Study of the antibody response in sheep to respiratory syncytial virus using an enzyme-linked immunosorbent assay and indirect fluorescent antibody tests

by

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GENERAL INTRODUCTION

Respiratory syncytial virus (RSV) has been recognized as an important respiratory tract pathogen of humans (1-4) and several animal species in natural and experimental infection (5-9).

Following RSV outbreaks, some investigators could easily demonstrate a significant 4-fold rise in serum antibody titers (10, 11). However, one study (12) reported no seroconversion in cattle with clinical disease.

Smith et al. (8) studied a limited number of ovine sera and found RSV antibodies. A serological study in Canada (13) indicated that not only cattle (14%) but also sheep (81%) and horses (6%) have complement fixing antibody titers to RSV. Recently RSV has been isolated from an adult sheep with mild rhinitis (14).

The objectives of this study were to 1) investigate the serum and secretory antibody response following RSV inoculation of lambs, 2) determine if reexposure to the same virus increases RSV antibody titers, and 3) compare the enzyme-linked immunosorbent assay (ELISA) results to the indirect fluorescent antibody test (IFA) results in sera and nasal secretions.

This thesis is presented in alternate format which includes one manuscript to be submitted to the American Journal of Veterinary Research in the style required. References cited in the manuscript are included with it in journal format. A general introduction, objectives of the study and a literature review precede the manuscript. Additional literature cited refers to citations in the general introduction and

literature review and is listed in the journal format. Acknowledgements follow the additional literature cited. The M.S. candidate, Fátima Esposti Cabanillas, was the principal investigator of the study and is the senior author of the manuscript. She also performed all of the experimental procedures which are reported in the manuscript with limited involvement of the co-authors.

LITERATURE REVIEW

Respiratory Syncytial Virus

Historical aspects In 1956, Morris, Blount and Savage (15) described the isolation of an agent suspected of causing respiratory disease in chimpanzees. Because of the production of coryza in these animals, they termed the virus: the chimpanzee coryza agent (CCA). This isolate was unrelated to other viruses of importance at that time.

In 1957, Chanock, Roizman and Myers (16) isolated two agents very similar to the CCA from infants with severe respiratory disease. The production of syncytia in the KB strains of human epidermoid carcinoma and human liver cells was characteristic of both the human isolates and the CCA.

Further studies using complement fixation and cross-neutralization tests showed a very close antigenic relationship between the human agents and CCA. Due to their antigenic similarities they were grouped together and given the name respiratory syncytial virus (RSV) because of their association with the respiratory tract and the production of syncytia in vitro (16).

In 1968, Doggett et al. (17) reported the presence of neutralizing antibody against the human RSV in bovine sera. They speculated that there was an antigenically similar virus present in cattle.

In 1970, Paccaud and Jacquier (11) reported the isolation of a virus from cattle during an outbreak of respiratory disease. This virus had a syncytia-forming effect in embryonic calf kidney (ECK) and embryonic calf lung (ECL) cell cultures and was non-hemagglutinating or hemadsorbing.

Cross-neutralization and complement-fixation tests confirmed a close antigenic relationship between this bovine isolate and human RSV. Subsequently, bovine respiratory syncytial virus (BRSV) has been isolated from cattle with respiratory disease in Japan (18), Belgium (19), England (20), and the United States (8) as well as many other countries. More recently, RSV has been isolated from goats (21) and sheep (14) with respiratory tract disease. Antibodies to RSV have been demonstrated in cats, dogs, horses, pigs and several non-human primates, but as yet virus has not been isolated from these species (13, 17, 22-24).

The RSVs are members of the genus Pneumovirus of the Paramyxoviridae family (25, 26). As indicated, the virus produces syncytia (multinucleate protoplasmic masses) both in cell culture and tissue of infected animals and these syncytia contain eosinophilic inclusions. The virions of RSV are highly pleomorphic and contain single-stranded RNA. The roughly spherical virions are 80 to 500 nm in diameter and the filamentous forms are 60 to 110 nm in diameter and may be up to 5 μm in length. The envelope contains surface projections that are 10 to 12 nm long and spaced 8 to 12 nm. The nucleocapsid symmetry is helical and is 12 to 15 nm in diameter. The virus is sensitive to lipid solvents, heat, and acid (pH 3.0). No neuraminidase or other

enzyme has been described for the Pneumovirus genus. Unlike the pneumonia virus of mice which is the other member of the genus, RSV does not contain hemagglutinin.

The most striking characteristic of RSV when it replicates in cell culture is the formation of syncytial masses. Syncytia usually have the nuclei clustered centrally. Cytoplasmic inclusions surrounded by a clear halo can be seen after staining with Giemsa's or hematoxylin-eosin (27, 28). Armstrong et al. (29) observed multinucleated cells as early as 24 hours after inoculation with the Long strain of human RSV in Hela cell culture and at 48 hours the syncytia were large and numerous. Jordan (30) studied the replication characteristics of RSV using various cell types and media and found that the virus capacity to produce syncytia was related to the composition of the medium and to the cell type used. Bennet and Hamre (27) and Norrby et al. (31) also reported the importance of the cell type used in the degree to which syncytia occur. No major nuclear change was reported in a study by Norrby et al. (31) but some cells had chromatin margination. Accumulation of glycogen and compact inclusions with a granular to "thread-like" composition was observed in the cytoplasm (31).

Armstrong et al. (29) using Hela cell cultures to study the inclusions of the ultrastructural level found the cytoplasmic inclusions to be of two types. The first one was small and paranuclear with a fine fibrillar matrix; the other was bigger, widely distributed in the cytoplasm and had a dense amorphous or granular matrix. Syncytia had both types of inclusions but mononuclear cells had only the fibrillar type.

Another characteristic of RSV is that it is extremely labile and this makes virus isolation from frozen samples difficult (32). Hambling (33) studied the stability of the virus using variable temperatures and serum concentrations and observed that using the lowest practical temperature (-65° C) and avoidance of refreezing resulted in the highest infectivity for a long period of time. Using more than 5 percent of serum did not result in improvement of virus survival time. It was also shown that it is possible to lose virus infectivity even at low temperatures. Virus stored at -65° C during three months had a 50 percent decrease in infectivity.

Clinical signs, pathogenesis and pathology Antibody to RSV is widespread in the human population (2, 34, 35). The virus is considered to be the major cause of bronchiolitis and pneumonia in infants and young children and is the leading cause of fatal respiratory tract disease in children less than a year old (9, 36). In older children and adults, the infection is primarily upper respiratory tract and produces symptoms similar to those of the common cold caused by other viruses. Reinfection is common in older children and adults with clinical response to subsequent infections less than that of the primary infection (37).

The mechanism by which RSV produces the most severe disease manifestations, which usually occur during the first few months of life, is unknown. An interesting feature is that serious disease from RSV infection occurs in these infants in the presence of circulating antibody, indicating that passive immunity is not protective (36, 38, 39).

The role of cell-mediated immunity in the pathogenesis of RSV infection is also unknown (9, 39-41). Several investigators believe that antibodies may be associated with allergic reactions in the lung and exacerbate the disease in infants and children (42-46). However, in a recent review by McIntosh and Fishant (39), they state that "no firm conclusions are possible about immunopathologic mechanisms."

The clinical and pathologic response of RSV infections in cattle has been extensively studied also. This has been through observations of natural infections and experimental infectivity studies. The isolation of RSV from cattle has been associated with mild to acute respiratory tract disease (6, 8, 10-12, 18-20, 47, 48). The disease in cattle is characterized by anorexia, depression, pyrexia, respiratory distress, cough, nasopharyngeal secretions and lacrimation. Cattle with fatal infections often show emphysema, edema and consolidation of the lungs. Experimental infectivity studies in cattle have not resulted in a response similar to that thought to occur naturally (5, 8, 19, 49-52) although studies by Bryson et al. (53) have come close. Smith et al. (8) and Wellemans and Leunen (54) described a more serious illness in those calves with circulating antibody to RSV prior to experimental infection. Whether or not there is an immune mediated component to RSV infection in cattle is speculative.

Experimental infectivity studies in other species indicated that ferrets, mink, chinchillas, guinea pigs, AKR mice, cotton rats and white-lipped marmosets were susceptible to infection with human RSV but did not develop clinical signs of disease (55-57). A rise in RSV

antibody titer in swine sera occurred with several disease problems suggesting that an as yet unisolated virus antigenically similar to RSV may be present and pathogenic for swine (24). Lambs can be infected experimentally with bovine RSV (58, 59) and recently RSV was isolated from an adult sheep with mild rhinitis (14). A RSV has been isolated from goats during an outbreak of acute respiratory tract disease (21). The significance of RSV in naturally occurring respiratory tract disease in sheep and goats has not been determined.

Serology Serologic means are usually used to diagnose RSV infection (32) because virus isolation is frequently unsuccessful (8, 12, 32, 54). Serologic diagnosis of RSV infection can be made by comparison of antibody titers of acute and convalescent serum samples. Tests used for the detection of RSV antibody in cattle, sheep, and goats include serum virus-neutralization (SVN) (6-8, 32), complement-fixation (CF) (60), immunodiffusion (ID) (61), indirect fluorescent antibody (IFA) (62), enzyme-linked immunosorbent assay (ELISA) (63), and indirect hemagglutination (IHA) (64).

While some form of the SVN test is the most frequently used for RSV serology, it is not without problems. The SVN test requires at least 5 days before the results can be read and it is difficult to determine an endpoint because complete neutralization of RSV is rarely achieved. The search for a more sensitive and reproducible SVN test for antibody to RSV resulted in the plaque-reduction SVN test (8, 65-70).

Indirect fluorescent antibody test is another test that has been extensively used to detect RSV antibody (28, 32, 38, 50, 62, 71). Potgieter and Aldridge found the IFA test to be simple to perform and more rapid and sensitive than the microtiter SVN test (62). In addition, they felt that it was as sensitive as the plaque-reduction SVN test.

The more recent development of an ELISA test for the detection of RSV antibodies shows promise (63, 72-74). Richardson et al. (73) found the ELISA test to be more sensitive, specific and reproducible than the CF and plaque-reduction SVN tests. Gillette (63) found that the ELISA test compared favorably with the plaque-reduction SVN and better than the CF test. The ELISA offers the advantage of being sensitive, rapid, and reproducible, and can be performed on minute amounts of serum (75, 76).

STUDY OF THE ANTIBODY RESPONSE IN SHEEP TO RESPIRATORY SYNCYTIAL VIRUS USING AN ENZYME-LINKED IMMUNOSORBENT ASSAY AND INDIRECT FLUORESCENT ANTIBODY TESTS

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Summary

The antibody response to respiratory syncytial virus (RSV) in lambs was followed using an enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) test. Two groups of isolation-reared lambs were exposed transtracheally and intranasally to a bovine isolate of RSV; one group of 5 lambs three times and the other group of 5 lambs one time. The antibody response in serum and nasal secretions was followed weekly for 22 weeks.

After the initial exposure to RSV, the geometric mean titer (GMT) of the serum antibody rose 32.5 and 8.4-fold when assayed by the ELISA and IFA tests respectively. With the second exposure, there was a 19.7-fold rise in the GMT by the ELISA test and a 4.9-fold rise in the GMT by the IFA test. The third exposure resulted in a 3-fold rise in GMT to RSV with both tests. The ELISA test gave an earlier 4-fold rise in GMT after the second exposure to RSV than did the IFA test.

After the initial inoculation, nasal secretions were negative for antibodies in both tests. The group that received second and third exposures became positive in both tests after the second exposure.

No clinical signs of illness were observed after RSV inoculation and virus was not isolated from the lambs.

Introduction

Both complement-fixing antibody and neutralizing antibody to respiratory syncytial virus (RSV), a known respiratory tract pathogen, have been found in sheep serum (1-3) and recently RSV was isolated from an adult sheep with mild rhinitis (4). These findings along with results of experimental infectivity studies using bovine strains of RSV indicate involvement of RSV in respiratory tract disease of sheep (5-7).

The objectives of this study were to investigate the serum and secretory antibody response after exposure to respiratory syncytial virus, to compare the sensitivity of the ELISA and IFA tests, and to quantify the levels of serum antibody response after single vs. multiple exposures to RSV.

Materials and Methods

Experimental design Ten 4-week-old lambs were used in this study. The animals were reared in isolation and divided into two groups at week 10 post-inoculation. Group 1 composed of 5 lambs was given one dose of inoculum intratracheally and intranasally at the beginning of the experiment (week 0). Group 2 was given three doses of inoculum intratracheally and intranasally at weeks 0, 10 and 16.

Sera and nasal secretions were collected at weekly intervals beginning the day of inoculation with RSV, continuing for a period of 22 weeks after inoculation. Antibody titers to RSV were determined using an ELISA technique and an IFA test.

Following each inoculation, lambs were observed daily for clinical signs of illness and their rectal temperatures recorded for 9 days. Nasal swabs were taken for viral isolation on the day of inoculation, then daily for 9 days.

Cell culture Ovine fetal turbinate (OFTU) cells were grown as described by Lehmkuhl and Cutlip (5). Briefly, cells were grown as monolayers in Eagle's minimal essential medium (EMEM) supplemented with 5% fetal calf serum, gentamicin sulfate (50 µg/ml) and L-glutamine. Cell cultures were incubated at 37° C in a 5% CO_2 atmosphere.

Virus Seventh laboratory passage of strain 375 of RSV which had been isolated from calves with acute respiratory tract disease was used (8).

Inoculum The virus was grown in OFTU cells until approximately 80% of the cells showed cytopathic effects (CPE). The cultures were then frozen and thawed. This tissue culture fluid was frozen at -70° C until needed for inoculation. Each lamb was inoculated with 15 ml of tissue culture fluid intratracheally and 5 ml intranasally. The inoculum contained 10^4 plaque-forming units per ml.

Specimens for serology Serum samples were prepared and nasal secretions were collected once a week from the day of inoculation through 22 weeks post-inoculation. Nasal secretions were collected with sterile cotton balls on hemostats which were inserted into the nostril and held there for approximately 2 minutes. The cotton balls were placed in sterile bottles containing 2 ml of cell-culture medium. The fluid was expressed from the cotton ball by using a 10 ml syringe and

the fluid was stored at -70° C. Before use, the nasal secretions were thawed and centrifuged at 8320 xg in a Microfuge 12[a] for 2 minutes and the supernatant was used in the ELISA and IFA tests.

ELISA antigen preparation Three different RSV antigens were used in preliminary ELISA tests; a crude antigen prepared directly from infected tissue culture, a semi-crude antigen obtained after precipitation with $(NH_4)_2SO_4$ and an antigen prepared as reported by Brůcková et al. (9).

ELISA test The method used was that described by Voller et al. (10) with the modifications of Gillette (11) for the preparation of buffers, substrate and solutions. The test was done in flat-bottom microELISA plates.[b] Optimal dilutions of conjugate[c] and viral antigen were determined according to the method of Snyder and Erickson (12). Diluent, negative serum, and weak and strong positive sera at 1:20 dilution were included in each plate. The strong positive serum had a titer by the plaque-reduction test $\geq$1,024; weak positive serum between 8 and 32; and negative serum <4. Correction for plate to plate variations was made by using the ratio of the average of all reference negative serum readings during the experiment and the reference negative serum reading of each plate.

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[a]Beckman Instruments, Inc., Palo Alto, CA.

[b]Immulon 2 plates, Dynatech Laboratories, Inc., Alexandria, VA.

[c]Peroxidase conjugated IgG Fraction Rabbit Anti-Sheep IgG (heavy and light chains), Cappel Laboratories, Cochranville, PA.

Duplicate samples of serial two-fold dilutions were made with serum samples starting with a 1:20 dilution. Nasal secretions were diluted 1:20. Plates were read on an ELISA reader[a] at λ ratio of 405/450 nm. Using the method of Hornsleth et al. (13), samples with absorbance values >0.112 were positive.

IFA test The method was modified from the procedure of Potgieter and Aldridge (14).

Strain 375 of bovine RSV was inoculated into OFTU cells and allowed to replicate until CPE was seen in approximately 50% of the monolayer. The cells were then trypsinized, resuspended in EMEM supplemented with 5% BFS, and dropped onto 8-well heavy teflon coated (HTC) microscope slides[b] using a 50 µl microtiter dropper. The slides were incubated in a humid CO_2 incubator until the cells attached to the surface, then air dried and fixed in cold acetone for 5 minutes. The slides were placed in slide boxes and stored at -70^{o} C until they were used.

Serial dilutions of serum samples starting from 1:2 were used to determine dilution extinction endpoints. Nasal secretions were tested without any further dilution than that following sampling. A commercial rabbit anti-sheep immunoglobulin G conjugated with fluorescein isothiocyanate[c] was used for antibody detection.

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[a]Dynatech MR-580, Dynatech Laboratories, Inc., Alexandria, VA.

[b]Cel-Line Associates, Inc., Newfield, NJ.

[c]Miles Laboratory, Inc., Research Division, Elkhart, IN.

Viral isolation Nasal secretions collected with cotton swabs were used for RSV isolation attempts. The procedure used was that described by Lehmkuhl and Cutlip (5) except OFTU cells were used.

Results

ELISA test Experiments to evaluate the ELISA antigens showed that neither crude antigen nor semi-crude antigen were appropriate for this study due to their high background reaction (Fig. 1). Antigen prepared using the method of Brůcková et al. (9) was the most suitable. This antigen (optimal dilution 1:400) showed a greater difference between the absorbance reading of the positive control serum and the negative control serum. It also gave a minimum background reaction as proved by the low absorbance reading corresponding to the diluent control (PBS).

Because the control antigen reacted very weakly with reference sera in several preliminary tests, all serum samples from week 5 were absorbed with OFTU cells and the absorbed and unabsorbed serum tested against the control antigen. No difference was seen in the absorbance readings indicating no participation of cellular antigens in the production of the humoral response.

During the standardization of the ELISA test, non-specific reactions were observed on several occasions. Those reactions were suspected to be due to the use of tape to cover the microElisa plates during the incubation period. Comparison studies between tape and parafilm confirmed that idea and showed that parafilm was the better

choice. Parafilm gave more consistent absorbance readings of ELISA controls and also minimum non-specific reactions.

Preinoculation sera collected at the beginning of the experiment had titers of 20 or 40 (GMT 32).

Following the first inoculation with RSV, Groups 1 and 2 were treated as a single group up to week 10. Extrapolating from the plot of the GMT of the serum antibody to RSV (Fig. 2), a four-fold rise in GMT was produced by day 10 postinoculation. After the second week, titers $\geq$160 were observed with the peak titers at week 7. There was a 32.5-fold rise in GMT between weeks 0 and 7. By the end of the first observation period, however, there was a slight decrease (2.3-fold between weeks 7 and 9) in the GMT. The serum antibody levels in Group 1 lambs remained relatively constant from week 10 through week 22. The GMT fluctuated from 422 to 735 and the titer range was from 320 to 1280.

The second inoculation of RSV in Group 2, 10 weeks after the first, resulted in a quicker response than that observed with the primary challenge. From the curve, a four-fold rise in GMT was reached by day 4 PI. One week after the second exposure, the titer of all 5 lambs was 5120. There was a 19.7-fold rise in GMT three weeks after the second exposure.

With the third inoculation of Group 2 with RSV at 16 weeks, the maximal GMT reached was 13,512. This occurred 2 weeks after the inoculation and was only a three-fold rise. There was a four-fold decrease in GMT between weeks 19 and 22.

Nasal secretions which were tested for RSV antibody only at 1:20 dilution were negative from the beginning of the study through week 10. Following the second inoculation of Group 2 with RSV, the nasal secretions became positive for RSV antibody and remained so through the 22 weeks of the study.

IFA test At week 0, the GMT to RSV by the IFA test was 10. As with the ELISA test, the lambs were treated as a single group up to week 10. There was a four-fold rise in GMT 11 days following the first inoculation of the lambs with RSV (Fig. 3). There was an 8.4-fold rise in the IFA GMT by week 3 postinoculation. The GMT of Groups 1 and 2 were relatively constant from week 3 through week 10 at which time Group 2 received the second inoculation of RSV. The response of Group 1 remained relatively constant at a GMT of 56 to 60 through the 22 weeks of the study. There was less than a two-fold decrease in GMT from week 3 to week 22.

The second inoculation of Group 2 resulted in a four-fold rise in GMT by day 7 PI. The maximum GMT (294) was observed 2 weeks after the second exposure. This was a 4.9-fold rise in IFA GMT. As with the first exposure, there was less than a two-fold decrease in GMT from week 12 to week 16.

Following the third inoculation of Group 2, the maximum rise in GMT was three-fold. This occurred week 18, 2 weeks after inoculation. The GMT at that time was 776. There was less than a two-fold decrease in GMT from week 18 to week 22.

The results of the IFA test for the nasal secretions were the same as with the ELISA test. The lambs of Group 1 were negative for RSV antibody during the 22 weeks of the study as where the lambs of Group 2 became positive following the second inoculation of RSV.

Virus isolation and clinical response Attempts to isolate RSV from all lambs were unsuccessful. The lambs did not develop clinical signs of infection following inoculation and the rectal temperature remained normal.

Discussion

The presence of low antibody titers to RSV previous to the first inoculation could be due to maternal antibodies passively transferred via colostrum to these lambs.

All lambs in the experiment developed an antibody response following RSV inoculation. Serum results with both testing procedures after first inoculation were similar in several aspects. First, both tests detected a four-fold rise in antibody titer at the same time. Second, both tests detected the stabilization of maximum antibody titers at the same time. Finally, the antibody response curves of both tests have the same shape. The difference between the results with both techniques was that ELISA titers rise were four-fold higher than those with IFA.

The IFA results in serum after first inoculation agree with those reported by El Azhary et al. (15).

Second and third exposures to RSV did result in an increase in antibody titers (that was monitored by both procedures) confirming the idea that sheep are susceptible to reinfection. This finding is in agreement with that of Mohanty et al. in cattle (16).

After each inoculation, antibody titers in serum were notably increased and then remained high as long as the experiment allowed. The same kind of response, but in calves, has been reported by El Azhary et al. (17).

Following second exposure of lambs to RSV, ELISA detected a four-fold rise in antibody titer earlier and had a mean rise in antibody titer four-fold higher than IFA. This observation, together with the fact that ELISA titers were higher than IFA titers after the first exposure, led to the conclusion that ELISA is more sensitive than IFA for detecting the serologic response of lambs to RSV. After the third exposure, both tests had identical results measuring antibody rise and time of maximum antibody stabilization may be because both tests were unable to detect minimal changes in antibody titers at such high dilutions. The high sensitivity of the ELISA has been previously reported (9, 18, 19). In addition, ELISA was found more efficient than IFA for detection of early RSV antibody.

In nasal secretions both testing procedures failed to detect an antibody response following a single exposure, perhaps due to the dilution of the samples or because the antigen dose was not enough to produce a primary response. Lehmkuhl and Cutlip (5) speculated that replication of RSV does not occur in the upper respiratory tract, thus

not stimulating a local immune response in the sheep following one exposure. On the other hand, second and third challenges gave increased antibody titers which were detected by both tests. These observations are compatible with those previously reported by Wellemans et al. (20).

As reported by Brůcková et al. (9), we also found that ELISA is able to detect long-persisting antibodies. In our study, the sensitivity of the method persisted up to 5 months following a single inoculation.

Another advantage of the ELISA as already reported by Steinhoff et al. (21) is that its results are numerical and that makes possible the use of statistical methods to determine the degree of positivity of the samples. Consequently, the results of the ELISA are more reliable and objective.

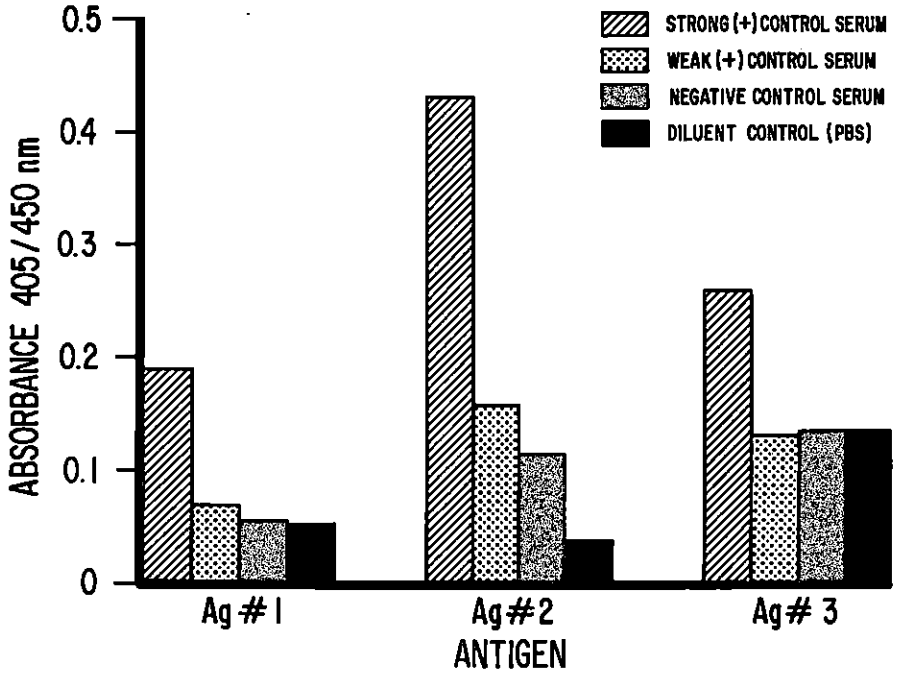
The indirect fluorescent antibody test was easier and faster to perform compared to the ELISA test. The IFA presents the following disadvantages: non-specific fluorescence and subjective results. Non-specific fluorescence can be reduced by dilution of the conjugate as previously reported (22). We used a 1:16 dilution of the conjugate that gave minimal background and high specific fluorescence.

Our failure to demonstrate a clinical illness following RSV inoculation is in agreement with a previous report (2) that described RSV seroconversions in lambs without any clinical signs.

Isolation attempts to recover RSV from nasal secretion were negative, confirming the great lability of the virus and the difficulty of its isolation (8, 23).

In summary, we have monitored a serum antibody response to both a single and multiple RSV inoculation and found increased antibody titers following second and third exposure. In nasal secretions we were able to detect antibody only after re-exposure to the virus. Both ELISA and IFA tests had a satisfactory performance with ELISA slightly more sensitive and efficient at detecting early and long-persisting antibodies to RSV infection.

Fig. 1. Comparison of absorbance values obtained for three different respiratory syncytial virus antigens. Ag #1 Crude antigen prepared directly from infected tissue culture fluid. Ag #2 Purified antigen prepared as reported by Brücková et al. (9) and Ag #3 semicrude antigen prepared after precipitation of Ag #1 with $(NH_4)_2\ SO_4$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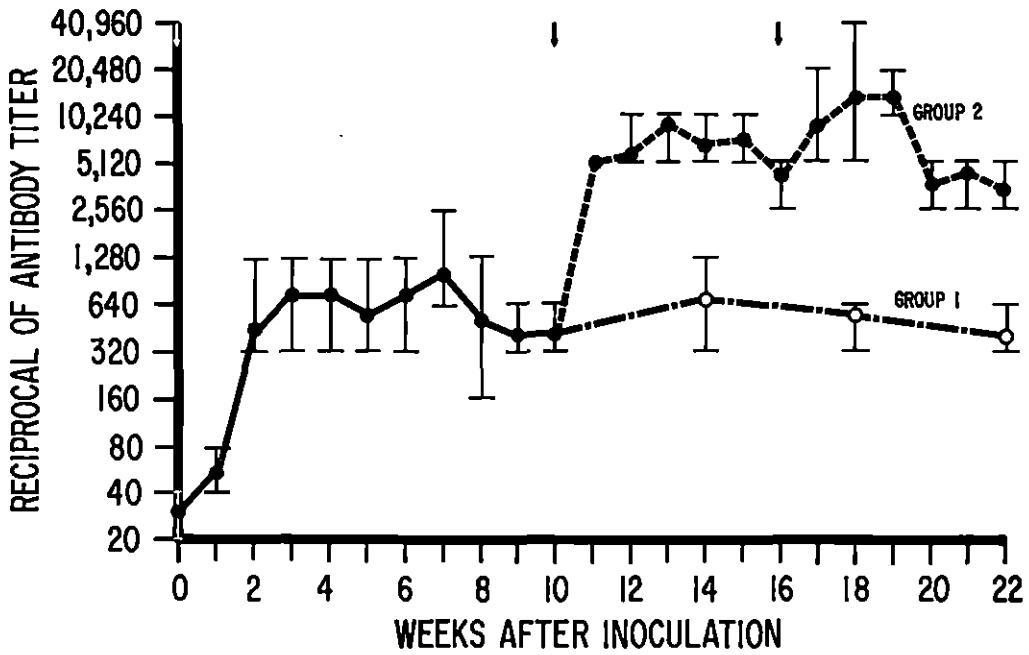
ABSORBANCE 405/450 nm
STRONG (+) CONTROL SERUM
WEAK (+) CONTROL SERUM
NEGATIVE CONTROL SERUM
DILUENT CONTROL (PBS)
Ag # 1
Ag # 2
Ag # 3
ANTIGEN

Fig. 2. Geometric mean and range of serum antibody titer to respiratory syncytial virus in two groups of lambs as determined by an ELISA test. White arrow indicates inoculation of Groups 1 (N=5) and 2 (N=5). Black arrows indicate further inoculation of Group 2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14
16
18
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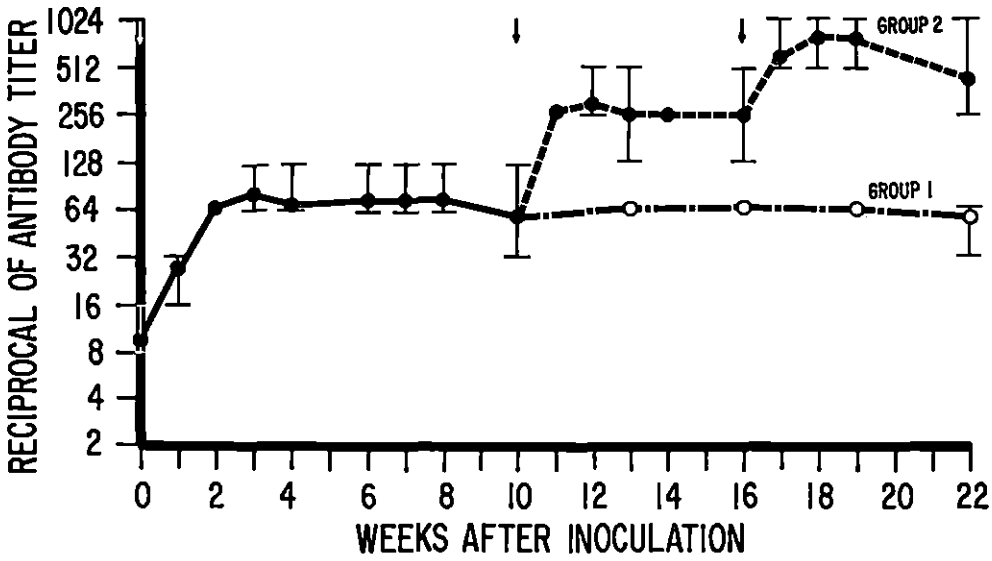
22
WEEKS AFTER INOCULATION
GROUP 2
GROUP 1

Fig. 3. Geometric mean and range of serum antibody titer to respiratory syncytial virus in two groups of lambs as determined by an IFA test. White arrow indicates inoculation of Groups 1 (N=5) and 2 (N=5). Black arrows indicate further inoculation of Group 2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22
WEEKS AFTER INOCULATION
GROUP 2
GROUP 1

References

1. Berthiaume L, Joncas J, Boulay G, Pavilanis V: Serologic evidence of respiratory syncytial virus infection in sheep. Vet Rec 89:337-338, 1973.

2. Woods GT: Bovine parvovirus 1, bovine syncytial virus, and bovine respiratory syncytial virus and their infections. Adv Vet Sci Comp Med 18:273-286, 1974.

3. Smith ML, Frey ML, Dierks RE: Isolation, characterization and pathogenicity studies of a bovine respiratory syncytial virus. Arch Virol 47:237-247, 1975.

4. LeaMaster BR, Everman JF, Mueller GM, Prieur MK, VanderShalie J: Serologic and virologic studies on naturally occurring respiratory syncytial virus and Haemophilus somnus infections in sheep. Proc Ann Am Assoc Vet Lab Diagn 26:265-276, 1983.

5. Lehmkuhl HD, Cutlip RC: Experimentally induced respiratory syncytial viral infection in lambs. Am J Vet Res 40:512-514, 1979.

6. Cutlip RC, Lehmkuhl HD: Lesions in lambs experimentally infected with bovine respiratory syncytial virus. Am J Vet Res 40:1479-1482, 1979.

7. Lehmkuhl HD, Cutlip RC: Experimental respiratory syncytial virus infection in feeder-age lambs. Am J Vet Res 40:1729-1730, 1979.

8. Lehmkuhl HD, Gough PM, Reed DE: Characterization and identification of a bovine respiratory syncytial virus isolated from young calves. Am J Vet Res 40:124-126, 1979.

9. Brůcková M, Svandová E, Syrůcek L: Detection of respiratory syncytial virus serum antibodies by an ELISA system. Acta Virol 25:41-48, 1981.

10. Voller A, Bidwell DE, Barllett A: Enzyme immunoassays in diagnostic medicine. Theory and practice. Bull Wld Hlth Org 53:55-65, 1976.

11. Gillette KG: Enzyme-linked immunosorbent assay for serum antibody to bovine respiratory syncytial virus: Comparison with complement fixation and neutralization tests. Am J Vet Res 44:2251-2255, 1983.

12. Snyder ML, Erickson GA: Recommended minimum standards for an enzyme-linked immunosorbent assay (ELISA) in pseudorabies serodiagnosis. From the National Veterinary Services Laboratories, USDA, APHIS, P. O. Box 844, Ames, IA 50010, 1981.

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

14. Potgieter LND, Aldridge PL: Use of the indirect fluorescent antibody test in the detection of bovine respiratory syncytial virus antibodies in bovine serum. Am J Vet Res 28:1341-1343, 1977.

15. El Azhary MASY, Galina M, Roy RS, Fontaine M, Lamothe P: Experimental infection of calves with bovine respiratory syncytial virus Quebec strain. Can J Comp Med 44:351-355, 1980.

16. Mohanty SB, Lillie MG, Ingling AL: Effect of serum and nasal neutralizing antibodies on bovine respiratory syncytial virus infection in calves. J Infect Dis 134:409-413, 1976.

17. El Azhary MASY, Silim A, Roy RS: Interferon, fluorescent antibody and neutralizing antibody responses in sera of calves inoculated with bovine respiratory syncytial virus. Am J Vet Res 42:1378-1382, 1981.

18. Richardson LS, Jolken RH, Belshe RB, Camargo E, Kim HW, Chanock RM: Enzyme-linked immunosorbent assay for measurement of serological response to respiratory syncytial virus infection. Infect Immun 20:660-664, 1978.

19. Engvall E, Perlmann P: Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J Immunol 109:129-135, 1972.

20. Wellemans G, Van Opdenbosch E, Boucque CV, Leunen J, Stobbe R: Vaccination des bovins contre le virus respiratoire syncytial (RSB) au moyen d'une souche atténuée. A. Epreuves d'inocuité et résultats sérologiques. Ann Med Vet 122:527-535, 1978.

21. Steinhoff MC, Hall CB, Schnabel K: Respiratory syncytial virus serology by a simplified enzyme-linked immunosorbent assay. J Clin Microbiol 12:447-450, 1980.

22. Clark HW, Bailey JS, Fowler RC, Brown TM: Identification of Mycoplasmataceae by the fluorescent antibody method. J Bacteriol 85:111-118, 1963.

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

24. Stott EJ, Thomas LH, Collins AP, Crouch S, Jebbett J, Smith GS, Luther PD, Caswell R: A survey of virus infections of the respiratory tract of cattle and their association with disease. J Hyg 85:257-270, 1980.

ADDITIONAL REFERENCES CITED

1. Bruhn FW, Yeager AS: Respiratory syncytial virus in early infancy (circulating antibody and the severity of disease). Am J Dis Child 131:145-148, 1977.

2. Chanock R, Finberg L: Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II. Epidemiologic aspects of infection in infants and young children. Am J Hyg 66:291-300, 1957.

3. Chanock RM, Kapikian AZ, Mills J, Kim HW, Parrott RH: Influence of immunological factors in respiratory syncytial virus disease. Arch Environ Health 21:347-355, 1970.

4. Chanock RM, Kim HW, Vargosko AJ, Deleva A, Johnson K, Cumming C, Parrott RH: Respiratory syncytial virus. I. Virus recovery and other observations during 1960 outbreak of bronchioloitis, pneumonia, and minor respiratory diseases in children. J Am Med Assoc 176:647-653, 1961.

5. Mohanty SB, Ingling AL, Lillie MG: Experimentally induced respiratory syncytial virus infection in calves. J Infect Dis 134:409-413, 1976.

6. Rosenquist BD: Isolation of respiratory syncytial virus from calves with acute respiratory disease. J Infect Dis 130(2):177-182, 1974.

7. Rossi CR, Kiesel GK: Serological evidence for the association of bovine respiratory syncytial virus with respiratory tract disease in Alabama cattle. Infect Immun 10:293-298, 1974.

8. Smith MH, Frey ML, Dierks RE: Isolation, characterization, and pathogenicity studies of a bovine respiratory syncytial virus. Arch Virol 47:237-247, 1975.

9. Tyeryar FJ Jr., Richardson LS, Belshe RB: Report of a workshop on respiratory syncytial virus and parainfluenza viruses. J Infect Dis 137:835-846, 1978.

10. Inaba Y, Tanaka Y, Sato K, Omori T, Matumoto M: Bovine respiratory syncytial virus: Studies on an outbreak in Japan 1968-1969. Jpn J Microbiol 16(5):373-383, 1972.

11. Paccaud MF, Jacquier C: A respiratory syncytial virus of bovine origin. Arch gesamte Virusforsch 30:327-342, 1970.

12. Lehmkuhl HD, Gough PM, Reed DE: Characterization and identification of a bovine respiratory syncytial virus isolated from young calves. Am J Vet Res 40:124-126, 1979.

13. Berthiaume L, Joncas J, Boulay G, Pavilanis V: Serological evidence of respiratory syncytial virus infection in sheep. Vet Rec 89:337-338, 1973.

14. LeaMaster BR, Evermann JF, Mueller GM, Prieur MK, VanderSchalie J: Serologic and virologic studies on naturally occurring respiratory syncytial virus and Haemophilis somnus infections in sheep. Proc Annu Meet Am Assoc Vet Lab Diagn 26:265-276, 1983.

15. Morris AJ, Blount RE, Savage RE: Recovery of cytopathogenic agent from chimpanzee with coryza. Proc Soc Exp Biol Med 92:544-549, 1956.

16. Chanock RM, Roizman B, Myers R: Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation, properties and characterization. Am J Hyg 66:281-290, 1957.

17. Doggett JE, Taylor-Robinson D, Gallop RGC: A study of a inhibitor in bovine serum active against respiratory syncytical virus. Arch gesamte Virusforsch 23:126-137, 1968.

18. Inaba Y, Tanaka T, Omori T, Matumoto M: Isolation of bovine respiratory syncytial virus. Jpn J Exp Med 40:473-474, 1970.

19. Wellemans G, Leunen J, Louchsenger E: Isolement d'un virus (220169) serologiquement serublable au virus respiratoire syncytial humain. Ann Med Vet 114:89-93, 1970.

20. Jacobs JW, Edington N: Isolation of respiratory syncytial virus from cattle in Britain. Vet Rec 88:694, 1971.

21. Smith MH, Lehmkuhl HD, Phillips SM: Isolation and characterization of a respiratory syncytial virus from goats. Proc Annu Meet Am Assoc Vet Lab Diagn 22:259-268, 1979.

22. Pringle CR, Cross A: Neutralization of respiratory syncytial virus by cat serum. Nature 270:501-502, 1978.

23. Wyatt LSR, Belshe RB, London WT, Lewis Sly D, Camargo E, Chanock RM: Respiratory syncytial virus antibodies in nonhuman primates and domestic animals. Lab An Sci 31:413-415, 1981.

24. Woods GT: Bovine parvovirus 1, bovine syncytial virus and bovine respiratory syncytial virus and their infections. Adv Vet Sci Comp Med 18:273-286, 1974.

25. Kingsbury DW, Bratt MA, Choppin PW, Hanson RP, Hosaka Y, ter Muelen V, Norrby E, Plowright W, Rott R, Wunner WH: Paramyxoviridae. Intervirology 10:137-152, 1978.

26. Melnick JL: Taxonomy of viruses. Progr Med Virol 26:214-232, 1980.

27. Bennet RC, Hamre D: Growth and serological characteristics of respiratory syncytial virus. J Infect Dis 110:8-16, 1962.

28. Kisch AL, Johnson KM, Chanock RM: Immunofluorescence with respiratory syncytial virus. Virology 16:177-189, 1962.

29. Armstrong JA, Pereira HG, Valentine RC: Morphology and development of respiratory syncytial virus in cell cultures. Nature 169:1179-1181, 1962.

30. Jordan WS: Growth characteristics of respiratory syncytial virus. J Immunol 88:581-590, 1962.

31. Norrby E, Marusyk H, Orvell C: Morphogenesis of respiratory syncytial virus in green monkey kidney cell line (Vero). J Virol 6:237-242, 1970.

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

33. Hambling MH: Survival of the respiratory syncytial virus during storage under various conditions. Br J Exp Pathol 45:647-655, 1964.

34. Adams MO, Thompson KM, Tobin JO'H: Respiratory syncytial virus infection in the North West of England. Arch gesamte Virusforsch 13:268-271, 1963.

35. Hambling MH: A survey of antibodies to respiratory syncytial virus in the population. Br Med J 1:1223-1225, 1964.

36. Chanock RM, Kim HW, Brandt CD, Parrot RH: Respiratory syncytial virus, in Evans AS (ed): Viral Infections of Humans. New York and London, Plenum Medical Book Company, 1982, pp 479-489.

37. Johnson KM, Bloom HH, Mufson MA, Chanock RM: Natural reinfection of adults by respiratory syncytial virus. Possible relation to mild upper respiratory disease. New Eng J Med 267:68-72, 1962.

38. McIntosh K, Masters HB, Orr I, Chao RK, Barkin RM: The immunologic response to infection with respiratory syncytial virus in infants. J Infect Dis 138:24-32, 1978.

39. McIntosh K, Fishant JM: Immunopathologic mechanism in lower respiratory tract disease of infants due to respiratory syncytial virus. Prog Med Virol 26:94-118, 1980.

40. Kim HW, Leiken SL, Anobio JO, Brand CD, Chanock RM, Parrot RH: Cell mediated immunity to respiratory syncytial virus induced by inactivated vaccine or infection. Pediatr Res 10:75-78, 1976.

41. Tyeryar FJ: Report of a workshop on respiratory syncytial virus and parainfluenza viruses. J Infect Dis 148:588-598, 1983.

42. Chin J, Magoffin RL, Shearer LA, Schieble JH, Lennette ED: Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. Am J Epidemiol 89:449-463, 1969.

43. Craighead JE: Disease accentuation after immunization with inactivated microbial vaccines. J Infect Dis 131:749-754, 1975.

44. Fulginiti VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn JW: Respiratory virus immunization. I. A field trial of two inactivated respiratory virus vaccines; An aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. Am J Epidemiol 89:435-448, 1969.

45. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff A, Stewart CE: An epidemiological study of altered clinical reactivity to respiratory syncytial virus infection in children previously vaccinated with an inactivated RS virus vaccine. Am J Epidemiol 89:405-421, 1969.

46. Kim HW, Canchola JG, Brandt CD, Pylese G, Chanock RM, Jensen K, Parrott RH: Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am J Epidemiol 89:422-434, 1969.

47. Odegaard CA, Krogsrud J: A field outbreak caused by bovine respiratory syncytial virus. Acta Vet Scand 18:429-431, 1977.

48. Bryson DG, McFerran JB, Ball HJ: Observations on outbreaks of respiratory disease in calves associated with parainfluenza type 3 virus and respiratory syncytial virus infection. Vet Rec 104:45-49, 1979.

49. El Azhary MASY, Galina M, Roy RS, Fontaine M, Lamothe P: Experimental infection of calves with bovine respiratory syncytial virus (Quebec strain). Can J Comp Med 44:351-355, 1980.

50. El Azhary MASY, Silim A, Roy RS: Interferon, fluorescent antibody, and neutralizing antibody responses in sera of calves inoculated with bovine respiratory syncytial virus. Am J Vet Res 42:1378-1382, 1981.

51. Jacobs JW, Edington N: Experimental infection of calves with respiratory syncytial virus. Res Vet Sci 18:299-306, 1975.

52. Moteane M, Babrick LA, Schiefer B: Studies on occurrence and significance of bovine respiratory syncytial virus in Saskatchewan. Can J Comp Med 42:246-248, 1978.

53. Bryson DG, McNulty MS, Logan EF, Cush PF, FIMLS: Respiratory syncytial virus pneumonia in young calves: Clinical and pathologic findings. Am J Vet Res 44:1648-1655, 1983.

54. Wellemans G, Leunen J: The respiratory syncytial virus and the respiratory troubles of cattle. Ann Med Vet 119:359-369, 1975.

55. Coates HV, Chanock RM: Experimental infection with respiratory syncytial virus in several species of animals. Am J Hyg 76:302-312, 1962.

56. Prince GA, Jenson AB, Horswood RL, Camargo E, Chanock RM: The pathogenesis of respiratory syncytial virus infection in cotton rats. Am J Pathol 93:771-791, 1978.

57. Prince GA, Porter DD: The pathogenesis of respiratory syncytial virus infection in infant ferrets. Am J Pathol 82:339-350, 1976.

58. Lehmkuhl HD, Cutlip RC: Experimentally induced respiratory syncytial viral infection in lambs. Am J Vet Res 40:512-514, 1979.

59. Lehmkuhl HD, Cutlip RC: Experimental respiratory syncytial virus infection in feeder-age lambs. Am J Vet Res 40:1729-1730, 1979.

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

61. Zygraich N, Wellemans G: Immunologic markers of an attenuated bovine respiratory syncytial vaccine. Zbl Vet Med B 28:355-362, 1981.

62. Potgieter LND, Aldridge PL: Use of the indirect fluorescent antibody test in detection of bovine respiratory syncytial virus antibodies in bovine serum. Am J Vet Res 38:1341-1344, 1977.

63. Gillette KG: Enzyme-linked immunosorbent assay for serum antibody to bovine respiratory syncytial virus: Comparison with complement-fixation and neutralization tests. Am J Vet Res 44:2251-2255, 1983.

64. Martin HT: Indirect hemagglutination test for the detection and assay of antibody to bovine respiratory syncytial virus. Vet Rec 113:290-293, 1983.

65. Coates HV, Alling DW, Chanock RM: An antigenic analysis of respiratory syncytial virus isolates by a plaque reduction neutralization test. Am J Epid 83:299-313, 1966.

66. Hauthorne JD, Albrecht P: Sensitive plaque neutralization assay for parainfluenza virus types 1, 2 and 3 and respiratory syncytial virus. J Clin Microbiol 13:730-737, 1981.

67. Kim HW, Anobio JO, Pyles G, Brandt CD, Camargo E, Chanock RM, Parrott RH: Clinical and immunological response of infants and children to administration of low-temperature adapted respiratory syncytial virus. Pediatrics 48:745-755, 1971.

68. Kisch AL, Johnson KM: A plaque assay for respiratory syncytial virus. Proc Soc Exp Biol Med 112:583-589, 1963.

69. Taylor-Robinson D, Doggett JE: An assay method for respiratory syncytial virus. Br J Exp Pathol 44:473-480, 1963.

70. Trépanier P, Payment P, Trudel M: A simple and rapid microassay for the titration of human respiratory syncytial virus. J Virol Meth 1:343-347, 1980.

71. Gardner PS, McQuillin J: Rapid virus diagnosis: application of immunofluorescence. Butterworth, London, p. 103-117, 1974.

72. Brůcková M, Svandová E, Syrůcek L: Detection of respiratory syncytial virus serum antibodies by an ELISA system. Acta Virol 25:41-48, 1981.

73. Richardson LS, Yolken R, Belshe R, Camargo E, Kim H, Chanock R: Enzyme-linked immunosorbent assay for measurement of serological response to respiratory syncytial virus infection. Infect Immun 30:660-664, 1978.

74. Steinhoff MC, Hall CB, Schnabel KC: Respiratory syncytial virus serology by a simplified enzyme-linked immunosorbent assay. J Clin Microbiol 12:447-450, 1980.

75. Leinikki P, Prsiila S: Quantitative, semiautomated enzyme-linked immunosorbent assay for viral antibodies. J Infect Dis 136 (suppl.):5294-5299, 1977.

76. Voller A, Bidwell D, Bartlett A: Microplate enzyme immunoassays for the immunodiagnosis of virus infections, in Rose NR, Friedman H (ed): Manual of clinical immunology. Washington, DC, American Soc. Microbiol. 1976, pp 506-512.

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