STUDIES ON THE LIFE CYCLE OF Cryptosporidium COCCIDIA IN EXPERIMENTALLY INFECTED CHICKENS

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Abstract. Experimental infections of 7–28-day-old chickens with Cryptosporidium oocysts isolated from spontaneously infected chickens demonstrated that the endogenous development of this parasite takes place simultaneously in the organs of digestive, respiratory and excretory systems and in bursa of Fabricius. It was demonstrated for the first time that the oocysts of Cryptosporidium are shed through the respiratory tract into the beak cavity. A novel rapid and simple method has been developed for the detection of oocysts. Its principle is the raising of the beak cavity. This method enabled to isolate the oocysts from experimentally infected chickens and immediately use them in the dose of $6 \times 10^4$ for peroral infection of a 37-day-old chicken. The prepatent period was 8 days, patent period 12 days. On days 4–7 after the first detection of Cryptosporidium oocysts in chicken excrements, the oocysts were detected also by the method of beak cavity raising. This indicates that the oocysts released from the respiratory tract are infective. This fact is important from the epizootological viewpoint in relation with possible spreading of Cryptosporidium infections in chicken farms.

Already 79 years have elapsed since the first Tyzzer's description of Cryptosporidium coccidium, C. muris Tyzzer, 1907 in gastric gland of laboratory mouse. During this period, another 19 species have been described, most of them on the basis of a supposed host specificity of these protozoans. The life cycle of some of them, with the description of individual developmental stages, has been well documented. However, the number of species has been much reduced in recent studies dealing with experimental cross transfers of oocyst isolates obtained from various host species (Reese and Current 1982, Tzipori 1983, Levine 1984, Upton and Current 1985). The original opinion about Cryptosporidium coccidia has been significantly changed by the evidence of their involvement in respiratory diseases of turkeys, chickens and pheasants. In spite of the fact that a lot of information has been obtained about Cryptosporidium coccidia, our knowledge of these protozoans is still insufficient.

The first finding of Cryptosporidium in chicken was reported by Tyzzer (1929), who identified the parasites as C. parvum. However, Levine (1961) determined the coccidia from chickens as an independent species which he named C. tyzzeri. Fletcher et al. (1973) found Cryptosporidium in the bursa of Fabricius in chickens. Respiratory cryptosporidiosis with the presence of parasites in mucosal epithelium of trachea and in inner surface of mucous glands of chickens was reported by Dhillon et al. (1981). Itakura et al. (1984) described Cryptosporidium infection in chicken broilers. In Czechoslovakia, coccidia of the genus Cryptosporidium were found in chickens in the mucosal epithelium of sinus infraboritalis (Pavlásek 1985). Pavlásek and Palkovič (1986) detected oocysts of Cryptosporidium in the excrements of spontaneously infected chickens. The parasites morphologically differed from C. parvum from calves and therefore the authors considered them to be a new, hitherto undescribed species differing from that infecting the mammals. This species occurs mostly in some domestic birds and sometimes also in wild birds. Pavlásek et al. (1986) described experimental
infection of chickens with this species and its endogenous development. Current et al. (1988) described the life cycle of _C. baileyi_ infecting chickens.

The purpose of this paper is to describe the course of experimental infection of chickens with _Cryptosporidium_, with the first record of oocysts in the organs of respiratory tract. A description is given of a novel method for the detection of oocysts from respiratory organs of live infected chickens.

**MATERIALS AND METHODS**

Isolation of oocysts and preparation of inoculum. Oocysts of _Cryptosporidium_ were isolated from the excrements of spontaneously infected chickens (Gallus gallus L. dom.) obtained from a chicken farm (South Bohemia) in 1985. The isolate was maintained in the laboratory of the Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice by repeated experimental infections of chickens. The oocysts were recovered from infected chickens by the method described in a previous paper (Pavlášek 1987) and kept in 2.5% solution of KClO₃ at the temperature of 4°C. Before the experimental infection (pe or ov) the oocysts were concentrated by saturated sucrose solution and washed in water. The infection dose of 5–6 × 10⁵ oocysts/chicken was counted in a Burker’s chamber.

**Experimental chickens.** Chickens of the race Brama (18 specimens) at the age of 21 days were infected perorally. For the detection of the endogenous development of the parasite always one chicken was washed daily and dissected on days 1–4, 8, 16, and 11, two chickens on days 5, 6, and 8, and three chickens on day 7.

Chickens of the race Ross (110 specimens) were divided into 5 groups of 22 animals each and infected perorally at the age of 7, 14, 21, 24, and 28 days; 22 chickens served as controls. _Cryptosporidium_ oocysts were detected in the chickens at 2–4 days intervals.

**Description of oocysts.** _Cryptosporidium_ oocysts were described in the excrements of infected chickens by coprological examination using the methods after Breslau (1857) and after Pavlášek (1987). Oocysts from the respiratory tract were detected in the beak cavity by the following method: 1.5 ml and 3 ml of water (87:13) were gently dripped into the beak cavity by means of a syringe, the injection needle of which was replaced by a rubber hose (inner diameter 2 mm, length 5 cm). The fluid was immediately sucked back into the syringe. One to two drops were placed on a slide, covered with a cover glass and the preparation was examined at the magnifications of 400–1,000 ×. In case of negative finding, a control examination was performed: the fluid was transferred to a tube and water was added to obtain the volume of about 10 ml. The fluid was then centrifuged at 3,000 rpm for 1 min, the supernatant was removed (mucosa often occurs on the surface) and the oocysts were then detected in two ways: 1. some drops of the sediment were examined as a native preparation, or 2. 10 ml of any flotation solution was added to the sediment and further processed using the common flotation and centrifugation procedures. The oocysts were collected by means of a loop (4 mm in diameter) from the superficial membrane, put on a slide and examined in the microscope at the magnifications of 400–1,000 ×.

Impacts from the surface of mucosal epithelia of the digestive, respiratory and excretory systems were taken from the dissected chickens. The samples were then fixed in methanol and stained after Giemsa. For the detection of _Cryptosporidium_ developmental stages, samples for histological methods were fixed in 10% neutral formalin, embedded in paraffin and the sections were stained after Giemsa.

Samples of the lungs of chickens were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For SEM, small pieces of tissue were fixed in 2.5% glutaraldehyde at 4°C for 2 h, washed in cacodylate buffer, postfixed in 2% OsO₄, stabilized in 1% potassium ferrocyanide and dehydrated in ethanol series (30–100%), and critical point dried by the method of critical point. Then they were coated with gold and examined in TESLA BS 300 scanning electron microscope (produced in Czechoslovakia). For TEM, the tissue was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 h and postfixed in 1% OsO₄ in 0.1 M cacodylate buffer, followed by dehydration through alcohol series and embedded into Epon. Ultrathin sections were cut with Reichert’s OM U2 ultramicrotome, contrasted with 2% uranyl acetate and Reynold’s solution of lead acetate, and examined with PHILIPS EM 420 transmission electron microscope.

**RESULTS**

1. The course of cryptosporidiosis in experimentally infected chickens

Experimentally infected chickens started to shed the oocysts, in relation to their age, on days 4–14 p.i. A large number of developmental stages of _Cryptosporidium_ coccidia, particularly of trophozoites and first generation of meronts, were found in the distal parts of small intestine, ileum, but also in caecum, during the first 24 h p.i. Single trophozoites could be detected in the large intestine, cloaca and bursa of Fabricius. While the intensity of infection markedly dropped in the small intestine during 48 h p.i., further development of the parasite, including all hitherto known developmental stages, concentrated particularly into the mucosal epithelium of bursa of Fabricius (Plate I, Figs. 1, 2, 3), cloaca, and large intestine (72–120 h p.i.). In some chickens, developmental stages of _Cryptosporidium_ were found in imprints of sinuses infraorbitals since day 5 p.i., in trachea since day 6 p.i. (Plate II, Fig. 4), in bronchi (Plate II, Fig. 5), and lung imprints since day 7 p.i., and in kidneys and ureter on day 8 p.i. Coccidia were found also in smears from the mucosa of conjunctival sac of two chickens on days 7 and 11 p.i., respectively.

During the series of experimental infections of Ross chickens, there appeared panting on days 8–10 p.i. The breathing was then very loud and turned to strong typical hoarseness of all infected chickens on day 14 p.i. (Pavlášek et al., unpublished). On this way, we managed to obtain _Cryptosporidium_ oocysts (Plate II, Fig. 7), as products of the endogenous development, from the respiratory organs of a live 35-day-old chicken using the method of beak rinsing. The presence of oocysts and their shedding through the air passages into the beak cavity was evidenced by the detection of a dead 40-day-old chicken on day 16 p.i. Zygotes and arising oocysts were found in the smears from the mucosal epithelium of trachea and, at a relatively high intensity, also in the larynx (Plate II, Fig. 6). Typical oocysts of this bird species of _Cryptosporidium_ (Plate II, Fig. 7), morphologically almost identical with those excreted with the excrements of infected chickens (mean size 6.2 × 4.8 μm), were found before the dissecting also in the beak cavity, if the method of beak rinsing was used. The parasites were found also in the bursa of Fabricius and cloaca of the same chicken.

2. Inoculation of chicken with oocysts isolated from beak cavity

Using the method of beak rinsing, a sufficient number of oocysts were obtained from a group of 22 experimentally infected 35-day-old chickens on day 16 p.i. Immediately after infection, the oocysts at the dose of 6 × 10⁵ were used for peroral infection of a 37-day-old chicken from the control group free from _Cryptosporidium_ infection (as confirmed by regular examinations during the experiment). First oocysts appeared in the excrements of the infected chicken on day 8 p.i. The period of latency was 12 days. The disease duration was 7 days after the first finding of oocysts. At that time, the oocysts could be detected also in the beak cavity. The other control chickens were free from _Cryptosporidium_ infection during the whole experiment.

**DISCUSSION**

Coccidia of the genus _Cryptosporidium_ were found in the respiratory tract of birds (turkeys) probably for the first time by Hoerr et al. (1978). Respiratory cryptosporidiosis in turkeys was described also by Ranek (1979). Dhillon et al. (1981) observed different
ИЗУЧЕНИЕ ЦИКЛА РАЗВИТИЯ КОКИДИЙ РОДА
CRYPTOSPORIDIUM У ЭКСПЕРИМЕНТАЛЬНО ЗАРАЖЕННЫХ КУР
И. Павлак

Резюме. С помощью экспериментального заражения 7—28-дневных кур опоносты Crypto-
споридиум, инъектированные в спонгиозные хрящи кур, было доказано, что эндогенное
быстро и воспалительный метод обнаружения курицы, принцип которого заключается
в промывании полости курицы. С помощью этого метода были выделены овощи из экси-
мента 7—28-дневных куриц и в 6 х 10^{-5} раза в редуцированных материалах (петушиные
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FINDING OF Coccidia OF THE GENUS CRYPTOSPORIDIUM IN THE ORGANS OF CALF EXCRETORY SYSTEM

Coccidia of the genus Cryptosporidium have been increasingly dealt with in the literature during the last years, particularly after the year 1982. New methods of the diagnostics enabled to discover Cryptosporidium in various animals. This infestation and the host spectrum is still increasing. Tyssere's original and excellent scheme of the life cycle of Cryptosporidium parasiticum was modified by Blix (1912: Arch. Protistenk. 26: 394—412), which is valid also for other described species of this genus. Cryptosporidium in the organs of the calf excretory system was found by the author. The validity of autoinfestation is ascertained to the coccidia not leaving their host and since they do not differ in their morphology from the coccidia extracted from the host. Data for example, Ieiki (Ieiki M., 1979: Jap. J. Parasit. 28: 295—307) and the ultrastructure of C. felis, Current and Long (Current W. L., Long P. L., 1983: J. Infect. Dis. 148: 1108—1113), described the course and characteristics of the life cycle of Cryptosporidium in the basis of experiments performed with chicken embryo.
Figs. 1–3. Developmental stages of Cryptosporidium coccidia in the mucosa of bursa of Fabricius from experimentally infected chicken. Fig. 1. SEM of developmental stages, meront and merozoites—arrow (×3,000). Fig. 2. TEM of the trophozoite (×18,000). Fig. 3. TEM: A—meront with merozoites (m), B—unidentified developmental stage (×10,500).

Fig. 4. Trachea of a 12-day-old experimentally infected chicken. Cryptosporidia on the surface of epithelial cells (arrows). Giemsa stain (×300). Fig. 5. Section through bronchus with numerous cryptosporidians (arrows). Giemsa stain (×1,000). Fig. 6. Zygotes and arcing oocysts, arrow from the larynx. Giemsa stain (×1,400). Fig. 7. Cryptosporidium oocysts isolated from the beak cavity of chicken by the new method (×1,000).