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Chapter 34

Bovine Respiratory Syncytial Virus

G. WELLEMANS

VIRUS PROPERTIES

Physical and chemical characteristics

The bovine respiratory syncytial virus (BRSV) is morphologically similar to the human RSV and pneumonia virus of mice, the only known members of the pneumovirus group (Cash et al., 1977).

The virus is of great pleomorphism. Many particles appear roughly spherical with an overall diameter of 80–450 nm (Ito et al., 1973; Mohanty, 1978). The RSV genome codes for ten virus proteins. Four proteins are associated with the virus envelope: the large glycoprotein (G, 84 k), the fusion-protein (F, 68 k), the matrix (M, 26 k) and the 22 k protein. The F protein consists of two disulphide-linked polypeptide fragments (F1, 48 k, and F2, 20 k) and causes fusion of infected cells (Walsh and Hruska, 1983). The G protein is the attachment protein (Levine et al., 1987). Three proteins constitute the virus nucleocapsid: the major nucleocapsid protein (N, 42 k), the phosphoprotein (P, 34 k) and the large protein (L, 200 k). A small protein (9.5 k) is found in association with purified virions and is candidate to be a nonstructural protein (Huang and Wertz, 1983). The nucleocapsids have a density of 1.26–1.27 g/ml, and structures of 1.32–1.36 g/ml appear during the disintegration of nucleoids. Virion RNA has a sedimentation coefficient of 52 S (Zhdanov et al., 1974).

Cell-culture-adapted virus survives quick freezing to −70°C, but Mohanty (1978) reports that isolations are more difficult from frozen than from fresh material. BRSV is sensitive to low pH, ether and chloroform and is destroyed by heating at 56°C for 30 min (Inaba et al., 1970; Paccaud and Jacquier, 1970; Rosenquist, 1974). Application of the least squares method to survival values obtained at 0.5 and 15 min indicated estimated half-lives of 1.8 and 2.8 min (Rosenquist, 1974).

The infectivity titer of a BRSV suspension decreases by about 1 log per 24 h at 37°C. The virus remains infectious for at least 10 years when stored in liquid nitrogen.

Antigenic properties

Evidence of a close antigenic relationship between the bovine agent and human RSV is confirmed by CF and IF tests (Paccaud and Jacquier, 1970; Wellemans et al., 1970).

Cross-neutralization experiments indicate that antiserum against human RSV neutralizes the homologous virus at significantly higher titers than the bovine virus (Inaba et al., 1970; Paccaud and Jacquier, 1970; Smith et al., 1975).

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Some differences, e.g. the spectrum of susceptible cells and an antigenic dissimilarity, suggest that the two viruses have different natural hosts (Inaba et al., 1970). Moreover, virus could not be recovered from the respiratory tract of mice inoculated with bovine strains (Taylor et al., 1984). However, Jacobs and Edington (1975) succeeded in an experimental infection of a calf with a human strain. This experiment was successfully repeated by Thomas et al. (1984).

The protein composition of human and bovine RSV strains is very similar, with only minor differences in mol. wt. (Cash et al., 1977). Two subgroups of human RSV can be distinguished; the major antigenic differences are located on the G protein (Mufson et al., 1985). BRSV can be classified as a separate group, sharing epitopes with most proteins of the two human subgroups, but being distinct with respect to the epitopes on the G protein (Orvell et al., 1987).

Monoclonal antibodies to the F protein showed neutralizing activity and prevented spread of the virus in vitro (Walsh et al., 1985). Passive transfer of monoclonal antibodies to the F and G proteins offered protection against challenge with RSV, while antibodies to internal proteins did not (Walsh et al., 1984).

Cultivation

In tissue culture, BRSV shows a wide range of susceptible host cells. It replicates in all cell types (kidney, testicle, thyroid, thymus, duodenum, rectum) of bovine origin as well as in cells from swine (embryonic kidney), hamster (lung, kidney), monkey (Vero) and human (embryonic lung and kidney, Hela, HEp-2). Better growth is observed in bovine than in human cell cultures (Matumoto et al., 1974). This is in contrast to the observations of Paccaud and Jacquier (1970), who did not succeed in cultivating their BRSV strains in a BHK-21 cell line and in various human cells.

Larger amounts of virus are obtained in calf kidney and testicle cell culture, and higher titers are scored in secondary than in primary cultures of the same cells (Mohanty, 1978; Wellemans, 1977). The direct IF test in which the immunoglobulins of BRSV antiserum are conjugated with fluorescein isothiocyanate detects the antigens as soon as 16–18 h after inoculation. Fluorescence is always confined to the cytoplasm. Within 24 h, fine fibrils appear, usually parallel to the long axis of the cell, and cytoplasmic granules are formed around the nucleus (Rossi and Kiesel, 1977b). After 24 h, coincident with rounding of the cells, fluorescence slowly moves to the periphery of the cytoplasm. By IF and as determined by the release of BRSV into the supernatant fluid, the minimal time for a single cycle of infection was between 24 and 26 h (Rossi and Kiesel, 1977b).

BRSV grows in organ cultures of bovine fetal trachea explants at 37°C and pH 7.2. It reaches maximum titers of 10^5 PFU/ml between 11 and 21 days after inoculation. Virus growth does not affect ciliary activity of the cultured cells (Thomas et al., 1976; Rossi and Kiesel, 1977a).

EPIZOOTIOLOGY

BRSV has been isolated in most European countries, North America, Australia, Japan and more recently in North Africa (Mahin and Wellemans, 1982).

The virus generally appears in a BRSV-free country after the introduction of an infected animal. Although the presence of virus carriers has not yet been proven (probably because of the difficult isolation of the virus), one should be aware of this possibility. Humans, especially veterinarians and animal handlers, could play a role in the transmission of the virus. Considering the success-

ful infection of a calf with a human strain (Jacobs and Edington, 1975) and the similarities between human and bovine strains, humans cannot be excluded as a virus reservoir (Berthiaume et al., 1973). However, Inaba et al. (1970) and Paccaud and Jacquier (1970) do not endorse this view.

Once a herd in a region is affected, the disease rapidly spreads from farm to farm. In an already infected region, the disease becomes endemical and affects the same herds almost every year. The animals, especially beef cattle, are most susceptible from 3 up to 9 months of age, though older animals are not always resistant (Paccaud and Jacquier, 1970; Wellemans et al., 1970; Van Bekkum et al., 1977). On the other hand, younger calves of only a few weeks of age can also be affected, and their protection by vaccination is still questionable (Wellemans, 1982). Differences in housing and other aspects of management probably have some effect; in some herds the virus is present without causing any disease, as shown by occasional seroconversions, in others one or even two outbreaks occur every year (Van Bekkum et al., 1977). However, severe disorders can be observed also in animals at pasture.

Most of the severe cases appear from October to January (Van Bekkum et al., 1977; Wellemans, 1977), but recently BRSV outbreaks have also occurred in spring and summer. Therefore, disease caused by BRSV can be expected during the whole year, as in the case of IBR. The weather, particularly a fall in atmospheric pressure, plays an important role in the outbreak of the disease (Wellemans, 1982).

PATHOGENESIS

The disease symptoms observed after experimental inoculation are not as severe as in cases of natural infection (Inaba et al., 1972; Jacobs and Edington, 1975; Smith et al., 1975; Mohanty, 1978; Elazhary et al., 1979; Thomas et al., 1984) and the infection frequently remains inapparent. Stott (1985) suggests that virus unpassaged in tissue culture may have a greater virulence for the natural host. Treatment with dexamethasone enhances lung lesions produced by bovine strains, extends the period of virus shedding and increases peak titers (Thomas et al., 1984). The concomitant presence of BVDV as well as a sudden fall in atmospheric pressure or a drop of the minimum temperature seem to aggravate the disease (Verhoeff and Van Nieuwstadt, 1984).

The first symptoms are observed 2–8 days after inoculation. The virus can be reisolated from nasal secretions for 4–10 days after inoculation, and from the nasal, tracheal and bronchial mucosae for 7–13 days after infection (Jacobs and Edington, 1975; Thomas et al., 1984).

It appears that BRSV infection can occur in the presence of circulating antibodies (Mohanty, 1978; McNulty et al., 1983). There is no evidence, however, that preexisting serum antibody causes exacerbation of the disease in young calves, as was reported in infants (Kim et al., 1969); other conclusions have been drawn by Smith et al. (1975). However, the protective effect of nasal neutralizing antibodies against infection with BRSV has been shown by Mohanty (1978). This author found that young calves with traces of neutralizing antibodies in the nasal secretions remained solidly immune to challenge. The quick appearance and the pathogenic action of BRSV on the nasal mucosa would indicate that the nasal cavity might be the point of departure to the target cells; Baskerville (1981) disagrees with this hypothesis. BRSV is unable to suppress ciliary activity, even though the virus replicates within ciliated epithelial cells of tracheal rings. It was therefore suggested that the tracheal epithelium may not be important in the pathogenesis of BRSV infection (Thomas et al., 1976; Rossi and Kiesel, 1977a).

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The accumulation of cellular debris and exudate favors bacterial proliferation, which may lead to extensive purulent pneumonia chiefly in young calves. However, the sudden onset of pulmonary emphysema is not explained by BRSV infection. Immunological hypersensitivity reactions probably are involved as complicating factors, as has been shown in humans (Gardner et al., 1970).

McIntosh and Fishaut (1980) have reviewed several theories about the pathogenesis of RSV disease in infants: serum antibodies reacting with the virus, prior sensitizing infection, CMI, IgE-mediated reactions—no firm conclusions are possible about immunopathologic mechanisms in bronchiolitis.

DISEASE SIGNS

In typical outbreaks, the disease develops in two distinct episodes. Suddenly 80–90% of a certain age group may show symptoms. Affected calves are 3–9, sometimes up to 15 months old. The animals cough and there is nasal discharge and a conjunctivitis with lachrymation. The body temperature is about 40°C.

About 2 or 3 days later, when everything seems to be back to normal, the second episode begins and signs of lung emphysema appear. Some animals have difficulty in breathing, accompanied by bouts of dry coughing. Body temperature at the time these symptoms are first manifest is close to normal. The calves lose weight and have a rough hair coat. Respiration rates can go up to > 100. The breathing of the sick animals 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 froth at the commissure of the lips. Ill calves may stand with a stretched neck and extended forelimbs (Fig. 131). Symptoms of abdominal breathing can develop. The animals can neither lie down nor eat and make desperate efforts to breath through the open mouth. Constipation is common and there is complete loss of appetite. The mucosae become cyanotic. On auscultation, some harshness in the breathing can be detected.

There can be up to 20% mortality in the herd. Farms specializing in baby-beef tend to have the highest losses. Mortality often occurs within a few hours.

Surviving calves recover after a few days: breathing becomes easier and

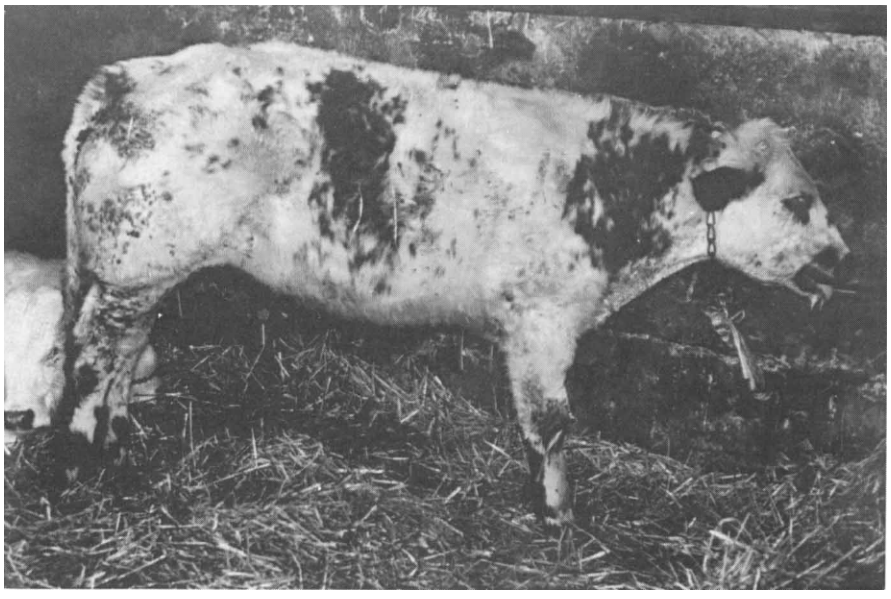

Fig. 131. Calf with BRSV infection; note the dyspnea.

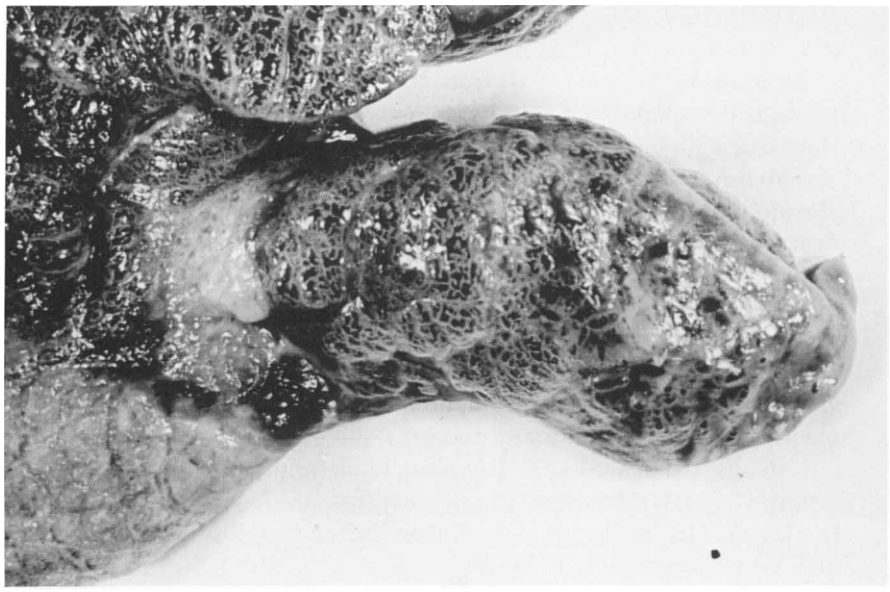

Fig. 132. Pulmonary emphysema (apical lobe) with hemorrhagic lesions.

appetite returns (Holzhauer and Van Nieuwstadt, 1976; Van Bekkum et al., 1977; Wellemans, 1977; Verhoeff and Van Nieuwstadt, 1984).

A slightly different symptomatology can be observed in 6-week-old calves, especially in fattening farms. Few or no emphysema lesions are encountered, but bacterial superinfections are frequently found. Cough, high fever and serous to mucopurulent nasal discharge are the most common symptoms.

Paccaud and Jacquier (1970) noted that all animals aged less than 7 years showed signs of acute respiratory disease. Pregnant animals or good milk producers tend to be affected (Inaba et al., 1972).

In Belgium, respiratory disorders are seldom observed in cattle more than 2 years old.

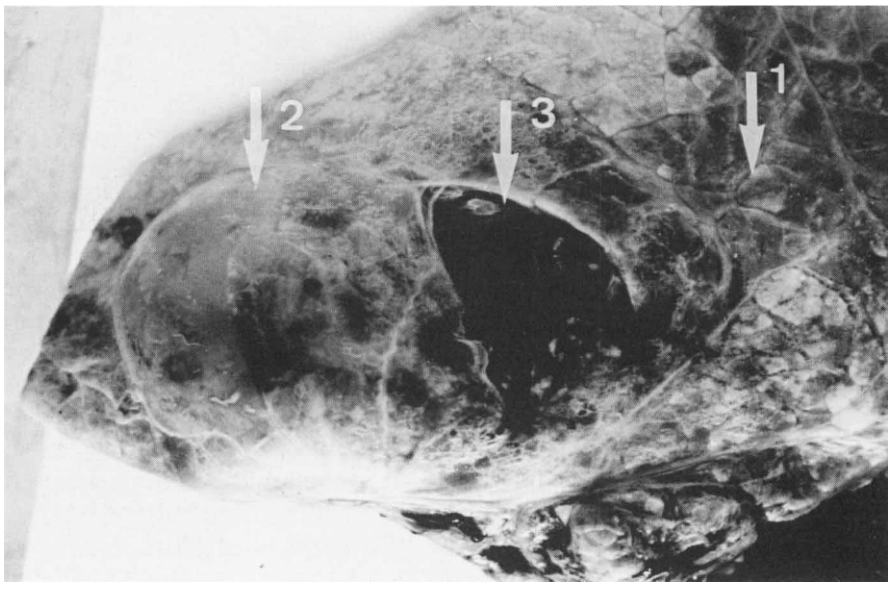

Fig. 133. Pulmonary emphysema (mediastinal lobe); (1) interlobular septa are distended; (2) large bullae; (3) pleural wall is broken.

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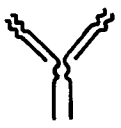
PATHOLOGY

In animals that have died from the disease, pathological changes are restricted to the respiratory tract; postmortem lesions are not really pathognomonic: they can also be found in cases of BVD and PI3 infection.

The lungs are voluminous and emphysematous. The apical and cranial lobes show lobular bronchopneumonia. Numerous large ecchymotic hemorrhages are present in emphysematous bullae and under the pleura (Figs. 132, 133); often foamy exudate covers the injected mucosae of the bronchi. The diaphragmatic lobes are edematous and interlobular septa are distended. Subpleural emphysema is present in all lobes but is more severe in the diaphragmatic lobe, where numerous large dissecting bullae are present. In some cases the pleural wall is broken, resulting in pneumothorax. In most cases there is also marked mediastinal emphysema and in some calves emphysema extends into the subcutaneous tissues of the shoulder, back and neck. The animals then show crepitating, asymetric swellings. Petechia are frequently seen, particularly on the larynx. In the heart, petechia are often found on the myocard (Holzhauer and Van Nieuwstadt, 1976; Wellemans, 1977; Bryson et al., 1983).

Calves of a few weeks of age present hepatization in the anterior lobes; emphysema is rare.

Histological lesions, e.g. mononuclear infiltration in the hyperplastic alveolar walls, bronchiolitis or lobular interstitial pneumonia with edema, are not pathognomonic of BRSV infection. However, in some cases, mainly in young calves, syncytia with eosinophilic inclusions are observed in the alveoles (Mohanty, 1978; Bryson et al., 1983). Two types of lesions can be found in ultrathin sections of apical and cranial lobes, with the aid of a fluorescein isothiocyanate (FITC) conjugated anti-BRSV serum. The lesions are referred to as types A and B. In type A lesions the antigen is distributed homogenously in the cytoplasm of the affected cells. These cells look undamaged, and syncytium formation is frequently seen. This type of lesions is found in calves of a few weeks of age and sometimes in older animals of the Charolais or Blonde d'Aquitaine breed (Wellemans, 1982). In type B lesions the antigen is clustered in packs. The cells are disrupted and antigen masses are spread i the alveolar or bronchial lumen. Rarely giant cells are found in these lesions (Wellemans, 1977). The type B lesions are encountered in emphysematous lungs of older calves of the Belgian Blue White breed.



IMMUNE REACTION

Epidemiological studies have shown that there is no solid protection in humans against nasal reinfection. However, cotton rats infected with RSV develop complete resistance to pulmonary infection lasting for at least 18 months. Nasal resistance was of shorter duration and decreased from 8 months on. Immunity to RSV infection is therefore more long lasting in the lungs than in the upper tract and the level of immunity to RSV in the upper respiratory tract does not necessarily reflect resistance in the lungs (Prince et al., 1983).

Preexisting maternal antibodies to BRSV did not protect calves from infection (Mohanty, 1978; McNulty et al., 1983). On the other hand, maternal immunity was effective but transient in the lungs of young cotton rats (Prince et al., 1983). Immune factors other than neutralizing antibodies may play a role in the maternal passive immunity (Prince et al., 1983).

In experimentally infected calves, high interferon titers are detected during the early stage of infection. This is followed by a period of at least 1 week during which interferon is not detectable. After this, moderate to low inter-

feron titers reappear in most animals and persist for a number of weeks (Elazhary et al., 1981). In contrast, in children no interferon or only low levels are found (Hall et al., 1978; McIntosh, 1978), and mean levels do not fluctuate significantly in relation to disease and recovery. These discordant results are probably due to the fact that the first blood sample in children is taken in the course of the disease, whereas in calves the first sample is taken at an early beginning stage.

Antibodies have been demonstrated by VN, CF, indirect IF, ELISA and precipitation tests in sera from cattle infected with BRSV. Neutralizing antibodies appear in serum of experimentally infected calves after 7 days at extremely low titers (4–8) (Mohanty et al., 1975). In natural infections, however, the SN titers score much higher (64: Rosenquist, 1974; 256: Inaba et al., 1972) and the maximum level is reached after 3 weeks. Using indirect IF, antibodies may be evidenced as early as 3 days p.i., with maximum titers at about 10 days p.i. (Elazhary et al., 1981). In natural infections, it is not uncommon to find highly seropositive calves in the acute stage of the disease. Experimentally infected animals can react in different ways: in some calves, antibodies develop very early, as described by Elazhary et al. (1981); in others their appearance is delayed (7–9 days). In nasal mucus antibodies evidenced by indirect IF are excreted from the first week on, and they are present for at least 3 months (Wellemans, 1977).

Results of leukocyte migration–inhibition tests under agarose indicate that a CMI response is elicited after infection of calves with BRSV. The calves also develop a delayed hypersensitivity skin response (Field and Smith, 1984).

Recovery of 6–7-month-old calves from severe BRSV-associated disease was accompanied by an antibody response that was mainly directed to the F and N proteins. Calves 2–3 weeks of age with moderate levels of maternal antibodies to BRSV particularly directed to the F and N proteins became seriously ill after infection. The antibody response in these calves was severely suppressed. In sera of 4–9-month-old calves that had died in the course of infections, high anti-F and anti-N antibody levels were found. Apparently, the presence or development of antibodies to the F and N proteins is not sufficient for protection against or recovery from infections with BRSV (Westenbrink et al., 1989).

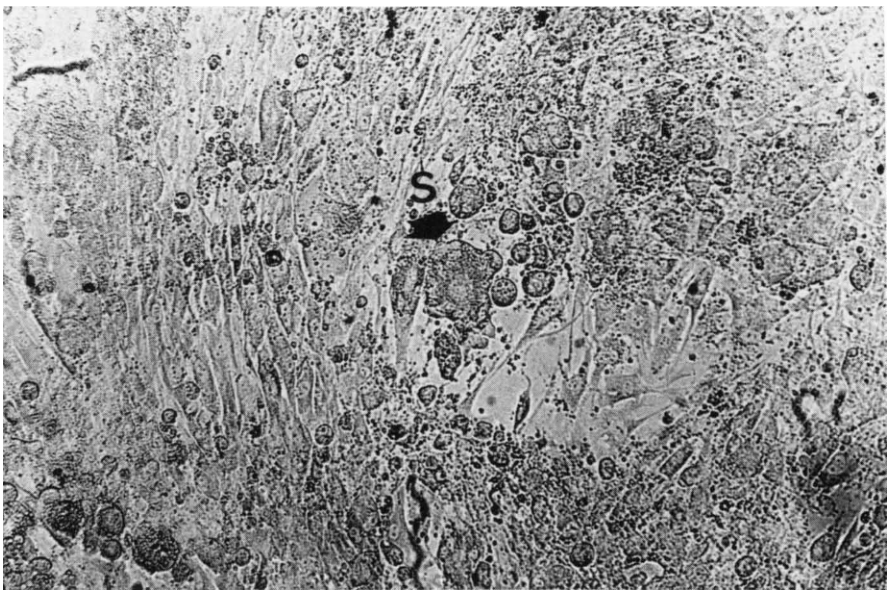


Fig. 134. CPE caused by a BRSV infection on BFK cell culture; note the presence of syncytia (S) and the "moth-eaten" aspect of the culture.

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LABORATORY DIAGNOSIS

A clinical diagnosis based on symptomatology is impossible. Only the laboratory can help the practitioner who suspects a BRSV outbreak in a farm. The laboratory diagnosis will be based on either BRSV isolation, the detection of viral antigen in suspected organs, or by evidencing a seroconversion in diseased animals.

The nasal mucus is taken with sterile cotton swabs during the initial stage of the disease (serous discharge, fever, conjunctivitis). The swabs are then put into a tube with a protein-rich medium (maintenance medium for cell cultures) and forwarded very rapidly in a cool box to the laboratory. Postmortem samples of lung tissue from very young calves should also reach the laboratory rapidly. In contrast to other viruses, e.g. IBRV, viral presence in the nasal mucous is of short duration and limited to the first stage of the disease, which often passes unnoticed.

The isolation of the virus in cell culture is difficult because of the late appearance of CPE; the time of incubation may be extremely long: 20 days (Inaba et al., 1970), 30 days (Paccaud and Jacquier, 1970), 45 days (Wellemans et al., 1970) or even 50 days (Smith et al., 1975). Secondary fetal calf kidney or testicle cells are most susceptible. Virus growth depends on the age of the cell culture, and the medium has to be replaced frequently.

The first changes noted are small areas where four or five cells become ballooned with shrinkage of cytoplasm. In the following days, syncytia develop in these cultures. They become opaque while contracting, and holes appear in the cell monolayer. The aspect becomes more and more granular, resembling a mycoplasma-infected cell culture (Fig. 134).

In preparations fixed with Bouin solution and stained with H & E, homogenous eosinophilic inclusion bodies are observed in the cytoplasm of syncytial cells. These inclusions are less polymorphic than in cells infected with PI3 virus.

The cells grown on glass slide of Leighton tubes are fixed in aceton at $-20°C$ and stained with an antiserum to BRSV conjugated with fluorescein isothiocyanate. Plaques of fluorescent cells confirm the presence of the virus (Fig. 135).

Isolation of BRSV is difficult and of long duration; it is not recommended as a routine procedure (Edwards et al., 1984).

Viral antigen can be detected in nasal mucus and in lung tissue. Nasal

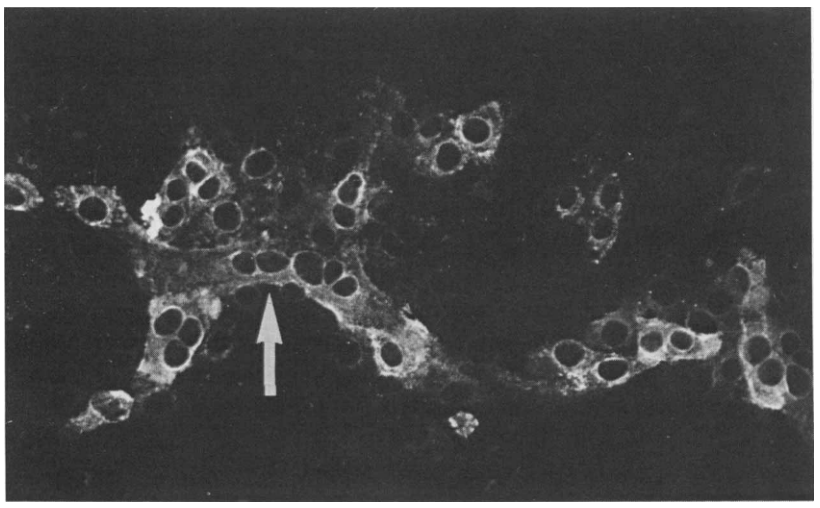


Fig. 135. Direct IF staining of a BRSV infected BFK culture; note the presence of a syncytium.

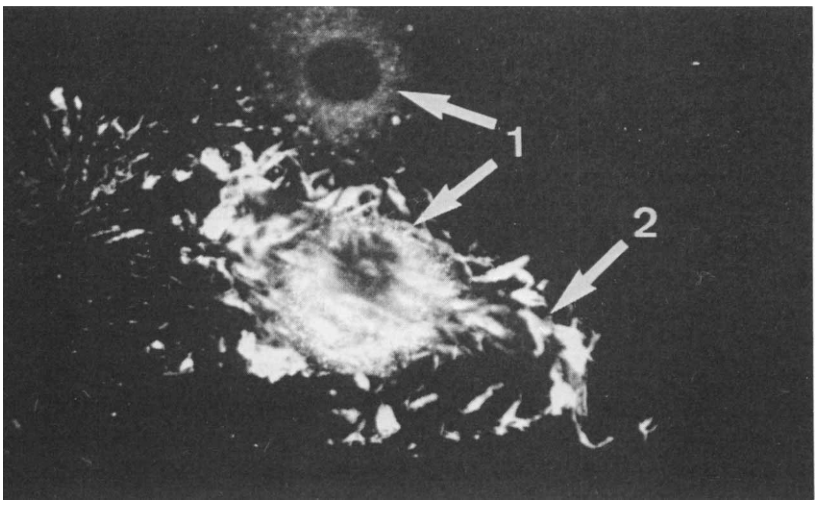
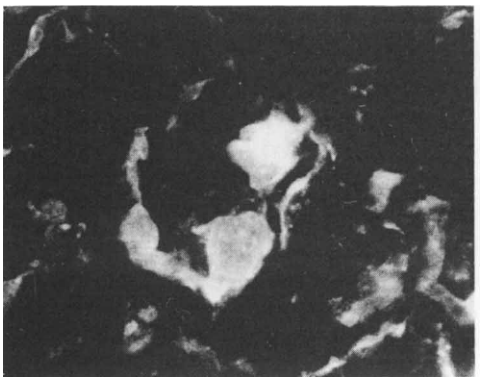


Fig. 136. Direct IF staining of an infected cell; two types of fluorescence present: (1) granular, around the nucleus; (2) filamentous, in the rest of the cytoplasm.

mucus can be taken with a swab. Though the most characteristic emphysema lesions are situated in the mediastinal lobes of the lungs, antigen has to be searched for mainly in the apical and cardiac lobes. The samples have to be forwarded quickly and under refrigeration to the laboratory. For the direct IF test, monospecific hyperimmune serum is required that is marked with fluorescein isothiocyanate using the classical technique.

Typical small, round cells in which fluorescence is confined to the cytoplasm are detected in the nasopharyngeal specimens (McNulty et al., 1983). Fluorescence is not seen in ciliated columnar epithelial cells, although many are present in most specimens (Thomas and Stott, 1981). Problems of nonspecific fluorescence are encountered with the method when applied to nasopharyngeal material but not in the examination of lung material (Thomas and Stott, 1981). Evans blue (1:10.000) can be used to reduce nonspecific fluorescence.

Ultrathin sections of lung fragments, preferably from the apical and cardiac lobes, are stained with conjugated serum. Fluorescent antigen is detected in this material for up to 48 h post mortem, even after freezing. However, it is noteworthy that the viral antigen disappears after longer freezing periods, even at low temperatures (– 100°C). In most of the lung specimens, antigen is found located in the alveoli and sometimes in the bronchioles. Two types of



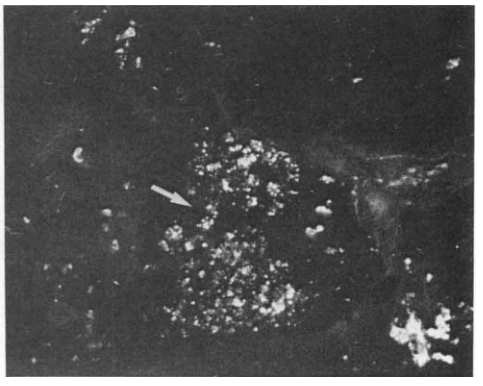


Fig. 137 (left). Direct IF staining on sections of the lung of a young calf; the affected cells of the alveolus appear intact (type A).

Fig. 138 (right). Direct IF staining on ultrathin sections of the lung of an older calf; lesions of alveolitis with disrupted cells (type B).

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fluorescence are distinguished (Fig. 136): type A, in which the antigen is spread homogenously throughout the cytoplasm. The cells look intact and syncytia are frequently observed (Fig. 137); and type B, in which the antigen is aggregated in granular packs. The cells are disrupted and antigenic masses are spread in the alveolar and bronchial lumen. Rarely giant cells are found in these lesions (Fig. 138). Type A lesions are encountered in 2–3-week-old calves and also in older cattle of the Charolais and Blonde d'Aquitaine breed; type B lesions are found in emphysematic lungs of older animals of the Belgian Blue White breed.

ELISA is applied more and more for the detection of viral antigens. Hornsleth et al. (1981) described its use for RSV diagnosis and confirmed its reliability.

An increase of anti-BRSV antibodies can be evidenced in paired sera taken from the same animals in the acute stage and 15 days to 3 weeks later. Neutralizing antibodies appear as a consequence of a BRSV infection. SN titers in convalescent animals rarely exceed 4–16 according to Bartha (1976), whereas Rosenquist (1974) and Inaba et al. (1970) report titers of 256. Despite the improved micromethods the SN test remains a time-consuming technique with difficult reading of the results; it is used in research work rather than for routine diagnosis.

Numerous authors have used the CF test (e.g. Takahashi et al., 1975; Holzhauer, 1978). Untreated infected cell culture fluid, fluorocarbon-treated and ether-treated material are equally suitable as antigen. Human RSV antigen is commercially available. Best specific reactions are obtained with 5% fresh normal calf serum added to the diluent of complement (Wellemans et al., 1970; Takahashi et al., 1975). Neutralizing and complement fixing antibody titers are closely related. The CF test is very reliable for the diagnosis of BRSV infection (Takahashi et al., 1975).

Seroconversion can be detected by AGID. After infection, more than 50% of the animals have immunodiffusion antibodies (Zygraich and Wellemans, 1981). However, this test is only seldom used because of the difficulty in obtaining precipitating antigen and the 3-day delay before the test can be read.

Espinasse et al. (1978) have adapted passive hemagglutination (PHA) to the detection of BRSV antibodies. The preliminary results are encouraging and appear equivalent to the SN scores.

Anti-BRSV antibodies in serum, nasal mucus and organ extracts can be evidenced rapidly and specifically using the indirect IF test (Wellemans, 1977; Potgieter and Aldridge, 1977). The production of a batch of slides covered with antigen-containing cells, which can be stored in a freezer for a long time, makes this test highly reproducible.

After experimental inoculation, the antibodies appear generally from the 7th day on and the titers rise quickly to levels of $\geqslant 1280$ at the end of the second week. Titers exceeding 5000 are frequently found. In other cases, however, a very slow titer evolution is noted, with a maximum score of 135 — although a BRSV infection could be confirmed on postmortem examination of the lungs.

No valid diagnosis can be made without paired sera. However, very high indirect IF or CF antibody levels in single samples are indicative of a BRSV infection in severe respiratory distress. The increase of anti-BRSV antibodies reflects an infection but no conclusions can be drawn about the pathogenic role of the virus. Striking seroconversions have been observed in animals vaccinated 2–3 months before, although no respiratory problems were noticed (Wellemans, 1982). Serological examination is useful but the possibility of atypical seroconversions exists.

PROPHYLAXIS AND CONTROL

BRSV carriers have not yet been proven to exist but it seems possible they will, as was shown for other bovine pathogens, e.g. IBRV (Bitsch, 1973), coronavirus (Van Opdenbosch et al., 1979) and BVDV (Coria and McClurkin, 1978). The fact that the disease becomes enzootic after its introduction in a BRSV-free area supports this hypothesis. Therefore it is advisable to forbid the introduction of diseased animals into a BRSV-free region.

Modification of breeding and fattening conditions (habitat, environment, feeding) has to be taken into consideration, although it is our experience that the most severely affected herds are not always the most badly managed ones.

The vaccination of young children with an inactivated RSV vaccine caused severe hypersensitivity reactions (Kim et al., 1969). In cattle, Mohanty et al. (1981) used a formalin-inactivated and Freund-adjuvanted vaccine. There was no evidence after challenge that vaccinal serum antibodies caused exacerbation of disease in young calves. The vaccine did not induce a nasal antibody response. All but one of the five vaccinated calves appeared to be protected against the disease after challenge exposure. Stott et al. (1984) used a vaccine containing cells persistently infected with BRSV and fixed with glutaraldehyde to retain viral antigens on the cell surface. The glutaraldehyde-fixed cells were combined with incomplete Freund's adjuvant or Saponine Quil-A. After challenge, virus was recovered from all control calves but from only one out of twelve vaccinated calves.

A BRSV strain (Poum 2) at the 94th passage level in BFK cell cultures is presently used in Belgium (Wellemans, 1982) and in the Netherlands (Holzhauer, 1982) for immunization of young cattle. The animals (at least 3 months old) are vaccinated intramuscularly in August–September and boostered 3 weeks later. Some vaccination problems that are not attributable to the vaccine virus strain have been described (Wellemans, 1982). This strain has been shown to be safe in over a million vaccinated animals (Zygraich, 1982) and the results of protection are satisfactory (Holzhauer, 1982; Wellemans, 1982).

REFERENCES

Bartha, A., 1976. Isolation of respiratory syncytial virus from cattle with a respiratory disease. Magy. Allatrv. Lapja, 31: 99–102.

Baskerville, A., 1981. Mechanisms of infection in the respiratory tract. N.Z. Vet. J., 29: 235–238.

Berthiaume, L., Joncas, J., Boulay, G. and Pavilanis, V., 1973. Serological evidence of respiratory syncytial virus infection in sheep. Vet. Rec., 93: 337–338.

Bitsch, V., 1973. Infectious bovine rhinotracheitis virus infection in bulls, with special reference to preputial infection. Appl. Microbiol., 26: 337–343.

Bryson, D.G., McNulty, M.S., Logan, E.F. and Cush, P.F., 1983. Respiratory syncytial virus pneumonia in young calves: clinical and pathologic findings. Am. J. Vet. Res., 44: 1648–1655.

Cash, P., Wunner, W.H. and Pringle, C.R., 1977. A comparison of the polypeptides of human and bovine respiratory syncytial viruses and murine pneumonia virus. Virology, 82: 369–379.

Coria, M.F. and McClurkin, A.W., 1978. Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhea virus. J. Am. Vet. Med. Assoc., 172: 449–451.

Edwards, S., Newman, R.H. and Stanley, M., 1984. Respiratory syncytial virus diagnosis. Vet. Rec., 114: 101.

Elazhary, M., Galina, M., Roy, R.S., Fontaine, M. and Lamothe, P., 1979. Experimental infection of calves with bovine respiratory syncytial virus (Quebec strain). Can. J. Comp. Med., 44: 390–395.

Elazhary, M., Silim, A. and Roy, R.S., 1981. Interferon, fluorescent antibody and neutralizing antibody responses in sera of calves inoculated with bovine respiratory syncytial virus. Am. J. Vet. Res., 42: 1378–1382.

Espinasse, J., Le Layec, C. and Faye, P., 1978. Hemagglutination passive: application de la méthode au diagnostic sérologique des affections respiratoires virales des jeunes bovins. Rec. Méd. Vét., 154: 227–232.

Field, E.W. and Smith, M.H., 1984. Cell-mediated immune response in cattle to bovine respiratory syncytial virus. Am. J. Vet. Res., 45: 1641–1643.

Gardner, P.S., McQuillin, J. and Court, S.D.M., 1970. Speculation on pathogenesis in death from respiratory syncytial virus infection. Br. Med. J., 1: 327–330.

Hall, C.B., Douglas, R.G., Simons, R.L. and Geiman, J.M., 1978. Interferon production in children with respiratory syncytial, influenza and parainfluenza virus infections. J. Pediat., 93: 28–32.

Holzhauer, C., 1978. Bronchopneumonia of Yearling Cattle. PhD thesis, Utrecht University, Utrecht, The Netherlands, 192 pp.

Holzhauer, C., 1982. Prevention of pinkengriep by vaccination, a field trial. Proceedings XII Congress on Diseases of Cattle, Amsterdam, September 1982, Vol. 1, pp. 166–170.

Holzhauer, C. and Van Nieuwstadt, A., 1976. The etiological role of bovine respiratory syncytial virus in "pinkengriep". Tijdschr. Diergeneesk., 101: 1023–1031 (in Dutch).

Hornsleth, A., Brenoe, E., Friis, B., Knudsen, F.U. and Uldall, P., 1981. Detection of respiratory syncytial virus in nasopharyngeal secretions by inhibition of enzyme-linked immunosorbent assay. J. Clin. Microbiol., 14: 510–515.

Huang, Y. and Wertz, G., 1983. Respiratory syncytial virus mRNA coding assignments. J. Virol., 46: 667–672.

Inaba, Y., Tanaka, Y., Sato, K., Ito, H., Omori, T. and Matumoto, M., 1970. Nomi virus, a virus isolated from an apparently new epizootic respiratory disease of cattle. Jap. J. Microbiol., 14: 246–248.

Inaba, Y., Tanaka, Y., Sato, K., Omori, T. and Matumoto, M., 1972. Bovine respiratory syncytial virus. Studies on an outbreak in Japan, 1968–1969. Jap. J. Microbiol., 16: 373–383.

Ito, Y., Tanaka, Y. and Omari, T., 1973. Structure of bovine respiratory syncytial virus. Arch. Ges. Virusforsch., 40: 198–204.

Jacobs, J.W. and Edington, N., 1975. Experimental infection of calves with respiratory syncytial virus. Res. Vet. Sci., 18: 299–306.

Kim, H.W., Canchola, J.G., Brandt, C.D., Pyles, G., Chanock, R.M., Jensen, K. and Parrot, R.H., 1969. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am. J. Epidemiol., 89: 422–434.

Levine, S., Klaiber-Franco, R. and Paradiso, P., 1987. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. J. Gen. Virol., 68: 2521–2524.

McIntosh, K., 1978. Interferon in nasal secretions from infants with viral respiratory tract infections. J. Pediat., 93: 33–36.

McIntosh, K. and Fishaut, J.M., 1980. Immunopathologic mechanisms in lower respiratory tract disease of infants due to respiratory syncytial virus. Prog. Med. Virol., 16: 94–118.

McNulty, M.S., Bryson, D.G. and Allan, G.M., 1983. Experimental respiratory syncytial virus pneumonia in young calves: microbiologic and immunofluorescent findings. Am. J. Vet. Res., 44: 1656–1659.

Mahin, L. and Wellemans, G., 1982. Serological evidence for the intervention of bovine respiratory syncytial virus in a respiratory disease outbreak in moroccan cattle. Zentralbl. Veterinärmed. B, 29: 76–79.

Matumoto, M., Inaba, Y., Kurogi, H., Sato, K., Omori, T., Goto, Y. and Hirose, O., 1974. Bovine respiratory syncytial virus: host range in laboratory animals and cell cultures. Arch. Ges. Virusforsch., 44: 280–290.

Mohanty, S.B., 1978. Bovine respiratory viruses. Adv. Vet. Sci. Comp. Med., 22: 83–109.

Mohanty, S.B., Ingling, A.L. and Lillie, M.G., 1975. Experimentally induced respiratory syncytial viral infection in calves. Am. J. Vet. Res., 36: 417–419.

Mohanty, S.B., Rockemann, D.D., Davidson, J.P., Sharabrin, O.I. and Forst, S.M., 1981. Effect of vaccinal serum antibodies on bovine respiratory syncytial viral infection in calves. Am. J. Vet. Res., 42: 881–883.

Mufson, K., Orvell, C., Rafnar, B. and Norrby, E., 1985. Two distinct subtypes of human respiratory syncytial virus. J. Gen. Virol., 66: 2111–2124.

Orvell, C., Norrby, E. and Mufson, M., 1987. Preparation and characterization of monoclonal antibodies directed against five structural components of human respiratory syncytial virus subgroup B. J. Gen. Virol., 68: 3125–3135.

Paccaud, M.F. and Jacquier, C., 1970. A respiratory syncytial virus of bovine origin. Arch. Ges. Virusforsch., 30: 327–342.

Potgieter, L.N.D. and Aldridge, R.L., 1977. Use of indirect fluorescent antibody test in the detection of bovine respiratory syncytial virus antibodies in bovine serum. Am. J. Vet. Res., 38: 1341–1343.

Prince, G.A., Horswood, R.L., Camargo, E., Koenig, D. and Chanock, R.M., 1983. Mechanisms of immunity to respiratory syncytial virus in cotton rats. Infect. Immun., 42: 81–87.

Rosenquist, B.D., 1974. Isolation of respiratory syncytial virus from calves with acute respiratory disease. J. Infect. Dis., 130: 177–182.

Rossi, C.R. and Kiesel, G.K., 1977a. Susceptibility of bovine macrophage and tracheal-ring cultures to bovine viruses. Am. J. Vet. Res., 38: 1705–1708.

Rossi, C.R. and Kiesel, G.K., 1977b. Bovine respiratory syncytial virus infection of bovine embryonic lung cultures: a kinetic study by the fluorescent antibody technique. Am. J. Vet. Res., 38: 1901–1904.

Smith, M.H., Frey, M.L. and Dierks, R.E., 1975. Isolation, characterization and pathogenicity studies of a bovine respiratory syncytial virus. Arch. Virol., 47: 237–247.

Stott, E.J. and Taylor, G., 1985. Respiratory syncytial virus from cattle with a respiratory disease. Arch. Virol., 84: 1–52.

Stott, E.J., Thomas, L.H., Taylor, G., Collins, A.P., Jebbett, J. and Crouch, S., 1984. A comparison of three vaccines against respiratory syncytial virus in calves. J. Hyg. (Camb.), 93: 251–261.

Takahashi, E., Inaba, Y., Kurogi, H., Sato, K., Goto, Y. and Omori, T., 1975. Diagnosis of bovine respiratory syncytial virus infection by complement fixation test. Nat. Inst. Anim. Health Q., 15: 179–185.

Taylor G., Stott, E.J., Hughes, M. and Collins, A.P., 1984. Respiratory syncytial virus infection in mice. Infect. Immun., 43: 649–655.

Thomas, L.H. and Stott, E.J., 1981. Diagnosis of respiratory syncytial virus infection in the bovine respiratory tract by immunofluorescence. Vet. Rec., 108: 432–435.

Thomas, L.H., Stott, E.J., Jebbett, J. and Hamilton, S., 1976. The growth of respiratory syncytial virus in organ cultures of bovine foetal trachea. Arch. Virol., 52: 251–258.

Thomas, A.P., Stott, E.J., Collins, A.P., Crouch, S. and Jebbett, J., 1984. Infection of gnotobiotic calves with a bovine and human isolate of respiratory syncytial virus. Modification of the response by dexamethasone. Arch. Virol., 79: 67–77.

Van Bekkum, J.C., Holzhauer, C., Straver, P.J. and Van Nieuwstadt, A., 1977. Viral infections of the respiratory tract in cattle in the Netherlands. Bull. Off. Int. Epizoot., 88: 69–79.

Van Opdenbosch, E., Wellemans, G., Dekegel, D. and Strobbe, R., 1979. Neonatal calf diarrheoa: complex viral etiology. Vlaams Diergeneesk. Tijdschr., 48: 512–526.

Verhoeff, J. and Van Nieuwstadt, A., 1984. BRS-virus, PI3 virus and BHV 1 infections of young stock on self-contained dairy farms: epidemiological and clinical findings. Vet. Rec., 114: 288–293.

Walsh, E. and Hruska, J., 1983. Monoclonal antibodies to respiratory syncytial virus proteins: identification of the fusion protein. J. Virol., 47: 171–177.

Walsh, E., Schlesinger, J. and Brandiss, M., 1984. Protection from respiratory syncytial virus infection in cotton rats by passive transfer of monoclonal antibodies. Infect. Immun., 43: 756–758.

Walsh, E., Brandiss, M. and Schlesinger, J., 1985. Purification and characterization of the respiratory syncytial virus fusion protein. J. Gen. Virol., 66: 409–415.

Wellemans, G., 1977. Laboratory diagnosis methods for bovine respiratory syncytial virus. Vet. Sci. Commun., 1: 179–189.

Wellemans, G., 1982. Evaluation du programme de vaccination anti-virus respiratoire syncytial bovin en Belgique. Proceedings XII World Congress on Diseases of Cattle, Amsterdam, September 1982, Vol. 1, pp. 146–152.

Wellemans, G., Leunen, J. and Luchsinger, E., 1970. Isolement d'un virus (220/69) sérologiquement semblable au virus respiratoire syncytial (RS) humain. Ann. Méd. Vét., 114: 89–93.

Westenbrink, F., Kiman, T. and Brinkhof, J., 1989. Analysis of the antibody response to bovine respiratory syncytial virus protein in calves. J. Gen. Virol., 70: 591–601.

Zhdanov, V.M., Dreizin, R.S., Yankevici, O.D. and Astakhova, A.K., 1974. Biological characteristics of respiratory syncytial virus. Rev. Roum. Virol., 25: 277–280.

Zygraich, N., 1982. Vaccination against RS virus: a five year experience with Rispoval. Proceedings of XII World Congress on Diseases of Cattle, Amsterdam, September 1982, Vol. 1, pp. 189–195.

Zygraich, N. and Wellemans, G., 1981. Immunological markers of an attenuated bovine respiratory syncytial virus vaccine. Zentralbl. Veterinärmed. B., 28: 355–362.