Health surveillance of imported embryo transfer in France*

M. THIBIER and M. NIBART**

Summary: The present paper reports the results of a field study involving 575 deep-frozen cattle embryos, imported from the USA. These embryos were transferred to 6 French regional AI Units. The recipients were introduced one month or more prior to transfer to an embryo transfer station at each of the 6 AI Units. They were isolated from other herds and were not vaccinated (except against FMD and, in some instances, against rabies). Serological tests were performed for brucellosis, IBR/IPV and enzootic bovine leukosis one month prior to and 1 and 3 months after transfer. In addition, one AI Unit required testing for trichomoniasis (vaginal washings) and campylobacteriosis one month before and 3 months after transfer. All tests were negative for all diseases, except one heifer which was serologically positive for IBR/IPV before transfer was done in error. This non-pregnant cow was discarded with no subsequent effect on the rest of the group.

These results clearly indicate that the measures taken by French authorities to permit embryo import into France (minimum requirements for donors and careful monitoring of recipients while in isolation) allowed embryo import to take place without risk to the French cattle population, which would not be possible otherwise.

Finally, the results demonstrate the absence of any infection of recipients by the transferred embryos.

KEY-WORDS: Cow - Disease control - Embryo - France - Legislation - Testing procedures - Trade in animals - Transplantation - USA.

INTRODUCTION

Breeders of French Black Pied bulls may well be proud of possessing animals of this breed, the genetic value of which is among the best in the world. Unfortunately, the same cannot be said for the female population, necessitating recourse to genetic sources available elsewhere in the world.

Up to last year, the source of genetic material introduced into France was essentially through the import of young bulls, the progeny of which were tested on French territory. This led to several difficulties particularly concerning health problems, due to the wide gap in health standards of sires between the French insemi-
nation centres and herds of origin of the imported bull calves, the former being the better. This is especially true of IBR/IPV and enzootic bovine leukemia, which are endemic on the other side of the Atlantic, whereas nearly all French stud farms are free of these diseases. It is therefore perfectly fair that the French authorities, together with those of the European Economic Community, forbade all import of breeding stock from the USA, any import being confined to cattle coming from Canada under stringent conditions.

Recourse to embryo transfer was, therefore, indispensable as a means of pursuing variety in stocking genes of this breed. The problem was, therefore, that of attempting to protect the health standards of French herds as far as possible while authorizing importation. For this purpose, and according to available literature and experimental data (11, 5, 8, 7, 9), a certain number of sanitary requirements were laid down, which made it possible to import several hundred embryos from the USA since mid-1984, sent to several selection Units.

Taking advantage of the fact that our services had, at the same time, the responsibility of a large number of surgical transfers to French recipients and of biological tests carried out on this occasion, we intend, after stating the French statutory health requirements for importation, to report on the results of biological tests performed before and after transfer in recipients for the following diseases: brucellosis, IBR/IPV, enzootic bovine leukosis and (to a lesser extent) trichomoniasis and bovine genital campylobacteriosis.

**FRENCH HEALTH REQUIREMENTS FOR THE IMPORT OF EMBRYOS**

Authorization for the import of bovine embryos is subjected to health requirements concerning donors from exporting countries and French recipients.

While requirements for donors correspond to minimum standards, offering guarantees to French livestock, compatible with the actual health status of exporting herds, strict control is still required for the recipient herds. This is intended not only to protect the herd from known and listed disease agents, but also from those which are known or unknown and which, at this stage, remain unclear.

**Requirements for donors**

Concerning the health status of the herd of origin from which the donors are chosen, the following conditions are required:

1. The country of origin must be free of rinderpest, foot and mouth disease caused by exotic virus types, and contagious bovine pleuropneumonia.

2. The donor animals must have been in the country for more than six months.

3. The herd of origin and the collection centre must be situated in the centre of an area 20 km in diameter, free of foot and mouth disease for over 30 days, of bluetongue, vesicular stomatitis and Q fever for more than six months.

This herd must not have been quarantined for disease control reasons during the previous six months. It must be officially free of tuberculosis and brucellosis.
There must have been no clinical sign of IBR/IPV, enzootic bovine leukosis, leptospirosis, trichomoniasis or bovine genital campylobacteriosis in the herd for two years.

With regard to donor animals, these must have remained on the territory of the exporting country for at least six months. Within 30 days prior to collection of embryos, the animals must have been submitted to tuberculin tests and also to serum-agglutination and complement fixation tests for brucellosis, all with negative results.

The semen with which the females have been inseminated must meet the French import standards.

Finally, the embryos must be duly identified and placed in sterilised sealed containers.

Requirements for recipients

The double requirement for recipients is, firstly, the complete isolation of such herds in order to protect the rest of the nation's livestock and, secondly, the taking of adequate steps to detect possible disorders; in particular, the animals must not have been vaccinated except against those diseases required by French regulations (foot and mouth disease and, possibly, rabies).

Thus, the recipient herd must be officially free of tuberculosis and brucellosis, and free of clinical signs of paratuberculosis and leukosis for at least two years. The recipients thus isolated are placed under the surveillance of the Departmental Veterinary Services for at least 3 months.

The following serological tests, showing negative results, must be carried out within the month preceding the transfer and then at 1 and 3 months afterwards:

- serum-agglutination and complement fixation tests for brucellosis;
- serum-neutralisation tests for IBR/IPV;
- gel diffusion tests for enzootic bovine leukosis (EBL).

The animals showing positive reactions before transfer are removed. In the case of positive tests after transfer, the females are isolated and slaughtered if they are not pregnant or, if they are, put in quarantine until calving and then sent to the abattoir.

The official authorities are naturally competent to take all necessary measures in the case of pathological incidents other than those mentioned above.

Some units also test their animals on their own initiative for trichomoniasis and campylobacteriosis. This is not obligatory since the risk is only for the owner of the animals and not for the community.

MATERIALS AND METHODS

Animals

This investigation concerned 581 frozen embryos transferred during 18 consecutive months. Various incidents occurred in six females independent of the transplantation and a total of 575 are included in this report. The embryos were acquired
by 6 large French regional breeding and insemination Units and the number, accordingly, varied between 25 and 210. All the females were heifers specially introduced into transfer stations. These generally comprised fifty heifers apart from two Units (Units E and F) which had only 10 to 20 females. Prior to transfer, no cattle had been kept in these premises for several months, and two of the stations were planned and built specially.

Those responsible for these regional AI Units deliberately chose animals from herds free, or officially free, from brucellosis and tuberculosis, most often free from IBR/IPV and EBL and, in any case, in which serological tests for these diseases proved negative. In this regard, it should be noted that blood samples for these tests were always taken before any purchase or transport, on the farm of origin and not during assembly of the animals.

Two lines of policy were followed with regard to the making up of the recipient herds. In the first case, the heifers purchased in the farms are grouped into lots and isolated in a quarantine station for one month before being sent to the transfer station, if all the tests for disease are negative. At the quarantine centre, a safety interval of at least one month was allowed for between each group.

In the second case, a contract is exchanged between the cooperative and the breeder who lends the recipient heifers. These animals, divided into groups, remain in the transfer centre for 5 months. Two groups follow on each year in such a way that there is an interval of 1 month between each lot.

The females which were not subjected to embryo transfer, because they were not seen to be in oestrus, or not found to be pregnant, were sometimes removed before the tests were started, which explains the lower number of serological results after transfer.

The laboratory tests used were typically Wright's tube serum-agglutination test and the complement fixation test for brucellosis (positivity threshold equal to 30 International Units and 20 EEC sensitizing units/ml, respectively). For IBR/IPV, the serum neutralisation test on calf embryo kidney cells was used, as adapted from Bitsch (1) by Goffaux et al. (4). The double-radial-diffusion test in agar as defined by the EEC expert panel in Rotterdam (see the review by Parodi, 13) was used as the serological test for enzootic bovine leukosis (EBL). Examinations for trichomoniasis and campylobacteriosis were carried out according to the methods respectively described by Florent (3) and Clark et al. (2).

Origin of embryos

All the embryos originated from the USA and came from about ten private embryo transfer groups, one of which supplied approximately a third of the imported embryos. We received no information on the health status of the male and female donors other than that officially required (see above). It should, however, be noted that no serological test for IBR/IPV or EBL is required. We were, therefore, led to believe that the incidence of these diseases is close to the average observed in the North-East, Mid-West or Californian states of the United States where large-scale IBR/IPV vaccinations are practised.

Before freezing, the embryos were placed in phosphate-buffered saline (PBS) to which 10% pure foetal calf serum (FCS) or 4 g/l of bovine serum albumin (BSA) and 10% glycerol (1.5 M) was added.
The medium containing the embryo(s) was either conditioned in sealed glass ampoules or in Cassou straws (IMV, France) stoppered with polyvinyl chloride or by an identification tab, or sealed by heat or ultrasonic waves.

After identification and freezing, the embryos thus conditioned were stored and transported in bottles containing liquid nitrogen until the moment of their being thawed in France.

**Thawing of embryos**

All manipulations with regard to thawing, dilution of glycerol and washing of embryos before transfer were carried out by specialised staff in stationary or mobile laboratories and with sterile material (Petri dishes, pipettes) and products (PBS, BSA, FCS, glycerol, saccharose) from American and French laboratories.

After thawing the embryos in a bain-marie at 37°C, they were collected and the glycerol was eliminated by plunging them either in 10 successive baths of a medium with decreasing concentrations of glycerol, or in 4 successive baths containing different concentrations of glycerol and saccharose, according to the method recommended by Elsden (personal communication, 1985).

**Method for examination and transfer of embryos**

After thawing and dilution of glycerol, the embryos were placed in the transfer medium and observed by microscope (magnification of 60 to 80). They were graded from 1 to 4, according to morphological criteria and to the recommendations of the International Embryo Transfer Society.

Only those embryos graded as 1, 2 and 3 were considered suitable for transfer.

The integrity of the zona pellucida was of particular interest, but the fact that it was broken had no bearing on the decision regarding the transfer of the embryo.

The embryos were put in place by specialised personnel employing the surgical flank approach, under strict aseptic conditions, using sterile transfer pipettes.

All the animals which came into oestrus at the expected time received an embryo and were subjected to early pregnancy tests at 21-24 days by assay of the progesterone level of plasma, according to Thibier and Saumande (15). Confirmation by rectal palpation was carried out at 60 to 90 days after transfer.

**RESULTS**

**Integrity of the zona pellucida and pregnancy rate**

Out of the 550 embryos transferred and subjected to full morphological examinations, 98 (17.8%) were found to have a broken zona pellucida after thawing. It should be noted that, for one single American transfer society, the comparison between pregnancy results for embryos with an intact (n = 280) or broken (n = 52) zona pellucida did not show any significant difference (49% and 52%, respectively; p >0.05).

The pregnancy rate varied significantly according to the unit of origin (10). On average, in the population studied and evaluated at 90 days, this was equal to 41%.
The extremes, according to the recipient groups, were 14% (n = 65) to 57% (n = 61). This variability is not, however, connected with the recipient stations, but depends on the viability of the embryos collected and sent by each of the different American Units to these stations.

**Serological tests for brucellosis**

Serum-agglutination and complement fixation tests were always carried out together and did not give rise to any discrepancy. As shown in Table I, no positive reaction was demonstrated either before or after embryo transfer. A number of tests for Unit A, made one month after transfer, were not carried out by our services and are not, therefore, taken into account.

**Table I**

**Negative results of serological tests for brucellosis (Wright’s serum-agglutination and complement fixation) of recipient heifers of imported embryos**

<table>
<thead>
<tr>
<th>Transfer Units</th>
<th>Samplings</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
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</tr>
<tr>
<td>A</td>
<td>205/205</td>
<td>16/16</td>
<td>190/190</td>
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<tr>
<td>B</td>
<td>61/61</td>
<td>61/61</td>
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<tr>
<td>C</td>
<td>51/51</td>
<td>51/51</td>
<td>51/51</td>
</tr>
<tr>
<td>D</td>
<td>165/165</td>
<td>165/165</td>
<td>164/164</td>
</tr>
<tr>
<td>E</td>
<td>68/68</td>
<td>—</td>
<td>68/68</td>
</tr>
<tr>
<td>F</td>
<td>25/25</td>
<td>25/25</td>
<td>25/25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>575/575</td>
<td>318/318</td>
<td>559/559</td>
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</table>

1: in the month preceding transfer; 2: in the month following transfer; 3: in the third month following transfer.

**Serological examinations for IBR/IPV**

Among the 575 animals tested (Table II), only one cow (Unit D) showed positive results to the serum-neutralisation test before the transfer was erroneously carried out. This cow did not become pregnant and, as examination revealed this on the 22nd day, she was eliminated from the herd. This exceptional case was without consequence on the rest of the group. All other tests were negative, particularly those following transfer.

**Serological examinations for EBL**

As shown in Table III, no sample was found to be positive.

**Examinations for trichomoniasis and bovine genital campylobacteriosis**

These examinations were simultaneously performed for the animals belonging to a single regional unit (Unit E) after samples had been taken by our mobile laboratory service, and kept in an incubator or under refrigeration at +4°C during transport to the laboratory.

No parasites nor specific colonies were found on examination (Table IV).
TABLE II
Negative results of serological tests for IBR/IPV
(serum-neutralisation)
of recipient heifers of imported embryos

<table>
<thead>
<tr>
<th>Transfer Units</th>
<th>Samplings</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>A</td>
<td>205/205</td>
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<tr>
<td>B</td>
<td>61/61</td>
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<td>C</td>
<td>51/51</td>
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<tr>
<td>D</td>
<td>164/165*</td>
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<tr>
<td>E</td>
<td>68/68</td>
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<tr>
<td>F</td>
<td>25/25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>574/575</strong></td>
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</tbody>
</table>

* One cow showed a positive test result before transfer and was subjected to a transfer by mistake. As its pregnancy test was negative, this cow was immediately culled without any consequence for the rest of the herd.

TABLE III
Negative results of serological tests for enzootic bovine leukosis
of recipient heifers of imported embryos

<table>
<thead>
<tr>
<th>Transfer Units</th>
<th>Samplings</th>
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<td></td>
<td>1</td>
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<tr>
<td>A</td>
<td>205/205</td>
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<td>B</td>
<td>61/61</td>
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<td>C</td>
<td>51/51</td>
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<td>D</td>
<td>165/165</td>
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<td>E</td>
<td>68/68</td>
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<tr>
<td>F</td>
<td>25/25</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>575/575</strong></td>
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</table>

TABLE IV
Negative results of examinations for trichomoniasis and campylobacteriosis
of recipient heifers of imported embryos

<table>
<thead>
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<th>Transfer Units</th>
<th>Samplings</th>
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<td></td>
<td>1</td>
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<tr>
<td>E</td>
<td>68/68</td>
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DISCUSSION AND CONCLUSION

We consider that this investigation of a large number of transfers, despite the removal of a number of non-pregnant animals before completion of the whole series of tests, gives interesting evidence.

First of all, the proportion of embryos with a broken zona pellucida after thawing is of the same order as that reported elsewhere (6). This does not necessarily mean that the zona pellucida was broken before freezing. It demonstrates, in any case, that even if this acellular membrane is not intact, this was without effect either on the pregnancy rate or on subsequent serological results in recipients.

We will not discuss here the pregnancy results, which are liable to be extremely variable according to the American embryo-producing units (10).

The present results clearly confirm the possibility of importing embryos without maximal health requirements for donors. The possibility of testing the consequences of such transfers in recipients submitted to sanitary requirements, similar to those suggested concomitantly by Hare and Singh (8), appears to be an effective means of ensuring the safety of such operations. In this investigation, all the negative serological results or biological tests demonstrate the validity of this method.

In addition, no clinical sign of any particular disease was found in the recipient stations, which emphasizes the feasibility of such grouping of females, and the absence of clinical contamination by unexpected slow, pathogenic viruses possibly introduced with the embryos, at least in the short term.

This field study confirms that, under these conditions, there is no risk of transmission of pathogenic agents, as has been confirmed by various research projects reviewed by Singh (14) and Hare (7).

Such sanitary conditions have, in addition, the great advantage of enabling selection units to choose young males born of these transfers, to introduce them into breeding stations prior to their entry into Artificial Insemination centres, while entirely respecting French regulatory measures (negative serological test for enzootic bovine leukosis in dams of bulls, for example).

In conclusion, this study justifies, in retrospect, the statutory decisions taken for the import of embryos. The existence of such stations for isolated recipients which have not been vaccinated, except against FMD and, sometimes, rabies has made it possible to demonstrate the lack of contamination of transfer recipients, as evidenced by the absence of any clinical signs and constant negative test results for brucellosis, IBR/IPV, enzootic bovine leukosis, as well as trichomoniasis and bovine genital campylobacteriosis.

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SURVEILLANCE SANITAIRE DU TRANSFERT D'EMBRYONS IMPORTÉS EN FRANCE. — M. Thibier et M. Nibart.

Résumé : Le présent travail rapporte le résultat d'une enquête portant sur 575 embryons congelés provenant des Etats-Unis et remis en place pour le compte de six grandes Unités régionales françaises d'insémination artificielle.
Les femelles receveuses, rassemblées dans des stations destinées à ces opérations de transfert, étaient isolées des autres troupeaux et non vaccinées (à l'exception de la fièvre aphtoise et parfois de la rage). Elles étaient soumises à des épreuves sérologiques vis-à-vis de la brucellose, l'IBR/IPV, la leucose bovine enzootique avant (dans les 30 jours) puis après (30 et 90 jours) le transfert. En outre, pour une Unité d'IA, des examens (lavage vaginal) de trichomonose et de campylobactériose étaient effectués avant, puis 90 jours environ après le transfert. Toutes les analyses se sont révélées négatives pour toutes les maladies recherchées, à l'exception d'une vache dont la sérologie vis-à-vis de l'IBR/IPV se révela positive avant le transfert effectué par erreur. L'animal non gestant fut ensuite éliminé sans conséquence pour le lot de ses contemporaines.

Ces résultats montrent que les dispositions prises pour permettre cette importation d'embryons (mesures minimales sur les donneuses et vérification attentive des conséquences du transfert dans des stations dûment isolées de receveuses) ont permis sans risques l'importation d'embryons, autrement impossible.

Enfin et surtout, ils montrent l'absence de toute contamination des femelles receveuses, à partir de ces embryons transférés.


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VIGILANCIA SANITARIA DE LA TRANSFERENCIA DE EMBRIONES IMPORTADOS EN FRANCIA. — M. Thibier y M. Nibart.

Resumen : En el presente trabajo se reseña el resultado de una encuesta sobre 575 embriones congelados procedentes de Estados Unidos y colocados por seis grandes Unidades regionales francesas de inseminación artificial. Las hembras receptoras concentradas en estaciones designadas para estas operaciones de transferencia estaban aisladas de los demás rebaños y sin vacunar (con excepción de la fiebre aftosa y a veces la rabia). Estaban sometidas a pruebas serológicas para la brucelosis, IBR/IPV, leucosis bovina enzootica antes (en los 30 días) y después (30 y 90 días) de la transferencia. Además para una unidad de IA, se efectuaban exámenes (lavado vaginal) de tricomonosis y campylobacteriosis antes, y seguidamente unos 90 días después de la transferencia. Todos los análisis resultaron negativos para todas las enfermedades investigadas, con excepción de una vaca cuya serología frente a IBR/IPV resultó positiva antes de la transferencia efectuada por error. Después se eliminó el animal no preñado sin consecuencia para el lote de sus coetáneas.

Estos resultados prueban que las disposiciones adoptadas para permitir esta importación de embriones (medidas mínimas sobre las donantes y atenta verificación de las consecuencias de la transferencia a estaciones debidamente aisladas de receptoras) permitieron sin riesgo la importación de embriones, que de otro modo sería imposible.

Por último y sobre todo, demuestran que las hembras receptoras no tuvieron ninguna contaminación, en base a estos embriones transferidos.
REFERENCES


