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Pathological, etiological, epidemiological and experimental examination of certain enteric diseases of poultry (intestinal spirochaetosis of geese and PEC/PEMS)

PhD dissertation thesis

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INTRODUCTION AND AIMS OF THESIS

Due to the achivement of genetics the production of poultry industry increases from year to year. However is not possible to take advantage of benefits of genetics without providing the optimal nutritional, husbandry and health care conditions of which essential part is prevention, or early diagnosis in case of development of diseases and establishing an efficient treatment on the grounds of diagnosis.

The occurence of multifactorial diseases represents a major problem for diagnostics. A number of enteric diseases of poultry such as poult enteritis complex (PEC), poult enteritis-mortality syndrome (PEMS) and avian intestinal spirochaetosis (AIS) fall in this group.

PEC/PEMS are infectious, multifactorial diseases affecting young turkeys between ages of 1-4 weeks. The symptomps of the diseases are diarrhoea, dehydration, growth depression and considareble mortality in certain cases. Their pathogenesis are similar. In the first phase intestinal mucosa is damaged by enteropathogenic viruses which results in absorption disorders. The damaged intestinal mucosa is colonized by bacteria, viruses and fungi. The transitional disorder of the immune system makes possible for the colonising bacteria to enter the blood flow causing considerable mortality. Gross and histopathological lesions are usually similar and non-pathognomonic. Intensive research is performed in order to identify the enteric viruses, playing an important role in damaging intestinal tract Improvement of diagnostic procedures (EM? IEM, PCR) has contibuted to an increase of knowledge as to these viruses.

Astroviruses and rotaviruses were the most frequent identified in faeces of diarrhoeal poult. Coronaviruses were known to play important role of pathogenesis of PEMS. Experimental data proves that these viruses may cause enteric disease in poults. However the number of potential pathogen viruses is larger than these.

Avian intestinal spirochaetosis (AIS) is a disease of birds characterized by colonisation of the caecum and colon by *Brachyspira* species and enteric symptoms. Besides the colonizing Brachyspira strains clinical symptoms are also influenced by genetics, husbandry and nutritional factors, which determine the microbial enviroment of the colonising *Brachyspira*. This also explains why there may be seriuos differences as to the clinical symptoms and pathological lesions in different stocks affected by AIS. Diagnosis is performed on the grounds of culturing and biochemical examinations of the pathogen. Because of the difficulties of culturing and the ambiguity of biochemical characteristics, efforts are made in order to improve the identification of *Brachyspira* with modern molecular biological methods. (PCR, ISH).

There used to be little data as to the occurence of PEMS/PEC in Hungary. The same referes to avian intestinal spirochaetosis. The occurence of the AIS was signalized by the fact that spirochaetes were found in fibrinous necrotizing typhlocolitis of geese and milder form of colitis affecting turkey. However, these pathogens were not accurately identified then.

The aim of this research is to study PEMS/PEC and intestinal spirochaetosis of geese. In the course of the research besides routine diagnostic examinations (gross pathological, histopathological, bacterial, parasitological) molecular diagnostic methods were performed to obtain data as to bacteria, viruses, and parasites occured in intestinal contents and changes caused by these. These data were complemented with results of the immunohistological examination and epidemiological data of the examined stocks. The analysis of the collected data made possible to draw conclusions regarding the pathological role of each agent.

Data as to the occurence of PEMS/PEC in Hungary were collected. In addition to routine diagnostic procedures molecular diagnostic methods were applied to identify turkey coronaviruses (TCoV), turkey astroviruses (TAstV-1, TAstV-2), and avian rotaviruses (ARoV) in birds suspicious of enteric virus infection on the ground of either case history or necropsy findings, The results of virus identification were compared to the results of gross pathological and histopathological examinations to establish whether single viruses or their combinations can cause changes identifiable by means of traditional diagnostic methods. The results of this research was completed with the data gained from the field vets in order to estimate the loss caused by the disease and to draw certain epidemiological conclusions referring the affected stocks

Geese died of fibrinous-necrotizing typhlocolitis were target examined for *Brachyspira*.species. Biochemical properties and antibiotic sensitivity of brachyspira cultured from affected large intestine (caecum colorectum) were analyzed. Further aims of this research were the study of epidemiological properties of the disease. Experimental infection was performed to study the pathogenicity of B. alvinipulli most often cultured from large intestinal changes and the pathogenesis of the disease caused by it.

MATERIAL AND METHODS

Enteric disease of poults

In the course of the research samples submitted to our institutes (CAO VDD Budapest and CAO VDD Kaposvár) for routine diagnostic examinations were processed.
Viral enteric infections were examined in poults (1-6 week), in which the case history or necropsy findings raised the suspicion of viral enteritis or targeted testing for these viruses was specifically requested. Turkey stocks older than 6 weeks showing the clinical symptomps of diarrhea were involved into testing for coronaviruses.

Gross pathological examination was performed according to the usual diagnostic procedure of our institutes. Samples for histopathological examination were fixed in 4% buffered formalin solution and used for making frozen or paraffin embedded sections stained with haematoxylin-eosin.

Bacterial culturing was attempted from heart blood, liver or bone marrow under aerobic conditions on common agar, 10% sheep blood agar and Drigalski's agar media. From a part of the examined birds culturing was attempted from small intestine under anaerobic condition on 10% sheep blood agar medium.

Pooled faeces samples were examined for the presence of parasites.

RNA extracted from the middle part of the small intestine was tested for the presence of enteric viruses (TCoV, TAstV, ARoV) by means of molecular diagnostic (RT-PCR) method. Small intestinal samples were pooled in groups of five samples each. After the homogenization of tissue samples the extraction of RNA was performed by a method using silica particles described in literature.
Specific primers were designed to amplify TCoV, TAstV-1, ARoV target sequences using the published GenBank sequence data. Primers described by Koci et al. 2000. were used to detect TAstV-2. Specificity of primers was controlled by sequencing of PCR products. Sequences were identified by means of a homology-identifying software (BLASTN, National Center for Biotechnology Information, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Intestinal spirochaetosis of geese

In the course of the research samples submitted to our institutes (MGSZH ÁDI Budapest and MGSZH ÁDI Kaposvár) for routine diagnostic examinations were processed.

Intestinal spirochaetosis of geese was diagnosed first in two breeder goose flocks at the end of first egg-laying season. Subsequently targeted testing was performed to culture brachyspirae from geese showing characteristic gross pathological changes.

Gross pathological, histopathological and bacteriological examinations were performed in the same way as described erlier. A certain part of histological sections were stained with Giemsa and with Warthin-Starry silver stain.

Localization of brachyspirae in large intestine was studied by immonohistochemical method. Detection of spirochaetes was attempted by using commercially available FITC labelled rabbit immun serum. This serum was shown to label the *B. alvinipulli* strain isolated from one of the geese and prepared on a glass side. Antigen-antibody binding was detected using a test kit containing a horseradish peroxidase labelled polimer. As chromogen, 3-amino-9-ethylcarbazole solution was used and the sectinos were counterstained with Mayer's haematoxylin.

Culture of brachyspirae from affected colon, caecum and rectum was attempted on selective medium (trypticase soy agar containing 5% sheep blood as well as antibiotics and yeast extract). The media were incubated under anaerobic conditions at 42^0C for 4-6 days. Inoculum taken from the selective medium from sites at the margins of swarming were surface streaked onto Columbia agar containing 5% sheep blood and the cultures were incubated under the condition described earlier.

Biochemical properties (hippurate hydrolysis, α-galactosidase, α-glucosidase, β-glucosidase) of pure cultures of *Brachyspira* strains were tested using Rosco tablets (Rosco, Denmark) according to the manufacturer's recommendations. Indole production of the strains was determined using filter paper impregnated with indole reagent (1 g para-dimethyl-amino-cyanoaldehyde in 100 ml 10% hydrochloric acid).

Lincomycin-, tiamulin, and amoxycillin sensitivity of *Brachyspira* strains were determined by agar dilution method, while those of tetracycline and erythromycin by E test (AB BIODISK, Sweden).

Experimental infection was performed to study the pathogenicity of *B. alvinipulli* most often cultured from large intestinal changes and the pathogenesis of the disease caused by it. 30 clinically healthy day-old geese were disrtibuted randomly in three groups 10 birds in each (A, B, C group). The ten goslings in Group A and Group B each were inoculated with a culture of *B. alvinipulli* and *B. hyodysenteriae* respectively, while the third group (Group C) served as uninfected controll. The goslings were weighed weekly. Three goslings from each group were killed by bleeding at the end of the first and second week and two goslings from each group at 21 and 35 days of age respectively. The goslings killed by bleeding were necropsied and samples were taken for histopathological and immunohistochemical examination and fixed in 4% buffered formalin solution. Culturing of brachyspirae was carried out from the mucous membrane of the ileum, caecum and colon and on postinfection day 14 also from kidney.

RESULTS AND CONCLUSIONS

Enteric disease of poults

Between the years of 2005 and 2007 young poults, the majority aged 1-3 weeks represented 214 stocks examined in the research. On the grounds of molecular diagnostic findings 57% of the stocks proved to be infected at least with one of the examined viruses. In 29% of the infected flocks combined virus infection occured. Simple ARoV or TAstV-1 virus infections occured most frequently and in the case of combined infections the combinations these viruses was the most frequent.

Regarding age related occurence pattern of turkey astro- and avian rotaviruses the simple TAstV-2 infecrtion emerged in flocks younger, than a week and in flocks over the age of two weeks this virus was not identifiable with the expection of a single flock. This pattern occured in the case of the combination of TastV-2 and other viruses. Simple TAstV-1 infection occured most frequently in flocks at the age of about three weeks. In the case of combined infections this virus also was identified at a younger age (two weeks). Regarding the simple ARoV infection similar pattern was not found. This viruses were found in stocks of varies ages.

From the point of view of stocks the infections occured under the age of one week, caused mainly by simple TAstV-2 or the combination of the latter with ARoV. Between the ages of 8-14 days the number of stocks infected with simple ARoV increased. Between the ages of 14-28 days simple ARoV, simple TAstV-1 or the combination of these two viruses were found most frequently in infected stocks. Around the age of three weeks a single TAStV-1 infection became dominant but over the age of four weeks the dominance of the simple ARoV infection was found.

TCoV infection occured in six stocks younger than six weeks. Taking in consideration the fact that the turkey is susceptible to TCoV infection at any age, we tried to identify the virus in the older stocks (> 6weeks) showing characteristical clinical symptoms (diarrhoea, growth depression). 36% of the 44 examined stocks older than six weeks proved to be TCoV infected.

In TCoV infected stocks the course of disease largely varied depending on whether the virus was identified in two weeks of age or younger or four weeks of age or older. The previous, on the grounds of its epidemiological characteristics corresponds the TCOV positive PEMS, while the latter corresponds the turkey coronavirus enteritis.

In case of single virus infections gross pathological changes (starvation, dehydration, catharral enteritis, foamy-watery caecum content*)* were similar regardless of the which virus identified later

In addition to chages in the intestinal mucosa mild to severe atrophy of lymphoid organs mainly the bursa Fabricii were identified by histopathological examination The most severe histological changes were identified in samples derivig from PEMS birds.

Paralell to identification of enteric viruses the mortality rate increased. Besides virus infection other factors too influenced the final mortality rate. In addition to virus infection simultaneous bacterial coinfection was identifiable in affected birds (most frequently E. coli sepsis). This had its most serious effect on mortality in stocks affected by PEMS but also may have contributed to an increasing mortality in other cases.

The growth of the infected birds declined and their body weight did not meet the expected standars of age and technology.

Intestinal spirochaetosis of geese

After the firs identification intestinal spirochaetois of geese in the period between 2005-2007 22 stocks proved to be positive.

The disease developed most frequently in one-year-old breeder stocks at the end of egg-laying. Gross pathologiocal changes such as severe fibrinous-necrotizing typhlocolitis and kidney fibrosis were identified in the affected birds. Most often *Brachyspira alvinipulli* was identified on the basis of the biochemical properties and fenotipical characteristics of the cultures deriving from the affected large intestine.

Experimental infection was performed with the isolated *Brachyspira* strains in one-day old geese. The strains applied colonised the large inestine, primarly the caecum. and could be cultured until the age of five weeks, the end of experiment. The colonisation caused mild clinical symptoms, gross and histopathological canges.

NEW SCIENTIFIC RESULTS

We was the first to collect data as to occurence of certain enteric viruses in turkey stocks showing characteristic clinical symptoms and pathological changes in Hungary.

We was the first to report on the occurence of TCoV positive PEMS in young turkey stocks, and also described the occurence of TCoV infection in affected older turkey stocks in Hungary.

We also found the marked presence of ARoV and TAstV-1 in the examined stocks frequently in combination. The virus infection did not cause as severe consequences as TcoV infection in stocks of similar age.

The applied molecular diagnostic method (RT-PCR) proved to be appropriate for etiological diagnosis of varies enteric virus infections.

We was the first to publish on the intestinal spirochaetosis of geese in the technical literature.

We established that most frequent *B. alvinipulli* can be cultured from the pathological changes in Hungarian goose stocks. We performed examinations to establish the frequency of the affected stocks.

We carried out experimental infections using isolated *Brachyspira* strains and proved, that the bacteria colonise the large intestine primarly the caecum as long as the fifth week of age (the end of the experiment) and causes moderate clinical symptoms and pathological changes.

LIST OF PUBLICATIONS

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