Serological and Parasitological Studies on Leucocytozoonosis of Chickens in South East Asian Countries, Especially in Malaysia

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Although six species of Leucocytozoidae have been described in domestic chickens,[9] a consideration of existing data suggested that *Leucocytozoon andrewsi*, *L. caulleryi*, *L. sabrazesi* and *L. schoutedeni* are valid species. Among them, *L. caulleryi* has the most widespread distribution and gives the highest damages to the chicken.[3,10,22] The control measures, therefore, are required more severely for *L. caulleryi* than for the other three species in chicken. The vector and life cycle of *L. caulleryi* are known to a more extent than that of other *Leucocytozoon*,[1,3] but its geographical distribution and some biological characters are still now controversial.

L. caulleryi was discovered by Mathis and Leger[25] from a chicken in Tonking in South East Asia for the first time. Since then, this has been found in Japan,[4] Korea,[2] Kwangtung[20] and Taiwan[23] of China, Ruzon of the Philippines,[24] Thailand,[6] West Malaysia,[30] Singapore,[7] Sumatra of Indonesia,[17] Burma,[16] Kerala of India,[34] and Sri Lanka.[31] As to Africa, however, its presence in Egypt[36] and Tanzania,[10] is questionable because it occurred under the absence of main vector species, *Culicoides arakawae*,[1] and its host in Tanzania was guinea fowl.[10] As regards *L. caulleryi* found in Asian countries, Garnham[15] claimed that the Burmese infection should be ascribed to *L. andrewsi* because of the distorted nuclei of the host cells remaining around the mature gametocytes, although *L. andrewsi* was discovered initially from chickens in South Carolina.[5] On the other hand, it has been suspected for a long time that the virulence of *L. caulleryi* might vary from place to place.[15] In fact, it was suggested recently that the severity of chicken leucocytozoonosis in Taiwan[22] and Malaysia[33] might be rather mild than that in Japan. The present authors also found another disparity in the biological characters among the different local strains of *L. caulleryi*; that is, the second generation schizonts in South East Asian strains were bigger in size, with a maximum diameter from 500[7,30] to 400[34] μm, than those of the Japanese strain, measuring 20 to 300 μm in the natural[4] and experimental infections.[18] Therefore, it might be needed to reexamine the prevalence of chicken leucocytozoonosis caused by the infection with *L. caulleryi* in various countries.

Recently, some seroimmunological methods, such as immunodiffusion,[26,27] counterimmunoelectrophoresis,[12] and immunofluorescence,[13] have been established and used frequently for the diagnosis of chicken leucocytozoonosis in Japan. These methods would make it possible to carry out immunologically the comparative study on the different local strains of *L. caulleryi* and elucidate the detail of their relationships. Furthermore, the laboratory colonization of *C. arakawae*, vector of *L. caulleryi* in Japan,[1] Taiwan,[21] and Malaysia,[8] has been carried out successfully for a long time in Japan,[28] and some strains of *L. caulleryi*

isolated from chickens naturally infected in Japan have been maintained by cyclic transmission in chickens and these colonized midges. It seems possible that, if the local strains of *L. caulleryi* in various epizootic areas also were isolated by using the colonized *C. arakawae*, they might be used for artificial infection at any desired time. Consequently, they might be compared under the same condition, and their bionomics would be manifested in detail.

Thus, the combined use of seroimmunological techniques and the colonized *C. arakawae* might be useful to clarify efficiently the above mentioned controversial problems on *L. caulleryi* and its infection in chickens. From this standpoint, some investigations were performed on chicken leucocytozoonosis in several Asian countries. In this paper, some results of them, mainly related to Malaysia, will be presented briefly.

Seroimmunological survey in leucocytozoonosis of chickens in Malaysia and other Asian countries

The existence and distribution of *L. caulleryi* in domestic chickens in Malaysia were described by Omar[30] for the first time. Afterwards, Fadzil and Cheah[8] showed *C. arakawae* to be a possible vector of *L. caulleryi* in Malaysia. Shanta et al.[32,33] reported some aspects of the epizootiology and the chemical control with sulfamonomethoxine on leucocytozoonosis of chickens in West Malaysia. Evidently, this disease has been one of the most serious diseases in poultry industry in this country as well as in Japan. Shanta et al., however, found differences in morbidity and mortality rate, the course of infection and some other aspects between the Malaysian and Japanese strains of *L. caulleryi*.[33] It seems possible that *L. caulleryi* occurring initially among jungle fowls within the confines of some parts of Southeast Asia might have spread throughout the Asian countries as the result of the increased traffic and poultry farming. If this is the case, some differences noticed between the Malaysian and Japanese strains of *L. caulleryi* might suggest that the nature of this protozoa has been changed partially with or after the expansion of its epizootic area.

Therefore, in order to compare the immunological characters and the general parasitological features of *L. caulleryi* in Malaysia and Japan, seroimmunological surveys on chicken leucocytozoonosis in Malaysia were conducted.[11]

As the results of the examination about 450 chickens from 10 to 500 days of age in the field around Ipoh, Malaysia, it was shown that 92.3% at the maximum, of the chickens were positive for antibodies against *L. caulleryi* by the immunodiffusion test (Table 1). But the rate of infection revealed by the blood smear examination was as fairly low as that reported by Shanta et al.[33] This finding suggested that almost all the chickens examined in this study have already recovered from *L. caulleryi* infection and the enormous prevalence of leucocytozoonosis of chicken in Malaysia might be equal to that in Japan. With regard to morbidity, there appeared to be no significant difference between the Malaysian and Japanese strains of *L. caulleryi*.

For the purpose of the seroimmunological investigation on the course of natural infection with *L. caulleryi* in Malaysia, ten 17-day chickens with no history of *L. caulleryi* infection were sentineled at Veterinary Research Institute in Ipoh, Malaysia.[11] At various times, these chickens were examined for *L. caulleryi* infection by means of the immunodiffusion test. Three chickens at the age of 28 days were positive for merozoites of *L. caulleryi* and for either antigens or antibodies against *L. caulleryi*. Although Shanta et al.[33] observed no outbreaks at ages younger than 45 days in the field by the blood smear examination, the age incidence on outbreaks is not in contrast with that described in Japan where chickens of all ages were susceptible.[4]

However, the period of anemia seen in the sentineled chickens infected with *L. caulleryi* was shorter than that with a similar degree

Table 1. Detection of haemoprotozoa in chickens in West Malaysia by immunodiffusion test and blood smear examination (Fujisaki et al., 1979)

Date of bleeding	Farm's name (Place)	Age of chickens	% Antigens positive for *L. caulleryi* (No. positive/ No. tested)	% Antibodies positive for *L. caulleryi* (No. positive/ No. tested)	Results of blood smear examination (No. positive/ No. tested)
July 20, '78	VRI (Ipoh)	90 days old	0	50 (6/12)	
		21 days old	0	6 (1/17)	No parasitemia
		10 days old	0	0 (0/12)	
July 27, '78	AHC (K. Koboi, S. Siput)	90 days old	1.8 (1/57)	12.3 (7/57)	L.c. M (2/57) L.s. G (1/57) P.j. T (4/57)
Aug. 2, '78	Tambun (Tambun)	90 days old	10.2 (5/49)	53.1 (26/49)	L.c. M (3/49)
Aug. 14, '78	Chemor (Chemor)	50 days old	2.0 (1/49)	61.2 (30/49)	L.c. G (10/49)
Aug. 16, '78	Ong (Jelapang)	1 year old	0	61.6 (24/40)	L.s. G (1/40)
Aug. 19, '78	Ong (Jelapang)	1.5 years old	0	92.3 (36/39)	L.s. G (9/39)
Aug. 23, '78	Phang Phoon (T. Rambutan)	150 days old	0	22.5 (9/40)	L.c. G (2/40) L.s. G (1/40) P.j. T (1/40)
Aug. 28, '78	AHC (K. Koboi, S. Siput)	180 days old	0	7.5 (3/40)	L.c. M (1/40) P.j. T (2/40)
Sept. 10, '78	Sena Ceong (Seremban)	40 days old	0	0 (0/40)	No parasitemia
Sept. 12, '78	PMC (Bukit Tengah)	90 days old	0	5 (1/20)	P.j. T (2/20)
		1 year old	0	5 (1/20)	P.j. T (1/20)

VRI : Veterinary Research Institute
AHC : Animal Husbandry Centre
PMC : Poultry Multiplication Centre
L.c. : *L. caulleryi*
L.s. : *L. sabrazesi*
P.j. : *P. juxtanucleare*
G : Gametocytes
M : Merozoites
T : Trophozoites

of parasitemia in chickens of the same age which were experimentally infected with the Japanese strain. From this result, it was suspected that the vilurence of the Malaysian strain might be rather mild to younger chickens.

In the immunodiffusion tests, antibodies in plasma of chickens naturally infected with *L. caulleryi* in Malaysia readily reacted with the serum-soluble antigens and the antigens derived from schizonts of the Japanese strain. The precipitin lines found by the Japanese

serum-soluble antigens, the Japanese schizont antigens and the antigens in plasma of chickens naturally infected in Malaysia appeared to be all similar in their characters. Therefore, the antigens of the Japanese strains of *L. caulleryi* could be said to be homologous and could be used for testing antibodies of the Malaysian strain and *vice versa*.

Morii et al.[29] also carried out the immunodiffusion test for *L. caulleryi* infection in chickens in Taiwan, the Philippines, Singapore, Malaysia and Thailand. In their study, 7.1 to 93.8% of chickens were positive for antibodies against *L. caulleryi*. This result seemed to indicate that antibodies or soluble antigens in the sera of chickens infected with *L. caulleryi* present in each Asian country, might have the same immunological characters.

In conclusion, the immunodiffusion test using the antigens and antibodies prepared from chickens experimentally infected with the Japanese strain is considered to be applicable to the epizootical surveys and diagnosis of *L. caulleryi* infection in chickens in Asian countries.

On the other hand, Fujisaki et al.[11] found the mixed infection with *L. sabrazesi* and *Plasmodium juxtanucleare* in the chickens recovered from *L. caulleryi* infection in Malaysia. This evidence shows that no common antigens for the protective immunity exist among the different species of *Leucocytozoon* in chickens, since the mixed infection of species of *Leucocytozoon*, that is, *L. caulleryi* and *L. sabrazesi* in Malaysia,[30] *L. andrewsi* and *L. sabrazesi* in Taiwan,[22] and *L. caulleryi* and *L. neavei* in Tanzania,[10] were reported so far from several epizootic areas. Moreover, some chickens infected with *L. sabrazesi* and/or *P. juxtanucleare* showed a negative antibody response against *L. caulleryi* in the immunodiffusion test.[11] This would suggest that the *L. caulleryi* antigens can have reacted only to antibodies produced by a previous infection with *L. caulleryi* in spite of that the antibody against *L. sabrazesi* or *P. juxtanucleare* was not demonstrated.

Parasitological and seroimmunological comparisons between Malaysian and Japanese strains of *L. caulleryi* isolated by the colonized *C. arakawae*

It was manifested by the seroimmunological survey on leucocytozoonosis of chickens in Malaysia that the biological characters of the Malaysian and Japanese strains of *L. caulleryi* were common in some characters. No comparative investigation, however, has been carried out in this study with regard to the reinfections with *L. caulleryi* in chickens recovered from its primary infection[33] and to the size of the second generation schizonts. It was considered that the artificial infection with the Malaysian strains of *L. caulleryi* isolated by using the colonized *C. arakawae* might be beneficial to elucidate these complications on *L. caulleryi*. Since the isolation of Malaysian strain of *L. caulleryi* had not been done, the authors in 1980,[19] transported by air to Japan the chickens infected with *L. calleryi*, immediately after they were confirmed to be infected naturally with the Malaysian strain, and that strain was isolated by exposing the chickens to the bloodsucking of the laboratory colony of *C. arakawae*. This isolated strain was designated Ipoh strain and maintained more than 20 generations by cyclic transmission in SPF chicken (Nisseiken Co., Japan) and colonized *C. arakawae*.

As the results of the parasitological and immunological comparison with the Japanese Koshigaya strain[12,13] under the same experimental condition, it was found that almost all the biological characters of the Ipoh strain, such as the appearance of antigens and antibodies in the sera, closely resemble the Japanese strain. In particular, no obvious differences in the virulence between two strains were observed in their experimental infections: the high motality was always shown in the younger chickens inoculated with a large number of sporozoites of either strain. Thus, it seems that the Ipoh strain isolated

and maintained using the colonized *C. arakawae* may cause severer damages to chickens than in the case of natural infection in Malaysia. Although the breed difference in chickens was suspected as one of the reasons for this difference in clinical manifestation, further detailed investigation on the epizootiology of chicken leucocytozoonosis in Malaysia will be needed to clarify this subject.

In this comparative study, the size of the second generation schizonts of both strains was significantly different in spite of the close resemblance in other biological characters.[14] About sixty chickens at various days after the sporozoite inoculation of the Ipoh strain were examined for the existence and the size of the second generation schizonts in their visceral organs. As the result, a fairly large number of schizonts showing the maximum size bigger than 500 μm in diameter were observed 16 days after sporozoite inoculation, and some schizonts were detected 21 days after the infection when higher titer of antibodies appeared in the sera. Such giant schizonts have never been observed in chickens infected with the Japanese strains except for the case of *in ovo* cultivation of *L. caulleryi*, in which the schizonts bigger than 500 μm in diameter were detected in the developing embryonated eggs, whose immunological function was immature, when infected with the Japanese Koshigaya strain.[35] Accordingly, the Japanese strain, especially its second generation schizont, might be more susceptible to host immunity than the Malaysian strain as to the supression of the growth of parasite. In this examination, some schizonts were detected in the liver and spleen of one chicken more than 200 days after infection of the Malaysian strain (Plate 1).[14] This suggested strongly that chickens clinically recovered from infection might reserve protozoa for a long time and become a source of new epizootics. Therefore, the reappearance of parasitemia in adult chickens in Malaysia which was regarded as the re-infection by Shanta et al.[33] might be due to the relapse phenomenon caused by the reserved parasites in chickens.

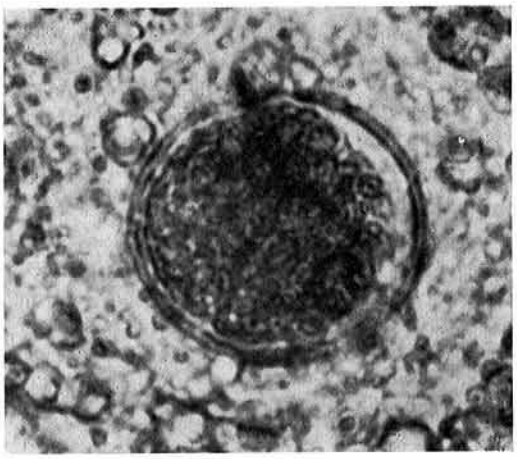

Plate 1. Photomicrograph of schizont detected in the crush preparation from liver of a chicken 203 days after sporozoite inoculation of the Malaysian strain of *L. caulleryi* × 400 (Fujisaki et al., 1982)

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