

## RESISTANCE OF CHICKENS TO REINFECTION WITH *LEUCOCYTOZOON CAULLERYI*

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**SUMMARY:** The effects of primary infection with *Leucocytozoon caulleryi* in specific-pathogen-free (SPF) chickens and their resistance to reinfection were studied. Three doses of  $2 \times 10^1$ ,  $2 \times 10^2$ , and  $2 \times 10^3$  *Leucocytozoon caulleryi* sporozoites were inoculated into three groups of 56-day-old SPF chickens resulted in 75, 100, 100% infection rates and 0, 25, 25% mortality rates, respectively. Surviving chickens had antibody titres of 2 to 32 on agar gel precipitation test and when reinoculated at 35 days after the primary infection showed 100% resistance.

**Keywords:** *Leucocytozoon caulleryi*, resistance

### INTRODUCTION

The role of *Leucocytozoon caulleryi* as the causative agent of chicken leucocytozoonosis has been well documented (Mathis and Leger, 1909; Fallis *et al.*, 1974; Morii *et al.*, 1981). Young chickens are highly susceptible (Morii and Kitaoka, 1969) while birds older than 30 days would survive the infection (Akiba *et al.*, 1958).

The degree of resistance of chickens to reinfection with *L. caulleryi* is also age related. Chickens that have recovered from primary infection at above 14 days of age showed solid immunity to reinfection. However, the degree of immunity, induced after primary infection, has not been studied. The present study was undertaken to determine the relationship between degree of primary immunity and degree of resistance of chickens to reinfection with *L. caulleryi*.

### MATERIALS AND METHODS

#### *Strain Used*

The VRI-strain of *L. caulleryi*, isolated from the Veterinary Research Institute (Rahmat and Parameswaran, 1991) was used in this experiment. Since its isolation, this strain was maintained in chickens and *Culicoides arakawae*, colonised at the authors' laboratory.

### *Experimental Chickens*

Fifteen SPF chickens of C-BE breed served as experimental chickens. The chickens were obtained from the SPF unit of the ASEAN Poultry Disease Research and Training Centre (APDRTC) and kept in an insect-free experimental chicken house throughout the experimental period. The chickens were fed with drug free diet obtained from the Feedmill Unit of the APDRTC.

### *Experimental Infections and Serological Tests*

#### **a. Primary Infection**

At primary infection, chickens were 56 days old. Varying doses of  $2 \times 10^1$ ,  $2 \times 10^2$ , and  $2 \times 10^3$  sporozoites were inoculated into three groups of four chickens each while one group of three chickens were uninoculated and kept as controls. The procedures for preparing sporozoite inoculum were as described previously (Rahmat and Parameswaran, 1991). Briefly, the inoculum was derived from the homogenate of *L. caulleryi* (VRI strain) sporozoites in Eagle's minimum essential medium supplemented with 10% chickens serum and 5% glycerol preserved at  $-80^\circ\text{C}$  for a period of one month before use.

#### **b. Serological Test**

The presence of specific serum soluble antigens and the antibodies against them in the blood of chickens infected with *L. caulleryi* were determined using the agar gel precipitation test (AGPT) according to the method previously described by Morii (1972). Blood samples were collected daily from day 20 after the primary infection with sporozoites and at alternate days from days 16 to 30 after reinoculation. Two-fold serial dilutions of serum samples using phosphate buffered saline was carried out in microplates. The titre was expressed as the reciprocal of the highest dilution that gave a precipitation in the AGPT.

#### **c. Reinfection**

To examine the relationship between antibody titres and resistance to reinfection, chickens that survived the primary infection were divided into four groups, namely group A consisting of three chickens with the highest titre of 32 each, Group B consisting of two chickens with medium titre of 8 each, group C consisting of three chickens with the lowest titre of 2 each and the control group. The control group included a chicken from group I that had neither antibody titre nor parasitemia following primary inoculation and two of the three chickens of the control group at primary inoculation. The remaining chicken served as a negative control in the reinoculation exercise. All chickens except the negative control were given 200 sporozoites per chicken at 91 days old.

### *Blood Examination*

Blood smears were made from blood samples drawn from day 10 to day 27 after first and second inoculations. The smears were fixed in methanol for three minutes and then stained in 8% Giemsa for 45 minutes. The smears were examined for presence of merozoites and gametocytes.

## RESULTS

The effects of primary infection on the chickens are summarised in Table 1. Infection of chickens, as indicated parasitemia, was first observed at day 15 in all the infected groups of chickens. Infection occurred at a rate of 75, 100 and 100% of chickens inoculated with  $2 \times 10^1$ ,  $2 \times 10^2$ , and  $2 \times 10^3$  sporozoites respectively. Chickens inoculated with the lowest dose did not die while chickens inoculated with medium and high doses had mortality rates of 25%.

Table 1. The rate and mortality of chickens after primary infection with *L. caulleryi*

Group	No. of sporozoites inoculated per chicken	First detection of parasitemia (DPSI)	Rate of infection	Mortality rate	Antibody titres	Range	Mean	GM
I	$2 \times 10^1$	15	3/4*	0/4*	2, -ve	0 - 8	3.5	2.8
II	$2 \times 10^2$	15	4/4	1/4	2, 2 8, 32	2 - 32	11.0	4.8
III	$2 \times 10^3$	15	4/4	1/4	32, 32 2, 32	2 - 32	24.5	16.0

- \* Number of chickens infected/no. of chickens used  
 + Number of chickens that died/no. of chickens used  
 GM Geometric mean calculated as  $\sqrt[n]{Y_1 Y_2 \dots Y_n}$   
 DPSI Days post sporozoites inoculation

The antibody titres of chickens are somewhat proportional with doses. Arithmetic and geometric means of various group titres revealed a linear relationship between doses and antibody titres.

Table 2. Degree of resistance of chickens to reinfection with *L. caulleryi* sporozoites

Group	Number of chickens	Number of sporozoites inoculated per chicken	Period of parasitemia	Rate of infection (%)	Rate of mortality (%)	AGPT antibody titre
A	3	200	-	0	0	32
B	2	200	-	0	0	8
C	3	200	-	0	0	2
Positive control	3	200	15-24	100	0	
Negative control	1	0	-	0	0	

The rates of infection in chickens subjected to reinoculation after a primary infection are shown in Table 2. Chickens that are primarily infected showed 100% resistance. The three chickens of the positive control group were all infected but showed no mortalities. Parasitemia was observed in the infected chickens from day 15 to day 24.

## DISCUSSION

Chickens that survived the primary infection and had no antibody titres ranging from 2 to 32 were not infected again by a second inoculation. Primary immunity persisted up to 35 days - the difference in time between primary and secondary infections. Failure of chickens to be reinfected was also reported by Morii and Kitaoka (1970). The workers observed that chickens primarily infected at 14 and 30 days old showed solid immunity to reinfection at 29 and 26 days after the first infection respectively.

The dose of 200 sporozoites per chicken was used at reinfection as this dose was adequate in producing 100% infection rate after primary inoculation. Similar infection rate was observed in positive control chickens after reinfection. In fact for the same infection rate, it was possible to inoculate with a dose as low as 10 sporozoites per chickens (Morii and Kitaoka, 1969).

The period of parasitemia of 15 to 24 days of the strain of *L. caulleryi* used for the present study was different from the Yamaguchi strain (Morii and Kitaoka, 1969, 1970) and the Shizuoka strain (Morii *et al.* 1988). The strain used in the present study closely resembled the Taiwanese strain (Morii *et al.*, 1986) although a previous study (Fujisaki, 1983) had reported that *L. caulleryi*, designated Ipoh strain, isolated from chickens imported to Japan, possessed similar biological characters as another Japanese strain.

It can be concluded that older chickens are able to sustain solid immunity irrespective of antibody levels attained after primary infection of *L. caulleryi*. The present study also indicated that humoral immunity is not related to resistance of chickens with leucocytozoonosis. This is in concurrence with earlier studies (Isobe *et al.*, 1989, 1991).

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## RINGKASAN

### KETAHANAN AYAM TERHADAP JANGKITAN SEMULA DENGAN LEUCOCYTOZOON CAULLERYI

Kesan-kesan daripada jangkitan primer dengan *Leucocytozoon caulleryi* pada ayam-ayam bebas patogen khusus (SPF) dan ketahanannya terhadap jangkitan semula telah kaji. Tiga dos,  $2 \times 10^1$ ,  $2 \times 10^2$ , and  $2 \times 10^3$  sporozoit *Leucocytozoon caulleryi* yang telah diinokulkan pada tiga kumpulan ayam SPF berumur 55 hari mengakibatkan kadar jangkitan masing-masing 75, 100 dan 100% dan kadar kematian masing-masing 0, 25 dan 25%. Ayam-ayam yang sembuh mempunyai titer antibodi di antara 2 hingga 32 pada ujian pempresipitatan gel agar-agar dan selepas diinokulkan semula pada 35 hari berikutan jangkitan primer menunjukkan ketahanan 100%.