# Bovine respiratory syncytial virus infection : can the diagnosis be confirmed by only histologic examination?

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#### **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 pattern closely rembles to the data of the literature, even if certain details are present with different frequency or intensity. That ist why

we believe that there are indeed many cases, which allow an etiologic diagnosis without demontration of the causative agent.

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#### PEROXIDASE-ANTIPEROXIDASE LABELING OF LEISHMANIA AMASTIGOTES IN TISSUE

#### E. Wunderlin, A. Pospischil

Formalin fixed and paraffin embedded tissue of 5 dogs experimentally infected with Leishmania infantum and 13 dogs with natural Leishmania infections contracted in Spain or Italy, were investigated using a PAP-technique. The primary anti-Leishmania antibody was diluted 1:400 and was incubated on the tissue sections for 18 hours at room temperature as had been determined in preliminary experiments.

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# ENDEMIC PAPILLOMATOSIS OF VIRAL ORIGIN IN WATER FOWL OF THE BASLE ZOOLOGICAL GARDEN

### N. Zangger, M. Müller

Papillomas, or warts, are benign proliferative epithelial tumors of the skin or mucosal epithelium. In man, many mammals and a few reptiles papillomas are induced by papilloma virus (Papovaviridae). Papilloma virus induced warts in birds are rare; they are known in the European chaffinch (Fringilla coelebs) and in an African gray parrot (Psittacus erithacus). The skin of the head, legs, feet and cloacal area ae predilected. Papilloma should be differentiated from cutaneous pox lesions and bumble-foot, an avian pododermatitis.

For the first time a papilloma virus induced papillomatosis on the plantar surface of the feet will be described based on an endemic in water fowl of the Basle Zoological Garden.

#### **Material and methods**

Twenty-one birds of the orders Anseriformes, Gruiformes, Ciconiiformes and Phoenicopteriformes presented proliferative vertucous skin lesions on the sole of the feet.

Specimens of the lesions were fixed in 4% formalin, embedded in paraffin, sectioned at 4  $\mu$ m and stained with hematoxylin and eosin. For ultrastructural examination samples were postifixed in 1% osmium tetroxide and embedded in Spur®. Ultrathin sections were contrasted with uranyl acetate and lead citrate; they were examined by means of a ZEISS EM 902 microscope.

#### Results

A variable number of firm, split or ulcerated warts were distributed on the sole of one or both feet. The 0,5–5 cm sized vertucous hyperkeratotic lesions were chiefly limited to the metatarso-digital and interdigital articulations.

The histological features were characterized by papillomatosis, acanthosis, para- and hyperkeratosis. In the stratum spinosum and granulosum vacuolated nuclei with asymmetrical margination of the chromatin contained homogenous basophilic inclusions of different size. Foci of ballooning cells with a small dark nucleus were in the highly sensitive identification of Leishmania amastigotes in tissue of experimentally and naturally infected dogs. This is of special interest for routine post mortem samples as well as biopsy material since in HE or in Giemsa stained sections parasites are recognized only when they occur in relatively high numbers.

The results of this study show that Leishmania amastigotes can be

labeled specifically by a PAP-technique which allows a fast and

upper stratum spinosum and granulosum. The focal necrotic epidermis was interspersed with dense colonies of coccoid bacteries. Clefts extended to the hyperplastic subcutis. The dermis was infiltrated with mononuclear cells, and in cases with necrosis, with heterophils. The arterioloes were regularly obstructed by thrombi.

Ultrastructural examination revealed in the upper epidermis intranuclear dense aggregates of virions in cristalline array corresponding to the basephilic inclusion bodies. The non-enveloped spherical viral particles had a diamter of 50 to 55 nm.

#### Discussion

The typical vertucous lesions on the sole of the feet in several orders of water fowl, associated with the histopathological and ultrastructural findings allow the first description of an endemic viral papillomatosis in birds.

In man and many domestic mammals several types of papillomaviruses affect specific parts of the body. As the papillomas were limited in chaffinches to the legs and in the water fowl to the sole of the feet a favored localisation of the papillomavirus is suggested in birds, too. Papilloma virus is relatively resistant to heat, drying, and cold, and can persist for a long time in desquamated epidermal cells; characteristics that enhance their spread in susceptible populations. Viruses penetrate the epidermis via microlesions, which occur frequently in the vulnerable plantar skin on the articulations of the sole. Morover the maceration of the skin may be an important predisposing factor as suggested in humans by the increased incidence of plantar warts in swimmers who frequent public pools.

The proliferative lesions seem to be very painful and caused ataxia. They would also render the birds vulnerable to predators. Most of the affected brids were emaciated, one duck had been attacked by a marten.

In poultry-farming, bumble-foot may be a result of inadequate floors (type, humidity) and confinement. To differentiate this pododermatitis from the virus-induced papillomatosis in feet, further investigations are necessary.