

THE PATHOGENITY OF *CRYPTOSPORIDIUM PARVUM* TYZZER, 1912 AND *C. BAILEYI* CURRENT, UPTON ET HAYNES, 1986 FOR CHICKENS

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Abstract. Clinical symptoms and pathological-morphological changes in the respiratory tract of chickens inoculated with *Cryptosporidium parvum* were described for the first time and compared with those induced by *Cryptosporidium baileyi*. Intratracheal inoculation with these two species caused symptoms of a respiratory tract disease in all chicken, but a disease of the digestive tract or death did not occur in any case. Pathological and morphological changes were observed only in the respiratory system and were characterized by petechiae in the mucosa of larynx, trachea, primary bronchi and in the wall of air sacs in chickens infected with *C. parvum*, or by diffuse hyperaemia of respiratory tract mucosa in chickens infected with *C. baileyi*. The pathological-histological changes, which were characterized by affections of various degrees in epithelial cells, mucous glands, and lamina propria mucosae, were more pronounced in the chickens infected with *C. parvum*.

Cryptosporidia were found for the first time in the glandular epithelium of stomach of laboratory mouse and were named *Cryptosporidium muris* (Tyzzer 1907). The range of hosts in which cryptosporidia have been found is still increasing. Tzipori et al. (1980) recorded 12 host species, O'Donoghue (1985) 14 host species, and Current (1986) already 31 species of mammals, 7 species of birds, 5 species of reptiles, and 2 fish species. Some authors assume that cryptosporidia are organisms with a great host specificity (Vetterling et al. 1971). Others, however, are of an opposite opinion and recommend to assign only one species to the genus *Cryptosporidium* (Tzipori et al. 1980). On the basis of papers dealing with the transmission of cryptosporidia among individual animal species Levine (1984) proposes to divide these parasites into four species: *Cryptosporidium crotali* (Triffitt, 1924) in reptiles, *C. meleagridis* (Slavin, 1955) in birds, *C. muris* (Tyzzer, 1907) in mammals, and *C. nasorum* (Hoover et al., 1981) in fish. Upton and Current (1985) found two morphologically different *Cryptosporidium* species in cattle, *C. parvum* and *C. muris*, *C. parvum* causing cryptosporidiosis in calves. Current et al. (1986) described *C. baileyi* as a new *Cryptosporidium* species specific for birds. Cryptosporidia with the same localization (cloaca, bursa of Fabricius) and oocyst morphology were detected in spontaneously infected broiler chickens by Pavlásek and Palkovič (1986) in Czechoslovakia. Peroral transmissions of *C. baileyi* to suckling laboratory mice and suckling kids were unsuccessful (Current et al. 1986). Unsuccessful was also the experimental intratracheal inoculation of suckling laboratory rat with oocysts of *C. baileyi* (Palkovič and Maroušek, unpublished results). Experiments with the transmission of cryptosporidia from mammals to chickens showed different results. Peroral inoculations of chickens with oocysts from infected calves were mostly unsuccessful (Pavlásek 1983; O'Donoghue 1985), similarly as the inoculation of chickens with mucosal scrapings from infected guinea pigs (Vetterling et al. 1971). However, Tzipori et al. (1980) succeeded in peroral infection of chickens with ileal homogenate from an infected calf. Lindsay et al. (1987) demonstrated developmental stages of cryptosporidia in the respiratory tract of 1- and 7-day-old chickens on days 4-12 after intratracheal inoculation with *C. parvum* oocysts. The oocysts were found in a mixed faeces sample on days 7 and 9 post infection (DPI).

The purpose of the present study was to compare the pathogenic effect of *C. parvum* and *C. baileyi* for chickens after intratracheal inoculation with oocysts.

MATERIALS AND METHODS

Twenty-four specimens of 3-day-old broiler chickens were divided into two experimental (A, B) and two control (C, D) groups of six specimens each. The chickens of experimental groups were inoculated intratracheally with 450,000 oocysts of *C. parvum* (group A) and *C. baileyi* (group B). The chickens of the control groups received intratracheally 0.20 ml of saline. The groups A, C and B, D were kept separately under the same conditions of the environment and feeding. In order to verify the infectivity of *C. parvum* for mammals, five 3-day-old white laboratory mice were inoculated perorally with 150,000 oocysts. Another group of mice of the same age served as control.

The inoculum of *C. parvum* ($5.10 \times 4.82 \mu\text{m}$ large spherical oocysts) was prepared from the faeces of naturally infected, 18-day-old calf. The oocysts of *C. baileyi* (oval, $6.47 \times 5.25 \mu\text{m}$) used in our experiment were obtained from faeces of naturally infected chickens and repeatedly passaged in experimentally infected chickens at the Institute of Parasitology, Czechoslovak Academy of Sciences. Faeces from the calf and chickens were suspended in 2.5 % potassium dichromate solution and stored at 4 °C for about 30 days. The oocysts from the samples were concentrated by flotation in Sheather's sugar solution, and stored in saline containing 100 IU/ml penicillin and 100 µg/ml streptomycin at 4 °C for 9 days. The number of oocysts in the infection dose was determined using Bürker's chamber. During the experiment, the chickens were observed daily for their clinical condition and the number of excreted oocysts was determined by the method of cloaca and beak cavity irrigation (Pavlíšek 1987a, b). Flotation-centrifugation method after Breza (1957) was used for daily examinations of mixed samples of chicken faeces from each group and faeces of both infected and control mice. One chicken from each group was dissected on 3, 5, 7, 10, 12, and 14 DPI. Mucosal scrapings of conjunctiva, adjacent nasal cavities, larynx, trachea, bronchi, duodenum, jejunum, ileum, caecum, large intestine, cloaca, and bursa of Fabricius, as well as imprint preparations of air sacs and lung parenchyma were stained after Giemsa. Excisions from conjunctiva, adjacent nasal cavities, larynx, trachea, bronchi, lungs, air sacs, cloaca, and bursa of Fabricius were fixed in 10 % neutral formalin and embedded into paraffin. Sections were stained with haematoxylin and eosin and after Giemsa. Samples of larynx, trachea, bronchi and air sacs were taken for scanning electron microscopy (SEM). Small tissue samples for SEM were fixed in 2.5 % glutaraldehyde at 4 °C for 2 h, washed in cacodylate buffer, fixed in 2 % OsO_4 , washed in redistilled water, dehydrated through graded alcohol series (30–100 %), and transferred to acetone. Then they were critical-point dried, coated with gold and examined with TESLA BS 300 scanning electron microscope. Organs of all chickens killed on 5 and 12 DPI were subjected to bacteriological examinations, cultivation and histological examinations for the presence of moulds, and cultivation and serological examinations for the presence of mycoplasmas at the Central State Veterinary Institute in Prague.

RESULTS

Clinical examinations

Chickens of both experimental groups did not exhibit any clinical symptoms of disease up to 5 DPI and behaved in the same way as those in the control groups. Since 6 DPI, the chickens from both inoculated groups were somnolent and less active. Since 10 DPI, a worsened clinical state was observed in chickens of group B (*C. baileyi*). Dyspnoea and wheeziness were noticed in them. The same clinical signs appeared in the chickens of group A (*C. parvum*) on 11 DPI and persisted up to 13 DPI. The changes in the clinical state of chickens in group B lasted up to the termination of the experiment, i.e. to 14 DPI. Neither a discharge from nasal cavities, beak or eyes nor signs of the digestive tract disease were observed in any chicken of both inoculated groups. The chickens of the control groups did not exhibit any clinical signs of disease during the whole course of the experiment.

Parasitological examinations

C. parvum oocysts (morphologically identical with those used in the inoculum) were found in chickens of group A only in the beak cavity washing of one chicken 5

and 7 DPI. None of the chickens had oocysts in cloaca washings or in mixed samples of faeces examined by flotation-centrifugation method. *C. baileyi* oocysts were found in all chickens of group B in beak cavity washing on 4—12 DPI and in cloaca washing on 3—14 DPI. In mixed faecal flotations, the oocysts were found on 4—14 DPI. In the stained mucosa scrapings of chickens of group A, *C. parvum* oocysts occurred in larynx and trachea from 3 DPI (particularly trophozoites and macrogametocytes), in primary bronchi from 5 DPI (trophozoites, meronts I and II, and macrogametocytes) (Table 1). *C. baileyi* oocysts were found in chickens of group B in larynx and trachea from 3 DPI (mainly trophozoites and meronts I and II), in primary bronchi, lung parenchyma, and bursa of Fabricius from 5 DPI (trophozoites — oocysts), and in cloaca from 10 DPI (trophozoites — oocysts) (Table 2). During both *C. parvum* and *C. baileyi* infection, all developmental stages were observed at various intensities. Whereas the number of numerous asexual (Pl. I, Fig. 1) and less numerous sexual developmental stages (Pl. I, Fig. 2) of *C. parvum* considerably decreased from 7 DPI, numerous asexual and sexual developmental stages of *C. baileyi* were detected up to the termination of the experiment (14 DPI).

No cryptosporidia occurred in the control groups of chickens.

The laboratory mice used for the verification of the inoculum infectivity for mammals passed *C. parvum* oocysts in their faeces from 7 DPI up to the end of the experiment (14 DPI). The control mice remained negative.

Pathological-morphological examinations

Pathological-anatomical changes in both chicken groups were observed only in the respiratory tract. In chickens inoculated with *C. parvum*, numerous petechiae were found in larynx and trachea from 5 DPI, in primary bronchi from 10 DPI, and on turbid and thickened air sacs from 12 DPI. On 14 DPI, the petechiae were observed only in trachea and larynx. The trachea contained a small amount of mucus (Table 1). The mucosa of upper respiratory tract in *C. baileyi*-inoculated chickens was diffusely hyperaemic from 5 DPI. Single minute petechiae occurred in larynx from 7 DPI, in trachea and primary bronchi and on air sacs only on 12 DPI. The lumen of trachea contained a greater amount of mucus than in chickens inoculated with *C. parvum* (Table 2).

Pathological-histological changes were characterized by various stages of affection of mucosa of the respiratory tract and of the air sacs of chickens of both groups. Developmental stages of cryptosporidia were demonstrated on the surface of epithelial cells and on secretory epithelium of mucous glands (Pl. I, Fig. 3). In *C. parvum*-inoculated chickens, the cilia disappeared from larynx, trachea, and primary bronchi, and the whole epithelial layer became markedly thicker. The larynx epithelium was hyperplastic, the mucous glands degenerated, lamina propria mucosae was focally hyperaemic and infiltrated with mononuclear cells and eosinophils, which penetrated through the epithelium up to the lumen (Pl. II, Fig. 4). A degeneration and destruction of epithelia, loss of mucous glands, strong cellulization of lamina propria mucosae mainly by an infiltrate consisting of mononuclear cells and eosinophils were observed in trachea. In primary bronchi, there was a focal hypertrophy and metaplasia of the cylindrical epithelium into a stratified columnar one, hyperaemia and marked infiltration of lamina propria mucosae with mononuclear cells (Pl. II, Fig. 5). The lung interstitium was hyperaemic and contained focal accumulation of eosinophiles. The findings of cryptosporidia in mesobronchi, parabronchi, and ventrobronchi were only occasional. The cilia of air sac epithelium were destructed at the places where cryptosporidia occurred and the middle connective tissue layer was focally infiltrated by lymphocytes and eosinophiles.

Table 1. Results of examinations of chickens inoculated with *Cryptosporidium parvum*

DPI	Clinical finding	Pathological-anatomical finding	Finding of cryptosporidia in preparations stained after Giemsa					
			Larynx	Trachea	Primary bronchi	Lung parenchyma	Cloaca	Bursa of Fabricius
3	Without clinical signs of disease	Without pathological-anatomical finding	+	+	0	0	0	0
5	Without clinical signs of disease	Single minute petechiae in larynx and upper third of trachea	+	+	+	+	0	0
7	Restricted motility	Large number of minute petechiae in larynx and upper third of trachea	+	+	+	0	0	0
10	Restricted motility, somnolence	Large number of petechiae in larynx, trachea and primary bronchi, the highest number in tracheal bifurcation	+	+	+	0	0	0
12	Restricted motility, dyspnoea, wheezing	Single petechiae in larynx; air sacs thickened, turbid, and with numerous petechiae	+	+	0	0	0	0
14	Without clinical signs of disease	Single minute petechiae in larynx and trachea	+	+	+	0	0	0

Table 2. Results of chickens inoculated with *Cryptosporidium baileyi*

DPI	Clinical finding	Pathological-anatomical finding	Finding of cryptosporidia in preparations stained after Giemsa					
			Larynx	Trachea	Primary bronchi	Lung parenchyma	Cloaca	Bursa of Fabricius
3	Without clinical signs of disease	Without pathological-anatomical finding	+	+	0	0	0	0
5	Without clinical signs of disease	Slight diffuse congestion of mucosa of larynx and upper half of trachea	+	+	+	+	0	0
7	Restricted motility, somnolence	Slight diffuse congestion of mucosa of larynx, trachea, and primary bronchi; single minute petechiae in larynx; mucus in trachea	+	+	+	0	0	0
10	Restricted motility, somnolence, dyspnoea, wheezing	Slight diffuse congestion of mucosa of larynx, trachea, and primary bronchi; single minute petechiae in larynx; mucus in trachea	+	+	+	+	+	+
12	Restricted motility, somnolence, puffy feathers, dyspnoea, half-open bill, wheezing	Diffuse congestion of trachea, single minute petechiae in larynx, trachea and primary bronchi and on air sacs; mucus in trachea	+	+	+	+	+	-
14	Restricted motility, puffy feathers, dyspnoea, wheezing	Diffuse congestion of trachea and primary bronchi; single minute petechiae in larynx; mucus in trachea	+	+	+	+	0	+

A loss of epithelium cilia, which were replaced by a continuous layer of cryptosporidia, was evident on the mucosa of respiratory tract of *C. baileyi*-inoculated chickens (Pl. III, Fig. 6). The epithelium was focally hyperplastic and the mucous glands were destructed. Lamina propria mucosae was hyperaemic and thickened due to the accumulation of the infiltrate consisting of mononuclear cells and single eosinophils. Focal accumulation of lymphocytes and eosinophils was observed also in the middle connective tissue layer of air sacs and interstitium of hyperaemic lungs. A large number of cryptosporidia were present also on the epithelial cover of mesobronchi, parabronchi and ventrobronchi (Pl. III, Fig. 7).

SEM examinations

Various developmental stages of *C. parvum* and *C. baileyi* were demonstrated by SEM on the surface of epithelial cells of the respiratory tract. A more massive infection was observed in *C. baileyi*-inoculated chickens, where the mucosa of larynx, trachea, and primary bronchi was at some places covered with a continuous layer of cryptosporidia (Pl. IV, Fig. 8). At the sites where cryptosporidia had separated, a loss of cilia was evident (Pl. IV, Fig. 9).

Results of other examinations

On the basis of bacteriological examinations of organs, cultivation and histological examinations for moulds, as well as cultivation and serological examinations for mycoplasmas, the possible presence of pathogenic bacteria, moulds and mycoplasmas was excluded.

DISCUSSION

Chickens inoculated with *C. parvum* and *C. baileyi* exhibited similar clinical signs indicating an affection of the respiratory tract. The excretions of oocysts were different. In *C. baileyi*-inoculated chickens, a large number of oocysts occurred in the washings from the beak cavity from 4 DPI and in cloaca washing from 5 DPI up to the termination of the experiment, whereas only one chicken from the group inoculated with *C. parvum* had single oocysts in the beak cavity washing on 5 and 7 DPI. The oocysts were morphologically identical with those used in the inoculum. Lindsay et al. (1987) found *C. parvum* oocysts in a mixed sample of chicken faeces on days 7 and 9 after intratracheal inoculation. Asexual and sexual developmental stages were demonstrated by them only in scrapings from the mucosa of respiratory tract. Since these authors did not find the developmental stages of cryptosporidia on the epithelium of digestive system or bursa of Fabricius, they assume that the life cycle took place in the respiratory tract and that the oocysts coughed out into the beak cavity were passaged through the digestive tract. Also in our experiment, *C. parvum* oocysts were found only in the scrapings from respiratory tract mucosa, where numerous asexual but only occasional sexual developmental stages were found. After 7 DPI, the number of the found developmental stages considerably decreased. In chickens infected with *C. baileyi*, numerous asexual and sexual developmental stages were found in the scrapings from the respiratory tract during the whole period of observation. On the basis of these results we assume that it will be suitable to compare the life cycle of *C. parvum* and *C. baileyi* in the chicken respiratory tract at the level of transmission electron microscopy. The results of pathological-histological examinations of the respiratory tract of *C. baileyi*-inoculated chickens, characterized by a loss of cilia, focal destruction of mucous glands and infiltration of lamina propria mucosae mainly by mono-

nuclear cells were identical with the results obtained by some other authors (Hoerr et al. 1978, Mason and Hartley 1980, Dhillon et al. 1981, Blagburn et al. 1987). In chickens inoculated with *C. parvum*, the number of cryptosporidia on the epithelial cells of respiratory tract mucosa was lower than in those inoculated with *C. baileyi* but the histopathological changes were more pronounced. Lamina propria mucosa, was markedly congested and thickened several times as a result of the infiltration with mononuclear cells and eosinophils. The changes in the trachea were characterized by the loss of cilia, degeneration and destruction of the epithelia, and loss of mucous gland. In primary bronchi, the cylindrical epithelium was hypertrophic and turned to a stratified squamous one.

Our results, as well as those obtained by Lindsay et al. (1987) indicate that after intratracheal inoculation, *C. parvum* can affect only the respiratory tract where it acts as a pathogen. *C. baileyi* inoculated intratracheally to chickens of the same age affects in addition to the respiratory tract also the cloaca and bursa of Fabricius, where its effect is probably non-pathogenic. Blagburn et al. (1987) inoculated perorally and intratracheally 1- and 7-day-old broiler chickens with *C. baileyi* oocysts. The intratracheally inoculated chickens developed respiratory cryptosporidiosis with marked pathological-morphological changes in the respiratory tract. Cryptosporidia were found in the cloaca and bursa of Fabricius of the perorally infected chickens, but did not induce there any pathological lesions. Though findings of Cryptosporidia in the respiratory and digestive tracts and bursa of Fabricius of spontaneously and experimentally infected birds have been reported, clinical cryptosporidiosis is usually associated with the infection of respiratory tract (Fletcher et al. 1975, Mason and Hartley 1980, Dhillon et al. 1981, Lindsay and Blagburn 1986, Lindsay et al. 1986). However, Hoerr et al. (1985) described serious diarrhoeas associated with a high mortality in spontaneously infected 5-day-old quails, in which the cryptosporidia were distributed only in the whole small intestine. The respiratory tract was not affected. The unsuccessful transmission of *C. baileyi* to 2- and 14-day-old quails performed by Current et al. (1986) suggests that another species of cryptosporidia might be involved in the case described by Hoerr et al. (1985).

In view of the diversity of localization, clinical signs, and pathological-morphological changes in spontaneously infected poultry and on the basis of unsuccessful experimental transmissions between some species we suppose that poultry cryptosporidiosis is not caused by a single species of cryptosporidia. The results of this study indicate that chicken respiratory cryptosporidiosis can be induced by two morphologically different species, *C. baileyi* and *C. parvum*, transmitted from chickens or calves.

The difference in the intensity of experimental infections with *C. parvum* and *C. baileyi* is conspicuous particularly in SEM examinations. The fact that only single sexual developmental stages could be demonstrated after *C. parvum* inoculation indicates a certain alteration in the development of *C. parvum* in an "atypical host". An elucidation of the cause of this alteration may contribute to the elucidation of the specificity of individual *Cryptosporidium* species.

From the epizootological point of view it is necessary to take into consideration the two possible etiologies of respiratory cryptosporidiosis in chickens (poultry) and to direct one's attention to an exact specific determination of the causative agent.

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Л. Палкович и В. Мароушек

Резюме. Впервые описаны клинические признаки и патологическо-морфологические изменения дыхательной системы кур, зараженных *Cryptosporidium parvum* и сравнены с изменениями, вызванными *Cryptosporidium baileyi*. Внутритрахеальное заражение этими криптоспоридиями вызывало признаки заболевания дыхательной системы. Ни в одном случае не наблюдали заболевания пищеварительного тракта или гибель подопытных животных. Патологическо-анатомические изменения встречались только в дыхательной системе и были характеризованы петехиями в мукозе глотки, трахеи, бронхов первого порядка и в стенке воздушных мешков у кур, зараженных *C. parvum*, или диффузной гиперемией мукоз дыхательной системы кур, зараженных *C. baileyi*. Патологическо-гистологические изменения, характеризовавшиеся разной степенью поражения эпителиальных клеток, слизистых желез и *lamina propria mucosae* были более выразительны, чем у кур зараженных *C. parvum*.

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I. A. Akimov, I. S. Starovir, A. V. Yastrebtsov, V. T. Gorgol': Kleshch Varroa — vzbuditel' varroatoza pchel. (The Varroa mite — the causative agent of the bee varroosis). Publ. House Naukova dumka, Kiev 1988, 118 pp., 44 Figs. (including 14 Plates), 4 Tables. Price 1.20 R.

Varroa jacobsoni belongs nowadays to the most important pests of honey bees in all countries of its occurrence where the economic losses caused by this mite parasite achieve very high values. Its continuing spread into new territories represents a very serious menace for the local bee breeders. Although many efforts have been oriented to find a successful way for its control, the results are not still satisfactory. A more profound knowledge of the biology of the parasite is necessary to find effective measures for its control. For this purpose a detailed information on the functional morphology of the organ systems can be useful. Especially to this aim is oriented the main part of this book.

The first chapter is devoted to general characteristics of the mite, to the morphology of individual stages, ontogeny, ecology and distribution. The following 8 chapters deal with individual organ systems: thagmosis and skeletal-muscular system, tegument, respiratory system, alimentary system, excretory system, exocrine glands, nervous system and endocrine system elements, reproductive system. In this text very many new data concerning *V. jacobsoni* obtained in course of authors' investigations are given. In the chapter 10 the morphological specializa-

tion of the mite's organ systems is treated. The chapter 11 discusses the ways of specialization of *V. jacobsoni* in comparison with another bee parasite — *Tropilaelaps clareae*. The book closes with a summary, list of references and 2 appendages: control measures for bee varroosis, and a glossary of acarological and morphological terms.

The matter of the book is well and lucidly presented. The drawings are very instructive, the photomicrographs are of good quality. But their numbering should be separate and not mixed with the numbering of figures in the text. Very useful are the tables giving comparison of morphological terms in the papers by individual authors. Also the terminological glossary may be welcomed by the readers. In the literature no paper by Czechoslovak authors is mentioned.

On the whole this one species monograph can be warmly accepted. It contains a great amount of important data accompanied by fine illustrations. The book has been intended first of all for specialists, but also the practitioners will find here many interesting facts, especially those concerning the control measures. The authors have done in it a great deal of work.

Dr. V. Černý, C.Sc.