

Short Note

BORDER DISEASE IN FRANCE

RUSSO P, DELOR Véronique and GIAUFFRET A

Ministère de l'Agriculture, Direction de la Qualité, Laboratoire National de Pathologie des Petits Ruminants et des Abeilles, 63, avenue des Arènes, 06051 Nice Cedex, France

Received, 12 th May, 1986/Accepted, 19 th June, 1986

Résumé

LA BORDER DISEASE EN FRANCE. — Des souches de pestivirus ont été isolées à partir de primocultures de rein d'agneaux provenant d'abattoirs et de cas cliniques typiques de Border Disease. De plus, des pestivirus ont été retrouvés dans des élevages atteints de formes atypiques ou de formes modérées de la maladie. La sérologie de ces troupeaux était systématiquement positive. Les premiers résultats d'une enquête sérologique menée sur l'ensemble du territoire français ont montré que si la moitié des départements étudiés est séro-positif, le nombre de cas cliniques demeure très faible (environ 2 %). La Border Disease ne représente donc pas, à l'heure actuelle, un problème majeur en France. Soulignons cependant la nécessité de contrôler rigoureusement les supports cellulaires utilisés dans le cadre de la production de vaccins vivants destinés à différentes espèces.

Border Disease (BD) is a transmissible congenital disease which affects sheep and occasionally goats (Russo 1986). Its name originates from the geographical area where the first viral strain was isolated, on the border hills between England and Wales (Hughes *et al* 1959). The agent responsible for it (Border Disease Virus or BDV) is a pestivirus and is antigenically related to viruses of the same genus (mucosal disease, hog cholera).

Although several articles have been published on the subject, (Brugère-Picoux and Consalvi 1983, Eloit and Toma 1983, Savey 1980), this disease had never been diagnosed in France.

Evidence of several pestivirus strains obtained from ovine cell lines and reacting clearly to an anti BDV reference serum led us to study this disease more extensively.

Materials and Methods

In vitro cultures

Primary cultures were established from the foetal kidney and testicles of slaughter house sheep and goats showing no necrotic lesions. The cell lines thus obtained were preserved in liquid nitrogen and used between 3rd and 7th passage *in vitro*.

The FLM cell line (Laude 1979) established from the muscle of an ovine foetus was used between 40th and 60th passage *in vitro*. These pestivirus-free cells are maintained with foetal calf serum free from anti-BVD antibodies and pestivirus (Boehringer-Mannheim).

Samples

Samples from organs of clinical cases with Border Disease-like symptoms were used for viral research on FLM cells. After three successive passages, the cells were examined by indirect immunofluorescence.

Primary kidney cells from «hairy lambs» and showing reduced growth were also studied by indirect immunofluorescence.

Sera

The sera used in our research came from different sources:

- an anti-Border Disease serum (against the Scottish strain BPII) provided by Moredun Institute.
- an anti-Border Disease national reference serum established in lamb at our Laboratory.
- sera from a serological survey carried out all over France and giving minimal representation of sheep farming in each region (4 to 5 clinically non affected flocks, 5 sera per flock).
- sera from the Alpes-Maritimes region (32 flocks, 5 to 20 sera per flock).
- sera received from other regions.

Indirect immunofluorescence

The cells are fixed in acetone at — 20 °C approximately 72 hours after infection. Porcine globulins anti-hog cholera or a lamb serum hyper-immunised with BPII strain were used initially. Secondly, anti-porcine or anti-ovine fluorescent globulins were used with an Evans Blue counter stain (Russo *et al* 1977).

Table 1. — Seroneutralisations in flocks with no pathological signs

	Studied	Positive	
		Number	%
Regions	35	17	48.6
Flocks	161	42	26.0

Seroneutralisation

Carried out on microplates on FLM cells, it involves fourfold dilutions of serum and 100 DCP50 of BPII virus. The serum titre can be obtained by simple logarithmic calculation. The positivity threshold retained is 1/5.

Questionnaire survey

In addition to the serological research mentioned above (cf sera), a clinical survey was also carried out. 540 questionnaires together with a summary of the main aspects of the disease were sent out. They were sent to regional veterinary services and veterinaries specialised in ovine and caprine pathology.

Results

Virological study

Eight ovine cell lines and four caprine cell lines were studied by indirect immunofluorescence. Three ovine cell lines (two from foetal kidney, one from foetal testicle) showed a specific very intense fluorescence with the anti-BD (BPII) reference serum (Russo and Giauffret 1984).

Only cell lines which were negative after testing were thereafter used for virological diagnosis.

In several regions of South of France, five diagnoses providing proof were set up for clinical signs, serology and isolation of the virus from organ cultures taken from typical cases of Border Disease (Brugère Picoux *et al* 1984, Russo and Giauffret 1985).

We also studied an atypical form characterised by numerous early abortions (200 out of 800 ewes), several later abortions with malformations (approx 40) and about forty viable lambs showing retarded growth, slight modifications in their fleece and bone deformations. Several lambs showed an unusual atrophic lesion which is very characteristic and gives the impression of a constriction between the thorax and the abdomen. The aetiology was confirmed by a highly positive serology of the flock and by the isolation of several viral strains.

A primary culture of lamb kidney with an attenuated clinical form of Border Disease was used to show a very clear fluorescence with the anti-BD reference serum.

All the viral strains isolated do not show a cytopathic effect *in vitro*.

Serological study

Current results of the serological survey throughout France are summarised in the table 1.

A more extensive serological study carried out in the Alpes-Maritimes showed that almost 90 % of the flocks were seropositive.

A smaller survey among 16 «pilot» flocks in South West France revealed that 45 % of them were seropositive. Only one case of clinical Border Disease was diagnosed in these flocks; on the other hand, attenuated forms of it were recorded.

Epidemiological study

216 of the 540 questionnaires sent out were answered. Only two clear clinical cases were recorded, that is, less than 2 % of the replies.

Discussion

The serological results obtained in seroneutralisation against the Border Disease virus appear to indicate a high frequency of pestivirus infections in sheep in France (about 50 %). Taking in account the cross-reactions in this group, all these reactions cannot be attributed with certainty to a BD virus infection. However, it is likely that this virus is present in a great number of cases. Study of the moderate forms observed in different regions of France (South-East, South-West) provide an argument in this field. In many flocks, owners notice small size lambs (practically zero growth) with hairy fleece (hairy lamb), and pigmented, to the exclusion of any other sign in the flock.

These animals («pellous» in Provence and «bourrus» in the South-West) are of no economic value. It is proven that these cases correspond to an attenuated form of the disease. In fact, in addition to the clinical signs, the anti-BDV serology of the flocks concerned is systematically positive and the isolation of pestivirus was obtained in our laboratory from renal cellular cultures.

In some cases, Border Disease can be diagnosed with certainty in presence of characteristic

signs, positive serology and isolation of a pestivirus which reacts very strongly to the anti-BDV reference serum. Taking into account the research carried out among the flocks and our work in the laboratory, the current incidence of the disease in France can be considered minimal (5 cases confirmed). The incidence of attenuated forms is difficult to specify on account of their low economic impact. We have not been able to evaluate the importance of BDV as an abortive agent for the present.

Considering the results of this study as a whole, we think that the clear clinical expression of the disease is probably linked to the intervention of more virulent strains or to undefined favourable conditions. The low occurrence of the disease in France compared with certain other countries remains inexplicable.

Border disease is not therefore, for the moment, a major problem in France (Russo *et al* 1985). It is

important however, that diagnosis laboratories and manufacturing companies observe strict control over the cells used *in vitro*, particularly when producing live vaccines for different species.

A pestivirus seems to be involved in the complex aetiology of a syndrome similar to the BVD-MD observed in sheep in France. It is too early to identify this virus as a BD virus. Current research should result in classification of this agent in its rightful place within the pestivirus group.

Acknowledgements

Our grateful thanks to: Aitken ID and Nettleton P (Moredun Institute, Edinburgh) for the BP11 strain and its immune-serum, Carnero R (LCRV Alfort) for the porcine γ globulins, Moussa A (LNBP Lyon) for the seroneutralisation technique.

References

- BRUGERE-PICOUX J, CONSALVI PJ, 1983. La «Border Disease» du mouton. Recl Méd Vét 159: 73-79
- BRUGERE-PICOUX J, MAES H, MOUSSA A, RUSSO P, PARODI AL, 1984. Identification de la «Border Disease» ou «Maladie de la frontière» chez le mouton en France. Bull Acad Vét 57:555-562
- ELOIT M, TOMA B, 1983. La border disease. Point Vét 15:55-59
- HUGHES LE, KERSHAW GH, SHAW IG, 1959. «B» or Border Disease. An undescribed disease of sheep. Vet Rec 71:313-317
- LAUDE H, GELFI J, 1979. Properties of Border Disease Virus as studied in a sheep cell line. Arch Virol 62:341-346
- RUSSO P 1986. La Border Disease. Bull Lab Vét (21):43-48
- RUSSO P, VITU C, LAMBERT M, GIAUFFRET A, 1977. Application d'une technique d'immunofluorescence au diagnostic sérologique de la chlamydiose des petits ruminants. Bull Acad Vét 50:415-424
- RUSSO P, GIAUFFRET A, 1984. Mise en évidence du virus de la Border Disease en France. Bull Acad Vét 57:563-565
- RUSSO P, GIAUFFRET A, 1985. Étude préliminaire de la Border Disease en France. Bull Lab Vét (19-20):70
- RUSSO P, GIAUFFRET A, FLECHE-SEBAN C, 1985. Occurrence of pestivirus infections in sheep in France. EEC Workshop on pestivirus of ruminants, Bruxelles, 10-11 septembre
- SAVEY M, 1980. La border disease est-elle une maladie méconnue en France? Sem Vét (159):3