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FOWL CHOLERA IN PHEASANTS (*PHASIANUS COLCHICUS*) – ETIOLOGICAL INVESTIGATION AND EFFECT OF THERAPY WITH THIAMPHENICOL

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Summary

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In an outbreak of fowl cholera that occurred in a pheasant (*Phasianus colchicus*) flock, seventeen per cent of about 1000 birds from the flock died of an acute disease. The established pathological alterations were typical of fowl cholera. *Pasteurella multocida* was isolated in pure culture from hearts, livers and spleens of three dead birds. Examined characteristics included phenotypic and biochemical determination of the biotype (subspecies) and the *in vitro* susceptibility of isolates to antimicrobial agents. Isolates were identified as belonging to the *multocida* subspecies. Their drug susceptibility was identical. All isolates were highly susceptible *in vitro* to tested amphenicols, including thiamphenicol. The application of thiamphenicol in the drinking water stopped the mortality.

Key words: antimicrobial therapy, fowl cholera, pheasants

INTRODUCTION

Fowl cholera is a frequently occurring disease which has settled in more than 100 species of domesticated and wild birds in most countries of the world (Sawada et al., 1990; Wilson et al., 1995). According to Sakuray et al. (1986), fowl cholera in pheasants is a fundamental problem of the infectious pathology of the semi-farm breeding. Single outbreaks of the disease in pheasants and half-savage ducks occur every year in our country (Mutafov, 1986) and the mortality reaches 25–34% for a short time (Lozenski, 1985). Clinically, the acute and peracute forms predominate. The delayed therapy or the use of an inappropriate medication may cause considerable economic losses.

The performance of an etiological investigation of a spontaneous fowl cholera in pheasants is a subject of scientific interest with regard to the determination of the subspecies affiliation of the agent. Such studies have not been performed in our country. This was one of the aims of our study, together with the assessment of the therapeutical efficacy of the antibiotic thiamphenicol.

MATERIALS AND METHODS

Diseased birds

The studies were performed in a flock of 1000 pheasants (*Phasianus colchicus*) at the age of 4-5 months, having no contact with other farms or wild birds. The investigations were initiated after the outbreak of fowl cholera in November 2000 that caused a death rate of 9.5% in affected birds prior to the treatment (at the first day of the appearance of the disease).

Isolation and characterization of bacteria

Bacteriological examination of heart, liver, lung and spleen specimens obtained from 3 pheasants dead from fowl cholera was performed on Mueller-Hinton agar with 5% sheep red blood cells (SRBC), grape sugar agar and broth and calf serum broth (National Centre of Infectious and Parasitic Diseases – NCIPD, Sofia). Inoculated nutrient media were incubated aerobically at 37°C for 24 hours. Six months later, pharynx specimens of 10 birds belonging to the same flock were analysed. One year later, after the change of the batch in the farm, the carriership was followed out in another 10 birds.

The bacteriological characterization of bacterial isolates was performed by morphological examination of Romanovsky-Giemza and Gram-stained preparations, determination of the cultural and the biochemical characteristics in stained differentiation media and Polymicrotests (NCPID, Sofia). A biological test was performed via subcutaneous inoculation of 4 albino mice (weighing 16–18 g) with 0.5 ml broth cultures of isolated strains.

The genus and species affiliation of isolates were identified according to accepted criteria (Carter, 1984). The subspecies determination was performed by biochemical investigations, including fermentation of trehalose, maltose, xylose, arabinose, mannitol, sorbitol, dulcitol and ornithine (Snipes et al., 1990; Bisgaard et al., 1991).

Serological characterization

The somatic serotype of isolates was determined by the gel diffusion precipitin test of Heddleston et al. (1972).

Susceptibility of isolates to antimicrobial agents

The classic agar diffusion method of Bauer et al. (1966) on Mueller-Hinton agar with 5% SRBC with standard discs for antibiotic sensitivity testing (NCIPD–Sofia) was used. Bacterial suspensions were inoculated at 2.10^6 cells/ml in agar plates and incubated at 37°C for 24 h. The diameters of zones of inhibition were interpreted according to the three–degree system for categorization of Bauer et al. (1966).

Pathological examination

After examination of gross lesions of 4 male and 3 female pheasants, killed by cholera, tissue specimens of their internal organs were collected, fixed in 5% formaldehyde, then embedded in paraffin, cut and stained with haematoxylin-eosin for microscopic observation.

Therapy

The broad-spectrum antibiotic thiamphenicol (Thiamphenicol, 20% injectable solution, Nikovet-Sofia), was applied in drinking water at a dose of 4 ml solution per 1 litre water throughout 5 days on the basis of our preliminary studies.

RESULTS

Bacteriological investigation

Pure bacterial cultures were isolated from hearts and livers of all examined birds as well as from the spleen of one of them. On solid nutrient media, the growth appeared as little, slightly mucoid and non-haemolytic S-colonies with a tendency towards fusion. In broth cultures, a weak muddiness and typical drags were observed.

The seven isolated strains showed identical cultural, morphological and biochemical features, typical for *P. multocida*. The serological test showed that all isolates belonged to *P. multocida* serotype 1. The results of biochemical tests (Table 1) were fairly uniform for all isolates, associated to the *multocida* subspecies.

Table 1. Biochemical characteristics of 7 *Pasteurella multocida* isolates from dead pheasants

Test	No of positive
Nitrate reduction	7
H_2S	0
Indole	7
Ornithine decarboxylase	7
Lysin decarboxylase	0
Arginine decarboxylase	0
Adonitol	0
Arabinose	0
Dulcitol	0
Dextrin	0
Esculin	0
Fructose	7
Galactose	7
D-glucose	7
Glycerol	0
Inositol	0
Inulin	0
Lactose	0
Maltose	0
Mannose	7
Mannitol	7
Melibiose	0
Rhamnose	0
Saccharose	7
Salicin	0
Sorbitol	7
Trehalose	0
Xylose	7
Urease	0

All seven isolates were identically sensitive to tested antimicrobial agents (Table 2). The amphenicols (chloramphenicol, thiamphenicol and florphenicol) produced the largest inhibition zones (26-32 mm in diameter). A high sensitivity (inhibition zones of 21–28 mm) was established to ciprofloxacin, sulfamethoxazole, trimethoprim and nalidixic acid. The sensitivity to aminoglycosides (gentamicin, kanamycin, amikacin) as well as to carbenicillin and cephalotin was relatively lower (inhibition zones 16–29 mm) while that to tetracycline – intermediate (inhibition zones of 16–19 mm). The middle-spectrum antibiotics lincomycin, novobiocin and erythromycin as well as the penicillins – ampicillin and oxacillin were not efficient in vitro (0–16 mm). The analysis of specimens obtained from 10 pheasants from the same flock 6 months later showed the presence of *Pasteurella multocida ssp*. in 5 birds. In the samples of the new batch of pheasants, taken another 6 months later in the same farm, no *Pasteurella spp*. were detected.

Table 2. Susceptibility of *Pasteurella multocida* ssp. *multocida* isolates from dead pheasants to antimicrobial agents

Antimicrobial agent	Disc content (μg)	Diameters of inhibitory zones (mm)
Chloramphenicol	30	26–30 (S)
Thiamphenicol	30	20–27 (S)
Florfenicol	30	26–32 (S)
Tetracycline	30	17–19 (I)
Gentamicin	10	23–29 (S)
Kanamycin	30	17–24 (S)
Amikacin	30	16–23 (I/S)
Lincomycin	15	10–15 (R)
Novobiocin	5	14–16 (R)
Erythromycin	15	0 (R)
Carbenicillin	100	24–26 (S)
Ampicillin	10	0 (R)
Oxacillin	1	0 (R)
Cephalotin	30	17–22 (S)
Ciprofloxacin	5	25–28 (S)
Nalidixic acid	30	21–24 (S)
Sulfamethoxazole + Trimethoprim	23.75 1.25	25–28 (S)

S = sensitive; R = resistant; I = intermediate

Pathological examination

At necropsy, macroscopical findings in liver showed multiple necrotic foci in all pheasants. The most characteristic

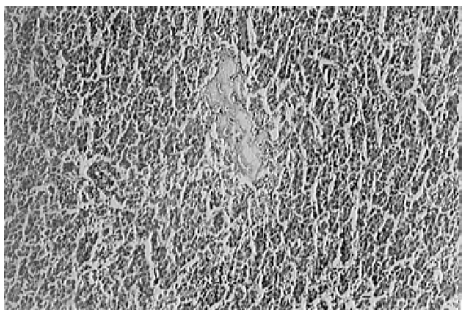

Fig. 1. Areactive micronecrosis in liver parenchyma. H/E; magnification × 250.

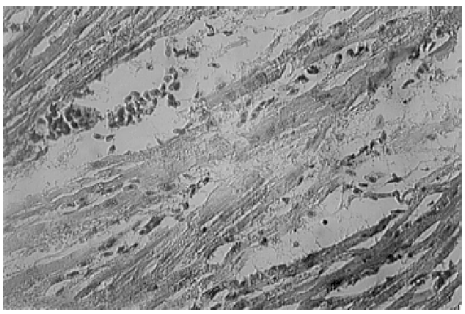

Fig. 2. Large necrosis with lysis of the sarcoplasma and myocardial haemorrhages. H/E, magnification × 400.

alterations were registered in hearts – many haemorrhages of 1–2 mm diameter in the coronary area. The spleen was not enlarged. It was scattered with multiple subcapsular haemorrhages. A catarrhal enterocolitis was present. Specific alterations were found out in the gizzards, which was soft, toneless, with dough-like consistence and massive haemorrhages under the cuticle. No macroscopic alterations were established in lungs or kidneys.

Histologically, the liver blood vessels were hyperaemic. The capilliaries were filled with lymphocytes and biliary thrombi. A parenchymal dystrophy and many areactive micronecroses were observed as well (Fig. 1). The largest necrotic foci were infiltrated by mononuclear cells. In the heart, hyperaemia and activation of the endothelium were established. Myocardial micronecroses as well as large necrotic areas with lysis of the sarcoplasms and haemorrhages were present (Fig. 2).

Therapy

Following the treatment, the death rate fell to 74 birds at day 2. On day 3, dead pheasants were only 2 and thereafter there were no lethal issues. The diseases caused the death of about 17% of pheasants within 48 h.

DISCUSSION

The results of the bacteriological and pathological investigations, confirmed the diagnosis fowl cholera in pheasants in a peracute form. The seven isolates were identified as *P. multocida* ssp. *multocida*. Three subspecies of *P. multocida* are known: *multocida*, *gallicida* and *septica* (Mutter et al., 1985). Accoding to literature data, the *multocida* subspecies predominates among the isolates from many domesticated and wild avian species in different countries (Snipes et al., 1990; Christensen et al., 1998). This subspecies was frequently encountered in mammals too (Bisgaard et al., 1991). The prevalence of *P. multocida* ssp. *multocida* in our country is of scientific interest. According to Christensen et al. (1998), the close genomic relationship among isolates from ducks, pheasants, chickens and turkeys indicate that *P. multocida* ssp. *multocida* may be interchanged among different populations of wild birds and flocks of back-yard poultry. Significant similarities among isolates of this subspecies in turkeys and wild birds are reported by Snipes et al. (1990). For the other *P. multicida* subspecies (*gallicida* and *septica*), such data are not known.

Sakurai et al. (1986) suggested that the carriership of the agent among healthy pheasants, let free with regard to chase purposes, was hazardous from the point of view of new outbreaks in pheasants and other avian species. Our investigations on pheasants from the same flock, performed 6 months after the outbreak showed carriership in 50% of the birds. It is known that in general, the virulence of isolates is related to host species. Our *P. multocida ssp. multocida* isolates were not pathogenic for mice. Their pathogenicity by respect to other birds could be interesting and subject to another study.

The microscopical and histological alterations do not differ from the findings, classic for fowl cholera. The severe lesions in the heart and the gizzard are impressive.

Thiamphenicol, applied by us, is a broad-spectrum antibiotic, semi-synthetic congener of chloramphenicol. Our preliminary studies and literature data show that thiamphenicol is highly active *in vitro* as well *in vivo* against the majority of gram-positive and gram-negative microorganisms, including *P. multocida* (Katoh et al., 1996; Kobayashy et al., 1996, Martelli, 1996). The results from the study with 61 strains of fowl cholera agent performed by Inamoto et al. (1994) show that most strains were highly *in vitro* sensitive to thiamphenicol.

The present results of the therapy manifest a high efficacy of thiamphenicol *in vivo* against a highly virulent *P. multocida* strain. This high antibacterial activity, as well as the suitable solubility are important priorities, which make the antibiotic suitable for fast and extensive application for treatment of fowl cholera as well as other bacterial infections.

CONCLUSIONS

P. multocida ssp. *multocida* was isolated as causative agent in a pheasant's enzooty. Thiamphenicol is highly active both *in vitro* and *in vivo* against *P. multocida* ssp. *multocida* and is appropriate for rapid and efficient therapy of fowl cholera in pheasants.

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