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# IN SITU HYBRIDIZATION: A METHOD TO CONFIRM THE ETIOLOGICAL DIAGNOSIS OF PARVOVIRUS ENTERITIS IN DOGS AND CATS?

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In situ hybridization was used to localize parvovirus in section from formalin fixed, paraffin embedded intestinal and lymphoid tissue from dogs with atrophic enteritis and cats with feline panleukopenia. The probe consisted of the pBR322 plasmid containing the entire VP1- and VP2-coding sequences of canine parvovirus. It was labelled by nicktranslation with biotinylated dUTP.

For the in situ hybridization the section was deparaffinized, endogenous peroxidase was inactivated by incubating with  $H_2O_2$  and the tissue was digested by proteinase K. The probe was denatured on the slide at 98°C for 10 minutes and hybridization was performed over night at 42°C. The hybridization product was localized by incubating the slide with an avidin-biotinylated horseradish peroxidase complex

and the peroxidase activity was detected by amino-ethyl-carbazol and  $\mathrm{H}_2\mathrm{O}_2$  as substrates.

The morphologic diagnosis of an atrophic enteritis correlated well with the presence of parvovirus in dogs. However, parvovirus could only be detected in 5 out of 9 cats with marked atrophic enteropathy. In this study we were able to confirm the etiologic diagnosis in several cases, but could not rule out a parvovirus infection, where the in situ hybridization ielded negative results. Whether this discrepancy between morphologic findings and results of the in situ hybridization reflected a) a lack of sensitivity or b) some cats had already eliminated the virus by the time of necropsy or c) the atrophic enteritis was caused by another agent, such as FeLV, still needed to be investigated.

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## PATHOLOGY OF SWINE - A PORTRAIT OF ECONOMIC LOSS IN PIG PRODUCTION IN SWITZERLAND

P. Wegmann

Economic loss in pig production due to runting or premature death is one of the most important economic factors in Swiss agriculture. In 1971 the costs were estimated at about SFr. 100 million. Half of this loss was due to the spontaneous death of younger pigs, the other half was due to bad feed conversion caused primarily by lung affections or unfavourable husbandry conditions. In 1988, 1447 pigs were necropsied at our institute. A statistic evaluation of the findings ist presented with an attempt to show a relationship between the diagnoses and the economic losses in Switzerland.

#### **Necropsy results**

75% of the pigs were younger than 2 months. An explanation for this age distribution is that deaths caused by infectious diseases predominate in pigs of this age and farmers thus show more concern for the aetiology. Runting is infrequent.

Intestinal infections with *E. coli* were of greatest importance. One peak occurs in the first two weeks after birth and is caused by *E. coli* 

strain O 149. The next peak is found in the second month after birth, i. e. after weaning. Here, the strains O 139 and O 141 dominate. In the next three months, intestinal infections with *E. coli* decrease and occur only after moving animals and the assembly of new groups.

The second major cause of death in younger pigs is septicemia. In the first month more than 60% are infections with *E. coli*, *Streptococcus* and *Erysipelothrix*. In the following months, *Haemophilus parasuis* (Glässer's Disease) often occurs. Affections of joints, skin or other diseases are relatively seldom, namely in only 13% of all the pigs under 2 months.

In the animals over 2 months, the respiratory tract was primarily affected; predominantly infections with *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Bordetella bronchiseptica*. The maximum incidence was in the 7 month-old pigs, where 100% had pneumonic infections. Respiratory diseases in SPF-pigs were rare.

Pigs with respiratory problems usually only tended to runt.

### Relationship: necropsy - economic loss

In 1988 approx. 650 000 pigs died before they were due for slaughter (3 991 370 born live, 3 326 553 slaughtered). Most deaths were a sequel of infectious diseases, starving or crushing by the sow, and occurred under the age of 2 months. The average value of a pig of this age group is Sfr. 90.— and the total economic loss per year is approx. SFr. 58,5 million. Particularly since runting is not frequent in this age group, it may be concluded that a major part of the economic losses in Swiss pig younger than two months production are directly correlated with this group of fatalities.

In the animals over 2 months, economic losses often result from chronic respiratory affections. The feed conversion rate of pigs in Non-SPF farms is approx. 0.3 to 0.5 under that of conventional farms and this leads to an estimated depreciation of approx. Sfr. 30.— per animal. Thus an additional SFr. 70 million loss arises from ill thrift within the 2 million Non-SPF pigs slaughtered yearly. Since the major proportion of all pigs with respiratory affections orginate from such Non-SPF farms, it may be concluded here too, that the necropsy findlings correlate well with the economic losses in Swiss pig production.

Animal Health Service of Bavaria, Germany

# BOVINE RESPIRATORY SYNCYTIAL VIRUS INFECTION: CAN THE DIAGNOSIS BE CONFIRMED BY ONLY HISTOLOGIC EXAMINATION?

J. Weikel

In the last two years the etiology of respiratory diseases in fattenig cattle changed essentially. First we had problems for many years caused by the so-called Bovine Influenza, then the Infectious Bovine Rhinotracheitis brought trouble to many farms. Now Bovine Respiratory Syncytial Virus (BRSV) is the most common infectious agent of the airways of young bulls. In the Animal Health Service of Bavaria 146 fattening cattle affected by pneumonia were tested for an infection by Bovine Respiratory Syncytial Virus using direct immunofluorescence between October 1988 and March 1990. The lungs of most of the animals were embedded in paraffin, stained with hematoxylin eosin and examined microscopically. Only the animals which were positive for BRSV were selected for this report. The purpose was to verify by histologic examination, if there are typical lesions caused by BRS-virus and if an etiologic diagnosis based only on microscopic findings is justified. The antigen of BRS-Virus was demonstrated in the pneumonic lesions of the apical lobes of the lungs of 59 animals. Slides were prepared of 56 animals and the subsequently presented changes were evaluated statistically.

Only in eight animals the inflammatory infiltrates were acute. 27 cattle, i. e. 48% were affected by chronic pneumonia. 21 respiratory inflammations (= 37.5%) were classified as subacute. As far as it is possible and suitable to quantify the extension by histological slides 38 pneumoniae (= 68%) were graded as circumscript. The quality of inflammation, which is mostly assigned to secondary bacteria, was purulent in 51 lungs (91%) and fibrinous in the remaining five ones. In search of changes typical for an infection by BRS-virus proliferative phenomena on the epithelium of bronchi were stated most frequently. The epithelium of mediate and small bronchi, which physiologically is a monolayer, was thickened in 52 animals (93%) into two or more rows. In 27 slides we found only circumscript or even inside of one bronchus localized propagation of the epithelial cells. 23 bovines hat a marked hyperplasia of the bronchiolar epithelium, which extended to most of the dissected bronchi. In two lungs nearly all bronchi were lined by a rim of extensively proliferated epithelium.

Syncytical cells, i. e. cells with much eosinophilic cytoplasm and more than one nucleus with different sizes and spongy chromatin,

were demonstrated in the alveolar lumina or close to proliferations of the bronchiolar epithelium in 47 animals (= 84%). 26 of them had just a few of those cells. Three lungs contained such a number of syncytia, that their shape like giant cells could be detected even by low magnification. If we found no syncytial cells then there was a localized or marked hyperplasia of the epithelium of the bronchi as a constant finding. Four cattle without proliferations of the bronchiolar lining displayed some syncytial cells each.

In more than one third of the lungs examined the thickened wall of the alveoli was separated from the lumen by a homogenous eosinophilic layer. Hyaline membranes were not demonstrated in  $36 \ (= 64.3\%)$  animals. Partial large amounts of mucus inside the bronchi were shown in a similar frequency, which caused a closing of the lumen of the bronchiolar region especially. The mucus was mixed frequently with neutrophilic granulocytes, some of them with regressive disorders of the nucleus. Hyaline membranes and aggregations of mucus in the bronchi are present in the same animal in a few cases (ten out of 56) only.

Mononuclear infiltrations of the interstitium and peribronchiolar lymphfollicles are a common findig in the course of the BRSV-infection too. Nine bovines (= 16%) only had no such lesions. In the slides of 27 of the remaining 47 lungs we could demonstrate peribronchiolar lymphfollicles alone, in three cattle the infiltrates were limited to the connective tissue between the alveoli.

Even though emphysema in 52 animals was grossly found in the distal lobes mainly, an increased content of air could be detected in the affected parts of the apical lobes of 49 lungs by histologic examination as well. The diagnosis was 29 times alveolar emphysema, 20 times an interstitial one.

Regarding the findings shown before the infection by Bovine Respiratory Syncytial Virus is to our opinion microscopically characterized as follows: bronchopneumonia dominant subacute or chronic, expanding in most of the cases over the entire slide. Outstanding attribute of the infection is the proliferation of the bronchiolar epithelium and the appearance of syncytial cells. Hyaline membranes, aggregations of mucus in the bronchi and bronchioli and mononuclear infiltrates of the interstitium are frequent findings too. This