COMPARISON BETWEEN THE IMMUNODIFFUSION AND THE IMMUNOFLUORESCENCE TESTS IN THE DIAGNOSIS OF BOVINE LEUKEMIA VIRUS (BLV)

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Résumé

COMPARAISON ENTRE LE TEST D'IMMUNODIFFUSION ET LE TEST D'IMMUNOFLUORESCENCE POUR LE DIAGNOSTIC DU VIRUS DE LA LEUCOSE BOVINE. — Les tests d'immunodiffusion et d'immunofluorescence ont été réalisés sur les sérums de 551 vaches provenant de troupeaux affectés de plusieurs cas de leucose. Les antigènes utilisés furent le gp70 et le p24 pour le test ID et une culture de BLV sur des cellules de rein d'agneau foetal pour le test IF.
En tout 307 vaches (56 %) ont réagi positivement à un, deux ou aux trois tests. Parmi les 307 vaches positives, 81,5 % ont réagi positivement avec l'antigène gp70, 0,6 % avec le p24, et 45 % avec le test IF. Dans 18,5 % des sérums testés, l'IF était positive tandis que les tests ID étaient négatifs.
L'absorption de ces sérums avec les antigènes ID n'empêche pas une réaction positive avec le test IF. Il semble donc que les antigènes qui s'expriment dans les tests ID soient différents de ceux qui s'expriment dans le test IF. Au vu de ces résultats, il semble que le diagnostic de routine dans les troupeaux pour la présence des anticorps au BLV doive être de préférence le test ID avec l'antigène gp70. Cependant le contrôle des cas individuels demande un test sérologique complémentaire. Le test IF est recommandé, puisqu'il met en évidence d'autres antigènes du BLV. Les résultats obtenus montrent que seulement 0,6 % de ces sérums réagissaient positivement avec l'antigène p24, tandis que dans le sérum d'animaux porteurs de tumeurs cliniques, les anticorps à cet antigène sont trouvés dans 15 cas sur 24 (62,5 %). La signification de ces résultats est discutée.

Since the BLV has been proven to be the causative agent in enzootic bovine leukemia, the need for a rapid and reliable diagnostic tool for the disease detection becomes essential. Various serological tests have been established in several laboratories, e.g., ID (Miller and Olson, 1972), IF (Ferrer *et al.,* 1972), CF (Miller and Van der Maaten, 1974) and RIA (Chander *et al.* 1976). The method of choice which is used by most laboratories and by the EEC, is ID; as detailed by Miller and Olson (1972).

BLV possesses multiple antigens, and the above mentioned serological tests are capable of detecting only one antigen at a time but cannot reveal them all simultaneously.

The question that arises is whether one serological test is sufficient for the detection of all infected animals. In order to clarify this

point we tested sera of cows, using the IF test and in parallel the ID test with 2 different antigens.

The first antigen we used for the ID test is prepared by freezing and thawing FLK infected cells, as described by Ressang *et al.* (1974). This antigen was found to be identical to the p24 (Portetelle *et al.* 1976). The other antigen was the gp70 prepared as described by Miller and Van der Maaten (1976). The IF test was carried out on FLK infected cells using the method of Ressang *et al.* (1974).

The tested sera originated from 551 cows from multiple case herds of leukemia and from 27 cows clinically affected with lymphosarcoma. All these sera were tested by the above mentioned techniques in parallel. The results obtained from the sera of cows originating from multiple case herds are summarized in Tables 1 and 2. As seen in Table 1,307 out of 551 sera reacted positively in one, two or in all three tests. From these, 81.5 % reacted positively with the gp70 antigen, 45.5 % in the IF test, and only 0.6 % with the p24 antigen. However, as seen in Table 2 only 27 % of the sera reacted positively in the ID and IF tests, while 18.5 % of the sera reacted positively in the IF test only.

Absorption of these sera with the gp70 and p24 antigens did not block a positive IF test. It seems therefore that the antigens expressed in the ID tests are not identical to those revealed by IF.

In view of these results, it seems that when screening of herds for the presence of antibodies to BLV is considered, the serological method of choice should be ID with the gp antigen. However, checking of individual cases requires an additional serological test. It is indicated because other BLV antigens are revealed in this serological technique.

Table 1 : Reactivity of sera of cows originating from multiple cases herds of leukemia as tested by ID and IF techniques.

Groups of animals	Number	% of positive
Cows tested	551	—
Negative	244	—
Positive	307	100
IF positive	140	45.5
p24 positive	2	0.6
gp positive	250	81.5

It was of interest to consider the reactivity of sera of cows affected with lymphosarcoma, when tested by the same techniques.

As seen in Table 3, all 24 clinically affected cows reacted with the gp70 antigen in ID and of these 15 cows were also p24 positive, while 14 were IF positive.

The comparison between these results and those obtained from multiple case herds of leukemia which is given in Table 4 reveals a strikingly different reactivity towards the p24 antigen. When screening of the above mentioned herds was carried out, only 0.6 % of the sera reacted positively, while 62.5 % of the lymphosarcoma affected animals exhibited specific antibodies to this antigen. The significance of these results has to be further elucidated.

Many questions can be raised concerning the presence of these antibodies and an active infective process in animals with viraemia, or as to the persistence of these antibodies as connected with a tumorous stage of the disease.

Table 2 : Reactivity of sera of cows originating from multiple case herds of leukemia divided into groups according to their specific response.

	Positively reacting cows	gp70 neg. p24 pos. IF neg.	gp70 neg. p24 neg. IF pos.	gp70 pos. p24 neg. IF pos.	gp70 pos. p24 neg. IF neg.	gp70 pos. p24 pos. IF neg.	gp70 pos. p24 pos. IF pos.
Number	307	0	57	81	167	0	2
Percent (%)	100	0	18.5	26.4	54.5	0	0.6

Table 3 : Reactivity of sera of cows affected with lymphosarcoma as tested by ID and IF techniques.

Groups of cows	Number	% of positive
Cows tested	27	—
Negative	3	—
Positive	24	100
IF positive	14	58
p24 positive	15	62.5
gp positive	24	100

An indication that such questions are relevant is based on the follow-up of 13 clinically healthy cows that react positively both with gp70 and p24. These cows continued to react positively with the gp70 for a period of over a year. When tested six months later 12 cows exhibited a negative reaction with the p24 antigen and only one remained positive. The same results were obtained when the sera of these cows were checked eight months later. Clinical examination of the 13 cows at this stage revealed enlarged peripheral lymph nodes in the case of the single cow which still reacted positively with the p24, while the others exhibited no pathological changes.

These limited data are far from being a proof that the persistence of antibodies to p24 indicate an active infective process which might lead to clinical manifestations. However this hypothesis cannot be ruled out without a thorough investigation of the various BLV antigens affecting the pathogenesis of this disease.

Table 4 : Comparison between the specific reactivity of sera of cows originating from multiple case herds of leukemia and those from cows affected with lymphosarcoma.

Type of herd	Total (%)	IF Positive	p24 Positive	gp70 Positive
Multiple case herd	100	45.5	0.6	81.5
Clinically affected cows with LS *	100	58	62.5	100

* LS = Lymphosarcoma.

Summary

The immunodiffusion (ID) and the immunofluorescence (IF) tests were carried out on the sera of 551 cows originating from multiple case herds of leukemia. The antigens used were the gp70 and the p24 for the ID test, while the entire BLV cultured fetal lamb kidney cells were used for IF.

In total, 307 (56 %) of the cows reacted positively in one, two or in all three tests. From the 307 positive cows, 81,5 % reacted positively with the gp70 antigen, 0.6 % with the p24 antigen, and 45 % were positive in the IF test. In 18,5 % of the tested sera, the IF was positive while the ID tests negative.

Absorption of these sera with the ID antigens did not prevent a positive reaction in the IF test to occur. It seems therefore that the antigens expressed in the ID tests are not identical to those which find expression in the IF. In view of these results, it seems that when screening of herds for the presence of antibodies to BLV is considered, the serological method of choice should be the ID test with the gp70 antigen. However checking of individual cases requires an additional serological test. The IF test is indicated because other BLV antigens are revealed in this serological

technique. The data obtained show that only 0.6 % of these sera exhibited a positive reaction with the p24 antigen, while in the sera of animals with detectable tumors, antibodies to this antigen were found in 15 (62,5 %) out of 24 cases. The significance of these findings is discussed.

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