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Bibliography

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ACTINOBACILLUS PLEUROPNEUMONIAE POLYSACCHARIDES

Atypical *Actinobacillus pleuropneumoniae* isolates that share antigenic determinants with both serotypes 1 and 7

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Journal of Veterinary Diagnostic Investigation, 2000, Vol. 12, No. 5, pp. 444–449.

The characterization of 3 atypical isolates of *A. pleuropneumoniae*, isolated from pigs in Quebec, Canada, in 1997, is presented. Two isolates (1B and 27E) showed positive reactions in coagglutination, immunodiffusion, and indirect haemagglutination tests for serotypes 1 and 7, whereas the third isolate (26B) reacted with antisera to serotypes 1, 4, and 7. These atypical isolates of *A. pleuropneumoniae* possessed a capsular polysaccharide (CPS) antigenically related to serotype 1 as well as an O-chain lipopolysaccharide antigenically related to serotype 7 or to serotypes 4 and 7, as shown by the use of monoclonal antibodies. Results of toxin profile and virulence assays for mice and pigs showed them to be more related to *A. pleuropneumoniae* serotype 7 field isolates. All 3 isolates induced antibodies mainly against serotype 7/4 O-long-chain lipopolysaccharide (LC-LPS) and, to a lesser extent, to the CPS of serotype 1, in experimentally infected pigs. Diagnostic laboratories that use a LC-LPS-based ELISA for serodiagnosis of *A. pleuropneumoniae* infection in pigs would probably diagnose herds infected with these atypical isolates as being infected by *A. pleuropneumoniae* serotypes 7 or 4, whereas those that use a CPS-based ELISA would probably consider them as infected by *A. pleuropneumoniae* serotype 1.

Identification of *Actinobacillus pleuropneumoniae* strains of serotypes 7 and 4 using monoclonal antibodies: demonstration of common LPS O-chain epitopes with *Actinobacillus lignieresii*

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Veterinary Microbiology, 1999, Vol. 65, No. 4, pp. 271–282.

Of 3 monoclonal antibodies (Mabs) directed against surface polysaccharides of *A. pleuropneumoniae* serotype 7 one recognized a capsular polysaccharide epitope (CPS) of *A. pleuropneumoniae* serotype 7, whereas the 2 other Mabs reacted with different epitopes of the LPS O-chain. One of the latter reacted with the reference strain of serotype 7 and the other one with serotypes 7 and 4. The 3 Mabs were used to test, by Dot-ELISA, 508 field strains of *A. pleuropneumoniae*. None of the strains belonging to other serotypes different from serotypes 4 and 7 were positive with the Mabs. Used in combination, the CPS and one of the LPS O-chain directed Mabs were suitable for serotyping detecting 100% of serotype 7 strains. It was confirmed for the first time that *A. pleuropneumoniae* serotype 4 is present in North America. Both O-chain specific Mabs also reacted with the O-chain of *Actinobacillus lignieresii*. The cross-reactivity between the species was confirmed using serum samples from pigs experimentally infected with *A. pleuropneumoniae* serotype 7 and *A. lignieresii*, by immunoblotting and ELISA. This is the first report of a specific cross-reactivity between the LPS of these bacterial species.

Biological activities of lipopolysaccharides extracted from porcine vaccine strains

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Journal of Veterinary Medical Science, 1999, Vol. 61, No. 12, pp. 1265–1269.

Lipopolysaccharides (LPSs) were purified from *Actinobacillus pleuropneumoniae* serotype 2, *Bordetella bronchiseptica* and *Haemophilus parasuis* serotype 5 in Japan. LPSs were used for vaccine production using a phenol-water procedure. In SDS-PAGE analysis, *A. pleuropneumoniae* LPS and *Escherichia coli* LPS demonstrated a typical ladder profile of a smooth-type LPS. *B. bronchiseptica* and *H. parasuis* LPSs lacked the ladder profiles. The biological activities of these LPSs were comparable to those of *E. coli* LPS in terms of activation of the clotting enzyme of *Limulus* amoebocyte lysate, mitogenic activity of mouse spleen cells and stimulation of TNF-alpha and nitric oxide production, but stimulation of IL-6 production was rare.

Cloning and mutagenesis of a serotype-specific DNA region involved in encapsulation and virulence of *Actinobacillus pleuropneumoniae* serotype 5a: concomitant expression of serotype 5a and 1 capsular polysaccharides in recombinant *A. pleuropneumoniae* serotype 1.

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Infection and Immunity, 1998, Vol. 66, No. 7, pp. 3326–3336.

A DNA region involved in *A. pleuropneumoniae* serotype 5 capsular polysaccharide (CP) biosynthesis was identified and characterized using a probe specific for the cpxD gene involved in CP export. The adjacent serotype 5-specific CP biosynthesis region was cloned from a 5.8-kb BamHI fragment and an 8.0-kb EcoRI fragment of strain J45 genomic DNA. DNA sequence analysis demonstrated that this region contained 4 complete open reading frames (ORFs), cps5A, cps5B, cps5C, and cps5D. Cps5A, Cps5B, and Cps5C showed low homology with several bacterial glycosyltransferases involved in the biosynthesis of lipopolysaccharide or CP. However, Cps5D had high homology with KdsA proteins (3-deoxy-D-manno-2-octulosonic acid 8-phosphate synthetase) from other gram-negative bacteria. The G+C content of cps5ABC was lower (28%) than that of cps5D and the rest of the *A. pleuropneumoniae* chromosome (42%). A 2.1-kb deletion spanning the cloned cps5ABC ORFs was constructed and transferred into the J45 chromosome by homologous recombination with a kanamycin resistance cassette to produce mutant J45–100. Multiplex PCR confirmed the deletion in this region of J45–100 DNA. J45–100 did not produce intracellular or extracellular CP, indicating that cps5A, cps5B, and/or cps5C were involved in CP biosynthesis. However, biosynthesis of the Apx toxins, lipopolysaccharide, and membrane proteins was unaffected by the mutation. J45–100 grew faster than J45, was sensitive to killing in precolostral calf serum, and was avirulent in pigs at an intratracheal challenge dose 3 times the 50% lethal dose (LD50) of strain J45. At 6 times the J45 LD50, J45–100 caused mild to moderate lung lesions but not death. Electroporation of cps5ABC into *A. pleuropneumoniae* serotype 1 strain 4074 generated strain 4074(pJMLCPS5), which expressed both serotype 1 and serotype 5 CP. Serotype 1 capsule expression was diminished in 4074(pJMLCPS5) in comparison to 4074. The recombinant strain produced significantly less total CP (serotypes 1 and 5 CP combined) in log phase but significantly more total CP in late stationary phase compared with 4074. Strain 4074(pJMLCPS5) caused less mortality and bacteraemia in pigs and mice following respiratory challenge than strain 4074, indicating that virulence was affected by diminished capsule production. These results emphasize the importance of CP in the serum resistance and virulence of *A. pleuropneumoniae*.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS AND PULMONARY MACROPHAGES

Pathogenesis of porcine reproductive and respiratory syndrome virus-induced increase in susceptibility to *Streptococcus suis* infection

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Veterinary Pathology, 2000, Vol. 37, No. 2, pp. 143–152.

Eighty 3-week-old crossbred pigs were randomly assigned to 6 groups (13–14 pigs/group). Group 1 pigs served as uninoculated controls, group 2 pigs were inoculated intranasally (IN) with *S. suis* serotype 2, group 3 pigs were inoculated IN with a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine, group 4 pigs were inoculated IN with the same vaccine and with *S. suis*, group 5 pigs were inoculated IN with VR-2385 (a high-virulence strain of PRRSV) and group 6 pigs were inoculated IN with VR-2385 and *S. suis*. Pigs exposed to both PRRSV and *S. suis* were inoculated with PRRSV 7 days before *S. suis* inoculation. The pigs were 26 days old

when inoculated with *S. suis*. Respiratory disease was significantly more severe in groups 5 and 6. Mortality rate was highest in group 6 (87.5%). This rate was significantly higher than that observed in all other groups except group 4 (37.5%). The mortality rate in group 2, inoculated with *S. suis* alone, was 14.3%. No pigs from groups 1, 3, or 5 died before the scheduled necropsies at 10 and 28 days postinoculation with PRRSV (DPI). To study the effect of PRRSV and/or *S. suis* on pulmonary clearance by pulmonary intravascular macrophages, 6 pigs from each group were intravenously infused with 3% copper phthalocyanine tetrasulfonic acid in saline before necropsy at 10 DPI. Mean copper levels in the lungs of pigs in groups 2, 5 and 6 were significantly lower than those in control pigs. The mean percentage of lung tissue grossly affected by pneumonia at 10 DPI was 0, 1, 0, 3, 64 and 62% for groups 1–6, respectively. Both gross and microscopic interstitial pneumonia lesions were significantly more severe in the VR2385-inoculated groups (5 and 6). PRRSV was isolated from bronchoalveolar lavage fluid collected at necropsy from 100% of the pigs in groups 5 and 6, 71.4% of pigs in group 4, 38.5% of pigs in group 3, and none of the pigs in groups 1 or 2. *S. suis* serotype 2 was cultured from the internal tissues of 7.7, 28.6 and 78.6% of the pigs in groups 2, 4 and 6, respectively. *S. suis* serotype 2 was isolated from whole blood at necropsy from 7.7, 35.7 and 78.6% of pigs in groups 2, 4 and 6, respectively. Significantly more pigs in group 6 had *S. suis* isolated from whole blood and internal tissues. It is concluded that both high-virulence PRRSV and *S. suis* decreased copper clearance, and the incidence of isolation of *S. suis* and PRRSV was higher in dually inoculated pigs. It is suggested that PRRSV-induced suppression of pulmonary intravascular macrophage function may in part explain PRRSV-associated increased susceptibility to *S. suis* infection.

Effects of porcine reproductive and respiratory syndrome virus (isolate tw91) on porcine alveolar macrophages *in vitro*

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Veterinary Microbiology, 2000, Vol. 71, No. 1/2, pp. 9–25.

To verify the role of porcine reproductive and respiratory syndrome virus (PRRSV) infection on pulmonary defence mechanisms, alterations in the viability, morphology and various functions of porcine alveolar macrophages (AMs) were evaluated *in vitro* for 2–72 h after exposure to a Taiwan isolate, tw91, at a multiplicity of infection (MOI) of 0.1. A low but constant rate of infection, around 5%, was seen in AMs from the PRRSV-infected group throughout the study. When compared with a mock-infected group, AMs from the PRRSV-infected group had a significantly lower viability at 18–72 h post-infection (PI) as determined by trypan blue dye exclusion. Also during this time period, the cells showed morphological changes, including rounding, bleb formation and rupture. The phagocytic and microbicidal capacity of AMs against *Candida albicans* was significantly inhibited at 6 h PI. Although the total amount of superoxide anion and hydrogen peroxide produced by the AMs was reduced after 18 and 12 h PI, respectively, the amount of production was enhanced in both reactive oxygen species on a per viable cell basis at 12 h PI. In contrast, the level of bioactive tumour necrosis factor alpha secretion, either total or on a per viable cell basis, was reduced soon after PRRSV infection, up to 36 h PI, followed by a rebound thereafter. Prostaglandin E2 production was enhanced, both in total and on a per viable cell basis, in the first 6 h of infection, especially at 2 h PI. However, it became lower than that of the control at 36 h PI. It is concluded that PRRSV infection could cause, directly and/or indirectly, not only death of AMs but also adverse alterations in their morphology and function, although some of the effects seemed to be reversible. Because AMs are crucial to the host against airborne pathogens, PRRSV infection may potentially predispose pigs to secondary pulmonary infections.

Preliminary characterization of protein binding factor for porcine reproductive and respiratory syndrome virus on the surface of permissive and non-permissive cells

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Archives of Virology, 2000, Vol. 145, No. 6, pp. 1099–1116.

In its natural host, porcine reproductive and respiratory syndrome virus (PRRSV) has been reported to have a restricted tropism for cells of the monocyte/macrophage lineage. To date, cloned monkey kidney cell lines, such as MARC-145 and CL2621 cells which have been established from MA-104 cells, are the only non-porcine cells known to support PRRSV replication. A binding assay was set up to follow by flow cytometry the attachment of PRRSV on the surface of porcine and non-porcine cells. PRRSV was able to bind permissive cells like porcine alveolar macrophages and MARC-145. Further binding assays with porcine peripheral blood leukocytes showed that only monocytes can attach the virus. By their lack of binding factor, lymphocytes appeared to be refractory to PRRSV infection. Pre-incubation of MARC-145 cells with chymotrypsin and pronase E, but not neuraminidase, blocked their binding activity for PRRSV. The binding activity of the protease-treated cells was regenerated 8 hours after treatment, but cells remained unable to bind PRRSV if maintained in the presence of cycloheximide, thus con-

firming the proteinic nature of the specific binding factor(s). Experiments with cells that have been previously characterized as non-permissive to PRRSV infection showed that many of them were able to bind the virus. Data obtained suggest that interaction of PRRSV with a specific binding factor on the surface of some cells is not sufficient to lead to a productive infection, and that a second putative receptor or other phenomena are probably required to pursue later events.

Porcine reproductive and respiratory syndrome (PRRS) virus down-modulates TNF-alpha production in infected macrophages

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Virus Research, 2000, Vol. 69, No. 1, pp. 41–46.

The effect of porcine reproductive and respiratory syndrome (PRRS) virus infection on the synthesis and secretion of tumour necrosis factor (TNF)-alpha and other pro-inflammatory cytokines by porcine alveolar macrophages (PAM) was investigated as well as the effect that TNF-alpha has on the replication of this virus. A clear reduction of phorbol myristate acetate (PMA)-induced expression of TNF-alpha mRNA was observed in cells incubated with PRRS virus. The presence of PRRS virus also induced a decrease in interleukin (IL)-1alpha and macrophage inflammatory protein (MIP)-1beta mRNAs expression with respect to PMA-stimulated uninfected cells. According to these results, exposure to the PRRS virus led to a reduction of the TNF-alpha protein in supernatants of PMA-stimulated PAM. On the other hand, addition of recombinant porcine TNF-alpha to cultures clearly reduced virus replication; however the addition of TNF-alpha to cultures containing IFN-alpha did not result in a further reduction of the produced by IFN-alpha alone. This indicates the lack of synergy in the effect of these cytokines on viral replication.

Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs.

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Journal of General Virology, 2000, Vol. 81, No. 5, pp. 1327–1334.

Twenty-two 4- to 5-week-old gnotobiotic pigs were intranasally inoculated with 106.0 TCID50 of porcine reproductive and respiratory syndrome virus (PRRSV) (Lelystad) and killed at different time intervals post-inoculation (p.i.). Bronchoalveolar lavage (BAL) cell populations were characterized, together with the pattern of virus replication and appearance of antibodies in the lungs. Total BAL cell numbers increased from 140 x 106 at 5 days p.i. to 948 x 106 at 25 days p.i. and remained at high levels until the end of the experiment. The number of monocytes/macrophages, as identified by monoclonal antibodies 74–22–15 and 41D3, increased two- to five-fold between 9 and 52 days p.i. with a maximum at 25 days p.i. Flow cytometry showed that the population of differentiated macrophages was reduced between 9 and 20 days p.i. and that between the same time interval, both 74–22–15-positive and 41D3-negative cells, presumably monocytes, and 74–22–15- and 41D3-double negative cells, presumably non-phagocytes, entered the alveolar spaces. Virus replication was highest at 7 to 9 days p.i., decreased slowly thereafter and was detected until 40 days p.i. Anti-PRRSV antibodies were detected starting at 9 days p.i. but neutralizing antibodies were only demonstrated in one pig killed at 35 days and another at 52 days p.i. The decrease of virus replication in the lungs from 9 days p.i. can be attributed to (i) shortage of susceptible differentiated macrophages, (ii) lack of susceptibility of the newly infiltrated monocytes and (iii) appearance of anti-PRRSV antibodies in the lungs. Neutralizing antibodies may contribute to the clearance of PRRSV from the lungs.

Porcine reproductive and respiratory syndrome virus (PRRSV): kinetics of infection in lymphatic organs and lung

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Journal of Veterinary Medicine. Series B, 2000, Vol. 47, No. 1, pp. 9–25.

Pigs were infected by the oronasal route with European isolates of the porcine reproductive and respiratory syndrome virus (PRRSV; I10 and Cobbelsdorf). The kinetics of infection in lymphatic organs and the lung were monitored by immunofluorescence detection of virus antigen, re-isolation of the virus and reverse transcription-polymerase chain reaction (RT-PCR) for PRRSV-specific RNA. The kinetics of PRRSV infection proceeded in 3 phases, irrespective of the varying infection of lymphatic organs within the first days after infection (p.i.). First, an early acute infection of lymphatic organs developed within the first week and was characterized by a high number of antigen-positive macrophages. Second, a delayed acute infection of the lung, which was most pronounced during the second and third week p.i. when a high number of infected alveolar macrophages was identified. The acute infection of lymphatic organs had resolved at this time. Infected cells in the lung were predominantly located in pneumonic lesions. Third, a persistent infection was

detected by RT-PCR and immunohistology at the end of the experiments on day 49 p.i. The virus persisted in lymphatic organs, especially in the tonsils, and in the lung. At this stage, indications for a re-occurrence of acute infection were seen in restricted areas of the lung.

Changes of leukocyte phenotype and function in the broncho-alveolar lavage fluid of pigs infected with porcine reproductive and respiratory syndrome virus: a role for CD8+ cells

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Journal of General Virology, 2000, Vol. 81, No. 2, pp. 497–505.

Porcine reproductive and respiratory virus (PRRSV) primarily infects and destroys alveolar macrophages of the pig. Changes of leukocyte populations was monitored in the broncho-alveolar lavage fluid (BALF) of PRRSV-infected pigs. Piglets were inoculated intranasally with PRRSV strain LV ter Huurne. The piglets were killed at intervals and the lungs removed, washed semi-quantitatively and examined by flow cytometry. The total number of recovered BALF cells increased approximately 10 times between day 10 and day 21 of infection and then decreased. The number of small low-autofluorescent cells (SLAC), (lymphocytic and monocytic cells), increased very strongly from day 2 until day 21 of infection; in contrast, the number of large highly autofluorescent cells (LHAC), (mostly macrophages), remained constant until day 14 of infection, increased slightly on day 21 and then decreased. On day 21 of infection in SPF piglets approximately 60% of the SLAC consisted of CD2+CD8+CD4-gammadeltaTCR – cells, which were partly CD8+CD6+ and partly CD8+CD6-. These phenotypes correspond to that of cytotoxic T-cells and natural killer cells respectively. It was concluded that during a PRRSV infection the total number of BALF cells increases mainly due to an influx of lymphocytic cells with a cytolytic phenotype.

The localization of porcine reproductive and respiratory syndrome virus nucleocapsid protein to the nucleolus of infected cells and identification of a potential nucleolar localization signal sequence

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Virus Research, 1999, Vol. 64, No. 1, pp. 1–12.

The nucleocapsid (N) protein of porcine reproductive and respiratory syndrome virus (PRRSV) possesses 2 regions in the N-terminal half of the protein that are enriched in basic amino acids. The PSORT computer programme identified the same regions as nuclear localization signal (NLS) sequence motifs. N protein localization to the nucleus of infected MARC-145 and porcine pulmonary macrophages was observed following staining with SDOW-17 and SR-30 anti-N monoclonal antibodies. Co-localization of SR-30 antibody with human ANA-N autoimmune serum identified the nucleolus as the primary site for N protein localization within the nucleus. Localization of the N protein in the absence of infection was studied by following fluorescence in MARC-145 cells transfected with a plasmid, which expressed the nucleocapsid protein fused to an enhanced green fluorescent protein (N-EGFP). Similar to infected cells, N-EGFP localized to the cytoplasm and the nucleolus. Results following the transfection of cells with pEGFP fused to truncated portions of the N gene identified a region containing the second basic stretch of amino acids as the nucleolar localization signal (NoLS) sequence. Another outcome following transfection was the disappearance of cells that expressed high levels of N-EGFP. However, cell death did not correlate with localization of N-EGFP to the nucleolus.

Effect of age, porcine reproductive and respiratory syndrome virus and *Salmonella choleraesuis* infection on the phenotype and phagocytic activity of porcine alveolar macrophages

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Journal of the Chinese Society of Veterinary Science, 1999, Vol. 25, No. 3, pp. 226–233.

Pulmonary alveolar macrophages (PAM) are important for clearing invading pathogens. The effect of age and pathogens on the phenotype expression and phagocytic activity of porcine PAM was investigated. Experimental pigs were weaned at 3 weeks old and then raised in an experimental control house. Bronchoalveolar lavages were collected from pigs of different ages for phenotype and functional assays. Surface molecule expression and phagocytic activity of PAM were measured by flow cytometry. There was no significant difference in the expression of MHC class I (monoclonal antibody, mAb, 74–11–10 & 7–34–1), MHC class II (mAb MSA3), and macrophage markers (mAb 74–22–15 & 2G4a) of PAM between 4- and 8-week-old pigs. However, the surface molecule expressions of PAM from 4- and 8-week-old pigs were significantly increased compared to 12-week-old pigs ($P<0.05$). A significant decrease in the RBC phagocytosis and rosetting activities of PAM from 12-week-old pigs was also observed as compared to the younger pigs ($P<0.05$). However surface molecule expressions of MHC class I and macrophage marker of PAM in *S. choleraesuis* and porcine

reproductive and respiratory syndrome (PRRS) virus infected pigs were significantly decreased (P<0.05). The RBC resetting activity of PAM from PRRS virus infected pigs was also significantly decreased when compared to pigs of the same age (P<0.05). Thus, age of the pigs may affect the phenotype and phagocytic activity of PAM. The decrease of RBC rosetting of PAM in PRRS virus infection may reflect a reduced state of pulmonary microenvironment in clearing invading pathogens.

Distribution of a Korean strain of porcine reproductive and respiratory syndrome virus in experimentally infected pigs, as demonstrated immunohistochemically and by *in-situ* hybridization

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Journal of Comparative Pathology, 1999, Vol. 120, No. 1, pp. 79–88.

Twenty-four specific pathogen-free pigs were inoculated intranasally with a Korean strain (SNUVR970501) of porcine reproductive and respiratory syndrome virus (PRRSV) at 3 days of age. 16 pigs were uninfected controls. Three infected and 2 control pigs were killed at 1, 3, 5, 7, 10, 14, 21 and 28 days after inoculation and the distribution of PRRSV was assessed by immunohistochemistry and *in-situ* hybridization. The most consistent and intense labelling for PRRSV was in the lung; the virus persisting in pulmonary macrophages for at least 28 days. The middle lobe of the lung was the optimum site for the detection of PRRSV antigens and nucleic acids, and the interstitial macrophage was the main cell type in which PRRSV was identified. Other tissues and cells in which the virus was detected included macrophages and dendritic cells in the tonsil, lymph nodes, spleen and Peyer's patches, and macrophages in the hepatic sinusoids and adrenal gland. It is concluded that initial entry of PRRSV through tonsillar and pulmonary macrophages is followed within 3 days by viraemia and subsequent interstitial pneumonia.

CELL MEDIATED IMMUNITY AND CATTLE VIRUSES

Effect of parainfluenza-3 virus challenge on cell-mediated immune function in parainfluenza-3 vaccinated and non-vaccinated calves.

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Research in Veterinary Science, 2000, Vol. 68, No. 2, pp. 197–199.

Four colostrum-fed calves were vaccinated with live parainfluenza type 3 (PI-3) virus vaccine at 1 and 5 weeks of age and 4 control calves were treated with cell culture medium at the same time. Two weeks after the second vaccination, both groups of calves were challenged with PI-3 virus by a combined respiratory route. Blood and nasal mucus samples were collected at intervals, and alveolar macrophages were recovered before and after challenge by bronchoalveolar lavage. The results demonstrated that clearance of virus, as indicated by presence of virus antigen was more rapid in previously vaccinated calves. Several alveolar macrophage functions were markedly reduced in all calves 5 to 7 days following virus challenge, although microbicidal activity was unaffected, compared with the controls. The production of neutrophil chemotactic factors by alveolar macrophages occurred more rapidly after virus challenge in the previously vaccinated calves and this correlated with a more rapid neutrophil influx into the lungs in these animals.

Effects of bovine herpesvirus type 1 infection in calves with maternal antibodies on immune response and virus latency

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Journal of Clinical Microbiology, 2000, Vol. 38, No. 5, pp. 1885–1894.

Infection of 7 passively immunized young calves with a virulent strain of bovine herpesvirus type 1 (BHV-1) was performed to determine whether they could become seronegative after the disappearance of maternal antibodies while latently infected with BHV-1. Four uninfected calves were controls. All calves were monitored serologically for 13 to 18 months. In addition, the development of a cell-mediated immune response was assessed by an *in vitro* antigen-specific gamma interferon (IFN-gamma) production assay. All calves had positive IFN-gamma responses as early as 7 days until at least 10 weeks after infection. However, no antibody rise was observed after infection in the 3 calves with the highest titres of maternal antibodies. One of the 3 calves became seronegative by virus neutralization test at 7 months of age. This calf had negative IFN-gamma results and was classified seronegative by ELISA at around 10 months of age. This calf was latently infected, as proven by virus re-excretion after dexamethasone treatment at the end of the experiment. It is concluded that BHV-1-seronegative latent carriers can be obtained experimentally. In addition, the IFN-gamma assay could distinguish calves with passively acquired antibodies from those latently infected by BHV-1, but it could not detect seronegative latent carriers. The failure to detect such animals presents an epidemiological threat for the control of BHV-1 infection.

The efficacy of modified-live bovine respiratory syncytial virus vaccines in experimentally infected calves

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Vaccine, 1999, Vol. 18, No. 9/10, pp. 907–919.

Clinical disease and pulmonary pathology were significantly reduced, relative to 9 unvaccinated controls, in 18 experimentally infected calves vaccinated with commercial multivalent modified-live bovine respiratory syncytial virus (MLV BRSV). *In vitro* assays of cellular immunity were more consistent correlates of vaccine-associated protection than presence of post-vaccination serum antibody. Most vaccinated calves shed virus, but peak virus titre was supressed compared with unvaccinated controls, with clearance coincident with the simultaneous appearance of mucosal antibody, cytotoxic cells in the lung and anamnestic or primary serum antibody responses. Virus clearance in unvaccinated calves was coincident with the appearance of BRSV-specific cytotoxic cells, before mucosal antibody was detected.

A comparative study on the immune response of cattle to sheep pox and lumpy skin disease viral vaccines

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Veterinary Medical Journal Giza, 1999, Vol. 47, No. 1, pp. 29–37.

Humoral and cell mediated immune responses of cattle to tissue culture adapted-lumpy skin disease (LSD) and sheep pox (SP) viral vaccines were evaluated. Both viruses induced high antibody titres as measured by serum neutralization test and ELISA. No significant difference in the ability of sera of cattle vaccinated with SP virus or LSD virus vaccines to neutralize both viruses was detected, indicating that epitopes responsible for humoral immune response are cross-reactive to a high degree in both viruses. Peripheral blood lymphocytes from animals vaccinated with LSD virus and stimulated with inactivated LSD virus (homologous mitogen) showed higher stimulation percentage than those stimulated with inactivated SP virus (heterologous mitogen), indicating that LSD virus as a vaccine produces better cell mediated immune response specific against LSD virus which has a significant role in protecting animals against virulent field LSD virus infection.

Semliki Forest virus vector carrying the bovine viral diarrhea virus NS3 (p80) cDNA induced immune responses in mice and expressed BVDV protein in mammalian cells

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JN-International, Inc., Oakland, NE 68045, USA.
Comparative Immunology, Microbiology and Infectious Diseases, 1999, Vol. 22, No. 4, pp. 231–246.

To generate a DNA vaccine against bovine diarrhoea virus (BDV), the gene for BDV-NADL NS3 was cloned into a eukaryotic expression vector of Semiliki Forest virus (pSFV-1). Five groups of 10 female BALB/c mice were used. Groups 1 and 2 were given vector-S3 (100 and 200 micro g), groups 3 and 4 received vector control plasmid (100–200 micro\g) groups 5 were infected with PBS. BALB/c mice injected with recombinant DNA generated statistically significant cytotoxic T-lymphocyte activity and cell mediated immune responses against cytopathic and non-cytopathic BDV. The BDV-NS3 did not generate neutralizing antibodies against BDV in mice. pSFV-1-NS3 DNA was subjected to *in vitro* transcription into mRNA. The mRNA was transfected into baby hamster kidney cells (BHK-21) and Madin-Darby bovine kidney cells. The recombinant cells were used in the detection of DNA antigen responses by immunological assays. This paper reports the ability of BDV-NS3 DNA inoculation to induce a strong cellular immune responses in mice.

Cell-mediated immune responses in cattle vaccinated with a vaccinia virus recombinant expressing the nucleocapsid protein of rinderpest virus

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Journal of General Virology, 1999, Vol. 80, No. 7, pp. 1627–1634.

A recombinant vaccinia virus expressing rinderpest virus (RPV) nucleocapsid protein (rVV-RPV-N) induced low levels of non-neutralizing anti-N antibodies in cattle. RPV-specific cell-mediated immunity induced by the recombinant was assessed by measuring both the lymphocyte proliferation and cytotoxic T-lymphocyte responses. The protective immune response was examined by challenging the vaccinated cattle with either a highly virulent (Saudi 1/81) or a mild (Kenya/eland/96) strain of the virus. The vaccinated cattle were not protected against challenge with the virulent RPV strain, except they showed a slight delay in the onset of disease when compared with the non-vaccinated controls. In cattle challenged with the mild strain, apart from a

transient fever, no clinical signs of rinderpest infection were seen in the vaccinated cattle. Of 2 control cattle, one showed a similar response the other died from classic rinderpest disease. Virus-neutralizing antibodies were induced more quickly following challenge with the mild strain in vaccinated cattle compared to the control animals. These results suggested that the cell-mediated immunity induced by rVV-RPV-N could stimulate the rapid production of neutralizing antibodies following RPV challenge but this response was not sufficient to protect against challenge with avirulent strain of the virus. Protection was seen in one of 3 animals challenged with a mild strain of the virus; however, a greater number of animals would need to be tested to estimate the significance of the protection afforded by the N protein.

Bovine herpesvirus 1 can infect CD4+ T lymphocytes and induce programmed cell death during acute infection of cattle

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Journal of Virology, 1999, Vol. 73, No. 10, pp. 8657–8668.

Calves were infected with bovine herpesvirus 1 (BHV-1) to examine whether BHV-1 can induce apoptosis of lymphocytes. *In situ* terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) staining of lymphoid tissues (pharyngeal tonsil, cervical, retropharyngeal, and inguinal) was used to detect apoptotic cells. Calves infected with BHV-1 for 7 days revealed increased apoptotic cells near the corticomedullary junction in lymphoid follicles and in the subcapsular region. Increased frequency of apoptotic cells was also observed in the mucosa-associated lymphoid tissue lining the trachea and turbinate. Immunohistochemistry of consecutive sections from pharyngeal tonsil revealed that CD2+ T lymphocytes were positive for the BHV-1 envelope glycoprotein gD. The location of these CD2+ T lymphocytes in the germinal center suggested that they were CD4+ T cells. Electron microscopy and TUNEL also revealed apoptotic and herpesvirus-infected lymphocytes from this area. Fluorescence-activated cell sorting analyses demonstrated that CD4+ and CD8+ T cells decreased in lymph nodes and peripheral blood mononuclear cells (PBMC) after infection. The decrease in CD4+ T cells correlated with an increase in apoptosis. CD4+ but not CD8+ lymphocytes were infected by BHV-1 as judged by *in situ* hybridization and PCR, respectively. Immediate-early (bovine ICP0) and early (ribonucleotide reductase) transcripts were detected in PBMC and CD4+ lymphocytes prepared from infected calves. In contrast, a late transcript (glycoprotein C) was not consistently detected suggesting productive infection was not efficient. It is suggested that BHV-1 can infect CD4+ T cells in cattle, leading to apoptosis and suppression of cell-mediated immunity.

"T" lymphocytes and humoral immune response to foot and mouth disease in cattle supplemented with different copper and molybdenum combinations

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Revista de Medicina Veterinaria (Buenos Aires), 1999, Vol. 80, No. 1, pp. 14–18.

A total of 19 crossbred heifers were divided into 4 groups: Group A, controls (4 animals); group B, given Mo supplements; group C, given Mo and Cu; group D, given Cu alone. The "T" lymphocyte rosette-forming test was carried out on days 1 and 100 (before vaccination against foot and mouth disease). The microneutralization test for antibodies to foot and mouth disease virus (FMDV) was carried out on the days 100 (day of vaccination) and 121. Normal cellular immunity was demonstrated on day 100 in all groups by the rosette-forming test and by relative and absolute leukocyte counts. There was no significant difference in humoral immune response between the 4 groups. Differences in liver and serum copper concentrations were confirmed by analysis of serum samples and liver biopsies. It is concluded that herds with copper deficiencies could be given parenteral copper in conjunction with FMD vaccination in order to facilitate herd management.

Phenotypic analysis of local cellular responses in calves infected with bovine respiratory syncytial virus

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Immunology, 1999, Vol. 96, No. 3, pp. 396–403.

Changes in lymphocyte subsets in the trachea, pulmonary tissue, bronchoalveolar lavage (BAL), peripheral blood and bronchial lymph nodes (BLN) of gnotobiotic calves (n=18) infected with bovine respiratory syncytial virus (BRSV) were analysed by flow cytometry. Following BRSV infection, virus titres in the nasopharynx reached a peak between days 5 and 7 and infection was resolving from day 10. Although calves did not develop signs of clinical respiratory disease, there was evidence of gross pneumonia and histological changes typical of BRSV bronchiolitis, which were most extensive from day 7–10 of infection. Following BRSV infection there was a recruitment of CD8+ T cells into the trachea and lung, which peaked on day 10 after infection. There were approximately equal numbers of CD8+ and CD4+ T cells in the lung and trachea of unin-

fected calves (n=4), whereas by day 10 of infection, CD8+ cells outnumbered CD4+ cells by 3:1 in the lungs and 6:1 in the trachea of the infected calves. Although the increase in CD4+ T cells into the lungs was less than that of CD8+ T cells, changes in expression of CD45R, CD45RO, L-selectin and interleukin-2 receptors all suggested that CD4+ T cells were activated during BRSV infection. Changes in gammadelta T cells were not observed in BRSV-infected calves. There was an increase in B cells in the BLN after infection and BLN CD4+ T cells changed from the majority expressing L-selectin and CD45R in uninfected calves to a predominance of L-selectin- CD45R- CD45RO+ phenotype, 10 days after infection. It is concluded that CD8+ T cells constitute the major lymphocyte subpopulation in the respiratory tract of calves recovering from BRSV infection.

Antibody response to glycoprotein E after bovine herpesvirus type 1 infection in passively immunised, glycoprotein E-negative calves.

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Veterinary Record, 1999, Vol. 144, No. 7, pp. 172–176.

This study was conducted to determine whether young calves with maternal antibodies against bovine herpesvirus type 1 (BHV-1) but without antibodies against glycoprotein E (gE) can produce an active antibody response to gE after a BHV-1 infection. Five calves received colostrum at birth from gE-seronegative cows which had been vaccinated 2 or 3 times with an inactivated BHV-1, gE-deleted marker vaccine. After inoculation with a wild-type virulent strain of BHV-1, all the passively immunized gE-negative calves shed virus in large amounts in their nasal secretions. All the calves seroconverted to gE within 2–4 weeks after inoculation and then had high levels of gE antibodies for at least 4 months. The development of an active cell-mediated immune response was also detected by *in vitro* BHV-1-specific interferon-gamma assays. All the calves were latently infected, because one of them re-excreted the virus spontaneously and the other 4 did so after being treated with dexamethasone. It is concluded that the gE-negative marker could distinguish between passively immunized and latently infected calves.

Demonstration of bovine CD8+ T-cell responses to foot-and-mouth disease virus

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Journal of General Virology, 1999, Vol. 80, No. 3, pp. 663–669.

This study investigated the importance of cellular immunity in foot-and-mouth disease in cattle, in particular whether a CD8+ T-cell response could be detected, as these cells may be involved in both immunity and virus persistence. As attempts to characterize classical cytotoxic T cells had yielded non-reproducible results, largely due to high backgrounds in control cultures, a proliferation assay was developed that was shown to detect antigen-specific, MHC class I-restricted bovine CD8+ cells responding to foot-and-mouth disease virus (FMDV). Proliferative CD8+ T-cell responses were detected consistently from 10 to 14 days following infection with FMDV and typically lasted 3–4 weeks. The role of CD8+ T cells in control of the disease, in particular their relevance for the establishment of persistence, may now be investigated.

SWINE VESICULAR DISEASE

Singleton reactors in the diagnosis of swine vesicular disease: the role of coxsackievirus B5

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Veterinary Microbiology, 2000, Vol. 76, No. 3, pp. 291–297.

Swine vesicular disease virus (SVDV) and Coxsackie B5 virus (CVB5) are closely related viruses that can infect pigs and man and give rise to cross-reacting serum antibodies. It is, therefore, possible that SVD antibodies found in serological screenings of pigs are induced by CVB5. Single positive animals found in screening programmes are generally referred to as singleton reactors (SR). To determine whether SR in SVDV screenings are induced by CVB5 infection, virus neutralization tests (VNTs) and radioimmunoprecipitation assays (RIPA) were carried out on sera of SR, sera of pigs experimentally infected with SVDV, and sera from pigs vaccinated with CVB5 isolates. The SR sera reacted repeatedly positive in the SVDV UKG/27/72 VNT, but reacted differently in three other VNTs (SVDV NET/1/92, CVB5A, and CVB5B). The VNT titres obtained with the SR sera revealed a correlation between both SVDV strains, and also between both CVB5 stains, but no correlation was found between SVD and CVB5 VNT titres. Sera of experimentally infected (SVDV) or vaccinated (CVB5) pigs showed titres in all four neutralization tests. In the RIPA, the reaction patterns of the SR sera varied considerably with all four antigens used, in contrast to sera from pigs experimentally infected with SVDV that reacted with all antigens used, and sera from pigs vaccinated with CVB5 that reacted only with CVB5 antigens. The results presented in this paper show that neither CVB5 nor SVDV infections are the only cause of the SR phenomenon. Testing for CVB5 specific antibodies can reduce the number of SR sera in the serodiagnosis of SVDV.

Differential diagnosis of swine vesicular disease virus (SVDV) by DNA amplification *in vitro*.

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Medycyna Weterynaryjna, 2000, Vol. 56, No. 8, pp. 513–516.

The aim of the study was to estimate the usefulness of RT-PCR for differential diagnosis of SVDV and other picornaviruses. Amplification of cDNA was performed using three pairs of primers selected from conservative and variable regions of the SVDV genome. A comparison was made between the effectiveness of amplifying chosen fragments of SVDV, antigenically related Coxsack virus (CAV-16D) and foot-and-mouth disease virus (FMDV) causing similar clinical symptoms. Our results indicate that the most suitable primers for SVDV detection were from the 2A (or 1D/2A) viral genome, whereas amplimers from the highly variable 1B coding region should be used to differentiate virus strains. Applying these primers resulted in differentiating SVDV isolates from the 1970s and 90s. A high sensitivity and specificity of RT-PCR was found. By means of this technique it was possible to detect RNA in virus preparations with a titre of 10 TCID50. The specificity was evaluated by restriction analysis (RFLP) with nuclease AluI of 1D/2A amplicon. One specific site was recognised by this enzyme and, as the result of digestion, two products (440 and 225 bp) were obtained.

Effects of chlorine, iodine and quaternary ammonium compound disinfectants on several exotic disease viruses

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Journal of Veterinary Medical Science, 2000, Vol. 62, No. 1, pp. 85–92.

The effects of 3 disinfectants (chlorine (sodium hypochlorite), iodine (potassium tetraglicine triiodide) and quaternary ammonium compound (didecyldimethylammonium chloride)) on 4 enveloped viruses (vesicular stomatitis virus, African swine fever virus, equine viral arteritis virus, and porcine reproductive and respiratory syndrome virus) and 2 non- enveloped viruses (swine vesicular disease virus (SVDV) and African horse sickness virus (AHSV)) were examined. Chlorine was effective against all viruses except SVDV at concentrations of 0.03 to 0.0075%. Iodine was very effective against all viruses at concentrations of 0.015 to 0.0075%. Quaternary ammonium compound was very effective in low concentration of 0.003% against 4 enveloped viruses and AHSV, but it was only effective against SVDV in combination with 0.05% NaOH. Electron microscopy revealed that chlorine caused complete degeneration of viral particles and destroyed the nucleic acid of the viruses. Iodine destroyed mainly the inner components including nucleic acid of the viruses. Quaternary ammonium compound induced detachment of the envelope of the enveloped viruses and formation of micelle in non-enveloped viruses. The effective concentration of quaternary ammonium compound was the lowest among examined disinfectants.

Recovery and assay of African swine fever and swine vesicular disease viruses from pig slurry

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Journal of Applied Microbiology, 1999, Vol. 87, No. 3, pp. 447–453.

Various techniques were compared for the recovery of African swine fever virus (ASFV) and swine vesicular disease virus (SVDV) in pig slurry. Extraction with Freon led to 80–100% recovery of SVDV added to pig slurry. The assay sensitivity enabled undiluted, centrifuged sample to be put directly onto monolayers of IB-RS2 cells, allowing a minimum detection level of 100.7 p.f.u. ml-1. ASFV was difficult to recover intact, and the best technique allowed a recovery of 60% with a minimum detectable level of 101.8 HAD50 ml-1, due to toxicity to the cells at low sample dilutions. Extraction with the addition of an equal volume of ox serum to inoculated slurry was best for recovering ASFV. It is suggested that poor recoveries with the other techniques may have been due to the inactivation of the virus while in the slurry rather than as a result of the inability of the method to extract ASFV.

Mapping the genetic determinants of pathogenicity and plaque phenotype in swine vesicular disease virus

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Journal of Virology, 1999, Vol. 73, No. 4, pp. 2710–2716.

A series of recombinant viruses were constructed using infectious cDNA clones of the virulent J1'73 (large plaque phenotype) and the avirulent H/3'76 (small plaque phenotype) strains of swine vesicular disease virus to identify the genetic determinants of pathogenicity and plaque phenotype. Both traits could be mapped to the region

between nucleotides (nt) 2233 and 3368 corresponding to the C terminus of VP3, the whole of VP1, and the N terminus of 2A. In this region, there are 8 nucleotide differences leading to amino acid changes between the J1'73 and the H/3'76 strains. Site-directed mutagenesis of individual nucleotides from the virulent to the avirulent genotype and vice versa indicated that A at nt 2832, encoding glycine at VP1–132, and G at nt 3355, encoding arginine at 2APRO-20, correlated with a large-plaque phenotype and virulence in pigs, irrespective of the origin of the remainder of the genome. Of these 2 sites, 2APRO-20 appeared to be the dominant determinant for the large-plaque phenotype but further studies are required to elucidate their relative importance for virulence in pigs.

Laboratory-scale inactivation of African swine fever virus and swine vesicular disease virus in pig slurry.

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Journal of Applied Microbiology, 1999, Vol. 87, No. 1, pp. 148–157.

Chemical treatment and heat treatment were both evaluated for the inactivation of African swine fever (ASV) and swine vesicular disease (SVD) viruses in pig slurry. The addition of NaOH or Ca(OH)2 at different concentration/time combinations at 4 deg C and 22 deg C was examined, as was virus stability at different temperature/time combinations. ASF virus (ASFV) was less resistant to both methods than SVD virus (SVDV). In slurry from one source, ASFV was inactivated at 65 deg C within 1 min, whereas SVDV required at least 2 min at 65 deg C. However, it was found that thermal inactivation depended on the characteristics of the slurry used. Addition of 1% (w/v) of NaOH or Ca(OH)2 caused the inactivation of ASFV within 150 s at 4 deg C; 0.5% (w/v) NaOH or Ca(OH)2 required 30 min for inactivation. NaOH or Ca(OH)2 (1% (w/v)) was not effective against SVDV at 22 deg C after 30 min, and 1.5% (w/v) NaOH or Ca(OH)2 caused inactivation of SVDV at both 4 deg C and 22 deg C. At higher chemical concentrations or temperatures, ASFV and SVDV inactivation was faster in slurry than in buffered medium.

Application of different diagnostic methods for the detection of SVDV infection in pigs

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Bulletin of the Veterinary Institute in Pulawy, 1999, Vol. 43, No. 1, pp. 11–18.

The persistence of swine vesicular disease virus (SVDV) in clinical samples and tissue samples from 5 experimentally infected pigs was investigated. Negative samples were taken from 3 uninfected pigs. Samples of epithelial tissue from vesicles, nasal swabs, blood, faeces and other organs were collected to examine for infectious SVD particles, genomes and antigen. Diagnostic tests applied included the QIAGEN RNeasy test for extracting virus RNA, the MAC-ELISA technique for detecting SVDV antigen, reverse transcription-polymerase chain reaction (RT-PCR, one-step PCR, the TITAN One Tube system) and RT-nested PCR (RT-nPCR, two-step PCR, using the external primers 1 AEX and 1 BEX). The reverse transcriptase RT-nPCR assay appeared to be the most sensitive for detecting SVDV in samples taken late in the course of infection. Only by nPCR could the presence of viral RNA in blood and nasal swabs be found for as long as 4 and 48 days after infection, respectively. Using virus isolation and RT-nPCR, it was possible to detect viral genomes in faeces up to 70 days after infection. By RT-nPCR, the viral RNA could be detected in somatic muscles and tonsils until 25 and 48 days after infection, respectively. The virus could not generally be found in other organs beyond 7 days after infection. It is suggested that the described RT-nPCR procedure can be useful for estimating the duration of SVDV infection in pigs.

GOAT POX AND SHEEP POX

Immunohistochemical detection of antigen in lamb tissues naturally infected with sheep pox virus

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Journal of Veterinary Medicine. Series B, 2000, Vol. 47, No. 3, pp. 173–181.

An outbreak of sheep pox was diagnosed in 3 sheep flocks in Van province, Turkey between January and April 1998. Various tissue samples from 6 naturally infected lambs were examined using the streptavidin-biotin peroxidase complex method. Sheep pox viral antigen was detected in the cytoplasm of sheep pox cells and degenerated epithelial cells of the skin, lungs and digestive tract involving typical sheep pox lesions. Nuclear staining was also observed in some typically deformed nuclei of sheep pox cells. The immunostaining of sheep pox virus showed a correlation with the presence of sheep pox cells and degenerated epithelial cells resembling sheep pox cells. To confirm the presence of sheep pox virus in the skin and lung samples, direct electron microscopy was performed and sheep pox virus was only demonstrated in 2 skin samples.

Detection of sheep poxvirus in skin biopsy samples by a multiplex polymerase chain reaction

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Journal of Virological Methods, 2000, Vol. 84, No. 2, pp. 161–167.

The development of a multiplex polymerase chain reaction (PCR) method with amplification of capripoxvirus in a single-step procedure from skin biopsies using 3 primer pairs, 2 specific for capripoxvirus and one specific for alpha-tubulin is described. A sensitive multiplex PCR was achieved by optimization of parameters such as the primer concentrations, magnesium and dNTPs concentrations. False negative results that sometimes arise due to inhibitors of DNA amplification may be avoided by the inclusion in the assay of alpha-tubulin primers. The results reported on 42 skin biopsies from sheep suspected to have poxvirus infection, indicated that the assay could monitor simultaneously DNA extraction from skin biopsy samples and allow improved detection of capripoxvirus within 24 h of specimen receipt in the laboratory.

Investigation of immunity in lambs born to ewes vaccinated with sheep and goat pox vaccine

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Pendik Veteriner Mikrobiyoloji Dergisi, 1999, Vol. 30, No. 2, pp. 55–62.

The duration of passive immunity in lambs of vaccinated pregnant ewes was investigated. Pregnant ewes were vaccinated s.c. with 0.5 ml of sheep and goat pox vaccine SP (Bk) LK65- 96.1. 21 days after the vaccination, average titre of neutralizing antibodies was log 10–1.81. Immunity in lambs of the vaccinated pregnant ewes was measured by serum neutralization titres after i.d. challenge. 15- and 30-day-old lambs which had received colostrum were challenged with 1000 I.D50/0.2 ml of Romanian sheep pox virus strain. No local reactions were seen in either age group. After lambing, the serum antibody titres in these lambs at 15, 30, 45 and 60 days were 10–0.95, 10–0.82, 10–0.53 and 10–0.00, respectively. After vaccination of the same lambs, the average antibody titres were 10–0.61, 10\-0.50, 10–1.28 and 10–1.42. These results show that lambs of vaccinated ewes can be protected by neutralizing antibodies for at least 4 weeks against sheep pox. If vaccination becomes necessary for newborn lambs from vaccinated ewes, treatment should be given at 6–8 weeks old.

An indirect IgM enzyme linked immunosorbent assay for diagnosis of sheep pox virus infection

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Indian Veterinary Journal, 1999, Vol. 76, No. 12, pp. 1050–1054.

An indirect ELISA for the detection of sheep pox virus (SPV) specific IgM antibodies was developed. Three groups of serum samples (4 samples from sheep artificially infected with SPV, Group I; 4 samples from naturally infected convalescent lambs, Group II; and 542 samples of sheep with unknown sheep pox status, Group III) were examined using IgM-ELISA. Samples were first screened at a single dilution (1:10) and then positive samples were examined using end-point titration. The mean absorbance value (OD 450) of 20 known sheep pox neutralizing antibody negative samples plus 3.29 standard deviation formed the cut-off value for distinguishing negative and positive samples in IgM ELISA for SPV. The mean titres of Group I, Group II and Group III samples were 440 plus or minus 103, 260 plus or minus 51 and 65 plus or minus 30.4, respectively. It is concluded that IgM-ELISA is a specific, sensitive and reproducible technique for diagnosing sheep pox.

Evaluation of avidin-biotin ELISA for the detection of antibodies to goat poxvirus using noninfectious diagnostic reagent

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Acta Virologica, 1999, Vol. 43, No. 5, pp. 297–301.

A noninfectious soluble antigen fraction of goat poxvirus (GPV) fractionated by ammonium sulfate precipitation was tested for its suitability as coating antigen in an ELISA. Accordingly, an avidin-biotin ELISA for the detection of GPV antibodies was optimized and evaluated using different groups of serum samples from goats with known or unknown immune status. A cut-off value higher by 60% than A492 reading of control negative sera gave a 91.8% specificity and a 94.1% sensitivity for the assay. Out of 90 goat pox-suspect sera obtained from the field, only 2 (2.2%) were found positive in the counter immunoelectrophoresis (CIE) test, which is so far the routinely used diagnostic test for goat pox, while 58 (64.4%) were positive in the avidin-biotin ELISA. The McNemar's analysis of these data showed that the avidin-biotin ELISA was significantly more efficient than the CIE test for the detection of GPV antibodies in goat sera.

A capripoxvirus detection PCR and antibody ELISA based on the major antigen P32, the homolog of the vaccinia virus H3L gene

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Journal of Immunological Methods, 1999, Vol. 227, No. 1/2, pp. 187–196.

Capripox diagnosis by classical virological methods dependent on live capripox virus is not suitable in countries such as Australia where the virus is exotic and live virus is not available. To develop diagnostic tests based on recombinant material, a 3.7 kb viral DNA fragment of sheep pox virus (SPV) that contained open reading frames homologous to the vaccinia virus J6R, H1L, H2R, H3L and H4L genes, was cloned and sequenced. A capripoxvirus specific polymerase chain reaction (PCR) assay was developed that differentiated between SPV and lumpy skin disease virus (LSDV) of cattle on the basis of unique restriction sites in the corresponding PCR fragments. The vaccinia virus H3L homologue was identified as the capripoxvirus P32 antigen. The P32 proteins of SPV and LSDV were expressed in *Escherichia coli* as a fusion protein with a poly-histidine tag and affinity purified on metal binding resin. The full-length P32 protein contained a transmembrane region close to the carboxy terminus and was membrane associated but could be solubilized in detergent and used as trapping antigen in an antibody detection ELISA. The ELISA was specific for capripoxvirus as only sera from sheep infected with capripoxvirus but not orf or vaccinia virus reacted with the capripoxvirus P32 antigen.

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