

実験的Eimeria tenellaおよびE.acervulina感染鶏の臓器および血液の変化について

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CHANGES OF ORGAN WEIGHT AND BLOOD
COMPONENTS IN AVIAN COCCIDIOSIS
CAUSED BY *EIMERIA TENELLA*
AND *EIMERIA ACERVULINA*

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Many papers have been published to deal with studies on the life cycle of *Eimeria* and on the chemotherapy of avian coccidiosis, while comparatively few reports have been made on the pathophysiological changes of the host in the course of infection with *Eimeria*.

Pathogenicity of the coccidium depends primarily upon the species of *Eimeria* and inoculum size. Moreover, the breed and age of the host are other factors as well as its nutritional condition. Mixed infection makes the clinical appearance more complicated.

For the screening program of coccidiostats, an experimental method with comparatively satisfactory reproducibility was devised, although all the factors needed for evaluation of a new substance were not fully covered. In this routine screening method, behavioral and pathophysiological changes in the host were traced in the course of infection with and recovery from coccidiosis.

MATERIALS AND METHODS

The chickens used in the present studies were of the Forsgate strain of the Single-comb White Leghorn breed. Fertilized eggs had been obtained from the Niigata Prefectural Poultry Experiment Station. Hatched chickens had been reared at the senior author's laboratory in such manner as to keep free from coccidia.

At 8 days of age the test chickens were moved to the room for infected birds which was filled with fresh air and maintained at a room temperature of 25~28°C and humidity of 45~60%.

A flock of 10 birds was placed in a sterilized cage with a wire floor which was 70 (W) × 40 (L) × 35 (H) cm in size. All the birds were given coccidiostats free chick feed under the following schedule. A diet consisting of 13 g of feed/day/chick was given at 9 days of age. Then it was increased by 1 g per day per chick with the advance in age.

A total of 280 chickens were divided into four groups. A group of 80 chickens was kept as an uninfected (normal) group. Another group of 55 chickens was inoculated orally with 500,000 sporulated oocysts per chick from a pure culture of *Eimeria acervulina* (abbreviated as the Ea group). Another group of 60 chickens was inoculated with 10,000 sporulated oocysts of *Eimeria tenella* per chick (Et-1 group) and another group

of 80 chickens was inoculated with 50,000 oocysts of this coccidium per chick (Et-5 group).

Five chickens of each of the infected groups and eight of the normal group were sampled at random on a certain day after infection. They were anesthetized with ether and sacrificed after collection of blood samples by cardiac puncture.

The blood glucose was measured in the heparinized whole blood sample by the glucostat method. Erythrocytes were counted by means of THOMA'S counting chamber after diluting blood with phloxin B solution. Hemoglobin was measured by means of ERMA'S hemoglobinometer after treatment of blood with cyanometo-reagents. For the determination of hematocrit value, heparinized blood samples were placed into capillaries and centrifuged at 12,000 rpm for 5 minutes. Total protein concentration in plasma was measured by the Hitachi hand protein refractometer.

At autopsy on the certain day after infection, the weight of the liver, kidneys, heart, lungs, spleen, and adrenals and the length of the caeca were measured. The weight of these visceral organs were converted to relative values to the body weight.

Parts of the small intestines and caeca were fixed in 10% formalin solution and stained with hematoxylin and eosin. They were examined for parasites in the digestive tract, lesion, and the process of recovery in host tissues.

The differences between the groups were tested for significance by way of analysis of variance and Tukey test every day after infection.

RESULTS

1. Clinical findings

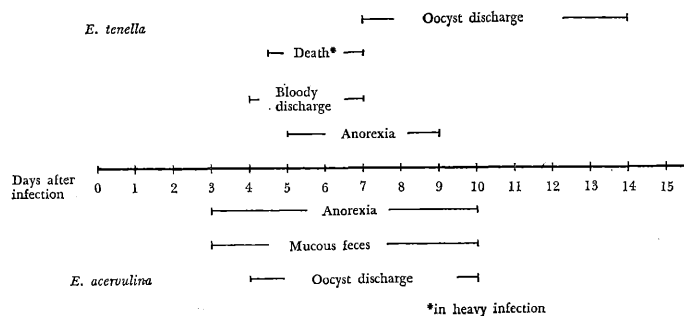
The clinical findings of the groups of a single experimental infection with coccidia are summarized in Fig. 1. From the 4th to 7th days after infection bloody dropping was noticed in both the Et-1 and Et-5 groups. They were especially severe in the later group. About 30% of the infected birds of the Et-5 group died on the 5th and 6th day. On the other hand, no mortality was observed in the Et-1 group.

Anorexia occurred to all the infected groups. Food consumption in the infected groups of the severest stage of infection was reduced to about half of that in the normal group.

2. Changes in weight of the body and visceral organs

Body weight showed a steady increase in the normal group in the course of the experiment. Retardation in body weight increase began on the 4th day in the Ea and Et-1 groups. A sharp falling in body weight was found in the Et-5 group from the 5th to 7th days. By the 15th day body weight in the two Et groups recovered to almost the

Fig. 1. Clinical findings in chickens after single infection with *Eimeria tenella* and *Eimeria acervulina*.



same level as that in the normal group. Despite of the recovery of food intake, no weight change returned to the normal level in the Ea group by the end of the experiment, or on the 31st day after infection (Fig. 2).

A marked increase in weight of the spleen was observed in the Et-1 and Et-5 groups from the 5th to 21st days. A slight increase in weight of the liver was noticed from the 5th to 15th days (Fig. 3).

In the Ea group, weight of the heart decreased strikingly from the 3rd to 10th days (Fig. 3), on the other hand, the changes in weight of the liver, lungs, kidneys and spleen were parallel to those in body weight. No changes in weight occurred to any other visceral organ.

3. Changes in the blood components

The erythrocyte count, hematocrit value, and hemoglobin concentration were observed to decrease extremely in both the Et-1 and Et-5 groups on the 5th and 6th day (Fig. 4). The grade of decrease was correlated with the severity of bloody dropping and the mortality which were dependent upon the inoculum size. By the 10th day, the changes returned to the normal range. Slight changes in hematocrit value and hemoglobin concentration were found in the Ea group. As shown in Table 1, mean corpuscular volume and mean corpuscular hemoglobin changed in the infected groups on the 5th day.

The total protein concentration in plasma presented a sharp drop in the Et-1 and

Fig. 2. Changes in body weight of chickens after single infection with *Eimeria tenella* and *Eimeria acervulina*.

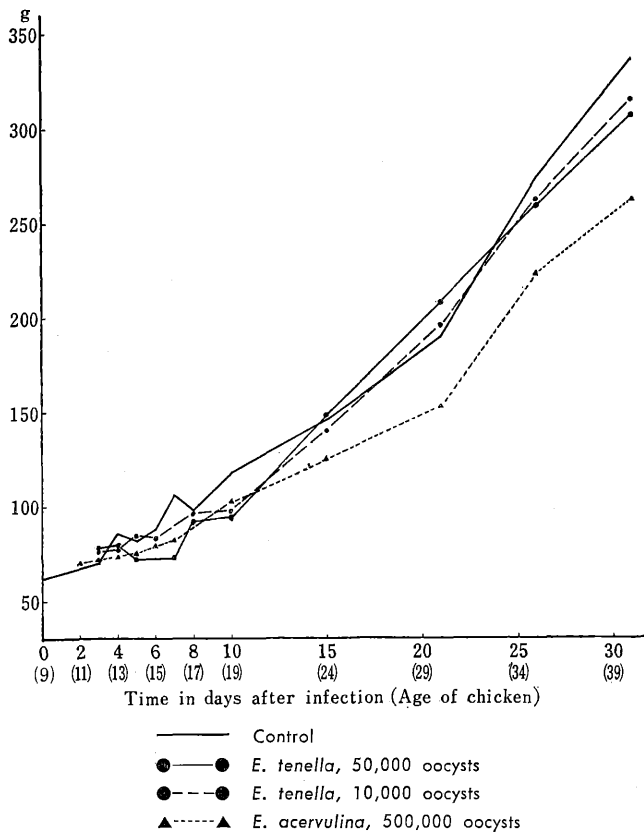
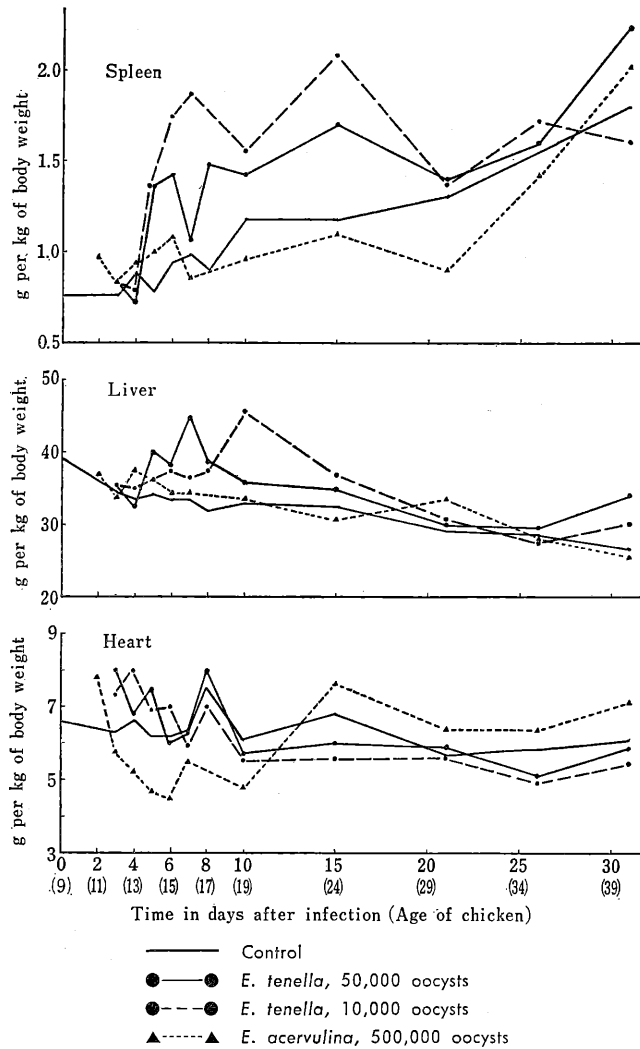


Fig. 3. Changes in organ weight of chickens after single infection with *Eimeria tenella* and *Eimeria acervulina*.



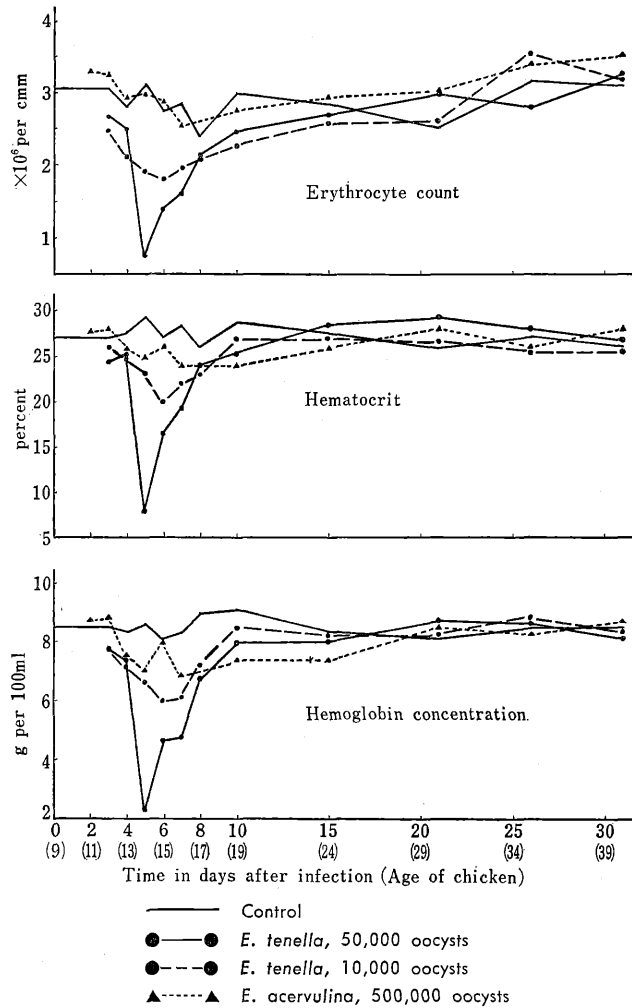
Et-5 groups on the 5th and 6th day, but it immediately return to the normal level on the 7th day after infection.

In the Ea group the total protein concentration in plasma was maintained at a low level for a somewhat longer time than in the Et groups, and recovered to the normal level on the 15th day (Fig. 5).

4. Pathological changes in the digestive organs

The caeca in length began to be atrophic in the two Et groups on the day after the onset of bloody discharge, and never returned to the normal level even by the 31st day after infection (Fig. 6). Severity of histological findings in the caecal tissue was almost parallel to the length of the caecum. In the Ea group, histological lesion disappeared almost completely by the 15th day after infection.

Fig. 4. Changes in erythrocyte count, hematocrit, and hemoglobin of chickens after single infection with *Eimeria tenella* and *Eimeria acervulina*.

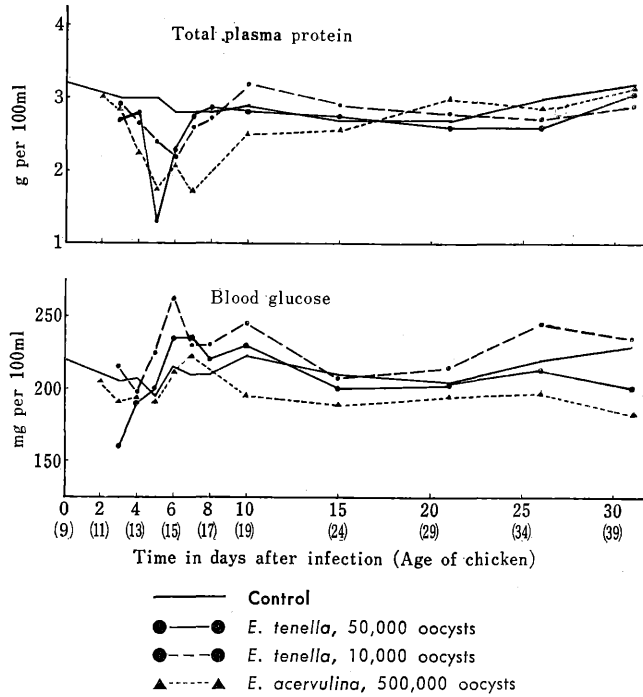


DISCUSSION

It has been recognized that the pathogenicity of coccidia parasitic to the chicken was variable depending not only upon the species of *Eimeria*^{3,6)} and inoculum size^{2,3)} but also upon the breed and age^{2,12)} of the host, and other factors.

In the screening programs of coccidiostats, the same method as shown in this report has been carried out routinely. Results, especially those of the clinical findings, almost reappeared. In all the 9 trials, 41 of the 82 chickens serving as infected-unmedicated controls died from a single infection with 50,000 sporulated oocysts of *E. tenella*. The grand total mortality was 50% ranging from 20~80%. The mean number of oocysts contained in one gram of feces (O.P.G.) was 500,000, on the 8th day after infection with 50,000 oocysts of *E. tenella*, and 20,000,000 on the 5th day with 500,000 oocysts of *E. acervulina*.

Fig. 5. Changes in total plasma protein and blood glucose of chickens after single infection with *Eimeria tenella* and *Eimeria acervulina*.



In contrast to the comparatively high mortality obtained from this investigation anorexia in the survived chickens was milder than that reported by REID¹¹⁾. It is supposed that the differences in mortality among the groups of infected chickens may be due to the differences in breed, age, and environmental conditions of these birds.

Although no changes in weight of the kidneys were observed in any infected groups, attention might be paid to the functional changes of the organ. BERTKE¹⁾ mentioned that the renal clearance function changed with the infection of *E. tenella*.

According to NATT⁹⁾, the total leucocyte count increased sharply on the 5th day after infection with *E. tenella*, and was maintained at the increased level until the 14th day at least. The increase in total leucocyte count was mainly due to that in lymphocyte count. The increase in weight of the spleen in this study seems to suggest the relationship between the bloody discharge and the hyperfunction of the spleen as a site of production of lymphocytes.

The changes in erythrocyte count of the Et-5 group were quite similar to those reported by JOYNER et al.³⁾ NATT⁷⁾ stated in this connection that the decrease in erythrocyte count on the 5th and 6th day after infection with *E. tenella* had resulted essentially from the loss of blood from ruptured blood vessels in the infected caeca, and that this symptom resembled a single mechanical bleeding caused by cardiac puncture. NATT⁸⁾ also reported in his succeeding paper that the decrease in blood volume was mainly due to the loss of erythrocyte and not due to any change in the plasma volume. The author does not support NATT's opinion completely, because it is suggested from Table 1 that the hemoglobin content and the size of red cells may vary with the stage of bloody discharge.

Fig. 6. Changes in caecal length and caecum-rectum ratio of chickens after single infection with *Eimeria tenella*.

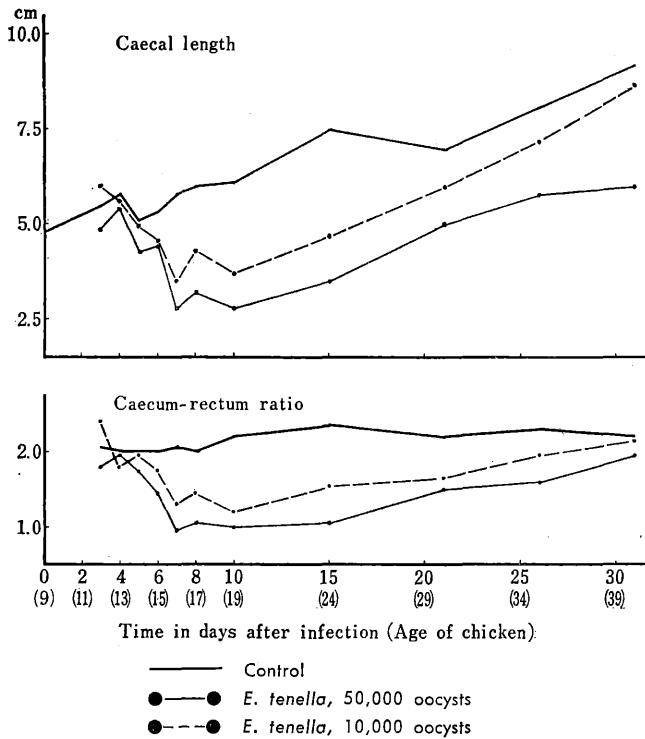


Table 1. Changes in mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration after single infection with *Eimeria tenella* and *E. acervulina*

Item	Group	Days after infection			
		0	5	10	15
MCV ¹⁾ (μ^3)	Normal	86.2 ± 4.4 ⁴⁾	107.8 ± 19.0	90.4 ± 11.5 ⁵⁾	98.5 ± 9.0
	Et-1		127.1 ± 38.6	112.9 ± 14.9	103.0 ± 7.2
	Et-5		123.9 ± 33.8	103.5 ± 13.5	103.8 ± 10.8
	Ea		84.9 ± 7.1	88.4 ± 4.2	89.1 ± 8.3
MCH ²⁾ ($\gamma\gamma$)	Normal	27.8 ± 1.3	34.4 ± 6.7	29.7 ± 4.6	39.7 ± 4.0
	Et-1		38.3 ± 12.9	35.9 ± 4.4 ⁶⁾	30.9 ± 2.9
	Et-5		34.7 ± 10.0	32.8 ± 2.5	29.4 ± 1.8
	Ea		24.0 ± 1.6	27.0 ± 1.8	25.6 ± 2.7
MCHC ³⁾ (%)	Normal	32.3 ± 1.5	30.0 ± 2.6	32.7 ± 1.0	30.0 ± 1.4
	Et-1		30.0 ± 1.2	31.8 ± 1.4	29.9 ± 0.9
	Et-5		28.0 ± 2.2	38.0 ± 2.8	28.4 ± 1.5
	Ea		28.3 ± 1.5	30.5 ± 1.1	28.7 ± 0.5

1) Mean corpuscular volume 2) Mean corpuscular hemoglobin 3) Mean corpuscular hemoglobin concentration 4) Mean ± standard deviation 5) P < 0.05 6) P < 0.01

As is clear from Fig. 5, the total protein concentration in plasma showed a more rapid recovery from a low level to a normal than the erythrocyte count. It is assumed from the result mentioned above that the loss of plasma may have been compensated more easily than the decrease in erythrocyte count in chickens.

In the case of *E. acervulina* infection, clinical manifestations and pathophysiological changes were clearly observed on the 3rd day after infection which was the day before the initial discharge of oocysts.

Anorexia in *E. acervulina* infection seemed to depend upon the severity of intestinal lesions where the parasite passed to another stage of its life cycle and induced the excretion of mucous feces. Food intake has been reported to return to normal by the 8th day (HEIN²⁾), the 9th day (REID et al.¹¹⁾), or the 10th day (in this work) after infection. In spite of normal food intake on and after the 10th day, the growth curve never returned to normal, and the differences in body weight gain between the infected and the normal group became greater with the advance in age during the period of the experiment.

The loss of heart weight in *E. acervulina* infection was quite interesting, although its mechanism is not cleared yet. The weight of any other visceral organ changed in proportion to the body weight, so that the relative weight of each organ to body weight was kept constant throughout the period of infection and recovery.

There was quite a marked differences in the erythrocyte count, the hemoglobin concentration, and total plasma concentration between *E. acervulina* and *E. tenella* infection. It is of interest to assume that the prolonged recovery of the total plasma concentration and mean corpuscular hemoglobin from a low level to a normal in *E. acervulina* infection may be due to the malnutrition caused by anorexia and successive disturbances in the intestinal function as suggested by KOUWENHOVEN et al.⁴⁾

SUMMARY

Pathophysiological changes were examined in chickens experimentally infected with *Eimeria tenella* and *E. acervulina* in the course of infection and recovery. Following the discharge of bloody feces on the 4th day of *E. tenella* infection, a marked decreases was found in such blood components as the erythrocyte count, hemoglobin concentration, and total plasma protein concentration until the 10th day. Anorexia, retarded growth, enlarged spleen and liver, and pathological changes of the caeca were also observed in the infected birds. It took about ten days after the onset of discharge of bloody feces for these birds to return to normal growth.

Prolongation of retarded growth and decrease in weight of the heart were striking findings in *E. acervulina* infection. Most of the clinical and pathophysiological changes were observed on the 3rd and 10th day after infection. The decrease in the components of blood was lower in severity but lasted a little longer in *E. acervulina* infection than in *E. tenella* infection.

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実験的 *Eimeria tenella* および *E. acervulina* 感染鶏の臓器 および血液の変化について

及川 弘・川口陽資
塩野義製薬油日ラボラトリーズ

角 田 清
農林省家畜衛生試験場

(昭和45年12月21日受付)

9日令のヒナ280羽を無感染対照, *Eimeria tenella* 孢子形成オーシスト1万個感染 (Et-5), 5万個感染 (Et-5), および *E. acervulina* 50万個感染 (Ea) の4群に分けた。感染後の経過に伴い各群5~8羽ずつ抽出して剖検し, 体重, 臓器重量, 血液所見, 消化管病変を測定ならびに観察した。

Etの各群では, 感染4日後に血便の排泄が始まり, それにひきつづき種々の症状が現われた。おもな所見は死亡 (Et-5), 摂食量の減退, 体重減少, 脾臓と肝臓の肥大, 盲腸の萎縮病変, 赤血球数・血色素濃度・ヘマトクリット値および血漿総蛋白濃度の低下であった。これらの症状の多くは, 感染10~15日後までに回復したが, 盲腸病変は30日後でも回復しなかった。しかし, 耐過生

存したものの成長は, 無感染対照群とほとんど変わらないほどに回復した。

Ea群では, 感染3日後より顕著な症状が現われ, とくに摂食量の減退, 成長抑制, 心臓の縮少が著しく, 粘着性に富む糞便を排泄した。血液では赤血球の変化は小さかったが, 血漿蛋白濃度は著しく低下した。これらの症状の多くは, 感染10日後までには回復し, これはオーシストの排泄が終わるのに一致した。血液成分にみられた変化はEt群に比べて小さかったが, 期間がやや長かった。Eaの群で最も注目すべき所見は, 発病期にみられた成長抑制が慢性的経過をたどり, 感染30日後でも回復しなかったばかりか, 無感染対照群との成長の差がさらに大きくなる傾向を示していることであった。