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PREVIEW ARTICLE

LABORATORY DIAGNOSIS METHODS FOR BOVINE RESPIRATORY SYNCYTIAL VIRUS*

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ABSTRACT

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Laboratory diagnosis of bovine respiratory syncytial (BRS) virus no longer poses a problem. Clinical diagnosis, based on signs of pulmonary emphysema manifest in autumn, should be confirmed by laboratory techniques. Direct isolation of the BRS virus from field samples in cell cultures is often unsuccessful, whereas detection of the viral antigens by staining ultra-thin tissue sections with fluorescein isothiocyanate antibody conjugates is highly effective. Complement fixation and especially indirect immunofluorescence tests are still very useful for the detection of BRS specific antibodies in serum and nasal mucus.

INTRODUCTION

Care should be taken to determine the aetiology of diseases before embarking on their treatment. Symptomatic treatments usually have little effect against bovine respiratory diseases. The symptoms observed in these disorders are the result of synergism in which various agents are predisposing conditions, followed by infection by one or more viruses and bacteria, and sometimes further complicated by the immune reaction of the body.

The bacterial infection can be overcome by the use of antibiotics and sulphonamides, but predisposing conditions are more difficult to counteract. Two important predisposing factors are due to the demands of

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over-intensification of modern beef production and the over-crowding of yarded animals, which for economic reasons are unavoidable. Measures to combat the viral component in the aetiology of these diseases depend on first isolating and identifying the agent or agents and then producing specific vaccines.

PRESENT STATE OF KNOWLEDGE

Respiratory disorders are causing considerable economic losses in cattle rearing, predominantly in intensive beef production. The aetiological agents in these disorders were formerly thought to be organisms of the genus Pasteurella. It has since been discovered on isolating them from the lungs that these bacteria are not the main factor in the disease.

In the USA a virus was isolated from the respiratory system by Madin and coworkers (1956). However, this virus, IBR (infectious bovine rhinotracheitis virus) is not involved in the general respiratory syndrome but causes a separate set of symptoms.

Again in the USA, Reisinger and coworkers (1959) isolated the parainfluenza-3 virus (PI-3) and the role of this organism in respiratory disorders was soon confirmed by numerous European investigators.

In 1969, researchers of this Institute (Wellemans and Leunen, 1969) demonstrated that BVD (bovine viral diarrhoea virus) played a part in respiratory disease. The Hungarian scientists Bartha and Aldasy (1964) isolated adenoviruses of type B, which came to be regarded as important factors in some cases of respiratory disorder.

Other investigators (Rosen and Abinanti, 1960; Bogel and Bohm, 1962) have isolated reoviruses, rhinoviruses and ECBO viruses with less clearly established pathogenic action.

Finally, Paccaud and Jacquier (1970), Inaba and coworkers (1972) and Wellemans and Leunen (1975) have established the importance of bovine respiratory syncytial virus as a causative agent in a great many cases of respiratory infection. This finding has been corroborated by researchers in various European countries.

In the Netherlands, researchers of the Virology Section of the CDI (Centraal Diergeneeskundig Instituut) have demonstrated the association of BRS virus with more than 70% of cases of respiratory infection occurring in autumn (Van Niewstad, personal communication, 1975). In Denmark, Bitsch and coworkers (1976) have repeatedly isolated a virus of the same type from the lungs of sick cattle.

The diagnostic methods so far used include such relatively laborious techniques as isolation of virus and serum neutralisation tests. The author and his team have therefore developed the diagnostic techniques described in this paper.

Clinical diagnosis

Over the last few years our laboratory has been carrying out examinations of conjugated sera of cattle affected with respiratory disorders caused by BRS virus. Veterinary practitioners suspect infection by this virus when they encounter a pattern of symptoms which may be summarised as follows: all the cattle on the affected farm, especially "late calves", at weights between 100 and 300 kg, are simultaneously found to have a body temperature of about 40°C. Apparently as a result of the effect of antibiotic treatment to combat secondary bacterial infection, there is then a rapid drop in the body temperature, together with very marked improvement in the general state of health.

About two or three days later, when everything seems to be satisfactory again, the animals suddenly present difficulty in breathing, accompanied by bouts of dry coughing. The body temperature when these symptoms are first manifested is close to normal. The breathing of the sick calves becomes increasingly rapid and shallow and the condition is aggravated by bouts of coughing. There is little or no discharge from the nostrils. Frequently there is frothing at the commissure of the lips. Constipation is a commonly occurring symptom and there is complete loss of appetite. The animals can neither lie down nor eat and they make desperate efforts to breathe through an open mouth. On auscultation some harshness in the breathing can be detected but rales are not often heard and towards the end of the disease state the classic signs of emphysema begin to appear. There can be up to 30% mortality in the herd and farms specialising in late calves tend to have the highest losses.

Opening up of the thoracic cavity in autopsy reveals widespread pulmonary emphysema and distended air spaces. In some cases the walls are broken, so that pneumothorax results.

The presence of tracheitis or of rhinitis is comparatively rare.

Laboratory diagnosis

Even where characteristic symptoms occur, the only means of determining

the aetiological diagnosis with certainty is laboratory investigation. Constant improvement in diagnostic techniques has led to easier identification of the virus. Infection by BRS virus can be ascertained by:

1. isolation of the viral agent, in cell cultures;
2. detection of the viral antigens in ultra-thin sections stained by a serum labelled with fluorescein isothiocyanate;
3. antibody detection in conjugated sera;
4. antibody detection in nasal mucus.

Isolation of BRS virus

Diagnosis from isolation of the BRS virus is the most laborious technique. Severe or fatal damage is not always directly due to the effects of the virus, but is often caused by complications such as bacterial infection or hypersensitive reaction of the body of the host animal. We have discovered that infection by BRS virus often produces very rapid immunological response in the animal, so that the maximum titre is attained within a week. Ground lung tissue from a dead animal may contain not only the virus and its antigens but also neutralising antibodies to the virus, which impede its isolation.

Attempts are also made to isolate the virus from animals which still seem to be healthy.

But even in favourable conditions there is no certainty that isolation will be achieved, because the BRS virus produces a very slow cytopathogenic effect in the initial passages. It sometimes takes more than a month to obtain discernible cell lesions.

Paccaud and Jacquier, in Switzerland, identified their virus after 32 days (1970) and Inaba et al., in Japan, had to wait 17 days (1972). Smith et al. (1975) and Jacobs and Edington (1971) do not seem to have been any more fortunate.

BRS virus causes the culture to form syncytia, with a very variable number of nuclei. The disappearance or bursting of these giant cells leaves vacuoles in the cell layers. Staining with haemalum-eosin reveals a predominance of circular inclusions and markedly fewer polymorphs than in PI-3 virus infection.

BRS virus in cell cultures is revealed by staining the viral antigens with a labelled serum. The virus isolated in culture is thus identified by immunofluorescence.

Ultra-thin section immunofluorescence

Improvement in ultra-thin section immunofluorescence techniques makes it possible to detect the presence of BRS virus more quickly and more efficiently than in the past. Sections 2 to 3 microns thick are made from lung tissue fragments in a cryostat. The tissue sections are mounted on slides and fixed with acetone for 20 minutes at -20°C. The sections are then coated with labelled hyperimmune sera specific to BRS, BVD, reo-like, Corona virus, parainfluenza-3, IBR and adenoviruses. We also use a serum for detecting the macrophages which are frequently present in the lung tissue of cattle suffering from chronic pneumonia and which may therefore lead to error in diagnosis. By means of our procedure we have been able to identify BRS antigen in about 40 cases. Each time we have succeeded in doing so we have monitored the other cattle on the farm concerned for the presence of BRS antibodies.

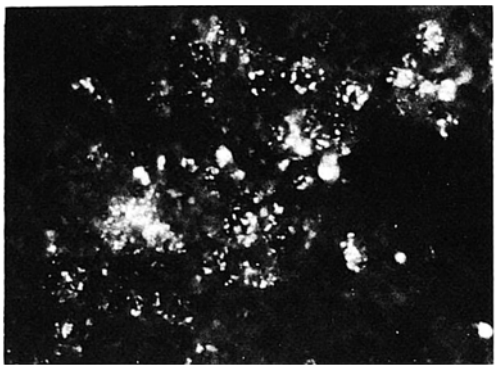
The diagnostic method using immunofluorescence enables anti-BRS virus antigen to be detected when the virus is no longer infectious. Bronchitic and bronchiolitic lesions are the most characteristic and are very useful for establishing the diagnosis. A single layer of positive cells separates intact portions of the mucous membrane from the broncho or bronchiolar tubes, which are often obstructed by cell debris. The findings in alveolar material are more difficult to interpret because of the presence of macrophages, sometimes in great abundance (Fig. 1).

Examination of conjugated sera

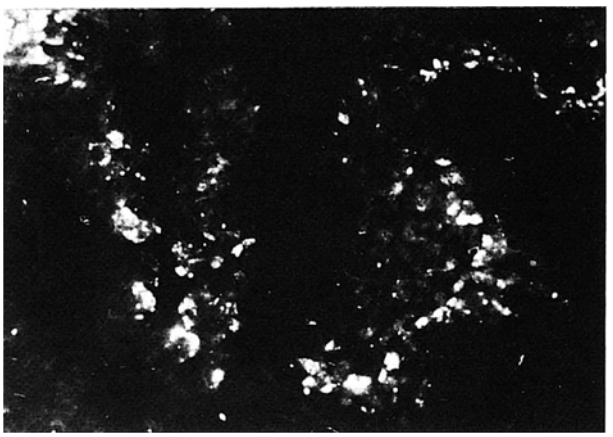
The investigation of antibodies in sera by means of complement fixation (CF) or indirect immunofluorescence (IIF) is the most reliable and straightforward diagnostic technique at present available. However, it usually necessitates the use of conjugated sera, which deters many veterinary practitioners from performing such tests at a time when they are more concerned with restoring the surviving animals to health than obtaining a belated diagnosis of the aetiology of the disease.

Complement fixation (C.F.). - If specific antigen is present, some antibodies form a stable complex into which complement is taken up. The CF test, which is simple to perform, demonstrates the existence of the antigen-complement-antibody complex, by fixing the complement which causes lysis of sensitised red blood cells.

The antigen is contained in the supernatant fluid of a cell culture lysed by the action of a strain of BRS virus adapted to cell cultures. This



(a)



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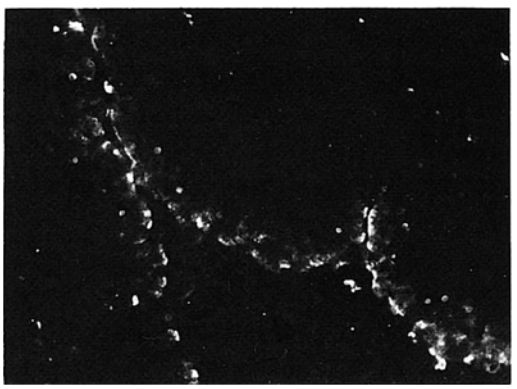

(c)

Fig. 1. Pulmonary lesions caused by BRS virus. (a), Alveolitis: disseminated fluorescence in the cells of the mucous membrane and in the alveolar lumen. (b) and (c), Bronchitis and bronchiolitis: showing BRS antigens in the mucous membranes.

supernatant fluid is used either without modification or after concentration by dialysis with a PEG 20,000 solution. The complement is guinea pig serum to which 5% of fresh calf serum has been added. The procedure for the antigen-complement-antibody combination is carried out at 4°C overnight and then for one hour at 37°C. Titres of 1:8 to 1:64 are obtained with the second set of sera. Where the blood specimens have not been collected until a few days after the onset of the disease it is already possible to detect high titres in the first set of sera. We have found that the antibodies to BRS virus develop very rapidly (Fig. 2a).

Indirect immunofluorescence. - Some antibodies can attach to the specific antigen and complex with it. A labelled serum which reacts with the immunoglobulins belonging to the species of animal under study reveals the complex. In serum tests the titre corresponds to the highest dilution with which fluorescence is obtained.

The indirect immunofluorescence (IIF) reaction enables diagnosis to be made very rapidly. In a successful test the results can be read within 24 hours. We have not found any cross-reaction with the viruses used in differential diagnosis (BVD, parainfluenza-3, IBR, rhinovirus, papular stomatitis, adenovirus). The antigen is produced on a PK 15 cell line. Its use obviates non-specific reactions due to the presence in the serum of antibodies which react with the cells of bovine origin. The titres obtained are as high as 1:405 to 1:3,735 and such titres are often obtained in the first set of sera (Fig. 2b).

We have used this technique for monitoring various farms where the cattle were affected with respiratory disorders caused by the BRS virus. The accompanying diagram illustrates the test results for 6 animals from which we had taken blood specimens by chance a few days before the disease was manifested.

We found average titres of 1:32 in CF and 1:810 in IIF in the first few days of the disease. The antibody level was elevated for a month and then the titre steadily declined. After two months the average CF titre was only 1:4 and the IIF titre was 1:135. Lowering of the CF titre continued and after the end of the third month the antibodies could no longer be detected.

Antibody detection in nasal mucus

There is some degree of excretion of antibodies in the nasal mucus, for protection of the respiratory mucosa. The availability of highly absorbent

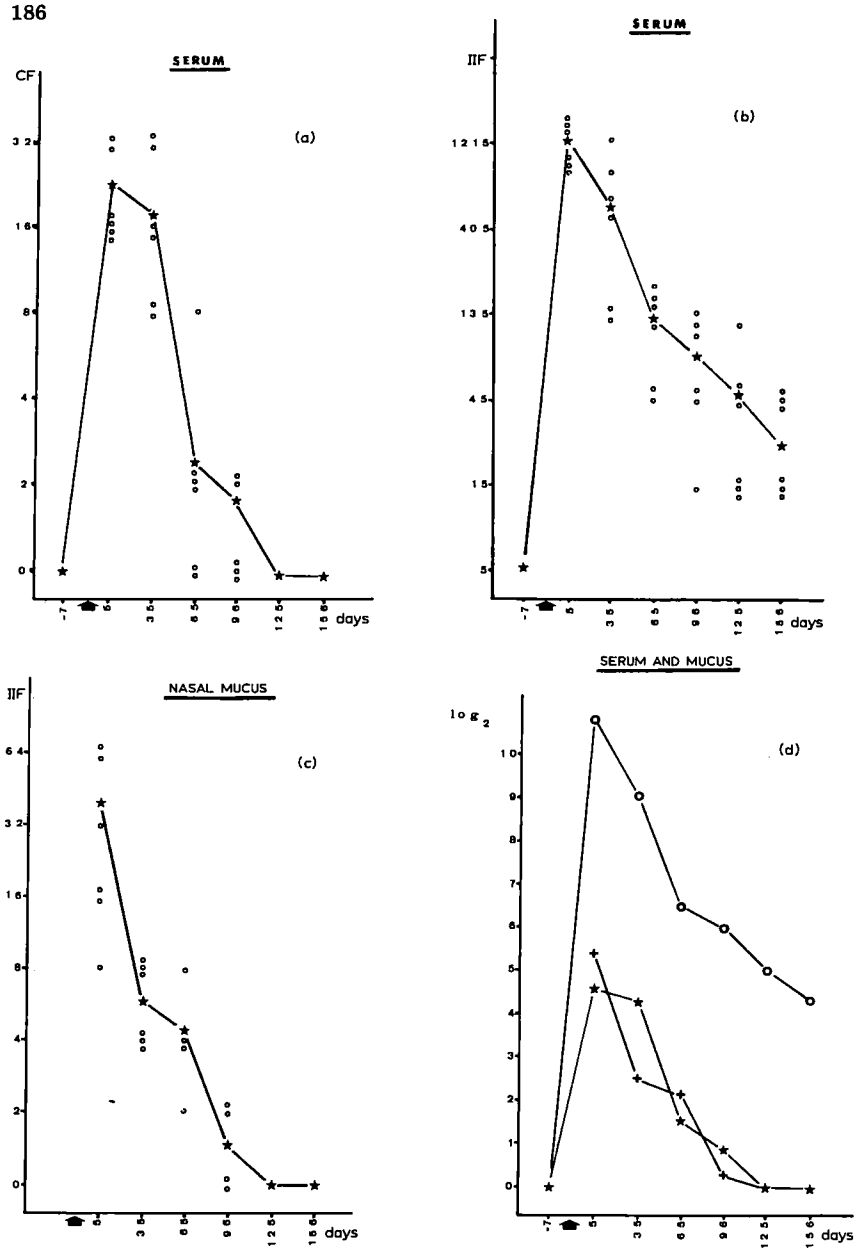

Fig. 2. (a), Bovine serum antibody levels after respiratory infection ☗ caused by BRS virus as shown by the CF test. (b), Serum antibody levels in the same animals as shown by the IIF test. (c), Antibody levels (IIF test) in nasal mucus. (d), Comparison of antibody levels shown by IIF (o) and by CF (★) in serum and in nasal mucus (+).

swabs makes it easy to take large enough samples of this mucus for the purpose of antibody titration. The IIF test revealed a titre of antibodies to the disease of 1:45 for at least a month. These antibodies disappeared about two months after the onset of the viral infection (see Figs. 2c and d).

SIGNIFICANCE OF THE FINDINGS

By adopting new techniques we have been able to speed up laboratory diagnosis of respiratory disorders caused by BRS virus. Identification of the active pathogen in the respiratory disorders explains the ineffectiveness of symptomatic treatments. The application of our methods in differential diagnosis has revealed the role of the various viruses in the disease states observed. This information enables the vaccine specific to the causative virus to be selected.

Differential diagnosis also supplies the explanation of some apparent lack of success in vaccination. There are various different aetiologies in respiratory diseases of cattle but the vaccines applicable are strictly specific.

CONCLUSIONS

There is difficulty in the clinical diagnosis of respiratory disorders caused by BRS virus. Only a limited number of experienced veterinary practitioners are able to decide on the diagnosis of this agent. Isolation of the virus by seeding cell cultures is problematic, because frequently the specimens no longer contain infectious particles.

Examination of ultra-thin lung sections stained with fluorescein isothiocyanate-labelled serum is a successful and reliable method. Its use is likely to increase.

However, serum testing remains the technique of choice. High antibody titres in CF and IIF demonstrate recent infection by the BRS virus.

Improvement in diagnosis of the causative virus in bovine respiratory disorders should result in better understanding of this type of disease and may lead to the production and testing of effective vaccines.

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KURZFASSUNG

Wellemans, G., 1977. Diagnostische Methoden des Sinzizialatmungsvirus **beim Rind bei Erkrankungen der Atemwege. Vet. Sci. Commun., 1: 179-189 (in Englisch).**

Die Erkrankungen der Atemwege die durch den Sinzizialatmungsvirus der Rinder hervorgerufen werden, können zur Zeit mit Leichtigkeit diagnostiziert werden. Die klinische Diagnostik, die auf die Anzeichen eines Lungenemphysems beruhen und im Herbst auftreten, muss durch eine Labordiagnose bestätigt werden. Die Sichtbarmachung der viralen Antigenen mittels Färbung ultradünner Schnitte mit einem durch Fluoreszeinisothiozianat markierten Serum erweist sich wirksam und zuverlässig. Die Isolierung des Virus in den Zellkulturen ist oft sehr schwierig. Bei der Aufstellung der Diagnose ist die Suche nach Antikörpern in den gekoppelten Seren, mit der Komplementbindungsmethode und besonders mit der indirekten Immuno-Fluoreszenz, von grosser Wichtigkeit.

RESUME

Wellemans, G., 1977. Les méthodes de diagnostic du virus respiratoire syncytial bovin (RSB) lors d'affections respiratoires. Vet. Sci. Commun., 1: 179-189 **(en anglais).**

En cas de troubles respiratoires dus au virus Respiratoire Syncytial Bovin (RSB) le diagnostic peut être posé actuellement sans grande difficulté. Le diagnostic clinique, basé sur les signes d'emphysème pulmonaire, apparaissant en automne, doit être confirmé par un diagnostic de laboratoire. L'isolement de l'agent viral sur culture cellulaire est souvent difficile. La mise en évidence des antigènes viraux par coloration de coupes ultra-fines à l'aide d'un sérum marqué à l'isothiocyanate de fluorescéine est efficace et fiable. La recherche d'anticorps dans des sérums couplés, par les méthodes de fixation du complément et principalement d'immunofluorescence indirecte, est de grande utilité pour l'établissement du diagnostic.

RIASSUNTO

Wellemans, G., 1977. I metodi di diagnostica del virus respiratorio **sinciziale del bovino (RSB) nelle affezioni respiratorie. Vet. Sci. Commun., 1: 179-189 (in Inglese).**

Attualmente la diagnosi sulle turbe respiratorie causate dal virus respiratorio sinciziale bovino (RSB) non presenta difficoltà di rilievo. La diagnosi clinica, basata sui sintomi di enfisema polmonare, che si manifestano in autunno, deve essere confermata mediante una diagnosi di laboratorio. L'isolamento dell'agente virale su coltura cellulare risulta spesso difficile. La messa in evidenza degli antigeni virali mediante colorazione di tagli ultrafini con un siero marcato all'isotiocianato di fluorescina è efficace e ed affidabile. Per stabilire la diagnosi è di grande utilità la ricerca di anticorpi nei sieri combinati, con i metodi del la fissazione del complemento e in particolare con l'immunofluorescenza indiretta.

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