Serological Diagnosis of Chicken Leucocytozoonosis

By SHIGEO KITAOKA

First Research Division, National Institute of Animal Health

Chicken leucocytozoonosis has been spread widely in Asian countries, Burma, India, Indonesia, Japan, Korea, Malaysia, the Philippines, Taiwan, Thailand, Singapore, Sri Lanka, and Vietnam. The causative agent was known a hemosporidian protozoa, *Leucocytozoon caulleryi* named by Mathis & Leger in 1909 from a chicken in Tonking. In Japan, this parasite was discoverd in 1954 for the first time. The epizootics of the disease by *L. caulleryi* (=*Akiba caulleryi*)[2] have broken out in summer every year, causing deaths and reduction of egg production rate in adult birds. The vector for *L. caulleryi* is biting midges of the genus *Culicoides*, in which the most important species is *C. arakawae*. The main habitat of the midge at immature stages is surface mud in paddy field, which are occupying the greater parts of farmland in Japan and some of other Asian countries. The general aspects of this disease were reviewed by Akiba.[1]

Although the clinical symptoms are partially useful for the diagnosis of infected chickens at living state, the detection of merozoites and gametocytes in blood smears stained with Giemsa is needed to be conclusive on it. The period of parasitemia is considerably regular and merozoites is appearing 14 to 19 and gametocytes 19 to 24 days after infection. Relapse of protozoa in chicken blood have never been observed as the other avian leucocytozoonosis[2]. Therefore, it is necessary to examine blood samples weekly during a supposed period of enzootics to confirm the infection of chickens with *L. caulleryi*. The serological diagnostic method has been proved useful through laboratory and field investigations to detect the infection from the very first stage by using antibodies, and probably for the whole life of infected chickens by using antigens.

Proportionality in the numbers of infective sporozoites and developed schizonts

With a total of 36 chickens inoculated with 1.0×10^1 to 1.2×10^4 sporozoites which developed in laboratory-reared *C. arakawae*[5] at 16 to 82 days of age, the number of schizonts of *L. caulleryi* per gram of tissue and the total number of schizonts in various organs and in the whole body were determined quantitatively[4], by preparing saline suspension (Plate 1).

Fig. 1 shows the relationship between the dose of sporozoites and the total number of produced schizonts in chickens inoculated at 25 days of age and examined on the 12th or the 13th day of infection. The summarized result apparently indicated that as the dose of sporozoites increased the number of schizonts produced was increased proportionally to a

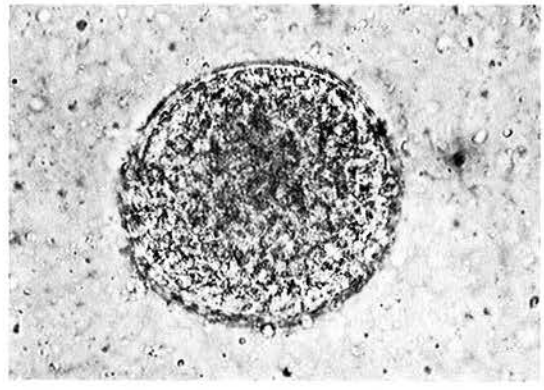

Plate 1. Free schizont in supension of tissue

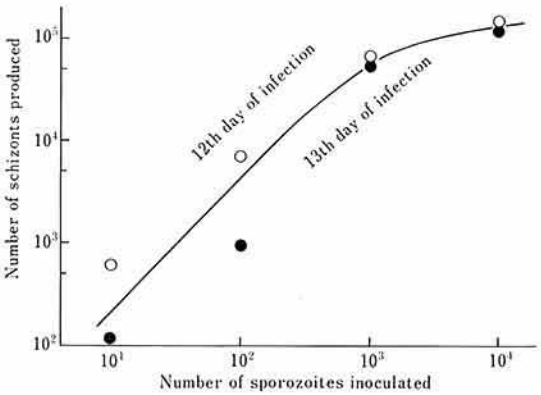

Fig. 1. Relationship between doses of sporozoites of *L. caulleryi* and the number of shizonts produced in chickens.

certain level beyond which an inhibitory tendency was shown in schizont production. The previous paper[6] showed that the mortality and the growth of chickens was governed by the dose of sporozoites. These harmful effects might be more directly affected by the amount of schizont production.

Production of soluble antigens in infected chickens

Soluble antigens were detected in the sera of chickens infected with *L. caulleryi* between the 10th and 15th days after sporozoite inoculation when they were allowed to react against sera collected from chickens infected with the same parasite after the 17th day following infection. A microscopic slide modification of the Ouchterlony double gel diffusion technique and the interfacial precipitation test were adopted for the present investigation[7]. The antigens prepared from schizonts were precipitated by the same sera tested for serum antigens. Antibodies against the antigens of merozoites and gametocytes were detected in sera collected from infected chickens 21 days after inoculation. No precipitation line was observed between antigens originated from normal chickens and chickens recovered from the infections with *Plasmodium gallinaceum, P. juxtanucleare,*

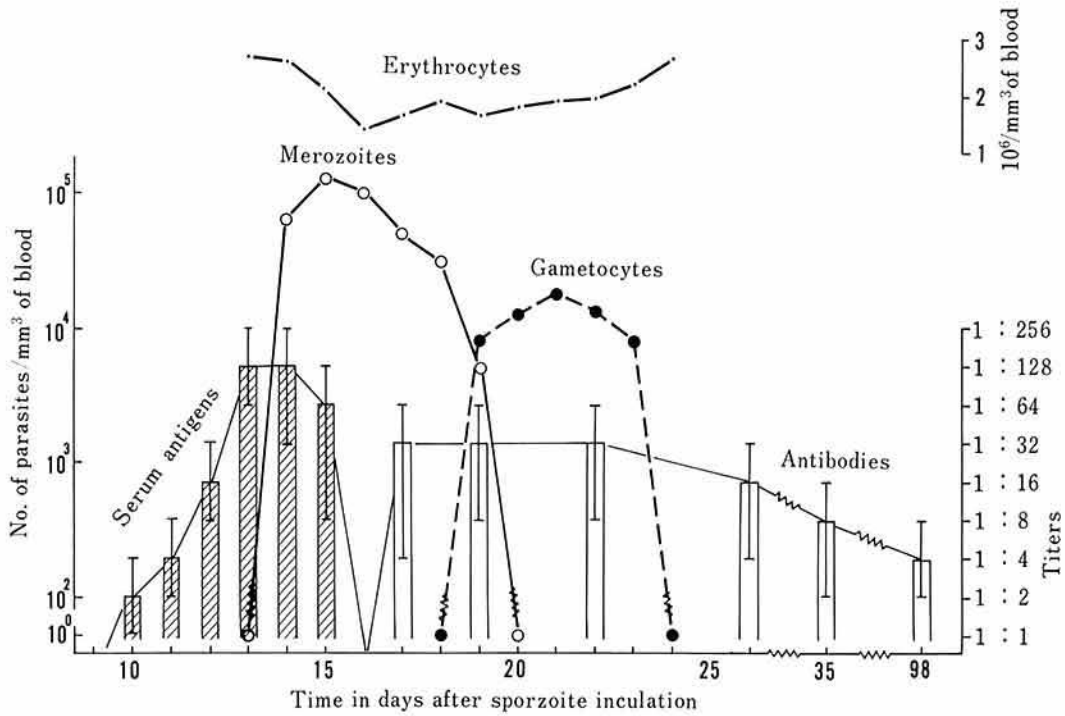

Fig. 2. Mean titers of serum antigens and antibodies in infected chickens with *L. caulleryi*.

Eimeria tenella, and *Mycoplasma gallisepticum*. Fig. 2 illustrates sequential appearance of serum-soluble antigens, their antibodies and parasitemia in infected chickens. The higher titer of serum antigens was recognized 2 days prior to the peak of parasitemia in all cases. The titers of serum antigens and their antibodies showed proportional rises with the numbers of sporozoites inoculated. It was proved that the serum-soluble antigens were originated from schizonts and proteinaceous in its nature and precipitin antibodies were of 7S class immunoglobulin by serological and biophysical procedures[8].

Application on enzootical survey and screening test of prophylactic drugs

The whole life-cycle of *L. caulleryi* among the populations of biting midges and chickens

Table 1. The Sequence in appearance of schizont- and gametocyte antiboides and parasitemia in blood of chickens under enzootics of leucocytozoonosis.

Chicken	V 16			VII 23			VII 30			VIII 6			VIII 13			VIII 20			VIII 27		
No.	SA	GA	P	SA	GA	P	SA	GA	P	SA	GA	P	SA	GA	P	SA	GA	P	SA	GA	P
1	+	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
2	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−	−	−	−	−
3	−	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
4	−	−	−	+	−	M	+	+	−	+	+	−	+	−	−	+	−	−	+	−	−
5	−	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
6	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	+	+	−
7	−	−	−	+	−	−	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
8	−	−	−	−	−	−	+	−	−	+	+	−	+	+	−	+	+	−	+	+	−
9	+	−	G	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
10	+	−	M	+	−	M	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−
11	+	−	M	+	−	G	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−
12	−	−	−	−	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−
13	+	−	−	+	+	G	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
14	+	−	M	+	+	G	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
15	−	−	−	−	−	−	−	−	−	+	+	G	+	+	−	+	+	−	+	+	−
16	−	−	−	−	−	−	−	−	−	+	−	G	+	+	−	+	+	−	+	+	−
17	+	−	M	+	+	G	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
18	+	−	−	+	+	M	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
19	+	−	M, G	+	−	−	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−
20	+	−	−	+	+	M	+	+	−	+	+	−	+	−	−	+	−	−	+	−	−
21	+	−	−	+	+	M	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
22	−	−	−	−	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−
23	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−
24	+	−	G	+	+	−	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−
25	−	−	−	−	−	−	+	−	M	+	+	G	+	+	−	+	+	−	+	−	−
26	−	−	−	−	−	−	−	−	−	+	−	G	+	+	−	+	+	−	+	+	−
27	−	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−
28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
29	−	−	−	−	−	−	+	−	M	+	+	−	+	+	−	+	+	−	+	+	−
30	+	−	G	+	−	−	+	+	−	+	+	−	+	+	−	+	+	−	+	+	−

SA—schizont antibodies, GA—gametocyte antibodies, P—parasite, M—merozoite, G—gametocyte

in the natural environment of Japan, especially its overwintering state, is still completely unknown. To elucidate this fundamental riddle, a continuous field survey has been carried out at a poultry farm, in Kakegawa, Shizuoka Prefecture from 1970 to the present[9]. Table 1 shows a part of a sequence in appearance of merozoites and gametocytes, schizont and gametocyte antibodies and parasitemia in the sentinel flock which had been fed on non-medicated feed from 18 April to 30 November, 1973. All the chickens showing parasitemia produced antibodies to each of schizont and gametocyte antigens. On the other hand, some birds producing antibodies to both antigens were missed to detected the parasite.

In the test conducted in parallel with the above test, the other 5 groups of chickens were fed on feed supplemented with pyrimethamine, sulfamonomethoxine, sulfadimethoxine or meticlopindol in various doses and combinations[3]. All the chickens in 5 medicated groups showed no parasitemia. Therefore, it was impossible to deduce the difference in preventative effect of each regimen. Each groups showed various rates of positive reactions to schizont or gametocyte antigens, except one group medicated with 1 ppm of pyrimethamine + 10 ppm of sulfamonomethoxine. The difference of attitude in the reactions against both antigens in this serological diagnostic method might present more informative data on the degree of inhibition and the developmental stages to be attacked by any drugs compared with the examination of blood smears.

Necessity for comparison with other Asian strains

Shanta & Wan[10] reported that the susceptibility of chickens to *L. caulleryi*—infection varys according to age and they paid attention to the differences in pathogenicity between the West Malaysian strains they observed and Japanese strains. The present author noticed that the morphological characters of sporozoites and schizonts of their materials were also slightly different from those of Japanese strains. It must be emphasized that the comparative study of different local strains in Asia is worthwhile to establish the validity of species identity of Japanese strains and to know their original area of distribution.

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