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RESISTANCE OF MALAYSIAN INDIGENOUS CHICKENS *(GALLUS GALLUS DOMESTICUS)* TO *LEUCOCYTOZOON CAULLERYI*

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SUMMARY **An experimental infection of *Leucocytozoon caulleryi* in the Malaysian indigenous chickens was carried out by the inoculation via wing-vein of forty 21-day-old chickens with varying doses of *L. caulleryi* sporozoites. Infection rates ranged from 60 to 100%. However, no clinical signs were observed. The period of parasitemia was observed from days 16 to 24 after inoculation and serum-soluble antigens and their antibodies were detected between days 14 to 16 and from day 18 onwards, respectively. The bodyweight gains of three groups of chickens heavily infected with *L. caulleryi* were significantly different (P<0.05) compared to uninoculated control.**

Keywords: ***Leucocytozoon caulleryi, Gallus gallus domesticus,*** **parasitemia, serum-soluble antigens**

INTRODUCTION

Leucocytozoonosis, a disease caused by *Leucocytozoon caulleryi*, was reported causing a major problem in poultry in several countries. In Malaysia, the disease occurs as a chronic low grade infection in the majority of cases (Omar, 1968). The disease is usually prevalent amount commercially bred chickens (Omar, 1968; Fujisaki *et al.*, 1979; Morii *et al.*, 1981).

In the Malaysian indigenous domestic fowls, in a survey by Rehana *et al.* (1986), only 1% of apparently healthy birds were found to be infected with the disease. The customary rearing system for the indigenous chickens being free range, implies that the birds are exposed to many parasitic infection and could therefore be carriers for the transmission of pathogens (Zaini and Kanameda, 1991). However, susceptibility of the chickens to *L. caulleryi* by experimental verification has not been reported. This paper reports an experimental infection of indigenous chickens with a local strain of *L. caulleryi*.

MATERIALS AND METHODS

Parasite

The VRI strain of *L. caulleryi* was used in this study. The protozoa was maintained by cyclic transmission in SPF chickens and a laboratory colony of *Culicoides arakawae* (Rahmat and Parameswaran, 1991).

Chicken

Day-old indigenous chicks were purchased from a breeding farm in Perak and reared in an insect-proof room at the ASEAN Poultry Disease Research and Training Centre (APDRTC). Throughout the experiment, the chicks were fed with a drug-free diet.

Experimental Infection

Forty 21-day-old chickens were divided into eight groups, A, B, C, D, E, F, G and H. Each of the groups contained five chickens. Chickens of groups A to G were each inoculated with 10, 100, 500, 1000, 2000, 5000 and 10,000 sporozoites, respectively whereas group H chickens remained as controls. Inoculation was done by intravenous route via wing-vein. In order to assess the effect of infection on weight gain, the chickens were weighed individually before and after experimental inoculation. The body weights determined before the experiment were used as the basis for the distribution of chickens into various groups in order to achieve almost equal total group weights.

Monitoring

Chickens were examined daily for clinical signs and various other parameters for a period of 30 days after the inoculation. Blood samples were collected in heparin from days seven to 30. Blood smears were made from blood drawn from day 10 to day 28. Blood samples collected from days seven to 19 were examined for serum soluble antigen (SSA) of *L. caulleryi* and samples drawn from days 14 to 30 were examined for antibodies against SSA, by agar gel precipitation test (AGPT) (Morii, 1972). The blood smears were stained with 8% Giemsa for 45 minutes after being fixed in methanol for 3-5 minutes and examined for the presence of merozoites and gametocytes of *L. caulleryi*.

RESULTS

The results of experimental infection with *L. caulleryi* are summarised in Table 1. Only 60% of chickens infected with 10 sporozoites were infected, while doses of 100 sporozoites and above resulted in 100% infection rate. However, no mortalities occurred and none of the infected chickens exhibited any clinical signs.

The period of parasitemia was observed between day 16 and day 24. Merozoites appeared from day 16 to as late as day 22, while gametocytes were found in blood smears between day 20 and day 24 after inoculation.

Soluble antigens were detected in the sera of infected chickens between the fourteenth and sixteenth day after sporozoite inoculation in all groups of chickens except group F. Some of the positive sera showed two to three clearly defined precipitin bands on AGPT and most bands were already visible after overnight incubation. Antibodies against the antigens were detected in the sera of infected chickens from day 18 onwards.

Table 1. Effect of experimental inoculation with varying doses of *L. caulleryi* sporozoites in the indigenous chickens

Group	Dose	Period of parasitemia		Appearance of		Rate of infection*	Mortality rate+
		merozoites	gametocytes	Antigens	Antibiotics		
A	10	16-20	20-24	14-16	18-30	3/5	0/5
B	100	16-22	20-24	14-16	18-30	5/5	0/5
C	500	16-20	20-23	14-16	18-30	5/5	0/5
D	1,000	16-21	20-23	14-16	19-30	5/5	0/5
E	2,000	16-22	20-24	14-16	18-30	5/5	0/5
F	5,000	16-22	20-24	15-16	18-30	5/5	0/5
G	10,000	16-21	20-24	14-16	18-30	5/5	0/5
H	Control	-	-	-	-	0/5	0/5

*Number of chickens infected over number of chickens inoculated

+Number of chickens that died over number of chickens inoculated

The bodyweight of the chickens before and after the experiment are shown in Table 2. By the one-way analysis of variance and followed by Duncan's new multiple-range test it was found that significant difference ($P<0.05$) in the bodyweight between each of groups E, F, G with group H, while no significant difference ($P< 0.05$) was observed among inoculated groups.

Table 2. Effects of *L. caulleryi* infection on bodyweight gain of indigenous chickens

Group*	Mean group weight		Weight Difference (g)
	Before (g)	After (g)	
A	85.5	351.4	265.8
B	86.1	342.6	256.5
C	86.2	346.8	260.7
D	86.2	365.2	279.0
E	86.5	313.5	227.1[a]
F	86.6	320.2	233.5[a]
G	86.4	304.3	217.9[a]
H	86.2	408.3	322.2[b]

[a]Significant difference ($P<0.05$) with b group

*Refer Table 1

DISCUSSION

Morii and Kitaoka (1969), reported 100% infection and mortality rates among chickens of 30 days old infected with 100 and above and 2,000 sporozoites of *L. caulleryi* per chicken, respectively. The chickens used by these workers were of the White Leghorn type. We had also observed somewhat similar results previously using White Leghorn SPF chickens and ISA commercial broilers (unpublished data). However, in the present study, indigenous chickens, despite being 100% infected at a dose of 100 sporozoites and above, did not exhibit clinical signs and did not die even with a dose as high as 10,000 sporozoites per chicken. This demonstrates that indigenous chickens are resistant to leucocytozoonosis.

The course of infection in indigenous chickens, in the present study, was notably different from that reported or observed in commercially bred chickens. Several workers using Japanese strains of *L. caulleryi*, (Morii and Kitaoka 1969; 1970; Morii *et al.*, 1989) consistently reported an earlier appearance of merozoites in the peripheral blood on the 14th day after sporozoite inoculation and that of gametocytes on the 19th day. In contrast, the present study showed a protraction of two days and one day in the appearances of merozoites and gametocytes, respectively. A protraction in the appearances of the two stages of the parasite was also reported by Morii *et al.* (1986) using Japanese strain inoculated in White Leghorn chickens - the merozoites and gametocytes appeared in the peripheral blood at day 15 and 20, respectively. The strain of *L. caulleryi* used in this study, in an earlier study (unpublished data), showed similar characters to that of the Taiwanese strain when inoculated in commercial breed of chickens. Therefore, the delay of one day in the appearance of merozoites as seen in this study in comparison to our earlier observation, demonstrated that it was not only related to strain difference but also to the type of breed of chickens used as experimental animals. We postulate that innate resistance of indigenous chickens might have caused a prolonged developmental rate of the parasites in the body. However, Morii *et al.* (1986), postulated the differences observed between the Taiwanese and Japanese strains as attributed to the differences in the developmental rate of schizogonic stages of the two strains.

In the present study, we were only able to first detect serum-soluble antigen and their antibodies at later periods of time as compared to the findings of Morii (1972) and Morii *et al.* (1986). In addition, the persistence of serum-soluble antigens in the peripheral blood was short. However, despite the short duration, both serum-soluble antigens and their antibodies were absent on day 17.

In conclusion, the remarkable resistance of the Malaysian indigenous chickens may present a criterion for genetic selection for extensive commercial poultry rearing in leucocytozoon endemic areas.

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RINGKASAN

KETAHANAN AYAM KAMPONG (GALLUS GALLUS DOMESTICUS) *TERHADAP* LEUCOCYTOZOON CAULLERYI

Suatu jangkitan ujikaji Leucocytozoon caulleryi *pada ayam kampong telah dijalankan dengan menginokulatkan 40 ekor ayam berumur 21 hari melalui vena sayap dengan dos-dos berbeza sporozoit* L. caulleryi. *Kadar jangkitan adalah dalam julat 60 hingga 100%. Bagaimananpun, tiada petanda-petanda klinikal yang dicerapkan. Tempoh parasitemia dicerapkan dari hari 16 hingga 24 dan antigen larut-serum dan antibodinya masing-masing dikesan di antara hari 14 hingga 16 dan dari hari 18 dan seterusnya. Tambahan berat badan ayam daripada tiga kumpulan ayam yang teruk dijangkiti* L. caulleryi *berbeza tererti ($P<0.05$) daripada kumpulan kawalan yang tidak diinokulatkan.*