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NECROTIZING LESIONS IN THE INTESTINE, GIZZARD, AND LIVER IN CAPTIVE CAPERCAILLIES (*TETRAO UROGALLUS*) ASSOCIATED WITH *CLOSTRIDIUM PERFRINGENS*

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ABSTRACT: During the period from 1982 to 1991, 863 captive and 32 wild capercaillies (*Tetrao urogallus*) were necropsied. The most common cause of death in captive capercaillies was necrotizing enteritis, diagnosed in 110 (13%) birds. Of these, 31 (28%) birds also had necrotizing lesions in the liver. Necrotizing gastritis occurred in 29 birds, two of which had concurrent necrotizing enteritis. In the capercaillies with necrotizing enteritis, *Clostridium perfringens* type A was isolated more frequently and in larger numbers than in birds which died from other causes. Thus, *Clostridium perfringens* type A may be of etiological importance in necrotizing enteritis. Necrotizing enteritis was not diagnosed in wild capercaillies.

Key words: Capercaillie, *Tetrao urogallus*, *Clostridium perfringens*, necrotizing enteritis, necrotizing gastritis, pathology.

INTRODUCTION

Since the beginning of the 1980's, considerable attention has been given to the breeding of capercaillies (*Tetrao urogallus*) in Norway. More than 100 small breeding units were established for the production and sale of live animals. The long term objective was production of high quality meat. One problem has been the occurrence of diseases which caused substantial losses.

Necrotic enteritis (NE) is a disease occurring primarily in broiler chickens (Parish, 1961). Necrotic enteritis also has been reported to occur in tetraonids (Aschenbrenner, 1987) and in wild geese (Wobeser and Rainnie, 1987). *Clostridium perfringens* is considered to be an important etiologic agent of NE (Shane et al., 1985). Ulcerative enteritis (UE) or "quail disease" is primarily a disease of quail, but also occurs in other gallinaceous birds (Peckham, 1959), and is caused by *Clostridium colinum* (Berkhoff et al., 1974). This disease has been recorded in captive capercaillies by Tschirch and Wilhelm (1983), although they made no attempt to isolate *C. colinum*.

In this study, we describe an enteritis characterized by focal necrosis and diphtheric membranes in the intestine, with occasional concurrent necrotizing lesions

in the liver. This disease is designated "necrotizing enteritis."

MATERIALS AND METHODS

A total of 863 captive capercaillies from 86 different breeding units, and 32 wild capercaillies, were submitted to the National Veterinary Institute, Oslo, Norway, for necropsy during the period of 1982 to 1991. The study is retrospective and thus the materials were not systematically collected. Approximately half of the captive bred birds had been frozen for a short period varying from a few days up to 8 wk, before being dispatched to the laboratory. A few birds had been frozen for up to 8 mo. Generally, only a few of the dead birds from epizootics with high mortality were submitted for necropsy. Information on mortality rates and use of antibiotics during these outbreaks were frequently not available. The wild birds included in the study all had been found dead and were sent to the laboratory for post-mortem examination.

Body condition and gross lesions were recorded. For histological examination, tissue specimens were collected and fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin. Gram-stained histological sections occasionally were prepared.

Routinely, bacteriological examination of intestinal contents from all birds with necrotizing enteritis was carried out by inoculation on heart infusion agar (Difco Laboratories, Detroit, Michigan, USA) with 5% bovine blood, and bromthymolblue lactose sucrose agar plates; this medium is composed of heart infusion agar (Difco) supplemented with 1% sucrose (Merck,

Darmstadt, Germany), 1% lactose (Difco), 0.1% Na-thio-sulphate (Merck), 0.008% bromthymol-blue (Merck) and 0.005% crystal violet (Merck). One of each plate was incubated at 37 C overnight, respectively, in aerobic and anaerobic (85% nitrogen, 10% hydrogen, and 5% carbon dioxide) atmosphere. This procedure also was employed for gizzard mucosa debris when gross pathological changes were observed in this organ. In addition, intestinal contents from 72 captive capercaillies submitted between 1988 and 1990, were subjected to quantitative examination for *C. perfringens*, coliforms, and lactobacilli/streptococci (Hofshagen and Kaldhusdal, 1992). The 72 birds were classified, according to necropsy findings, as birds without enteritis, birds with necrotizing enteritis, and birds with catarrhal enteritis.

Typing of *C. perfringens* was performed on 44 strains isolated from birds with necrotizing enteritis and 49 strains from birds without enteritis, based on the presence of alpha, beta or epsilon toxins. A strain was considered positive for alpha toxin (phospholipase C) when producing an opaque zone on egg yolk agar. The possible production of beta or epsilon toxin were measured by means of enzyme-linked immunosorbent assay tests described by Martin et al. (1988), and Naylor et al. (1987).

Chi-square tests (Dean et al., 1990) were used to test differences in occurrence of necrotizing enteritis and gastritis in relation to age and to *C. perfringens* infection in birds with different diagnosis. Results from quantitative bacteriological examinations were analyzed with a Wilcoxon two-sample test (Dean et al., 1990). *P*-values ≤ 0.05 were considered significant.

RESULTS

Necrotizing enteritis was diagnosed in 110 (13%) of 863 captive capercaillies, and was the most frequent cause of death in captive capercaillies (Table 1). Necrotizing lesions similar to those observed in captive birds were not found in any of the wild capercaillies examined.

Necrotizing lesions were found in the small intestine of 106 (96%) of the 110 captive birds with intestinal lesions. The duodenum, jejunum, and ileum all seemed equally affected; however, lesions normally were found in a confined portion of varying length within one or more of the small intestinal segments. In 35 (33%) of these 106 birds, necrotizing lesions were found concurrently in the large intestine.

TABLE 1. The occurrence of necrotizing gastritis and enteritis in relation to age in captive bred capercaillies submitted to the National Veterinary Institute, Norway, for investigation, 1982 to 1991.

Age	n	Number of birds (%) with	
		Necrotizing gastritis	Necrotizing enteritis
0 to 4 wk	356	0 (0) ^a	4 (1) ^a
Juvenile	275	9 (3) ^b	67 (24) ^b
Adult	232	20 (9) ^c	39 (17) ^c
Total	863	29 (3)	110 (13)

^{a,b,c} Different superscripts within column indicate significant difference by a Chi-square test ($P \leq 0.05$).

In these 35 birds, both colon and cecum were affected. Within the cecum, however, necrotizing lesions were confined primarily to the neck segment. Four (4%) of the 110 birds with necrotizing lesions had these lesions in the cecum only.

Two of the 29 capercaillies diagnosed as having necrotizing gastritis (Table 1) had concurrent necrotizing enteritis and necrotizing gastritis; the other 27 birds had lesions in the gizzard only.

In birds with necrotizing enteritis, the most common manifestations were multiple necrotic plaques, 1 to 3 mm in diameter, and confluent diphtheric membranes in the small intestinal mucosa. These lesions appeared as greyish-white spots from the serosal side of the intestine.

In acute cases of necrotizing enteritis, the intestine was hyperemic and congested, with hemorrhagic intestinal content. Scattered mucosal ulcerations and necrotic foci were observed in these birds. In subacute to chronic cases, affected parts of the intestines appeared slightly dilated and pale, and contained a watery fluid with agglutinated particles.

Microscopically, the necrotic foci consisted of demarcated areas of the mucosa with loss of villi, and frequent extension into the submucosa. These foci were composed of degenerate epithelial cells, mononuclear leukocytes, heterophilic granulocytes, fibrin, and gut content. Gram-positive bacilli in large numbers consistently were observed in Gram-stained

sections of affected areas. Occasionally, the necrotic foci were surrounded by a hemorrhagic border. In areas with confluent necrosis and diphtheric membranes, the intestinal mucosa was totally destroyed.

Necrotizing lesions observed in the gizzard usually were confined to the mucosal lining of the cranial or caudal diverticulum. Occasionally, the horny layer of the gastric cuticula was undermined and partly loosened. The majority of birds with necrotizing gizzard lesions came from one farm using artificially crushed quartz for grit.

Lesions were observed in the liver of 31 (28%) of the 110 capercaillies with necrotizing lesions in the gastrointestinal tract. The livers were swollen and congested with focal or coalescing, well-demarcated, pale, greyish areas on the cut surface. Histologically, focal and confluent, non-zonal, degenerative and necrotic lesions with mild leucocyte infiltration and reticuloendothelial proliferation were observed. In two birds, the liver was affected without any concomitant intestinal lesions being observed.

Clostridium perfringens was isolated from the intestinal contents in 100 (91%) of the 110 birds with necrotizing enteritis. *Clostridium perfringens* was isolated from the gizzard from all birds with necrotizing gastritis, usually in pure culture. All 93 strains of *C. perfringens* tested were type A.

Quantitative bacteriological examination was carried out on the intestinal contents from 13 birds with necrotizing enteritis, of which all had been frozen before being dispatched to the laboratory (five <1 week and none >4 months); 11 birds with catarrhal enteritis of which 8 had been frozen (six <1 week and none >4 months); and 48 birds without enteritis of which 26 had been frozen (twelve <1 week and none >8 months). *Clostridium perfringens* was isolated from 12 of the 13 birds with necrotizing enteritis; 6 of the 11 birds with nonspecific catarrhal enteritis; and 21 of the 48 birds without enteritis. The number of *C. perfringens* colony forming units

(cfu) were significantly different between the three groups. The group of birds with necrotizing enteritis had the highest number of *Clostridium perfringens*, the median number of cfu being 7.3×10^6 (Table 2).

DISCUSSION

The disease described in this paper has been designated "necrotizing enteritis" because of its resemblance to both UE and NE and the uncertainty regarding the etiology. The subacute to chronic disease described in capercaillies by Tschirch and Wilhelm (1983), referred to as UE, is similar to, if not identical with, necrotizing enteritis as described in the present study. The lesions also resemble pathological changes described in bobwhite quail and other gallinaceous birds with UE (Harris, 1961; Peckham, 1978). However, reports of lesions being localized predominantly in ceca and lower intestines, and perforating ulcers as seen in bobwhite quail with UE (Peckham, 1978), do not fit well with the pattern of pathological changes demonstrated in capercaillies. Culture media promoting the growth of *C. colinum* were neither employed in the present study nor by Tschirch and Wilhelm (1983). The possible role of *C. colinum* as an etiological agent causing necrotizing enteritis in capercaillies thus remains uncertain.

Gross lesions in NE include distended small intestine with diphtheric membranes and fluid intestinal content. Histologically, necrotic and diphtheric lesions with loss of villi tips are observed (Helmboldt and Bryant, 1971). This description resembles the subacute to chronic necrotizing enteritis in capercaillies. *Clostridium perfringens* type A, and sometimes type C, are considered causes of NE in broiler chickens (Al-Sheikhly and Truscott, 1977; Parish, 1961). The disease has been reproduced experimentally in chickens fed a diet to which *C. perfringens* was added, and by inoculation of *C. perfringens* intraduodenally (Al-Sheikhly and Truscott, 1977; Cowen et al., 1987).

TABLE 2. Number of birds examined, percentages of birds infected with *Clostridium perfringens*, and the number of colony forming units per gram of intestinal content given as 25th percentile, median and 75th percentile in three different groups of capercaillies.

Diagnosis	No. examined	Birds with <i>Clostridium perfringens</i> ^a	25th percentile	Median	75th percentile
No enteritis	48	21 ^c	<10 ²	<10 ² ^c	4.9 × 10 ³
Catarrhal enteritis ^b	11	6 ^c	<10 ²	2.0 × 10 ² ^f	5.4 × 10 ⁶
Necrotizing enteritis	13	12 ^d	5.5 × 10 ⁴	7.3 × 10 ⁶ ^e	6.2 × 10 ⁷

^a Detection level: 10² colony forming units per gram intestinal content.

^b Nonspecific etiology, no indication of necrotizing lesions.

^{c,d} Different superscripts indicate significant ($P \leq 0.05$) difference in a Chi-square test.

^{e,f} Different superscripts indicate significant ($P \leq 0.05$) difference in Wilcoxon two-sample test.

Berkhoff et al. (1974) isolated *C. perfringens* from quail with UE, but believed that the bacterium had no etiological significance in the development of UE. In contrast Ashenbrenner (1987) suggested that *C. perfringens* might be of importance as a secondary agent. In our study, *C. perfringens* type A was isolated more frequently and in higher numbers from birds with necrotizing enteritis, than in other capercaillies. We propose that *C. perfringens* type A is an etiologic agent for necrotizing enteritis in capercaillies.

In this study, we found necrotizing enteritis and gastritis to be serious causes of losses among captive capercaillies. Moreover, only a few of the birds which died during disease epizootics normally were sent for necropsy. Thus, necrotizing enteritis is of even greater importance than shown in this report.

Juvenile capercaillies appeared most susceptible to necrotizing enteritis, whereas adult birds had the highest occurrence of necrotizing gastritis; young capercaillie chickens did not seem susceptible to either necrotizing enteritis or gastritis. Age of the bird might be a significant risk factor.

Clostridium perfringens was not isolated from all birds with necrotizing enteritis. Freezing and storing of birds prior to examination might have reduced the likelihood of isolating the organism. Antibiotics, especially tetracyclines were commonly administered during epizootics of necrotizing enteritis, and in stress situations sus-

pected to induce such outbreaks. Information on whether antibiotics had been used was not systematically given, and isolation of *C. perfringens* might have been hindered by antibiotics administered prior to death.

The feed given to capercaillies reared in captivity differs from that of wild birds. The use of feed concentrates rich in protein might have promoted growth of *C. perfringens* in the intestine of captive capercaillies. Hanssen (1979) showed that the intestinal bacterial flora in captive willow ptarmigan (*Lagopus lagopus*) was very different from that in wild birds; the small intestine of wild ptarmigan scarcely contained bacteria, whereas birds in captivity had an intestinal flora quite similar to that of the domestic fowl. Although no comparable studies have been carried out on capercaillies, it is reasonable to assume that the bacterial flora in captive and wild capercaillies differ.

The grit used on the farm on which most of the birds found to have necrotizing lesions in the gizzard were kept was in the form of artificially crushed quartz. The sharp crystals might have caused traumatic lesions in the gizzard, thus creating favorable conditions for bacterial growth.

Necrotizing enteritis and gastritis have not been reported from wild tetraonids. The reason for this might be that free-living birds are not exposed to predisposing stress factors such as transportation or sudden environmental and dietary changes.

The number of captive capercaillies submitted for necropsy have dropped dramatically in recently years, due to a decline in the number of breeding units. Capercaillies do not seem to be very amenable to captive breeding. There are difficulties in husbandry and management practices, and the additional problems with necrotizing enteritis and gastritis pose a serious hazard to farmed birds.

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