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DETECTION OF VIRAL AGENTS IN DIARRHEIC PIGS IN AUSTRIA

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Introduction: Diarrhea in pigs is a complex problem resulting from interaction between infective agents, host immunity and management procedures. It causes considerable economic loss to the pig production, especially in suckling and weaner pigs. Bacteria are often assumed to be the primary causative agents of diarrhea; tests for viral infections are in general less initiated. The most agents of diarrhea in young pigs are enterotoxigenic *Escherichia coli*, *Clostridium perfringens*, Coccidia but also coronavirus (TGE, PEDV, SDCV) and rotavirus. In Austria, several recent investigations aimed to determine the occurrence of single pathogen outbreaks, but studies on mixed infections are rare or either reported many years ago. This part of the study concentrates on viral pathogens inducing diarrhea – see poster P197 for bacterial infections. Identification of the prevalent viruses in combination with other pathogens will help us to improve our understanding of the epidemiology of diarrhea, as well as providing information about the most appropriate therapies or vaccination.

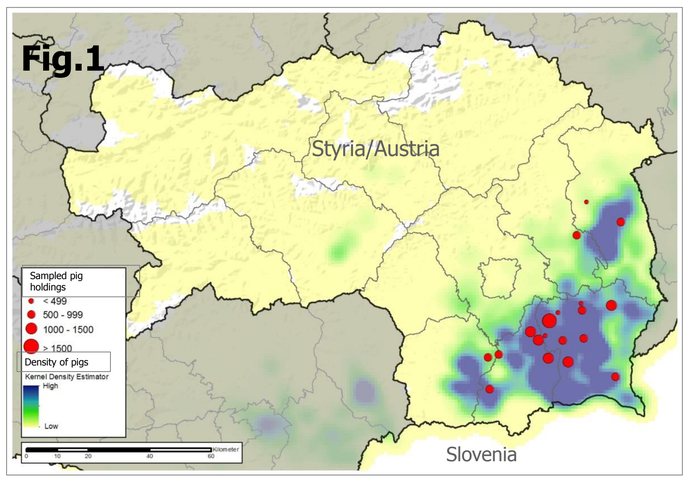

Fig.1: Sampled pig holdings in Styria (red)

Material and Methods: In this study different parts of the gastrointestinal tract (stomach (pars glandularis), duodenum, jejunum, ileum, colon and rectum) of 55 diarrheic pigs of different age from 19 commercial farms in Styria/Austria were submitted for diagnosis (see Fig.1,2). Virological diagnosis was performed by electron microscopy (TEM, negative staining) because of its "open view"; samples were different digestive tract contents and tissue suspensions. Digestive tract samples were also investigated by means of routine histology (H&E) and immunohistochemistry (ABC-technique) for rotavirus. A monoclonal antibody raised against recombinant rotavirus capsid 2B4 (Fa. Santa Cruz Biotechnology Inc./USA) was used. PCR was used to exclude Hepatitis E virus and to determine PCV1 and PCV2.

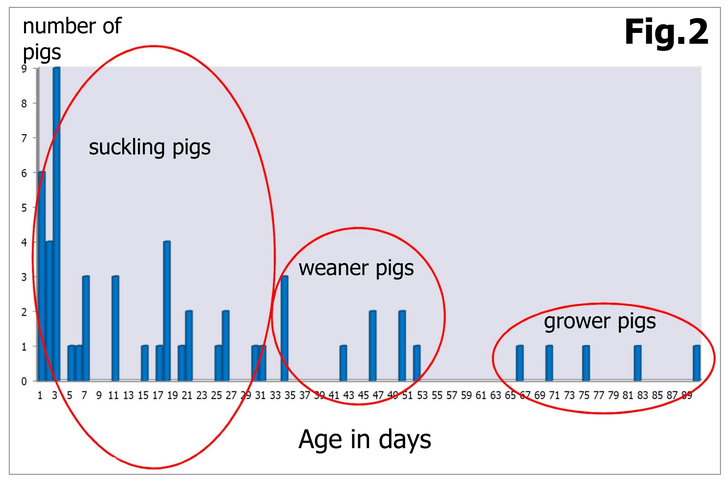

Fig.2: Age distribution of sampled pigs

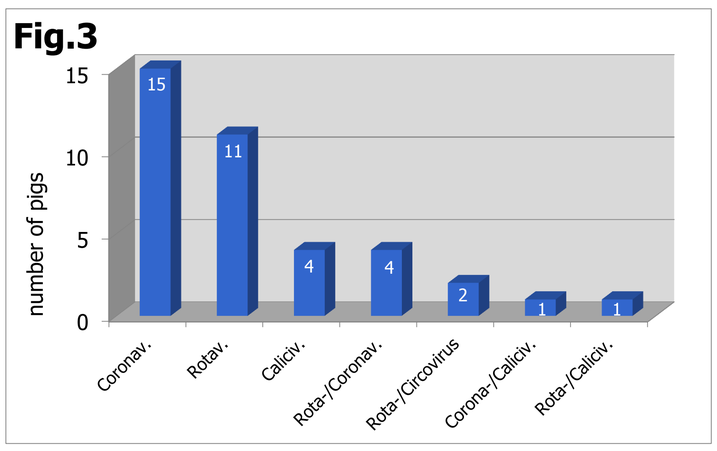

Fig.3: Single and multiple virusinfections

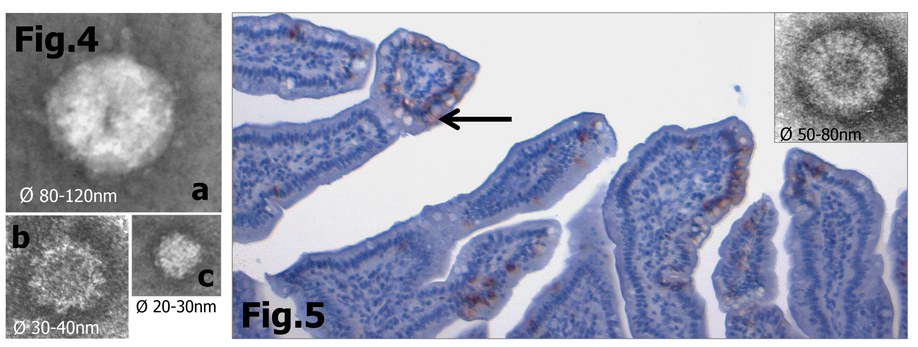

Fig.4: Negative staining/TEM: a) Coronavirus, b) Calicivirus, c) Circovirus
Fig.5: Immunohistochemistry (ABC technique; rotavirus mAB capsid 2B4): Rotavirus-antigen (arrow) as brown-red signals in jejunal enterocytes; insert: TEM/Rotavirus

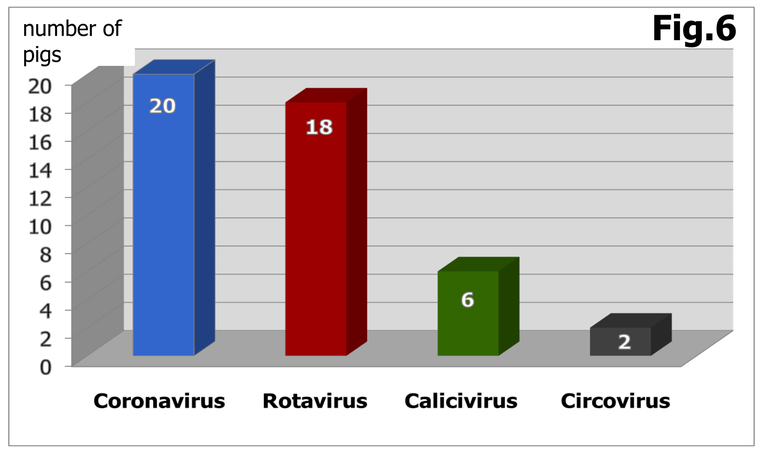

Fig.6: Frequency of virus types detected in sampled pigs

Results and discussion: The occurrence of viruses was proven in 80% of the diarrheic animals. 63,6% of the virus positive animals were infected with one virus type, 36,4% with more than one virus type (Fig.3). Corona- and rotavirus predominated (Fig.6). Presence of coronavirus (Fig.4a) was dominant in 45,5% of the sampled virus positive animals followed by rotavirus (in 40,9%, Fig.5). The occurrance of circovirus (in 43,2% of the samples) was excluded from the study as the minimal frequency of circovirus particles was of no importance for enteral infections in suckling, weaner and young grower pigs. In two diarrheic suckling pigs a high amount of circovirus could be detected and confirmed by PCR as PCV1-type and PCV2-type (Fig.4c). Caliciviruses were detected in 16,6% of the infected animals (Fig.4b). Caliciviruses belonged to the noro- or sapovirus group because Hepatitis E virus was excluded via PCR. No other virus type (e.g. entero-, toro-, adeno- and parvovirus) was detected in our samples. In the animals with coinfection the combination rota-/coronavirus was dominant, followed by rota-/circovirus, corona-/calicivirus and rota-/calicivirus (Fig.3). Virus concentration was high in colon, moderate in rectum and low in small intestine. Rotavirus was found more often in suckling pigs whereas coronavirus was detected both in suckling, weaner and grower pigs (Fig.7).

Histology gave information on the relevance of bacteria and viruses for the acute disease process. Intestinal lesions from diarrheic pigs could be grouped in four different types (A,B,C,D) by reason of histological examination (Fig.8,9,10). Mixed types (e.g. AB) were also present (Fig.10). Pathogenic bacteria were found in all cases of diarrhea with virus incidence. Therefore lesions induced by viruses or pathogenic bacteria were often detected simultaneously in the same sample (see Fig.10). Tissue lesions typical for virus infections were shortening and fusion of intestinal villi, like in Fig.9C. Viral lesions dominated in infected weaner and grower pigs whereas bacterial lesions were more often found in suckling pigs.

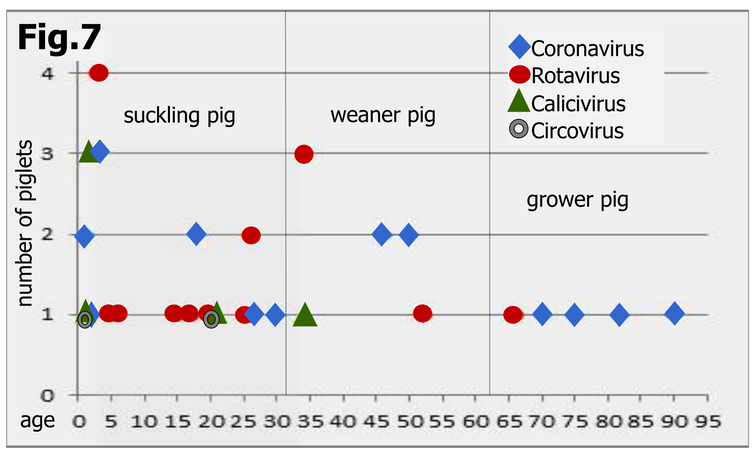

Fig.7: Frequency of virus types / pig age in days

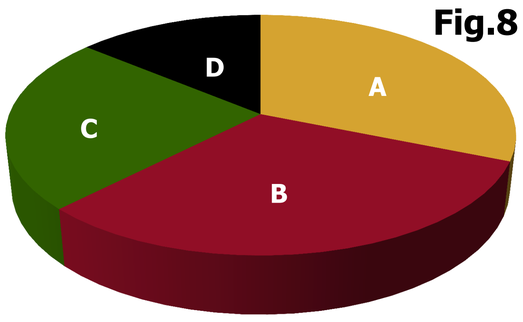

Fig.8: Frequency of the four intestinal epithelium types A,B,C,D detected in the infected pigs.

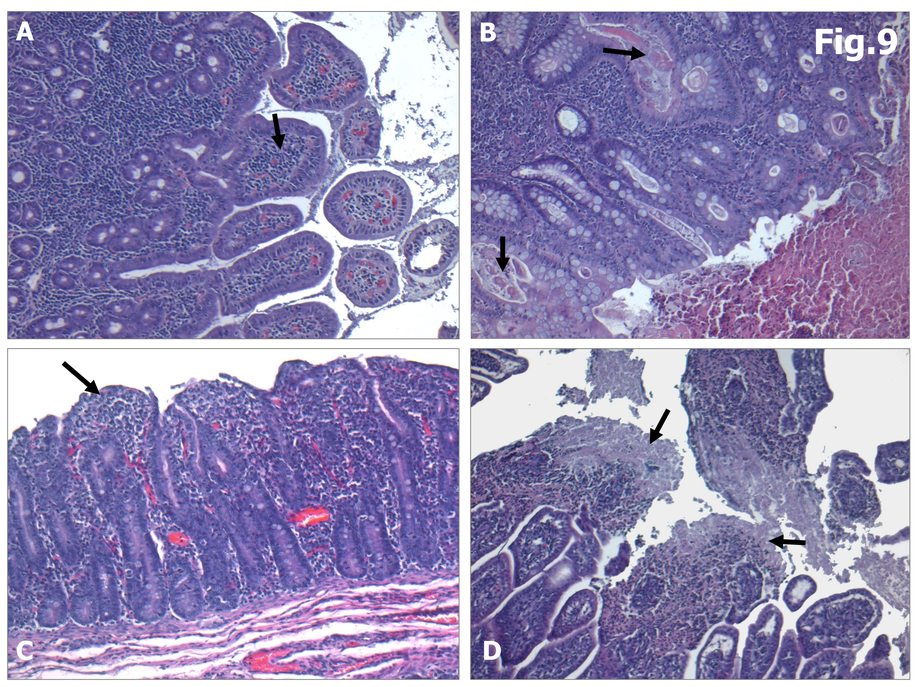

Fig.9: Different histological clusters of the digestive tract of diarrheic pigs (H&E): A) lymphoplasmocytic inflitration of the propria, oedema in the submucosa and subserosa – lesions without specifity, B) granulocytes and detritus around the lymphoglandular complexes, neutrophilic infiltrates in the propria – lesions caused primarily by bacteria and toxins, C) shortening and fusion of intestinal villi – viral lesions, D) apical necrosis of intestinal villi, ulcerations – bacterial lesions

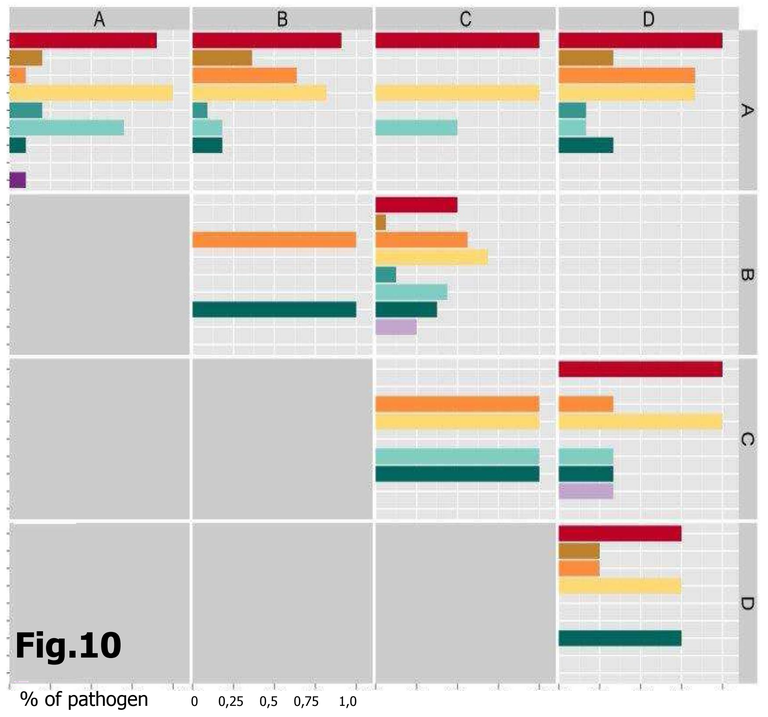

Fig.10: Different histological clusters (A,B,C,D and mixed types e.g. AB) in comparison with the enteral pathogens
• *Clostridium perfringens* A, • *Cl. dfficile*, • haem. *E. coli*, • n. haem. *E. coli*, • Calicivirus, • Coronavirus, • Rotavirus, • Coccidia, • *Trichuris suis*