ニワトリコクシジウムEimeria acervulina,E. hagani,E. maximaおよびE. tenellaのsporozoiteの細胞化学的観察

誌名	日本獣醫學雑誌 = The Japanese journal of veterinary science
ISSN	00215295
著者	中井, 裕
巻/号	43巻5号
掲載ページ	p. 767-769
発行年月	1981年10月

Jpn. J. Vet. Sci. 43, 767~769 (1981)

BRIEF NOTE

Cytochemical Observations on Sporozoites of Chicken Coccidia, *Eimeria acervulina, E. hagani, E. maxima,* and *E. tenella*

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(Received for publication December 22, 1980)

Cytochemical contributions on the intracellular stage of coccidia have been accumulated [2, 3]. However, data treated with the sporozoite of chicken coccidia are very few. Therefore, we performed cytochemical studies on the sporozoite of *E. acervulina, E. hagani, E. maxima* and *E. tenella* applying in vitro excystation technique.

Fresh oocysts of each species used for the experiments were isolated from the feces of chickens which were infected with *E. acervulina, E. hagani, E. maxima,* and *E. tenella* separately. Those oocysts kept in petri dishes with 2% potassium bichromate solution were allowed to sporulate in an incubator at 25–28°C for several days. After sporulation completed, the oocysts were isolated from the bichromate culture media. They were ground to rupture the oocyst wall in a homogenizer, and the liberated sporocysts were collected. Those sporocysts were transfered in a digestive solution composed of 1.0% of trypsin and 10% of chicken bile in 0.1 M pH 8.0 phosphate buffered solution, and were incubated at 37–39°C until most sporozoites were liberated.

The incubation period was 25 minutes on *E. acervulina* sporocysts, 40 minutes on *E. hagani,* 70 minutes on *E. maxima,* and 135 minutes on *E. tenella.* Those sporozoites were collected by centrifugation and used for the experiments. Smears of sporozoites fixed with methanol were used for Giemsa stain, those fixed with Carnoy's fixative for Feulgen's reaction and for pyronin-methylgreen stain, and those fixed with buffered formalin (pH 7.0) or Gender's solution for PAS stain. For vital staining, a suspension of sporozoites was mixed with an equal volume of 0.01% acridine orange phosphate buffered solution at pH 7.0, and was examined under the fluorescence microscope.

In Giemsa preparations, the cytoplasm of the sporozoite of each species was stained deeply at the anterior part and the middle part, and the nucleus was usually obscured by surrounding cytoplasm. Most sporozoites of each species had a pair of spherical refractile bodies (RB) which were stained weakly. One of them was in front of the

Table 1. Size of *Eimeria* sporozoites in Giemsa preparations (μm)

	E. acervulina	*E. hagani*	*E. maxima*	*E. tenella*
Length	7.7±1.0	13.0±0.9	10.8±1.2	12.4±1.2*
Width	1.6±0.4	2.0±0.5	3.0±0.7	3.3±0.8

*: Mean ± S.D. n=50

nucleus, and the other was at the back of it. The measurements of 50 sporozoites of each species are shown in Table 1. There was significant difference among four species on the average length and width. However, the alive sporozoites frequently change their size and shape by their own movement or by environmental conditions. Therefore, it is not easy to distinguish species by only measuring the size of the sporozoite.

In the fresh sporozoite in vital staining, the periphery of nucleus exhibited yellow-green and a central granule (the nucleolus) was stained red. The cytoplasm was evenly red and a pair of RB were green. Although it was easy to distinguish those organella, any obvious difference among these species could not be found, for their stainability was very similar each other in these four species.

In Feulgen preparations, a weakly positive reaction was confirmed in the nucleus and it formed a granular mass or irregularly circulated granules.

In pyronin-methylgreen staining, the nucleus and the cytoplasm of sporozoite were not stained with methylgreen. The nucleus was stained with pyronin, and was granular or circular in shape. The cytoplasm surrounding RB was also stained. Moreover, deeply stained granules were sometimes found at the end of the cytoplasm. The RB was not stained with either methylgreen or pyronin. We estimated the Feulgen positive granules in the nucleus to be DNA, and pyronin positive ones in the cytoplasm to be RNA. The specific difference in the degree of DNA polymerization might cause the nucleus to be stained with not methylgreen but pyronin.

In PAS staining, many positive granules surrounding RB were found, especially, abundant in the middle part of the sporozoite. This finding corresponds with other authors' reports in many points [1, 2, 5], and this granule might be amylopectin as was identified by Ryley et al. [4]. The content of granules was varied by individual sporozoite or by species.

For the purpose of more detailed examination, we set up six degrees of indices by content of amylopectin granules (Fig. 1). Those granules in 50 sporozoites of each species were examined and classified into the degrees. As a result, the sporozoites of

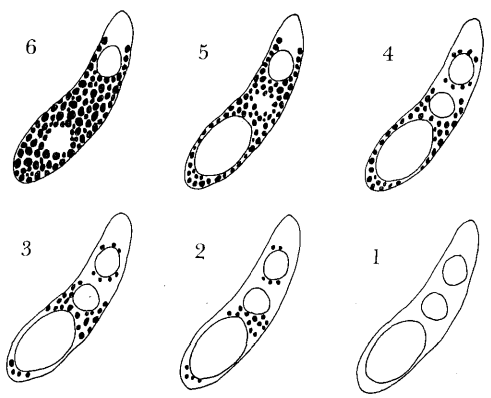

Fig. 1. Index of PAS postive granules

Table 2. PAS positive granule index of *Eimeria* sporozoites

E. acervulina	*E. hagani*	*E. maxima*	*E. tenella*
1.1±0.3	1.1±0.3	1.5±0.6	4.4±1.0*

*: Mean ± S.D. n=50

E. acervulina, E. hagani and *E. maxima* showed the index from 1 to 2. However, those of *E. tenella* showed the index from 3 to 6 (average 4.4) which was about three times as many (Table 2).

The reason why sporozoites of *E. tenella* contained more amylopectin than the other three species was estimated as follows: sporozoites of *E. tenella* excyst in the upper part of the small intestine and the liberated ones reach to the caeca after a distant migration. It is considered that the sporozoite consumes much energy for this, but that it does not take substrates from outside during the migration period. Therefore, amylopectin which was abundant in the cytoplasm may play an important role as an energy source for a long migration.

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要　約

ニワトリコクシジウム *Eimeria acervulina, E. hagani, E. maxima* および *E. tenella* の sporozoite の細胞化学的観察（短報）：中井　裕・勝野正則・扇元敬司（東北大学農学部家畜衛生学教室），角田清・伊藤進午（家畜衛生試験場）——ニワトリコクシジウム *Eimeria acervulina, E. hagani, E. maxima, E. tenella* の新鮮 oocyst より，人工脱殻によって sporozite を得，その細胞化学的な性質について比較検討を行なった．その結果，sporozoite の基本構造である核，細胞質，refractile body には種間差が認められず，虫体の大きさのみに差が見られた．PAS 染色陽性の顆粒が，供試 *Eimeria* すべてに観察されたが，*E. tenella* の sporozoite は，他種と比して，この顆粒を多量に含有していた．