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Central Agricultural Office, Veterinary Diagnostic Directorate, Tábornok u. 2, H-1149 Budapest, Hungary

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* Corresponding author: Ákos Thuma; E-mail: thuma.akos@gmail.com,
Tel: +36 1 460-6300, Fax: +36 1 252-5177

Abstract

The aetiology of increased mortality observed in two breeder duck flocks (Flock A consisting of 3500, and Flock B comprising 4300 laying ducks) during the first egg-laying season was studied. In Flocks A and B, 773 and 715 ducks (18.4% and 16.6%) died within a 24-week and a 20-week period, respectively. Death was preceded by clinical signs including movement difficulties, lack of appetite and depression lasting for one to two days. Diarrhoea was not observed. On gross pathological examination, the ducks were found to have haemorrhagic to fibrinonecrotic typhlocolitis, renal degeneration accompanied by fibrosis and mineralisation, hepatic and splenic amyloidosis, and swelling of some of the metatarsal and phalangeal joints. Histopathological and immunohistochemical examination consistently demonstrated spirochaetes in the mucous membrane of the affected large intestine. On the basis of their cultural and biochemical properties and PCR sequencing analysis, 4 out of 7 spirochaete strains isolated from the ducks (Flock A) by culture on special media under anaerobic conditions were identified as *Brachyspira hyodysenteriae*, and 5 out of 8 strains (Flock B) were identified as *B. pilosicoli*.

This is the first report on the isolation of *B. hyodysenteriae* and *B. pilosicoli* from laying ducks affected by fibrinonecrotic typhlocolitis.

Introduction

Among avian species, natural cases of intestinal spirochaetosis have been described in chickens (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Swayne *et al.*, 1992; McClaren *et al.*, 1997), rheas (*Rhea americana*) (Sagartz *et al.*, 1992), and turkeys (Dwars *et al.*, 1990).
The waterfowl industry, including goose and duck production, has considerable economic importance in Hungary. In an earlier paper, we reported intestinal spirochaetosis causing 18% and 28% mortality respectively, in two breeder goose flocks in the period of moulting at the end of the egg-laying season (Nemes *et al.*, 2006). Eight out of the nine *Brachyspira* strains isolated from the geese were identified as *B. alvinipulli* while the ninth isolate proved to be *B. hyodysenteriae*. *Brachyspira hyodysenteriae*, *B. intermedia* (Janssson *et al*, 2001), *B. alvinipulli* and *B. pilosicoli* (Swayne & McLaren, 1997) have been isolated from asymptomatic wild ducks (*Anas platyrhynchos*).

This paper reports the natural occurrence of intestinal spirochaetosis associated with increased morbidity and mortality in domesticated duck flocks.

Materials and Methods

In Flock A comprising of 4200 primary breeder ducks (3500 laying ducks and 700 drakes), the first egg production period lasted from 5 March to early July. From the end of March the daily mortality started to increase primarily in laying ducks (Figure 1), and a total of 773 ducks (18.4%) (703 laying ducks and 70 drakes) died within a 24-week period. Death was preceded by movement difficulties, lack of appetite and depression lasting for one to two days. Diarrhoea was not observed. In April, May and June the flock was treated with amoxicillin and doxycycline (10 mg/kg/body weight in the feed, for 5 days), without success.
In Flock B, comprising 5000 primary breeder ducks (4300 laying ducks and 700 drakes), the daily mortality increased during the first egg-laying cycle and a total of 715 ducks (16.63%) (550 laying ducks and 165 drakes) died within a 20-week period (Figure 2). The clinical signs observed were similar to those seen in Flock A.

Gross pathological and histopathological examination. From Flock A, a total of 16 dead ducks were submitted to our institute for examination on three different occasions at four-week intervals. From Flock B, a total of 12 ducks (10 laying ducks, 8 dead and 2 sick birds,

and 2 drakes) were submitted to the institute. After gross pathological examination, the kidney, liver, caecum, colon and rectum, and in some cases also the ileum, altered joint capsule, spleen, lungs, heart and brain were fixed in 4% buffered formaldehyde solution, embedded in paraffin, cut into 4 µm thick sections, and stained with haematoxylin and eosin.

Immunohistochemistry. The colon and caecum of all ducks were examined by this method. Detection of *Brachyspira* was performed using a commercially available, fluorescein isothiocyanate-labelled rabbit immune serum (National Veterinary Services Laboratories, Ames, USA). This serum reacts with *Leptospira* cultures belonging to 14 different serovariants and with 5 different *Leptospira* serovariants in the organs of experimentally infected golden hamsters. The specificity of the serum has been tested with a further 13 bacterium species; however, a cross-reaction of the same intensity as that obtained with leptospirae was found only with one of the bacteria tested, i.e. *Brachyspira hyodysenteriae* (Miller *et al.*, 1989). This serum was shown to label the *B. alvinipulli* strain isolated from one goose, and the *B. pilosicoli* and *B. intermedia* isolated from diseased ducks of Flocks A and B. After deparaffination, the sections were heated in citrate buffer solution (pH 6.0) in a microwave oven (750 W, 20 minutes) and subsequently treated in 3% H_2O_2 solution for 10 minutes and then with 2% milk powder solution at 37 °C for 10 minutes. Incubation with the labelled rabbit immune serum diluted 1:10,000 was done at 37 °C for 30 minutes. Antigen-antibody binding was detected using a test kit containing a horseradish peroxidase labelled polymer (EnVision™ + anti-rabbit HRP, Dako, Glostrup, Denmark). As chromogen, 3-amino-9-ethylcarbazole solution (Sigma Aldrich Co., St. Louis, MO, USA) was used and the sections were counterstained with Mayer's haematoxylin. The positive control was a colon section of a pig that had died of swine dysentery, while the negative control was a section on which *Brachyspira*-specific antibody had been replaced with phosphate buffer solution.

Bacteriological examination. Bacterial culture was attempted from heart blood, liver and altered joint fluid under aerobic conditions and from caecal contents and colorectal contents under aerobic and anaerobic conditions on common agar, 10% sheep blood agar and Drigalski's agar.

Culture and identification of Brachyspira. Culture of *Brachyspira* spp. from the affected caecum, colon and rectum was attempted in 28 ducks. After making a 1- to 2-cm-long incision in the affected part of the intestine with sterile scissors, the intestinal mucosa was

scraped with a sterile inoculating loop. The material thus obtained was inoculated onto a selective medium (trypticase soy agar containing 5% sheep blood as well as 6.25 µg/ml vancomycin, 6.25 µg/ml colistin, 25 µg/ml spiramycin, 12.5 µg/ml rifampicin and 200 µg/ml spectinomycin), and the medium was pierced in several places in the line of inoculation (ring test). The media were placed in a jar and incubated with a reagent providing anaerobic conditions (AnaeroGen, Oxoid) at 42 °C for 3–4 days. The presence and extent of ß-haemolysis was observed on the original plate. The shape and motility of the bacteria were examined by dark-field microscopy of fresh preparations made from the *Brachyspira* colonies. Inocula 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 conditions described earlier. *Brachyspira* strains were subcultured two to five times.

Phenotypic characteristics. The biochemical properties of the pure cultures (hippurate hydrolysis, α-galactosidase, α-glucosidase, β-glucosidase) were tested using Rosco tablets (Rosco, Taastrup, Denmark) as described for porcine isolates by Hommez *et al.* (1998). Indole production of the strains was determined using filter paper impregnated with indole reagent (1 g para-dimethyl-amino-cyanoaldehyde in 100 ml of 10% hydrochloric acid). The phenotypic definitions used to identify the species are shown in Table 1.

DNA extraction and PCR amplification. Sequencing and phylogenetic analyses. The fresh pure cultures of 15 putative *Brachyspira* strains from the affected ducks (Flock A and Flock B) were examined. Total DNA was extracted from the cultured bacterial strains using a modified Guanidine HCl based method described previously (Dán *et al.*, 2003). Partial PCR amplification of the nicotinamide adenine dinucleotide reduced oxidase (nox) gene was performed using the primers and protocol described by Townsend *et al.* (2005). Amplified PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen), then sequenced in both directions using the ABI PRISM BigDye Terminator Cycle Sequencing Kit V 3.1. on an ABI PrismTM 3130 Genetic Analyser (Applied Biosystems, Foster City, USA) with the primers used for amplification and two additional interior primers (Townsend *et al.*, 2005). Sequences were corrected, assembled, edited and checked for the presence of restriction endonuclease sites using the programs (EditSeq, SeqMan and SeqBuilder) included in the DNASTAR (Lasergene, WI, USA) software package. Nucleic acid and protein databases were searched using the programs BLASTN and BLASTX (Altschul *et al.*, 1990) at the National

Center for Biotechnology Information, Bethesda, Maryland, USA (http://www.ncbi.nlm.nih.gov). Multiple sequence alignments were built using the MUSCLE multiple alignment software (Edgar, 2004). With the exception of three *B. corvi* strains and uncultured strains all *Brachyspira* spp. sequences longer than 850 base pairs (bp) in the corresponding nox gene region were included in the alignment. The resulting partial nox gene alignment with an 850 positions in the final dataset was used as input to generate a tree using the Molecular Evolutionary Genetics Analysis (MEGA) software v.4.0 (Tamura *et al.*, 2007). The evolutionary distances were computed using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) implemented with the Maximum Composite Likelihood model (Tamura *et al.*, 2004). The topology of trees was confirmed by 1000 bootstrap replicates (Felsenstein, 1985).

Results

Gross pathological examination. The ducks 28 submitted from the two flocks to the institute for necropsy showed slightly poorer than medium body condition and pathological changes in the caecum, colorectum, kidney, liver, spleen and metatarsal and phalangeal joints (Table 2). In 26 cases (93%) the caecum and colorectum were slightly dilated and contained a reddish, foul-smelling content, often containing fibrin strands. The mucous membrane was swollen and hyperaemic, showed superficial necrosis in some segments and was covered by a fibrinous pseudomembrane (Figure 3). In six birds (21%) an acute catarrhal ileitis was observed. The kidneys of all the ducks were swollen and were pale yellowish-brown in colour (Figure 4). The liver and spleen of 23 ducks (88%) were swollen and greyish-brown. In 21 laying ducks (87%) follicular degeneration and atrophy were seen in the ovaries, while in the two drakes examined testicular atrophy was observed. In 18 ducks (69%) one or more metatarsal or phalangeal joints were swollen (Figure 5). The articular wall was thickened and a slightly increased volume of yellowish-brown fluid was seen in the joint cavity.

Histopathological examination. The colorectal and caecal mucosa was swollen, the epithelial cell layer was necrotic and had become detached in some parts or was covered by a muco-fibrinous exudate (Figure 6). The lamina propria contained scattered haemorrhages and was infiltrated with lymphocytes, histiocytes and heterophilic granulocytes. In 13 cases (46%), necrosis of the colorectal mucosa extended into the upper third of the lamina propria.

Segmental or diffuse cell injury of the renal tubular cells was observed in 19 cases. In addition, focal or diffuse intertubular fibroplasia with consequent atrophy of the glomeruli and tubules, accompanied by mineralisation, were detected in 9 ducks (Figure 8). In the livers and spleens showing gross pathological change, multifocal or diffuse deposits of amyloid were observed. The altered joint capsules showed lymphocytic, histiocytic and heterophil granulocytic infiltration with marked fibroblast proliferation. No significant changes were observed in the other organs examined.

Immunohistochemical examination. Bacteria with typical undulating morphology were detected in the caecum and colon of all the ducks examined (Figure 7). Spirochaetes stained with similar intensity in the pig colon that was used as the positive control, while in the negative control sections no labelling was observed. The spirochaetes were located singly or in groups in the intestinal lumen of the ducks, directly above the epithelial cells or within the intestinal glands. Occasionally they occurred among the necrotic and detached enterocytes as well as in the superficial lamina propria. They could not be detected within the enterocytes.

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Bacterial culture. Bacteria could not be cultured in any of the cases from the heart blood and liver under aerobic conditions. From the synovial fluid of the altered metatarsal and phalangeal joints *Erysipelothrix rhusiopathiae* (Flock A, 4 cases; Flock B, 1 case), *Escherichia coli* (Flock A, 1 case; Flock B, 1 case) and *Staphylococcus aureus* (Flock B, 1 case) were isolated. From the mucous membrane of the colorectum and caecum, *E. coli* and enterococci grew out under aerobic conditions.

Under anaerobic conditions, *Brachyspira* spp. was isolated in 15 cases from the mucous membrane of the affected intestinal segments (caecum, colorectum). No clostridial species were detectable in any of the intestinal specimens. The biochemical properties of *Brachyspira* isolates are demonstrated in Table 3. Ten out of the fifteen isolates could be identified to the species level on the basis of their phenotypic and genotypic properties. One strain (24916/2) proved to be *B. suanatina* by genotypic and then also by phenotypic analysis (it gave negative indole and positive α-glucosidase reaction, white background in Table 3). Four strains could not be identified on species level by either phenotypic or genotypic tests (white background in Table 3).

Sequence and phylogenetic analyses. Based on nox gene sequence similarity, computational restriction endonuclease analysis (Rhode *et al*., 2002; Townsend *et al.,* 2005) and

phylogenetic analysis, 10 out of the 15 *Brachyspira* spp. strains cultivated from affected ducks were reliably identified as known species (Figure 9, Table 4). One strain (24916-2; Farm A) was identified as *B. suanatina*, five strains from Flock B (10363: 1, 2, 3, 5 and 9757-2) were identical at nucleotide level and were identified as *B. pilosicoli*, while four strains from Flock A (21330-3, 29086: 1, 2, 3) were *B. hyodysenteriae*. These last mentioned strains were also identical at nucleotide level. The remaining five duck isolates could not be unequivocally assigned to any of the presently known *Brachyspira* spp. Sequences obtained from the present study have been submitted to the GenBank under the following accession numbers: HM462456, HM462457, HM462458, HM462459, HM462461, HM462462 and HM462463.

Discussion

Avian intestinal spirochaetosis (AIS) is a disease of birds characterised by a pronounced colonisation of the caecum and/or rectum with anaerobic intestinal spirochaetal bacteria of the genus *Brachyspira*. AIS has been recorded mainly in flocks of laying hens and broiler breeder hens, where it causes a mild to moderate, subacute to chronic disease (Davelaar *et al.*, 1986; Dwars *et al.*, 1989; McLaren *et al.*, 1997; Stephens & Hampson, 2001; Hampson & Swayne, 2008). AIS has not been diagnosed in broiler flocks, but has been reported as a sporadic condition in other domesticated poultry species such as turkeys (Dwars *et al.,* 1990) and in game birds including pheasants and partridges (Jansson *et al.*, 2001). Severe AIS has been reported in common rheas (Sagartz *et al.*, 1992) and in geese in Hungary (Nemes *et al.*, 2006). Subclinical colonisation either with pathogenic or apathogenic *Brachyspira* species is common in feral waterfowl, particularly ducks (Jansson *et al.*, 2004; Oxberry *et al*., 1998; Hampson & Swayne, 2008).

Of bacteria belonging to the genus *Brachyspira*, the pathogenicity of *B. hyodysenteriae, B. intermedia, B. pilosicoli* and *B. alvinipulli* has been documented and experimentally reproduced in various avian species. In a few cases, *B. innocens, B. murdochii* and certain hitherto unclassified spirochaetes have also been detected from the intestinal tract of birds; however, these were not pathogenic in experimental infections (Hampson & Swayne, 2008).

The investigations reported in this paper identified *B. hyodysenteriae* and *B. pilosicoli* associated with intestinal lesions of laying ducks and drakes. These ducks had fibronecrotic

typhlocolitis, nephrosis and interstitial renal fibrosis. In the majority of cases, spirochaetes were detected deep in the mucosa and were isolated from the large intestine. These observations suggest a pathological role of the spirochaete in the disease, but this does not exclude a potential synergistic role of anaerobic rods (clostridia) in producing the necrotic intestinal lesions (Hampson & Swayne, 2008). However, attempts to isolate clostridia failed.

The clinical signs, gross lesions and histopathological changes of intestinal spirochaetosis described in different avian species (Hampson & Swayne 2008) are similar to those of intestinal spirochaetosis occurring in mammals, especially in swine, and their main feature is a haemorrhagic-to-necrotic colitis. However, in no species has there been an indication of severe renal lesions similar to those observed in the sick and dead ducks from this study, and seen by us previously in geese (Nemes *et al.*, 2006). The development of severe renal lesions in geese and ducks may be attributed to certain species-related characteristics, e.g. the sensitivity of the excretory system of waterfowl to dehydration or to the adverse effect on the kidneys by toxins absorbed from the affected large intestine. In addition, the amyloid deposits seen in the spleen and liver and the high incidence of arthritic lesions especially in the metatarsal and phalangeal joints were remarkable in the sick and dead ducks in both Flocks A and B.

Brachyspira species typically have alkaline and acid phosphatase, esterase, lipase, ß-galactosidase, and phosphorylase activities (Stoutenburg, 1993). Differences in the patterns of haemolysis, indole production, hippurate hydrolysis and the presence or absence of α-galactosidase activities have been used to categorise isolates. As these phenotypic properties can vary, biochemical testing should be combined with more specific molecular techniques and phylogenetic analyses for species identification (Bano *et al.*, 2008; Feberwee *et al.*, 2008; Hampson & Swayne, 2008).

Feberwee *et al.* (2008) reported that less than half of 73 *Brachyspira* isolates could be identified on species level based on their biochemical phenotypes, while all but four isolates (5.2%) were defined by PCR and sequencing of the DNA extracted from the bacteria.

The lack of an accurate validated diagnostic technique for *Brachyspira* spp., especially in birds, is well known. Besides the traditional laboratory diagnostic procedures, several reliable molecular methods are available for characterising and typing these bacteria, but all of them have disadvantages and limitations (Jansson, 2009).

In the present study, 9 out of 15 *Brachyspira* species isolated from ducks were unambiguously identified as *B. hyodysenteriae* or *B. pilosicoli* by the use of traditional and DNA-based methods. Strain **21330-2** (Farm A) shows high similarity values (Table 4; 99%

amino acid similarity representing one amino acid difference out of 298 amino acids) with the reference *B. intermedia* isolate and is closely related phylogenetically to the distinctly clustering *B. intermedia* group (Figure 9). The *Bfm*I restriction profile corresponds to that described by Townsend *et al*. (2005) for *B. intermedia* (Table 4) strains. Due to one silent mutation, however, the *Dpn*II restriction site is missing, and therefore this *Brachyspira* spp. isolated from duck shows a pattern different (898, 41) from the rest of *B. intermedia* published and from all sequences available in the GenBank.

Strain 24916-1 (Farm A) showed a restriction pattern identical with that of avian isolates 701 and 802 analysed by Townsend *et al.* (2005), and suggested to be representatives of the proposed genus *'B. pulli'*. Unfortunately, there are no nox gene sequences available in the GenBank database for the strains in question to allow a sequence comparison. Strains 10363-4 and 10363-6 (Farm B) show a restriction enzyme profile identical with *B. hyodysenteriae.* All the same, the high sequence similarity with *B. alvinipulli* (91% and 95% at nucleotide and amino acid level, respectively) and the location of this strain on a distinct phylogenetic branch with *B. alvinipulli* (Figure 9) make it difficult to allocate this strain to any known *Brachyspira* spp. Strain **9757-1** (Farm B) shows high percentage similarities (Table 4) with, and is closely related to, the proposed genus *B. suanatina* isolated from pigs and mallards (Rasback *et al.*, 2007). Strain **9757-1** displays a new *Bfm*I enzyme nox gene restriction profile not found in previously characterised species. However, based on a predictive computational restriction site analysis of the six *B. suanatina* nox gene sequences deposited in the GenBank (accession numbers shown in Fig. 9), the restriction enzyme pattern (*Bfm*I: 691, 248; *Dpn*II: 684, 214, 41) obtained with the two enzymes used would be very similar to that of strain 9757-1 (Table 4).

Although the phenotypic profiles did not correspond, strain **24916-2** could be determined as *B. suanatina* by DNA-based methods. *B. suanatina* is a rather new species and its typical biochemical profile has not yet been defined by studying numerous isolates from this species. The strains isolated from ducks provide further evidence for the huge population diversity of *Brachyspira* spp. found in avian species.

Different *Brachyspira* spp. were found within a single flock and also in cultures from single birds, emphasising the need to obtain multiple samples when investigating outbreaks of avian intestinal spirochaetosis. It was not surprising to find a rather considerable correlation between the results of the phenotypic and genotypic characterisation of the isolates as they had been isolated from areas with intestinal pathology. Mixed cultures may occur but lesions are caused by a large number of one (or more) pathogenic strain(s) (Feberwee *et al.*,2008).

This is the first report on the isolation of *B. hyodysenteriae* and *B. pilosicoli* from laying ducks affected with fibronecrotic typhlocolitis.

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Legend of figures

Figure 1. *Change of the weekly mortality rate in Flock A comprising 3700 laying ducks and 700 drakes*

Figure 2. *Change of the weekly mortality rate in Flock B comprising 4307 laying ducks and 750 drakes*

Figure 3. *The membrane of the colorectum is swollen and hyperaemic, in some segments it shows superficial necrosis and is covered by a fibrinous pseudomembrane*

Figure 4. *The kidneys are swollen and show pale yellowish-brown discoloration*

Figure 5. *The metatarsal joint is swollen*

Figure 6. *The colorectal mucosa is swollen and infiltrated with lymphocytes, histiocytes and heterophilic granulocytes. The epithelial layer is necrotic and covered by a mucous-fibrinous exudate*

Haematoxylin and eosin (H.-E.) stain, Bar = 55 µm

Figure 7. *Brachyspira spirochaetes between the degenerated and detached mucosal epithelial cells*

Immunohistochemical reaction, Bar = 30µm

Figure 8. *Degeneration and atrophy of the tubular epithelial cells, and intertubular fibroblast cell proliferation in the kidney*

H.-E. stain, Bar = 55 µm

Figure 9. *Partial nox gene phylogenetic tree of 59 Brachyspira isolates including the sequences analysed in this study. The evolutionary history was inferred using the UPGMA method with 1000 bootstrap replicates. Bootstrap values over 83% are shown in nodes. The scale bar indicates genetic distance. GenBank accession numbers are assigned for each isolate. Sequences resulting from this study are indicated in bold*

Table 1. *Biochemical properties for differentiating of Brachyspira species*

species	haemolitic zone on blood agar	indol reaction	hippurat	α-galactosidase	α-glucosidase	β-glucosidase
B. aalborgi	weak	-	weak	-	-	-
B. alvinipulli	weak	-	+	+/-	-	+
„B. canis"	weak	-	-	+	-	+
B. hyodysenteriae	strong	+/-	-	-	+/-	+
B. innocens	weak	-	-	+	+/-	+
B. intermedia	weak	+	-	-	+	+
B. murdochii	weak	-	-	-	+/-	+
B. pilosicoli	weak	+/-	+/-	+/-	-	-
„B. pulli"	weak	+/-	NA	+	+/-	+
„B. suanatina"	strong	+	-	-	-	+

Table 2. Gross- and histopathological findings in two (A and B) duck flock

	FLOCK A	FLOCK B	TOTAL
No. of investigated animals	16 (♀)	12 (10♀ , 2♂)	28
Gross- and histopathological findings:			
fibrinonecrotic typhlitis and colorectitis	16	10	26 (93%)
swollen, yellowis-brown kidney	16	12	28 (100%)
tubularnephrosis	10	9	19 (73%)
chronic (fibrotic) nephropathy	6	3	9 (35%)
swollen, greyish-brown liver and spleen	14	9	23 (88%)
amyloid deposites in the spleen and liver	14	9	23 (88%)
arthritis (metatarsal and phalangeal joints)	10	8	18 (69%)
ovarial degeneration (follicular atrophy)	14	7	21 (87%)*
testicular atrophy	/	2	2 drakes

*Remark: % of laying ducks

Table 3. Biochemical properties of the Brachyspira strains isolated from duck flocks

Flocks	Flock A							Flock B							
Strains	21330/2	21330/3	24916/1	24916/2	29086/1	29086/2	29086/3	9757/1	9757/2	10363/1	10363/2	10363/3	10363/4	10363/5	10363/6
Haemolysis	weak	strong	weak	strong	strong	strong	strong	weak	weak	weak	weak	weak	weak	weak	weak
Ring test	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Indole	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Hippurate hydrolysis	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-
α-galactosidase	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+
α-glucosidase	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-
β-glucosidase	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+
phenotype	intermedia	hyodysentariae	spp	suanatina?	hyodysentariae	hyodysentariae	hyodysentariae	spp	pilosicoli	pilosicoli	pilosicoli	pilosicoli	spp	pilosicoli	spp
genotype	intermedia?	hyodysentariae	spp	suanatina	hyodysentariae	hyodysentariae	hyodysentariae	spp	pilosicoli	pilosicoli	pilosicoli	pilosicoli	spp	pilosicoli	spp

Table 4. Partial nox gene restriction endonuclease cutting site positions identified and sequence similarity of the Hungarian *Brachyspira spp*. isolated from ducks. Results are included in a Table containing the predicted fragment sizes of the *Brachyspira spp*. reference strains (grey background) published by Townsend *et al.* (2005).

Species	*Bfm*I (bp)	*Dpn*II (bp)	*Nucleotide and amino acid similarity
B. aalborgi	453, 248, 238	939	
B. alvinipulli	742, 197	898, 41	
B. pilosicoli	742, 197	898, 41	
10363: 1, 2, 3, 5 (Farm B) **9757-2**			99% and 100% *B. pilosicoli* strain P43/6/78 AF060807 and AAC78816
B. hyodysenteriae	742, 197	684, 214, 41	
21330-3 (Farm A) **29086: 1, 2, 3 (Farm A)**			100% and 100% *B. hyodysenteriae* strain B78 AF060807 and AAC78809
10363-4, 6 (Farm B)			91% and 96% *B.alvinipulli* strain C AF060814 and AAC78823
B. intermedia	504, 238, 197	684, 214, 41	
21330-2 (Farm A)	504, 238, 197	898, 41	97% and 99% *B. intermedia* strain PWS/A AF060811 and AAC78820
B. innocens	504, 211, 197, 27	684, 214, 41	
B. murdochii	504, 211, 197, 27	684, 157, 57, 41	
24916-1 (Farm A)	453, 238, 197, 51	939	91% and 95% Uncultured *B. spp.* Clone A_S4 FJ599592 and ACM41754
*B. suanatina*** **24916-2 (Farm A)**	691, 248	684, 214, 41	100% and 100% *B. suanatina* strain AN 3949:2/02 DQ487123 and ABF38943
9757-1 (Farm B)	691, 197, 51	684, 214, 41	97% and 98% *B. suanatina* strain AN 1418:2/01 DQ487124 and ABF38944

*Strains and their GenBank nox sequence accession numbers showing the highest BLASTN and BLASTX similarity with the sequences determined in this study.

** Restriction endonuclease cutting site positions identified in this study.

Figure 1. Change of the weekly mortality rate in Flock A comprising 4200 ducks

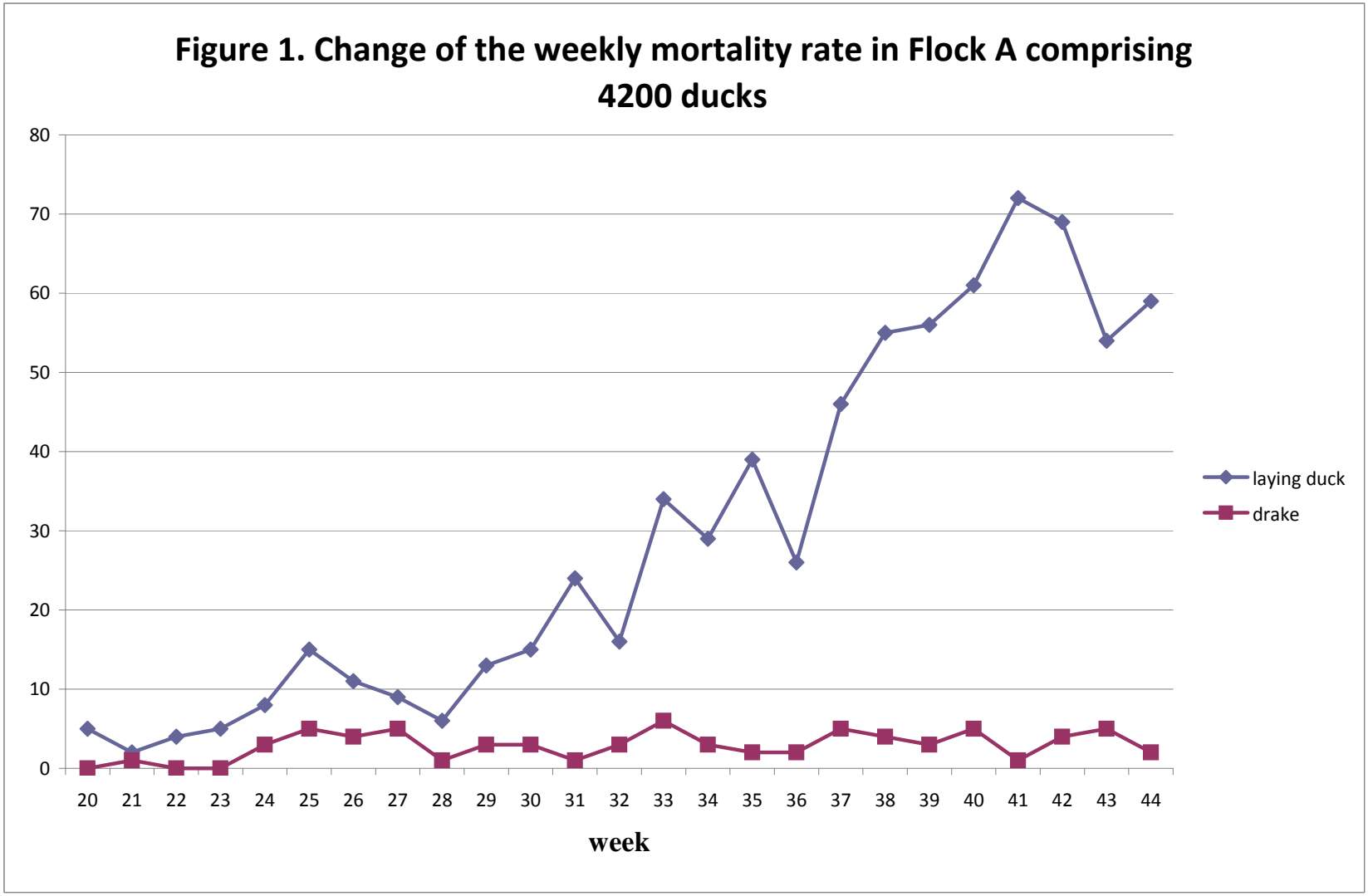
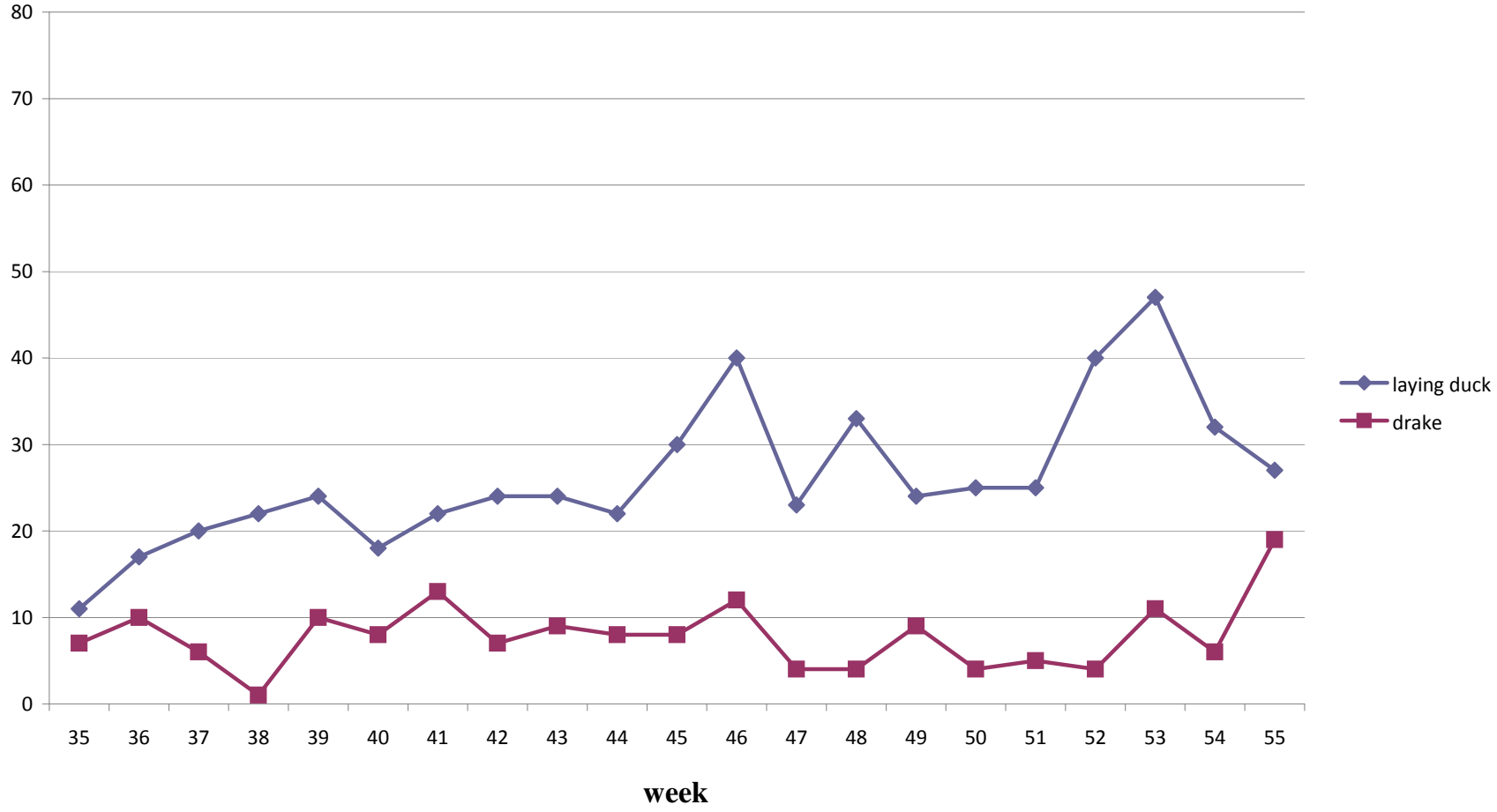
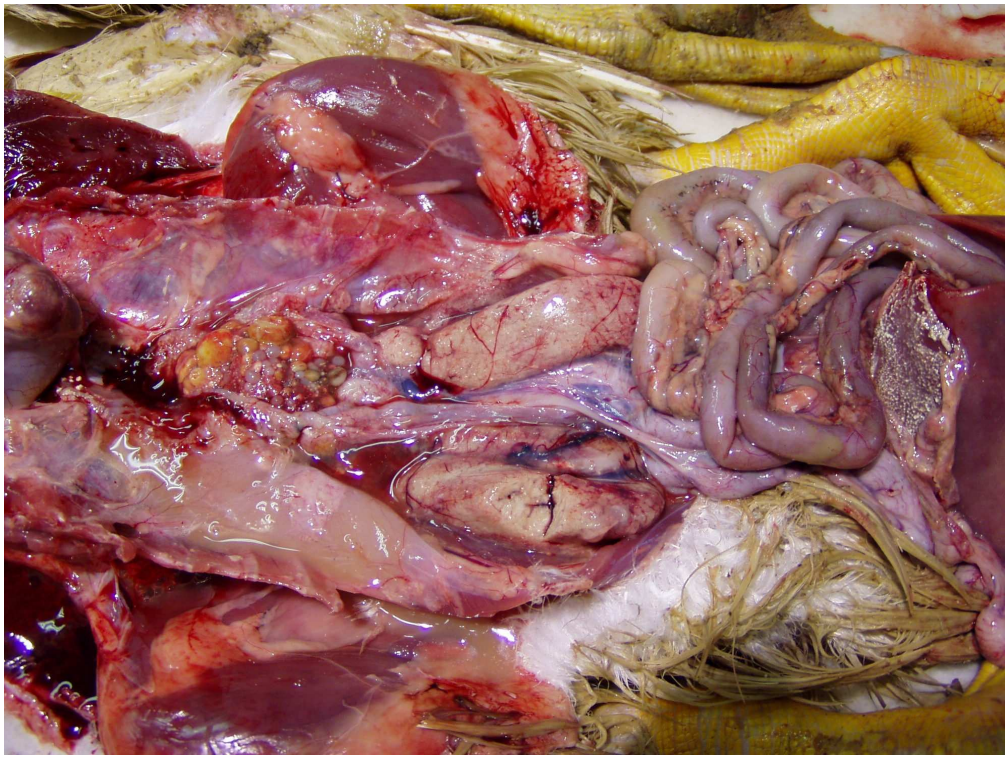


Figure 2. Change of the weekly mortality rate in Flock B comprising 4870 ducks



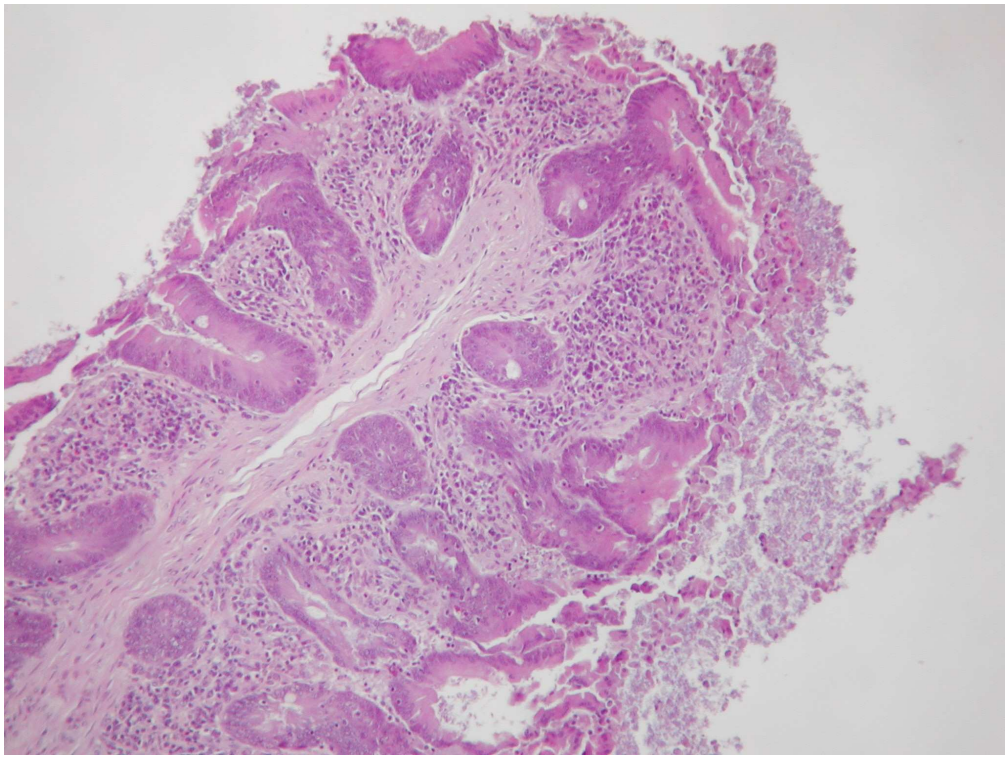
g duck

The membrane of the colorectum is swollen and hyperaemic, in some segments it shows superficial necrosis and is covered by a fibrinous pseudomembrane
219x164mm (300 x 300 DPI)



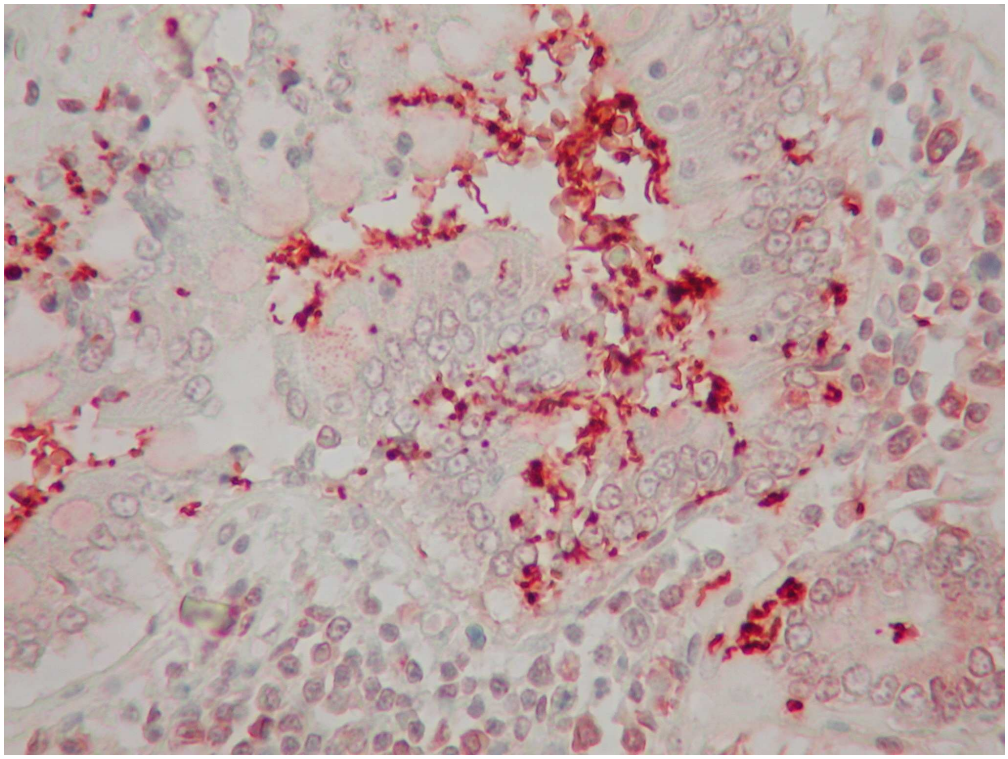
The kidneys are swollen and show pale yellowish-brown discoloration
349x262mm (144 x 144 DPI)

The metatarsal joint is swollen
914x685mm (72 x 72 DPI)



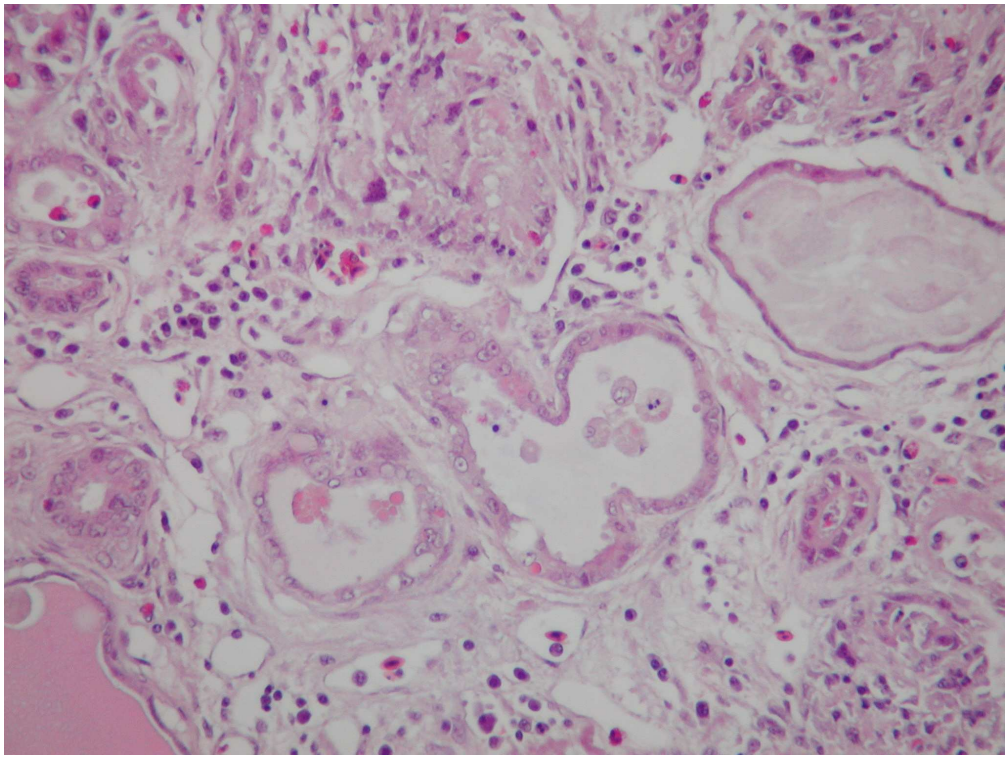
The colorectal mucosa is swollen and infiltrated with lymphocytes, histiocytes and heterophilic granulocytes. The epithelial layer is necrotic and covered by a mucous-fibrinous exudate Haematoxylin and eosin (H.-E.) stain, Bar = 55 µm

173x130mm (300 x 300 DPI)



Brachyspira spirochaetes between the degenerated and detached mucosal epithelial cells
Immunohistochemical reaction, Bar = 30µm

173x130mm (300 x 300 DPI)



Degeneration and atrophy of the tubular epithelial cells, and intertubular fibroblast cell proliferation in the kidney
H.-E. stain, Bar = 55 µm

173x130mm (300 x 300 DPI)