fold dilution of the previous wash. The embryos must be gently agitated in each of the washes, and as soon as this is completed, they are moved to the next wash. Only embryos from the same donor are washed together, and a maximum of 1O can be done at any one time.

The embryos are under low power observation during the washings, but it is finally necessary to use a higher magnification again (i.e., >5OX) to ensure that the ZP is still intact and free from extraneous cellular debris. If they are required, samples of collection fluid, uterine mucus and

debris, as well as any unfertilized and degenerated ova should be collected and stored at low temperatures for later testing for disease agents.

Bovine embryos can be frozen and preserved at low temperatures in liquid nitrogen. If freezing procedures are carried out correctly, pregnancy rates are not usually reduced by more than 10 percent below those expected for fresh embryos transferred under similar circumstances. For freezing, embryos are usually placed in a freezing medium containing 10 percent glycerol (or some other cryopreservative) for 10-20 minutes and then loaded into correctly identified straws for the controlled freezing process.

Storage of Embryos

Embryos may be stored frozen from the time of their collection until they are used. In the interval, while the whole batch of embryos for export is assembled and while export documentation is being prepared, the donor farm as well as the surrounding area may be kept under surveillance so that It can be officially certified to be tree at specified diseases. Typically. but depending on the incubation period of the pathogen, the interval would be at least one month after the last collection of embryos for tile export batch.

Embryo Transfer

An important step for the success of ET is the selection of recipients that are healthy and reproductively sound. Ideally, the recipient should be in oestrus on the same day as the donor when the embryo was collected, but asynichronies of up to 24 hours can yield acceptable pregnancy rates. ET can be done surgically or non-surgically, but the latter method is almost invariably used nowadays.

For non-surgical transfer an epidural anesthetic is considered essential by most ET technicians, both for animal welfare and to give consistently good results. Transfer equipment must be cleaned and sterilized and should be handled in a manner that will reduce the possibility of contamination. The vulvar area should be cleaned to ensure that the pipette (ET gun) containing the embryo enters the vagina with minimal contamination. The next step is to pass the ET gun through the cervix into the uterus. Because the cervix can be difficult to pass, heifers may present a special challenge arid sortie breeds are more difficult than others. The tip of the instrument is then passed smoothly and atraumatically into and along the uterine horn ipsilateral to the corpus luteum. Some people believe that surgical transfer gives a slightly higher pregnancy rate than nonsurgical transfer even when the latter is performed by very proficient technicians.

Record Keeping

Accurate record keeping and identification of the embryos is essential for the integrity of the ET program. Standardized forms developed by the IETS are recommended ant these have been adopted by many breed organizations as their official documents for registration of offspring resulting from ET.

CONSIDERATION OF RISK FACTORS IN EMORVO TRANSFER

While it is universally accepted that ET is the safest method for moving animal genetic material, few attempts have been made so far to **quantify** the levels of disease risk reduction that are achieved by the procedures described in the lETS Manual (33).

Stringfellow et al. (32) reviewed the potential "chain of infection in ET and indicated that if a single link in this chain is broken transmission is prevented. However, since it is impossible to prove that a biological event, such as disease agent transmission, **cannot** happen, data must be obtained on which to base a realistic estimate of the **probability** of its happening in a paper on risk assessment with regard to the importation of bovine embryos. Acree and Beal (1) state that a field trial involving the transfer of 300 embryos, obtained from 100 exposed parents, into 300 susceptible recipients and resulting in 150 healthy calves, would permit a prediction with 95 percent of confidence, that no more than 1 in 100 of these "exposed origin" embryos would infect a susceptible recipient upon transfer and no more than 2 in 100 would result in an infected offspring.

It may be argued that the factors which cumulatively determine the safety of bovine embryos for international movement are:

a. The disease situation of the country and area;
b. The health status the farm of origin and the embryo donor;
c. The pathogenesis of the disease agent;
d. The competence and efficiency of the ECT in processing the embryos;
e. The health status of the herd of origin, while the embryos are In post-collection storage;
f. Post-collection testing of collection fluid, including any unfertilized embryos;
g. Susceptibility of recipients to infection by the disease agent.

Factors (a) - (c) constitute the first line of defense against the introduction of exotic disease agents through embryo importation and factors (d) - e) constitute the second line of defense, In the third line of defense are factors (f) and (g) which although they might not be essential for diseases such as FMD, could be important for less obviously symptomatic conditions or for insect-borne diseases such as bluetongue. These factors will now be discussed in more detail.

Disease Surveillance and Health Status of the Donors

Of primary concern to regulatory officials are the measures taken in the exporting country to ensure a very low probability of the embryos having been exposed to catastrophic disease agents. While FMD is of utmost concern to most countries, the diseases that are considered to be of potentially catastrophic risk do vary between countries.

The efficiency of risk reduction measures taken in the country of origin mainly depend on the efficiency of the disease surveillance and reporting system of that country, and these have to be assessed for each individual country or area. The official approval arid control of the ECT is an important risk reduction factor. The ECT must not only have adequate facilities, equipment and training (23), but must also be committed to following all proper hygienic procedures and to

maintaining high levels of professionalism and integrity. Tile efficiency of the ECT should be maintained through regular official inspection to ensure compliance with sanitary collection, processing and storage of embryos. Thibier and Guerin (38) reported excellent results with a sanitary control system for ECTs that was used by the French Ministry of Agriculture (Veterinary Services) for a period of over six years. They concluded "So, such a control was therefore found to be feasible and proved very valuable for insuring a high level of health security involving ET procedures."

Pathogenesis of the disease agent

For a pathogen to be transmitted by an embryo, it must be able to associate itself with the embryo or the surrounding issues and fluid. The probability of this happening depends very largely on the pathogenesis of the disease, especially whether or not the disease agent is present in the genital tract during the disease and after, on the convalescent phase. For example, it is possible that FMD virus would reach the uterine environment and the embryo during viremia. Contamination of personnel, media and equipment could also occur during that period. However, collection of embryos intended for international movement in the middle of an FMD outbreak, when animals are viremic, is very unlikely.

After recovery of the cows, the probability that FMD virus will reach the embryo is remote (35), but this scenario may be possible with other disease agents. Even if the donor cow is a pharyngeal carrier of FMD virus, viremia and contamination of the embryo in the genital tract is highly unlikely because of the high FMD antibody levels (35). Again, with other diseases where there are latent infections, embryo exposure may be possible. Therefore, detailed information about the pathogenesis of diseases of concern to the importing country is of great importance for effective risk assessment.

Sanitary Handling and Washing Of embryos

Technical competence collecting, handling. washing arid microscopic evaluation of embryos is a vital ECT responsability. Washing of bovine embryos, provided that their ZPs are intact, has been shown to be highly effective for the removal of many pathogens (viruses and bacteria) from exposed embryos (reviewed by Singh [28], Singh and Thomas [30]. Stringfellow et al. [31,32]. Shisong et al, [27]. lETS Manual [33] and Wrathall [40]).

Bovine embryos exposed *in vitro* to large amounts of FMD virus were, for example, shown to be free of virus after 10 serial washings that were done according to the procedure laid down in the IETS Manual (33). The amount of virus used to contaminate the embryos prior to washing in this particular experiment was at least ten thousand times greater than that reported to be present in the ovarian follicular fluid of FMD viremic cows *(10,20). T*he embryo collection process itself, which involves flushing the uterine cavity with large quantities of fluid medium, introduces a further dilution factor of approximately 1,000-fold.

If should perhaps be emphasized that, by definition, when starting with a suspension of 10^8 infective virus units in the medium, there is at the fourth wash (the 10^{-8} dilution) a 50 percent chance of there being one virus unit in the washing medium. In wash number 5 the probability of

detecting virus is about 1:100, while in wash number 6 it is 1:10,000 etc. Following dilution to the tenth wash the probability of detecting virus would be 1:1.000,000,000,000. Caamano et al. (8) could not detect any FMD virus beyond the first wash, starting with the in vitro contamination of embryos in a medium with an infectivity of $10^{-6.8}$ unit/mI, but they may have lost some infectivity during the virus/embryo incubation period of 16 h at 37°C.

The effectiveness of washing embryos for removal of pathogens depends on whether or not any pathogen has adhered to the ZP or got trapped in crevices or submicroscopic defects in the ZP. In this respect it should be noted that after 10 washings no virus could be recovered from 169 embryos and oocytes that had been contaminated *in vitro* with FMD, despite the use of very sensitive test methods (29). The probability that FMD virus might be present **on** or **within** the embryos collected from a viremic donor has been shown to be low: thus when 48 washed embryos and oocytes that were collected from donors at the peak of FMD infection were tested (again using very sensitive test procedures) the results were negative (18,20). Apart from the possibility of a specific pathogens/ZP interaction, the effectiveness of washing embryos for removal of pathogens basically depends on' the integrity and competence of the ECT. Therefore, the official approval and regular monitoring of the ECT by the veterinary service are important factors for reducing the risks of disease transmission by embryos 36).

The studies on embryos exposed to FMD virus in vitro were followed by experiments involving their transfer to cattle. FMD virus-exposed ZP-intact embryos were washed according to the lETS Manual protocols and then transferred into the uterus of susceptible cattle (18). Later, embryos collected from cows that were in the viremic stage of FMD were transferred. Finally, a large field trial was done that involved transferring embryos collected from cows that had been naturally exposed to FMD were used 39). None of these in vitro experiments showed a positive result (i.e., transmission of FMD virus by the embryo) which is in agreement with the results of the a vitro experiments.

For the purposes of considering the disease transmission risk of embryos we define **"non-exportable embryos"** as those with a defect in the ZP, those with extraneous material adhering to the ZP, and hatched blastocysts without a ZP (figure 1, also see glossary).

Freezing and storage of embryos

Storage of frozen embryos prior to their transfer into susceptible recipient animals allows for the donor animals and the farm of origin to be observed for periods of time equivalent to or longer than the incubation periods of specific diseases that are of concern. If the donors and the other animals on the farm remain healthy, then obviously there is much less chance that the embryos will have had any exposure to those infectious diseases.

Naturally, it is an essential requisite for disease freedom to ensure that the embryos have been collected, processed, washed and frozen in sterile media, and using sterile

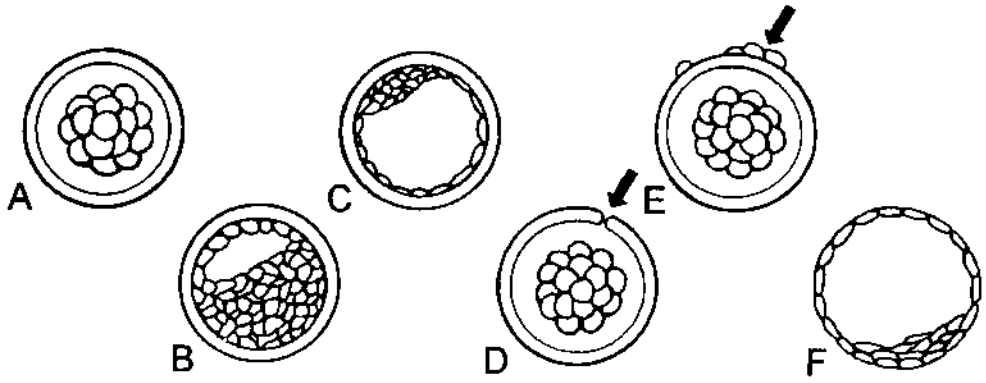

Figure I. Embryos must be evaluated after treatment for intactness of the zona peIIucida (ZP) and freedom from adherent material. A. ZP-intact morula, B.ZP-intact blastocyst, C. ZP-intact expanding blastocyst, D. Morula with a defect In the ZP, E. Morula with extraneous material adhering to the ZP, F. Hatched blastocyst without ZP. A, B and C are acceptable embryos in terms of disease control. D, E and F are unacceptable in terms of disease control.
Source: IETS Manual (Ref. 33) equipment. They must then he stored in sterile straws and in liquid nitrogen containers that have had no possibility of prior exposure to disease pathogens or to contaminants.

The probability that clinical disease would be detected if it did occur on the farm of origin during the storage of the embryo will depend on the efficiency of the local disease surveillance system and on the level of veterinary supervision. Judging the reliability of those involved in such duties can be politically sensitive, but it is a very important part of risk assessment.

Testing of Collection Fluids

The microbiological testing of collection fluids prior to transfer of the embryos has potential benefits for risk reduction. While it may not be necessary for diseases such as FMD, it could be important for conditions that tend to be clinically asymptomatic (e.g., bluetongue in cattle) and, for these, regulatory officials may want to include testing of collection fluids to show the absence of the disease agents from the uterine environment. Testing of collection fluids could also be an important risk reduction factor with diseases for which insufficient research data exist regarding the risk of disease transmission by embryos. In addition, it will help to ensure that the media used to collect and process the embryos were themselves free from pathogens.

Transmission of Pathogens into Susceptible Recipients

The actual transmission and establishment of a pathogen in the importing country depends on the ability of the pathogen to infect the recipient of the embryo and to produce the disease. For insect-borne diseases, such as bluetongue the presence 01 the specific vector is also required for the disease to become established in the importing country.

Table 1. Scenario Pathway and Consequences of Risk Events for Disease Transmission by Bovine Embryo Transfer.

Number of embryos to be imported (N_1)
Number of donor farms required (N_2)

		CONSEQUENCE
Animal Health status of the Region and Farm of Origin.		
P_1	Disease in the Region?	No ➔ No risk ➔ P_2
		Yes➔
P_2	Disease on donor Farm?	No ➔ No risk ➔ P_3
		Yes➔
P_3	Infection on donor farm observed by Animal Health Surveillance System?	Yes➔ No export ➔ P_4
		No ➔
P_4	Infection on donor farm observed by Embryo Collection Team?	Yes➔ No export ➔ P_5
		No ➔
Pathogenesis of the Disease Agent		
P_5	Disease agent reaches embryonic environment of infected donor?	No ➔ No risk
		Yes➔ **Contaminated** embryos ➔ P_6, P_7
Embryo Collection		
P_6	Non-exportable embryos (F_1) detected?	Yes➔ Individual embryos discarded ➔ P_9
		No ➔ **Infectious** embryos
P_7	Exportable embryos ($1-F_1$) properly washed?	Yes➔ **Infectious** embryos P_9
		No ➔ ➔ P_9
P_8	Disease agent adheres to zona pellucida?	No ➔ Washing effective➔ P_9
		Yes➔ **Infectious** embryos➔ P_9
P_9	Total risk of **Infectious** embryos (P_6 , P_7 and $P_{8)}$	Total risk infectious embryos ➔ P_{10}
Health status of Donors while Embryos in Quarantine		
P_{10}	Clinical disease on infected donor farm?	Yes➔ No export
		No ➔ ➔ P_{11}

Diagnostic Test

P_{11} Diseaseagent detected in infected Collection fluid — Yes➔ No export; No ➔ Export ➔

Import risk

SCENARIO PATHWAY FOR THE QUANTIFICATION OF RISK FACTORS IN EMBRYO TRANSFER

The scenario pathway (table 1) illustrates the flow of risk-associated events from the donor farm, to the time of exporting the embryos. The pathway showing the flow of events in the importing country is presented in table 2. The pathways are based on a knowledge of normal ET practices as described in Section 1 and consideration of the risk factors in Section 2.

Questions asked for each event are: "What can go wrong?", "How likely is that to happen? and "What are the consequences?". For instance, if FMD is defected on the donor farm, the export of embryos will be cancelled. Similarly, if non-intact ZP embryo is defected that embryo will be discarded for export. However, failure to detect in either case may mean that embryos will be processed and exported.

N_1 (table 1) is the proposed number of bovine embryos to be imported, from a Region where a disease exits, which must be prevented from being introduced into the importing country. The number of farms required to assemble the batch of embryos for import (table 1 N_2) depends on the number of embryos required, the number of transferable embryos produced per donor animal and the number of donors per farm. These numbers may vary in accordance with the livestock production system of the Region. The presence of the disease In the Region during the time that the batch of embryos for export is assembled (table 1, P_1) determines whether the disease may be on the donor farm (P_2).

The next events in table 1 are the detection of the disease in the herd by the animal health surveillance system (P_3), and/or by the ECT (P_4), during the period prior to the collection, as well as the detection of disease signs by the ECT during the actual collection process. Event P_5 is the contamination of embryos by a disease agent in the genital tract of the donor. **Contaminated** transferable embryos can be divided into two fractions:

F_1 = non-exportable embryos which, for reasons of potential disease transmission (as defined in Section 2) consist of those with a defect in the ZP, with extraneous material adhering to the ZP, and hatched blastocysts without a ZP (figure 1), and, $1\text{-}F_1$ = exportable embryos.

Table 2. Scenario Pathway and Consequences of Risk Events for Disease introduction and Establishment by the importation of infectious Bovine Embryos.

Receiving Country (IMPORT RISK)		CONSEQUENCE
P_{12}	Transmision of disease agent by transfer of infectous embryos?	No ➔ *No* risk Yes ➔ infection of recipient ➔ P_{13}
P_{13}	Disease agent transmitted by vectors	No ➔ disease spread Yes ➔ ➔ P_{14}
P_{14}	Vector present in importing country?	No ➔ No risk Yes ➔ Disease spread

P_6 is the failure to detect and remove non-exportable (F_1) embryos. When a non-exportable embryos detected it is discarded. However, since undetected non-exportable contaminated embryos are not affected by the washing procedures, they are now referred to as **infectious** embryos, meaning that they carry at least one infective unit of the pathogen. When contaminated exportable embryos ($1-F_1$) are not property washed (P_7), these also may remain **infectious**. Even if embryos are washed properly, the effectiveness of the washing procedure depends on the characteristics of the disease agent: i.e., when a disease agent specifically adheres to the ZP (P_8) it may not be completely removed so again the embryo may remain **infectious**. Thus, events P_6, P_7 and P_8 lead from **contaminated** embryos P_5 to **infectious** embryos P_9. The likelihood that embryos will still carry at least one infective dose following the events described under P_6 ,P_7 and P_8 is the "sum"[2] of the following three fractions: non-exportable embryos not removed, embryos not properly washed, and disease agent not removed by the washing.

The observation of disease on the donor farm while the embryos are in storage (table 1, P_{10}) can be made by the official veterinary service or by the local animal health surveillance system. If the disease is detected and notified the export of the embryos will be cancelled.

Diagnostic tests (P_{11}) are included in the pathway scenario (table1) because some countries may, for instance, require the testing of collection fluids. For catastrophic diseases most importing countries will wish to consider only the **import risk** (table 1), which is the probability that at least one infectious embryo is contained in the lot for export. However, for other diseases, including those that are exclusively vector transmitted, the scenario pathway of table 2 might also be considered. For event P_{12} the question is asked whether the disease agent is transmitted by ET to the recipient of the embryo.

The minimal infectious dose of the pathogen must be considered in this regard. If the recipient becomes infected and vectors are not required for spread to other members of the population, then there is a possibility that the disease will establish itself. Of course the disease may also establish itself if a vector is required and the vector is present.

[2] The "Sum" of a Probability A (P_a) and Probability B (P_b) is not simple the addition of these Probabilities, but $1-(1-P_a) \times (1-P_b)$ (Ref. 15).

Next, each event (e.g., the **number** of embryo donor farms, or the **probability** of selecting an infected farm) is considered as a unit to be ascribed a mathematical value (table 3). What are shown in table 3 are conditional probabilities. For example, given that a disease is not detected the first time, what is the probability that it is also not detected the second time? And given that it is not defected the second time, what is the probability that it is not detected the third time?

Sequential probabilities can be combined. For Instance, if the probability is 0.07 that FMD on the farm of origin is not detected by the Animal Health Surveillance System and the probability is 0.02 that the ECT does not detect the disease during their presence on the farm, then the combined probability that FMD is not detected by the Animal Health Surveillance System and the ECT is 0.07 x 0.02 = 0.0014. In this way the final import risk and the risk of disease establishment can be calculated.

It is not usually possible to state with certainty what is the likelihood that an event will occur. For instance, we do not know with certainty how often the ECT will miss a non-intact ZP, or how many infective doses of a pathogen will actually remain attached to the ZP. However, on a scale from 0 to 100%, it usually is possible to agree on a range of frequencies that an event will occur. For instance, the minimum and maximum estimates of likelihood of the occurrence may be 0.1% and 30%, respectively. Experts and evidence will point to the most likely frequency somewhere within this range, for instance 10%. Such a frequency distribution is often not "normal" and it can be quite asymmetrical.

The minimum, maximum and most likely values form what is known as a 'three-point estimate', which not only expresses the best knowledge about the event, hut also provides a guide to the degree of uncertainty of information related to the event. Examples of three-point estimates are given in table 8 of Part II, which lists all the events in an embryo ml ports ion risk scenario pathway for FMD. The estimated values are based on currently availabIe scientific and practical evidence. Simple mathematical accumulation of the three-point estimates for all the events provides a final three-point estimate for the importation risk.

Three-point estimates do not indicate the relative Chance that the event will occur, but the Lotus 123/@RISK computer program[3] used for the present risk study, is a powerful software package which permits the construction of a so-called Probability Density Function (PDF) for each event (for examples see Figures 3 and 4). A PDF shows the range of probabilities that the event will occur and also the relative chance that it will occur. A narrow range for the PDF indicates a greater confidence in the estimates than a wider range.

The Lotus 123/@RiSK computer program constructs a PDF for each event through a large number of recalculations, based on a random number generated for each calculation, and the distribution parameters, such as the minimum, maximum and most likely probability values. The results of such simulations are presented graphically (for examples, see

[3] Lotus and 1-2-3 are registered trademarks of Lotus Development Corporation. @Risk, Risk Add-in for Lotus 1-2-3, Version 2.01. 1902. Palisade Corporation, 31 Decker Rd.. Newfield, NY 14867.

TABLE 3. Mathematical Units of Risk for Disease Transmission by Embryo Transfer.

N_1	Number of embryos to be imported
N_2	Number of donor farms required for N_1
P_1	Probability of disease in the Region during the time N_1, is being assembled for export
P_2	Probability of disease on a donor farm*
P_3	Probability that the Animal Health Surveillance System fails to detect an infected farm
P_4	Probability that the Embryo Collection Team fails to detect an infected farm
P_5	Probability of embryo donors with infection and contamination of the embryos and/or genital tract
F_1	Fraction of non-exportable embryos
P_6	Probability that the ECT fails to detect and remove non-exportable embryos (F_1)
P_7	Probability of exportable embryos ($1-F_1$) being inadequately washed
P_8	Probability of residual pathogen (at least one infective dose) adhering to adequately washed exportable embryos ($1-F_1$)
P_9	Probability of collecting infectious embryos from an infected donor ** (a function of P_6, P_7 and P_8)
P_{10}	Probability of failure to detect disease on the farm of origin while the embryos are being stored in quarantine
P_{11}	Probability that laboratory tests fail to detect the disease agent in the collection fluid from an infected donor
P_{12}	Probability that the disease agent is transmitted by the infectious embryo to a recipient
P_{13}	Probability that the disease is vector transmitted
P_{14}	Probability that the disease vector is present

IMPORT RISK* =** $P_2xP_3xP_4xP_5xP_9xP_{10}xP_{11}$
RISK OF DISEASE SPREAD IN THE IMPORTING COUNTRY = Import Risk x $P_{12}xP_{13}xP_{14}$

* The probability P_2 that at least one infected farm is included in the number of farms required can be calculated by the binomial $1-(1-P_1)^{N2}$. However, in the range of the values being used N_2xP_1 gives a similar numerical result. (Ref. 15)
** $P9 = 1-\{[1-(P_6xF_1)]x[1-(P_7x(1-F_1))]x[1-(P_8x(1-F_1))]\}$ (Ref. 15)
*** Probability of at least one infectious embryo included in the import batch (N_1).

See Figures 3, 4 and 5) and as statistical reports (example In table 10). Of particular interest are the Expected Results, which are the most likely occurring outcomes of the simulation, and the Percentile Probability Values, indicating the chances at particular outcomes. The Lotus 123/@RISK computer program also accumulates the PDFs of all individual events to form a final PDF for the import risk, giving a most likely import risk, as well as, the maximum risk at the 95 percent chance level. The present model for the quantitative assessment of the risk of disease transmission by bovine El is applied to FMD in Part II of this paper.

PART II

APPLICATION OF THE OUANTITATIVE RISK ASSESSMENT MODEL TO THE EXPORT OF BOVINE EMBRYOS FROM A REGION WITH FOOT-AND-MOUTH DISEASE

GENERAL INFORMATION

Epidemiology and Control of FMD in the Exporting Country and Region

For the purpose of illustration the Region selected (or collection of the embryos is the FMD endemic region in the State of Sao Paulo, Brazil. Cattle populations of Brazil, the State of Sao Paulo and of the selected Region are listed in table 4. In the Region some of the best Zebu breeds can he found and the Region has been a source for this genetic material for carry parts of the Americas.

In the Region there is a systematic FMD vaccination program for the cattle population, which currently covers about 90% of the farms. Cattle of one year of age or older are vaccinated bi-anually during March and September with commercially available inactivated FMD vaccines.

Table 4. Numbers of farms and cattle in Brazil and selected Region.

	No. of Farms	**No. of Cattle**
Brazil		147,000,000
State of Sao Paulo	133,000	12,200,000
Selected Region Areas		
Pres. Prudente	14,800	2,260,000
Marilia	11,300	1,210,000
Aracatuba	11,000	1,770,000
S. José Rio Preto	25,000	2,150,000
Riverao Preto	15,300	1,170,000
Bauru	9,100	1,100,000
Total selected Region	86,500	9,660,000

Sources: Animal Health Yearbook, FAO/OIE/WHO, 1992 (Ref. 12) Combate a Febre Aftosa no Estado de Sao Paulo, March, 1994, Secr. Of Agriculture/CATI/DDA (Ref. 9)

Younger cattle which only received one vaccination are revaccinated three months later during May or November. An efficient Vesicular Disease Surveillance System Is operated by the Secretary of Agriculture of the State of Sao Paulo. This system is part of the Continental Vesicular Disease Surveillance System, coordinated by the Pan American Foot-and-Mouth Disease Center (5, 6,24).

In comparison to the period prior to 1980, the number of outbreaks reported in recent years has decreased considerably (Figure 2). As can be observed in Table 5 the Region continues as a secondary endemic area, and it is characterized by high cattle density, a high rate of renewal of

susceptible young cattle, and much cattle movement particularly during certain periods of the year. Although, there are frequent outbreaks of the disease, the number of diseased animals Is relatively low, with morbidity being S-IC percent of the affected herd in most cases.

The Embryo Collection Teams

The ET industry is well developed in Brazil, The Brazilian Embryo Transfer Association (ABTE) has 365 members (August, 1993) which, as shown in table 6, performed a total of more than 51.000 ETs (7). In addition it is estimated that non-affiliated ET practitioners performed some 5.000 transfers. In 1992 and 1993 approximately 10,000 embryos were frozen annually. The results of the ETs are summarized in Table 7.

There is no doubt that several of the ECTs are capable of adhering to the standards for the sanitary handling of embryos for export as recommended by the lETS and the International Office of Epizootics (OlE) (23,33). However, there is no system of official approval or control of ECTs in place at the time of writing,

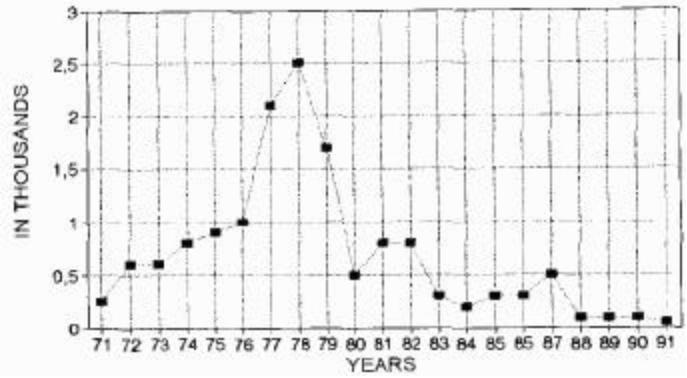

Figure 2. Number of herds with FMD in ma *State* of SA0 Paulo during 1071-1 OGI
Source: Ref. 9

Table 5. Number of FMD Infected Herds in Brazil and the Selected Region.

Year	**1992**	**1993**
Brazil	1.199	1.417
State of Sao Paulo	225	196
Selected Region Areas:		
Pres. Prudente	5	27
Marilia	13	26
Aracatuba	18	14
S. José Rio Preto	76	25
Riberao Preto	61	36
Bauru	18	7
Total selected Region	191	135

Sources: Situation of The FMD Control Programs, South America, 1992 and 1993. Pan American Foot—and—Mouth Disease Center, Caixa Postal 589, CEP 20001-970, Rio de Janeiro, Brazil. Combate a Febre Aftosa no Estado de Sao Paulo, March, 1994, Secr. de Agrlc./CATI/DDA (Ref. 9).

Table 6. Statistics on the Embryo Transfer in Brazil: Members of the Brazilian Embryo Transfer Association (ABTE)*.

	Number	%	ETs performed
University and research	91	25	2,730
Graduate students and trainees	25	7	250
Government officers	12	3	0
Private, solely ET 28,800		36	10
Private, ET + clinics	201	55	20,000
Total	365	100	51,780

* August 1993. **Source**: Ref. 7

Table 7. Statistics on the Embryo Transfer Industry in Brazil: Estimated Number of Bovine Embryo Transfers performed during 1892.

Number of donors	3,000
Number of viable embryo collected	69,000
Number of embryos transferred	51,000
Number of pregnancies	29,000
Pregnancy rate*	56,8 %

* Pregnancy usually determined at 60 days. **Source**: Ref. 7

QUANTIFICATION OF RISK FACTORS FOR FOOT-AND-MOUTH DISEASE TRANSMISSION BY BOVINE EMBRYO TRANSFER

In Table B estimates are presented (or he risk of each event In the scenario pathway (tables I, 2 and 3) for the transmission of FMD by bovine ET.

N_1 *Number of* embryos to *be imported.* The initiating event for this scenario pathway is the proposed importation into art FMD free country of 200 bovine embryos from tire selected Region in the State of Sao Paulo Brazil.

N_2 *Number* of *donor farms required.* The embryos are to be collected by the non-surgical method on selected farms. The predicted number is four transferable embryos, stages 4-7 grades 1 and 2 (33) collected from each superovulated cow. The number of embryos obtained from artificially

infected viremic donors kept under laboratory conditions were lower than would normally be expected (20).

The assembly of a batch of 200 embryos for export probably would require the flushing of some 50 donor cows. However, it has beer, reported that Zebu breeds tend to produce fewer transferable embryos hail the European breeds (11). Based on these numbers and local estimates, the batch of 200 embryos is likely to require 15 farms, with a minimum and maximum of 10 and 30 farms. respectively.

P_1 Probability of disease in the Region. These estimates relate to the likely incidence of FMD in the selected Region and are based on the information reported weekly by the Vesicular Disease Surveillance and Information System, coordinated by the Pan American Foot-and-Mouth Disease Center (5, 6, 24) and the reports from the disease surveillance system at the State level.

The incidence of FMD for the years 1992 and 1993 in the selected Region were 0.002 and 0.0015, respectively. Since in the near future a slight improvement of the epidemiological situation can be expected in the selected Region, the must likely incidence is estimated to be 0.001. It is unlikely that the incidence of FMD will surpass 0.005, but if it does. embryo export activities will probably be suspended. The minimum incidence is estimated to be 0.0001.

P_2 Probability of disease on *the donor farm.* The Probability that FMD occurs in at least one farm of the number of farms required for embryo collection can be calculated by the binomial 1 - (1 -p r)N2. However, in the range of the numbers being used, N_2xP_1 basically gives the same numerical result (15).

*P_3 Probability that the animal health surveillance system fails to detect FMD o*n *the donor farm.* The probability of detection of FMD on the donor farms depends on the efficacy of the local animal health surveillance system of the selected Region and the sensitivity of the detection system.

In this particular Region of Brazil it is unlikely that a significant number of outbreaks would go undetected The estimates of the proportion of donor farms with FMD that will go undetected by the animal health surveillance system given in table take into account the fact that embryos for export will probably be collected on well managed farms with high animal health standards.

P_4 Probability that the ECT fails to defect FMD on *the* donor farm. If the herd was in the pre-clinical stage at the time of the first epidemiological evaluation, it is most likely that clinical signs will be evident by the time the ECT arrives at the farm for the actual collection of the embryos.

While it is unlikely that the ECT will collect embryos on a farm with clinical FMD, this could happen when all animals on the farm are still asymptomatic. Since FMD is on acute febrile disease, the likelihood of this happening appears remote. Furthermore, if an embryo donor cow has clinical FMD at the time of embryo collection, it is unlikely that the ECT would fail to detect any abnormality.

Table 8. Quantification, of Risk Units for Foot and Mouth Disease Transmission by Embryo Transfer.

		PROBABILITY		
		Minimum	**Most likely**	**Maximum**
N_1	Number of embryos to be imported = 200			
N_2	Number of donor farms required for	10	15	30
P_1	Probability of Foot and Mouth Disease (FMD) in the selected Region	.0001	.001	.005
P_3	Probability Animal Health Surveillance System fails to detect FMD on donor farm	001	.01	.05
P_5	Probability of FMD virus reaching the embryonic environment and contaminating the embryos	.001	.01	.1
F_1	Fraction of non-exportable embryos	.005	.02	.1
P_6	Probability that the ECT fails to detect and remove non-exportable embryos (F_1)	.01.	05	.5
P_7	Probability of exportable embryos ($1-F_1$) being washed inadequately	.0001	.00	.01
P_8	Probability of at least one infective dose of FMD virus adhering to exportable washed embryos	10^{-16}	10^{-15}	10^{-14}
P_{10}	Probability that FMD on the donor farms in not detected during post-collection surveillance	.001	.01	.05
P_{11}	Probability that laboratory tests fail to detect FMD virus in the collection fluid from an infected donor	.01	.05	.1

P_5 Probability that FMD reaches the genital tract. In the case of FMD the frequency with which the virus reaches the embryonic environment depends on the likelihood of viremia, which in turn depends on the immune status of the embryo donor. The probability of the virus reaching the ovary, oviduct or uterine lumen is remote in a well vaccinated animal with high levels of neutralizing antibodies. Viremia may be common in poorly animals and virus has been recovered from the uterine tract (20) and ovaries and Graafian follicles (10) from experimentally infected heifers. Published work (18) indicates that viremic donor cattle in the clinical phase of FMD produce a high percentage of contaminated ova and embryos.

The estimates given in table 8 are based on the fact that, as a rule, the herds from which donor cattle originate are well vaccinated. The FMD carrier animal is unlikely to be relevant to be question of disease transmission by embryos, since such cattle have high levels of circulating antibodies. Thus the virus will not circulate in the blood stream and will not reach the genital tract (35). Collection *of embryos.* F_1 is the fraction of embryos that are "non-exportable" in terms of disease transmission, as defined in Part 1, Section 2 (see Figure 1). Therefore, $1-F_1$ is the fraction of exportable embryos. Estimates of the number of non-exportable embryos embryos, according to expert opinion are given in Table 8.

Human error is an important source of risk ant estimated for this section must reflect the level of confidence which officials in the importing country have in the competence and integrity of the ECT in the exporting country. It is unfortunate that in Brazil no official system for the approval

and control of the ECT is established, since this would greatly enhance the confidence or regulatory officials in the importing country. The estimate of the maximum probability of 50 percent that the ECT may fail to detect and remove non-exportable embryos is a reflection of this general lack of confidence and not of the quality of work by any individual ECT.

Risk units P_6 and P_7 relate to the collection and handing of the embryos by the ECT, whereas P_8 relates to the characteristics of the virus species and biotype. It handling procedures are done according to the lETS Manual (33), any existing risk will be greatly reduced. The main sources of risk are that "non-exportable" embryos are not remove (P_6) and "the washing of exportable embryos is not done correctly" (P_7). With respect to P_8, "virus that adheres to the ZP cannot be removed to the same extent as non-adhering virus" is risk but it is not one that is applicable to FMD.

P_6 *Probability at non-exportable* embryos *not removed.* The estimates given in this paper reflect a consensus of the opinions of commercial and non-commercial ET practitioners. One practitioner mentioned that while hatched embryos Stage 8 or 9) cannot be washed effectively, and most not be used for international trade, if such an embryo looks good on microscopic inspection and the donor is valuable, there might he a temptation to proceed with its transfer surreptitiously. Another practitioner noted that he had missed one embryo with a non-intact ZP out of approx. 1000 embryos which were examined and photographed. Yet another indicated that there are so many opportunities to examine the embryo for zona damage in the course of normal embryo handling procedures that this would never be missed. Whether the multiple opportunities for detailed embryo examination are actually taken depends on many factors, including the workload and integrity of the ECT, but ills unlikely that most non-exportable embryos (see glossary) would not be detected by an experienced, responsible ECT during the course of normal embryo processing.

Finally, there is a possibility that FMD virus could be trapped in submicroscopical debris attached to the embryo. Obviously, this debris will not be observed during inspection of the ZP, but in vitro and in *vivo* experiments *(20,28)* have shown that the probability of that occurring with FMD virus is remote. As noted above, the rather high estimate of a maximum probability of 50 percent that the ECT may fail to detect and remove non-exportable embryos is due to the absence in Brazil of an official system of approval and control of lie ECTs.

P_7 Probability of *exportable* embryos *being inadequately washed.* As mentioned earlier the recommended washing procedures are laid down in the lETS Manual (33) Basically the embryos are taken through ten one-hundred-fold serial dilutions in embryo medium. Depending on the infectious agent, it mayor may not adhere to the ZP, but FMD virus does not normally do so (20,29). The quantitative estimates which are shown in table are again based on the opinions or several commercial and non-commercial ET practitioners and are intended to reflect the variable degrees of competence and integrity of the ECT.

With regard to washing it should perhaps be reiterated that there are built-in safety margins because embryos are actually washed several additional times in the course of normal embryo handling procedures. For example quite large volumes of fluid may be sin through the uterus, collection tubing and filter during the embryo recovery. The filter is also rinsed with fresh

medium after uterine flushing. There are further dilutions during separation and classification of embryos into different dishes, during the pre-freezing movement of embryos from one cryostatic fluid into another, loading the embryos into straws, and finally during the removal of the cryostatic agent.

P_8 Probability that FMD virus adheres to *PP and is not removed.* While a few pathogens (e.g. IBR virus and vesicular stomatitis virus) do tend to adhere to the ZP after in *vitro* exposure, research has shown that FMD virus does not. The recommended ten times washing of embryos contaminated with a non-adhering virus like FMD will cause a 10^{-20} dilution of the virus (34). Since under natural conditions the amount of FMD virus in the uterus, and liable to gain access to the unwashed embryos, is not likely to exceed 10^5 viral units *(20),* the washing should result in a probability of not more than 10^{-15} that one infective dose of FMD virus would remain adhered the embryos.

P_9, composite risks P_6, P_7 and P_8. If the ECT fails to detect and remove non-exportable embryos, or fails to wash exportable embryos adequately, or if residual virus adheres to the ZP, **contaminated embryos** now become **infectious embryos** (embryos carrying at least one infective dose of FMD virus). It is clear that, with a non-adhering virus such as FMD virus (P_8), the "human error risk" P_6 and P_7 become the predominant risks in this section.

P_{10} Probability that FMD is not detected on the donor farm while the embryos are *in post-collection storage.* Ideally, farms from which embryos for export have been collected should afterwards remain under official supervision, which would make it unlikely that FMD on the farm or the area would go unreported. In the case of the Region selected here there are no official requirements in this regard. The estimates given in table 8 are based on the assumption that the importing country requests post—collection surveillance as part of the export health certification process.

Laboratory tests of *infected donors.* The question *has* been raised as to whether serological testing —virus neutralization or the so-called "VIA antibody" tests *(19)— of* embryo donors would be useful as a risk reduction measure. However, since the donor cows are regularly vaccinated against FMD, it is to be expected that they will have high levels of FMD virus neutralizing antibodies. Similarly the VIA antibody may be raised by FMO vaccination alone. Thus, neither test will be useful for the detection of FMD viral activity on the form or in the embryo donor cow (3).

P_{11} Probability that laboratory tests fail to detect FMD virus in the collection fluid from an infected donor. When collection fluids are tested for FMD virus in cell cultures or by polymerase chain reaction (PCR) techniques the probability of false negative test results is remote. For example, use of baby hamster kidney cell cultures En roller bottles for the defection of residual infective FMD virus in inactivated FMD virus suspensions for FMD vaccine production has been shown to be very effective *(25).* Hundreds of millions of doses of FMD vaccine have been successfully safety tested using this method in the absence of actual statistics for the testing of embryo collection fluid for FMD virus, the estimates we have given for false negative results is 5 percent, but this may be unduly pessimistic. The testing of collection fluid would not only

indicate whether FMD was present in the genital tract, but would also show if contaminated media had been used.

Establishment of FMD in the receiving country. This section, which relates to the probability of tie establishment of FMD in the importing country, is probably irrelevant to the veterinary authorities and decision makers of the importing country. They are primarily interested in the **importation risk**, without or with diagnostic tests, and less in the **risk *of* disease establishment.**

P_{12} Probability that an infectious embryo will cause FMD in a recipient cow. Cottral et al. (10) have shown that FMD virus can be transmitted experimentally to susceptible heifers by the vaginal/uterine route. They reported that five of 16 heifers that were artificially inseminated with semen from infected hulls and S of 10 heifers inseminated with various dilutions of FMD virus developed FMD. The smallest dose of virus resulting in infection was $I0^3$ mouse LD_{50} while the highest dose that was given without producing clinical infection was 10^8 mouse LD_{50}. While no such data are available for ET, it appears that a rather high infective dose of FMD virus would be required to initiate infection of a recipient by ET.

FMD is not transmitted by biological vectors, so risk factors P_{13} and P_{14} are not applicable to FMD virus.

COMPUTER MODEL RISK SIMULATION

Table 9 shows the Lotus 123/@Risk spreadsheet we have used to assess the risk of the importation of 200 embryos from the selected Region in Brazil where FMD occurs. The three-point probability estimates are according to fable S for which supporting evidence and data were provided in the previous section.

The "expected value" is the result of one iteration of the @Risk computer program for a triangular probability distribution of the three-point estimates. The "cumulated expected value" for an event is the product of the expected value of the event multiplied by the accumulated value of the previous event along the pathway scenario. The cumulated expected value indicates the progressive risk reduction.

For the present simulation study 10,000 iterations were executed, with a different random number for each of the calculations. Figure 3 illustrates the PDFs *for* each event resulting from those iterations. The frequency of the event occurring is on the X-axis (horizontal), while the probability of the frequencies is on the Y-axis (vertical). The "expected result" is the midpoint of the distribution. A narrow range of the frequencies on X-axis indicates a greater degree of confidence in the estimates than a wide range. For instance, there is only a 10-fold difference between the minimum and maximum values for F_{11} (Probability that laboratory tests will fail to detect FMD virus in the collection fluid), while P_5 (Probability of contamination of embryos in the genital tract) has a 100-fold difference between the minimum and maximum value. This indicates a higher degree of uncertainty for the estimates for P_5.

Figure 4 shows the PDFs for some of the cumulated risks and indicates that in the present example, with embryos collected in a FMD endemic Region of Brazil, the predicted probability

of having a batch with at least one contaminated embryo is about 10^{-6}. The probability of an infectious embryo is approximately $10^{-8.}$ If testing of collection fluid and post collection surveillance of the donor farm is included, the expected import risk, defined as the probability of one or more infectious embryos in the imported batch of 200 embryos, is 10^{-11} The same results are presented in figure 5 in a cumulative manner. On the X-axis is the range or frequencies of the event. On the Y-axis, on a scale from 0% to 100%, is the probability that the value is equal to or smaller than that on the X-axis value.

Finally, a statistical summary report on the simulation results is given in Table 10. This shows the expected, the minimum and the maximum probabilities, as well as the probabilities of important risk at different percentile levels.

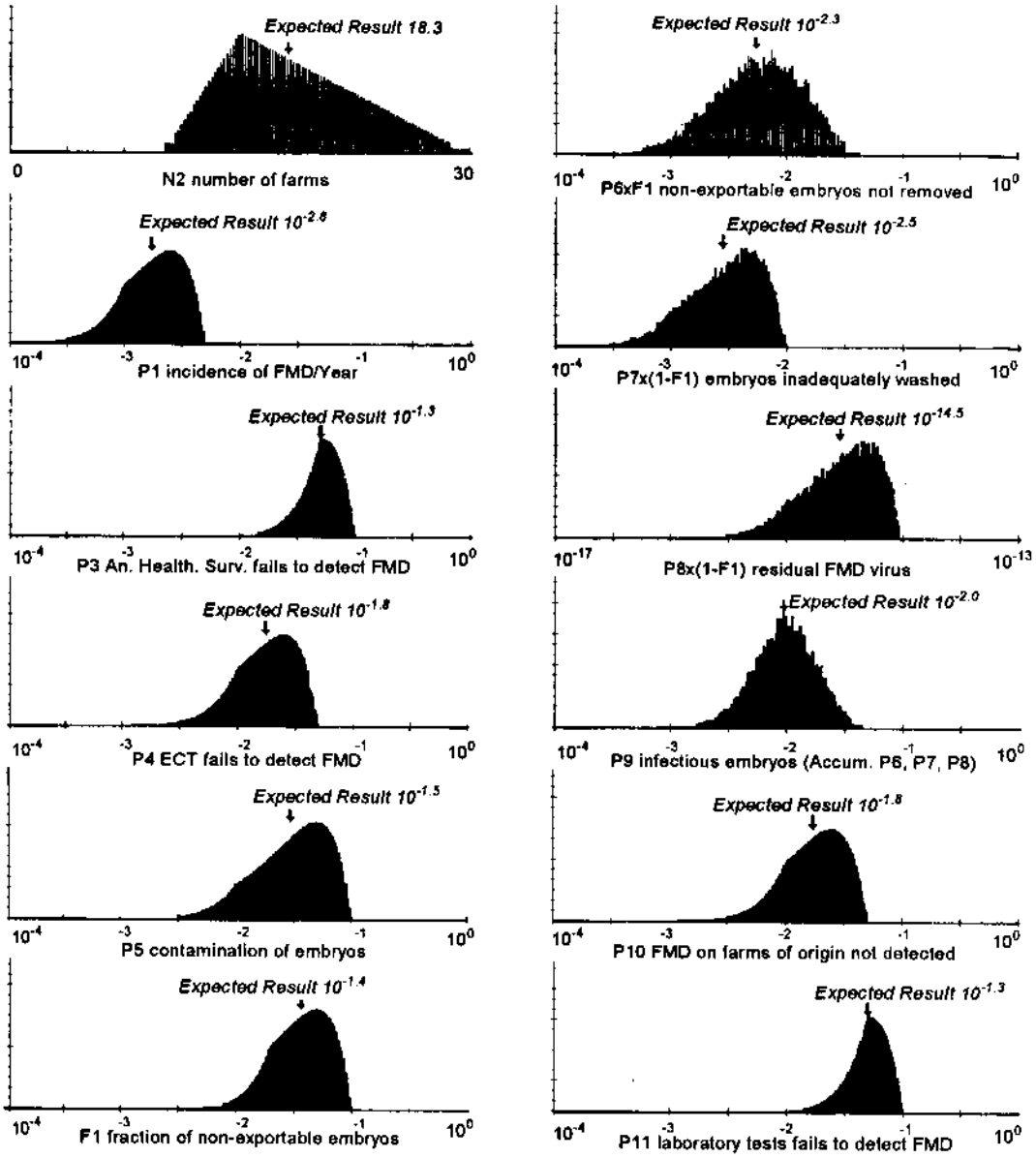

Figure 3. Probability Density Functions of the Three-Point Estimates for the Risk of FMD Transmission by Bovine Embryos.

X-axis (horizontal): Frequency of the Event occurring

Y-axis (vertical): Probability of Frequency

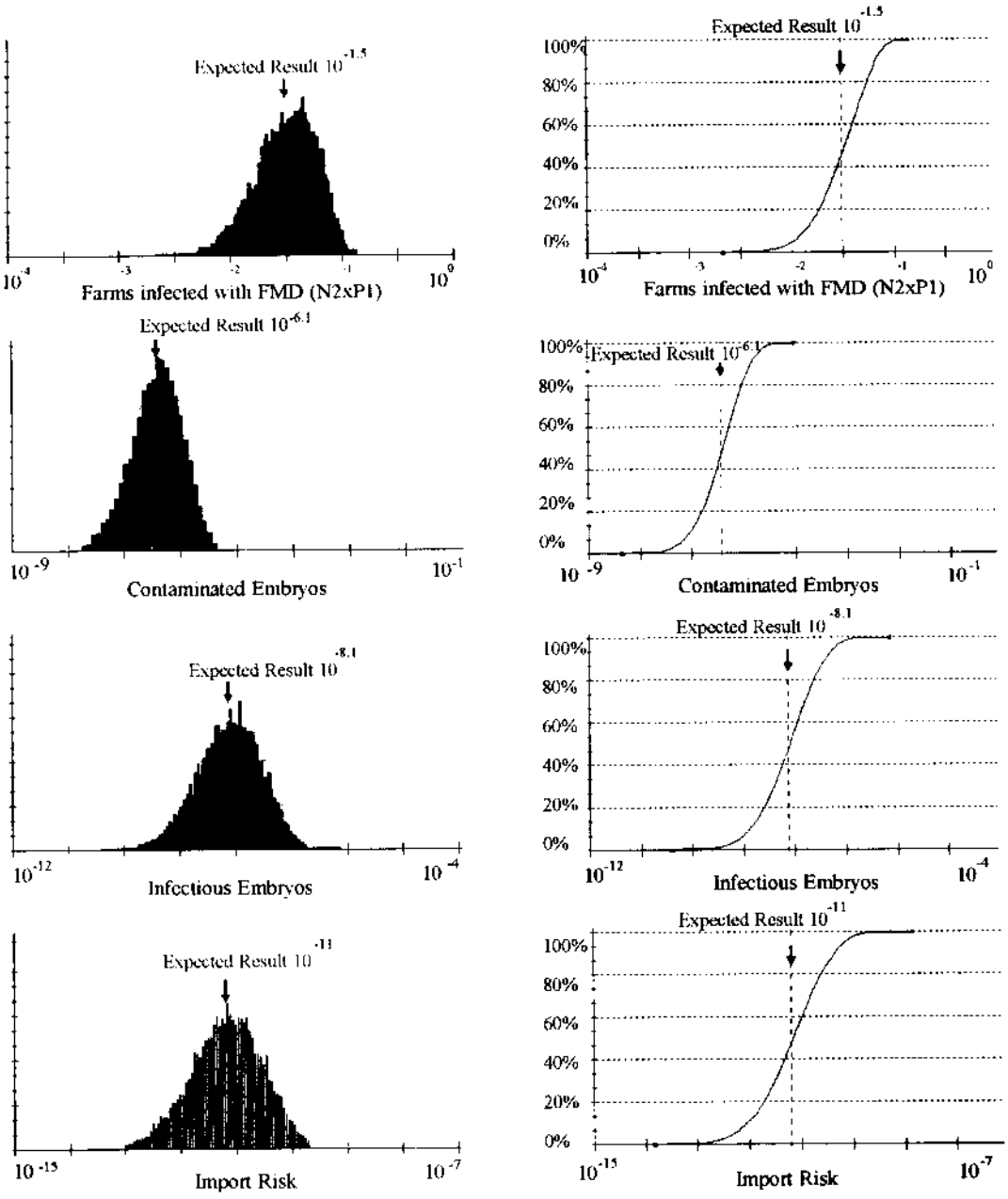

Figure 4. Probability Density Functions. **of the Risk of FMD Transmition by Bovine Embryos.**
X-axis Frequency of the event
Y-axis Probability of Frequency

Figure 5. Probability Density. **Functions of the Risk of FMD Transmition by Bovine Embryos.**
X-axis Frequency of the event
Y-axis Probability equal to or smaller than value on X-axis

DISCUSSION AND CONCLUSIONS

The import risk is defined as the probability that one or more infectious embryos (i.e., embryos carrying at least one infective dose of FMD virus) would be included in a batch, of 200 embryos ready or import. According to the figure 4 and table 10 the most likely risk of that happening a batch of 200 embryos from a FMD endemic Region in Brazil would be 10^{-11}. In other words: The risk of importing a lot of 200 embryos with one or more infectious embryos, collected and processed as described, is one in 100 billion. There is a 5 percent chance, however, that this could be as high as one in 10 billion.

Table I0. @RISK. Simulation Results for the Risk of the Importation of 200 Embryos from a FMD endemic Region in Brazil.

	P2 Farms infect with FMD	P5 Contaminated Embryos Log (10)	P9 Infectious Embryos Log (10)	Export Risk Log (10)
Expected/Mean Result	0.037	-6.11	-8.13	-11.19
Maximum Result =	0.136	-4.52	-6.19	-9.05
Minimum Result =	0.002	-8.28	-10.61	-13.92

Percentile Probability
(Chance less than or equal to Shown Value)

0 %	0.002	-8.28	-10.61	-13.9
5 %	0.010	-7.04	-9.14	-12.34
10 %	0.013	-6.81	-8.90	-12.07
15 %	0.016	-6.66	-8.74	-11.89
20 %	0.018	-6.54	-8.61	-11.76
25 %	0.020	-6.44	-8.52	-11.65
30 %	0.023	-6.36	-8.42	-11.53
35 %	0.025	-6.28	-8.33	-11.43
40 %	0.028	-6.21	-8.26	-11.34
45 %	0.030	-6.14	-8.18	-11.25
50 %	0.033	-6.07	-8.10	-11.16
55 %	0.036	-6.01	-8.03	-11.08
60 %	0.039	-5.94	-7.95	-10.99
65 %	0.042	-5.87	-7.88	-10.91
70 %	0.046	-5.80	-7.80	-10.82
75 %	0.050	-5.73	-7.72	-10.73
80 %	0.055	-5.65	-7.62	-10.62
85 %	0.060	-5.56	-7.53	-10.50
90 %	0.067	-5.45	-7.39	-10.36
95 %	0.079	-5.30	-7.20	-10.13
100 %	0.136	-4.52	-6.19	-9.05

As described in Part I, section 2, the first line of defense against the introduction 01 an exotic disease through embryo importation includes the disease situation in the country and Region, the health status of the farms and donor cows from which the embryos are collected, and the pathogenesis of the disease agent. In the present example dealing with the import of a batch of 200 embryos from the State of Sao Paulo. Brazil, the predicted probability of failure of this defense is 11.000.000, with a 5 percent confidence level that this would be not more than 1:200,000. Clearly, the first line of defense is quantitatively most important for preventing the introduction of FMD through bovine ET.

The second line of defense is constituted by the proper handling and processing of embryos by the ECT. The present consensus among veterinary scientists is that, in tile case of FMD, the lETS Manual (33) washing protocol for bovine embryos reduces the import risk practically to zero. However, the QRA shows that the following factors also must be considered:

- The probability of failure by the ECT In detect and remove non-exportable
- Embryos (those with a debris attached to the ZP and hatched blastocysts);
- The probability of failure to adequately wash the embryos;
- The probability that embryos remain infectious in spite of being adequately washed.

The first two points relate mainly to human error, but might also arise from incompetence or dishonesty on the part of ECT personnel. establishing an effective system in Brazil for the official approval of collection teams and regular controls on their operational procedures would reduce the chances 0' inadequate handling and processing of embryos. The third factor depends On specific pathogen/embryo interactions, and, in the case of FMD virus which can be efficiently removed by washing, this is virtually irrelevant compared to the "human error" factors. Consequently, in accordance with the estimates and calculations in Tables 8 and 9. The risk reduction in this part of the scenario pathway is approximately a 100-fold.

The third line of defense Includes post-collection quarantine of donor farms and testing of collection fluids, if these are deemed necessary. The resulting risk reductions by these procedures are approximately 100-fold and 20-fold, respectively. For FMD it can be argued that eliminating the testing of collection fluid and post-collection surveillance of donor farms may be justified, since the import risk would still be only one In 100 million.

The risk estimates that are presented in this document can be easily recalculated if justified by further consideration of existing data and information, or when experience or research generate flew information, in practical terms, however, if is unlikely that such adjustments would substantially affect the main conclusions of this QRA for FMD. More likely is that new information will remove some of the uncertainties that necessarily have had to be incorporated in the present estimates. This should serve only to increase confidence in the safety of ET for the international exchange of bovine genetic material.

In the present case the risk of importing of bovine embryos from a FMD endemic Region in Brazil has been assessed. The QRA methodology should be applied to other pathogens and different animal species to assess the safety of FT under those circumstances.

PART Ill

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GLOSSARY

Risk Assessment

Hazard. Elements or events that pose potential harm: an adverse event or adverse outcome. Hazard is specified by describing what might go wrong and bow that might happen.

Negligible risk. (also known as tolerable risk, de minimis risk) a mutually agreed upon measure of risk so low that all parties agree to accept risks at or below this level under most circumstances.

Risk. The likelihood and magnitude (of the consequences) of occurrence of an adverse event; a measure of the probability of harm and the severity of the adverse effects. Objective measurement and scientific repeatability are hallmarks of risk, In risk studies, it is common, especially in oral communication to use risk synonymously with the likelihood (probability or frequency) of occurrence of a hazardous event. In such instances, the seriousness of the consequences is assumed to be significant.

Risk Analysis. The process that includes risk assessment, risk management and risk communication.

Risk assessment. The process of identifying a hazard and evaluating the risk of a specific hazard, whether in absolute or relative terms. It includes estimates of uncertainty and is an objective, repeatable, scientific process.

Risk communication. Open, two-way exchange of information and opinion about risk leading to better understanding and better risk management decisions, It is a tool to provide a forum for interchange of Information with all concerned, both inside and outside the veterinary authority, about the nature of hazards, the risk assessment and how the risks should be managed; a tool to assure the unambiguous interchange of information among those affected by the outcome of risk assessment activities.

Risk management. The pragmatic decision-making process concerned with regulating the risk. Risk management is a tern used in at least two ways. It refers to risk policy in a political sense It is also used to describe a risk mitigation procedure (e,g,, quarantine or serological testing) which is required before an Import can be completed. It is important to recognize the context of he discussion when the term risk management is used. Risk mitigation treasures of risk reduction measures - any action(s) which reduces the risk of an agent to cause harm. Examples Include quarantine, diagnostic testing, inspections, restricted use, processing and sentinel monitoring.

Safety. The degree to which risks are judged acceptable; a subjective decision of the acceptability of a risk. In the literature, It is generally used when discussing safety for human health, What one individual views as safe, another may view as presenting unacceptable risk. in a regulatory context, managers make decisions about, for example. an importation based on their evaluation of the safety of the action for the health of the national herd,

Unrestricted risk estimate. The measure of risk to animal health it a commodity were to be imported in its usual commercial form with no risk mitigation measures applied.

Lotus 123/@ Risk

Cumulative frequency distribution. A cumulative distribution constructed by cumulating the frequency across the range of a frequency distribution. On the X-axis (horizontal) is the range of frequencies of the event. On the Y-axis (vertical) - on a scale from 0% to 100% - is the probability that the value is equal to or smaller than that on the X-axis value.

Expected result. The midpoint of a PDF curve, The expected result of a triangular distribution usually does not coincide with the peak of the curve.

Probability density function (PDF). Statistical term for a frequency distribution constructed from an infinitely large set of values where the class size is infinitesimally small, The frequency of an event is plotted on the X-axis (horizontal), while the probability of the frequencies is on the Y-axis (vertical).

Simulation. Repeated recalculation of the risk model with different input values with the intent of getting a complete representation of all possible scenarios that might occur in an uncertain situation.

Three-point estimate. Estimates for the minimum, most likely and maximum frequency of occurrence of an event.

Triangular distribution. A distribution determined by the three-point estimate of an event This distribution curve expresses the best knowledge about the frequency of occurrence of an event and the level of uncertainty of information related to the event.

Embryo Transfer

Contaminated Embryo. Embryo carrying at least one infective dose of a disease agent prior to sanitary handling as recommended by the IETS Manual (33).

Import Risk. The probability that one or more contaminated or infectious embryos would be included in a batch of embryos ready for import.

Infectious Embryos. Embryo carrying -at least one infective dose of a disease agent following sanitary handling as recommended by the IETS Manual (33).

Non-Exportable Embryo. Embryo with a defective zona pellucida, embryo with debris attached to the zona pellucida, and the hatched blastocyst (see Figure 1).