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A Review of *Leucocytozoon caulleryi* Infection in Chickens

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ABSTRACT

Leucocytozoon caulleryi is an etiological protozoan of chicken leucocytozoonosis commonly found in various Asian countries. *Leucocytozoon caulleryi* is transmitted by *Culicoides* biting midges and shows high infectivity and pathogenicity to chickens. The severity of clinical symptoms and mortality depend on the number of sporozoites inoculated. Severely infected chickens often die of hemorrhage. Almost all of the chickens challenged with sporozoites after a primary infection at various ages show complete resistance to reinfection and the acquired immunity is expressed against the second generation of schizogony. Soluble antigens are found in the sera of chickens between the 10th and 15th day after sporozoite inoculation. These antigens originate from second-generation schizonts. Antibodies against the antigens of each developmental stage of *L. caulleryi* are recognized in the sera of infected chickens. Each stage is antigenically distinct.

INTRODUCTION AND HISTORY

Many species of *Leucocytozoon* belonging to the suborder Haemosporina are known from various kinds of birds throughout the world. Since *Leucocytozoon caulleryi* as an etiological agent of leucocytozoonosis of chickens was first described by Mathis and Léger (1909) in Tonkin in Southeast Asia, the prevalence of chicken leucocytozoonosis caused by infection with this parasite has been recognized in various Asian countries (Fallis et al., 1974). Akiba et al.(1958) reported the first case of chicken leucocytozoonosis in Japan.

Because of its severe infectious effects causing deaths due to hemorrhage and anemia, retarded growth and reduction of egg production rate in chickens, *L. caulleryi* is considered as one of the highly pathogenic protozoa of chickens.

Akiba (1960) demonstrated that *L. caulleryi* is transmitted biologically by *Culicoides* biting midges belonging to the family Ceratopogonidae of Diptera. It has been clarified up to this time that most of the species of *Leucocytozoon* are

transmitted by *Simulium* black flies. Bennett et al. (1965) proposed that those species which are transmitted by *Simulium* black flies are to be classified into the genus *Leucocytozoon* and those which are transmitted by *Culicoides* biting midges into a genus *Akiba*. Although such generic names were used for some time, Hsu et al. (1973), Levine (1973), and Fallis et al. (1974) had equally proposed that the genus *Akiba* should be a subgenus when the biological character of each species belonging to the family Leucocytozoidae had yet to be investigated through more detailed comparisons.

Leucocytozoon caulleryi is not easily transmitted by blood passage because this protozoan does not undergo schizogony in circulating blood cells of chickens. Now, experimental infections with *L.caulleryi* in chickens are initiated by the inoculation of sporozoites obtained from infected *Culicoides* biting midges. Morii and Kitaoka (1968a) devised a laboratory colonization method for *Culicoides arakawae* serving as a vector for *L.caulleryi*. Therefore, it became possible to maintain *L.caulleryi* in the laboratory and to perform more extended studies on the experimental infections in chickens.

Chicken leucocytozoonosis caused by infection with *L.caulleryi* has been studied by many investigators, and the results of these studies have been reviewed extensively (Akiba 1970; Fallis et al., 1974). This review will focus on studies of life cycle, specificity, and immune responses of chickens to *L.caulleryi*.

LIFE CYCLE

Leucocytozoon caulleryi has three growth stages such as schizogony and gametogony in chickens, and sporogony in *Culicoides* biting midges. *Leucocytozoon caulleryi* infection starts when an infected *Culicoides* biting midge takes a blood meal from a chicken, simultaneously injecting sporozoites into the bloodstream of the chicken (Akiba 1960; Morii et al., 1965; Morii and Kitaoka 1968b). Such sporozoites invade the capillary endothelial cells in the spleen, lung, liver, and bursa of Fabricius and develop into round first-generation schizonts, which mature and release first-generation merozoites on day 5 postinfection (Morii and Fukuda 1992). Mature first-generation schizonts are variable in size ranging from 40 to 65 μm in diameter. Mature first-generation merozoites measuring 7.1 μm in length are long and slender, and are less than 1 μm in width. These merozoites, in turn, invade the capillary endothelial cells of various tissues and organs and become large second-generation schizonts (= megaloschizonts) that release second-generation merozoites on day 14 or 15 postinfection (Akiba 1970; Akiba et al., 1971; Morii et al., 1986). Mature second-generation schizonts are found extracellularly in various organs and tissues and reach sizes of up to 400 μm in diameter. A great variation in the size of mature second-generation schizonts is shown (Kitaoka et al., 1972). The second-generation merozoites measuring about 1.7 μm in length and 1 μm in width are oval in shape and invade erythrocytes and grow into gametocytes. Mature gametocytes completely freed from host cells appear in the peripheral blood of the chicken on days 18-24 postinfection. The macrogametocytes are about 15 μm in diameter and spherical or ovoid in shape. The microgametocytes are a little smaller in size than the macrogametocytes.

Culicoides biting midge that takes a blood meal therefore ingests mature gametocytes. The microgametocyte forms 8 slender microgametes (Morii et al., 1984a). The motile microgamete fertilizes the macrogamete to produce zygote. The spherical zygotes remain within the contents of the blood meal and transform into elongate motile ookinetes 30 to 60 minutes after blood meal. The ookinetes cross the biting midge midgut wall and continue development to form round oocysts. The oocysts measure at maturity about 10 μm in diameter and produce many sporozoites which mi-

grate to the salivary glands of biting midge in 2 and 3 days at 25℃ after blood meal (Akiba 1960; Morii et al., 1965; Morii and Kitaoka 1968b; Morii et al., 1984b). The sporozoites measuring about 10 μm in length and 1.2 μm in width are long and slender. High infectivity to chickens of sporozoites develops during their residence in the salivary glands of biting midge (Morii at al., 1984b). The sporozoites enter the chicken during biting midge feeding.

SPECIFICITY

Leucocytozoon caulleryi was first observed by Mathis and Léger (1909) in the peripheral blood of chickens. Since this original report, natural infections with *L. caulleryi* in chickens have been recognized by many investigators. Mathis and Léger (1910) kept chickens, ducks, geese, turkeys, and pigeons in a pen with chickens with *L. caulleryi*. Only chickens infected with *L. caulleryi*. Morii and Kitaoka (1971) reported that pheasants, Japanese quails, bob-whites, quinea fowls, and bamboo partridges were inoculated intravenously with sporozoites of *L. caulleryi*, but none of these birds was infected. These results suggest that the chicken is the only host for *L. caulleryi*.

The pathogenicity of *L. caulleryi* has been studied in experimentally infected chickens, and the results of these studies were reviewed by Akiba (1970) and Fallis et al., (1974). Morii and Kitaoka (1969) reported that chickens were infected with a single sporozoite of *L. caulleryi*, and that the severity of clinical symptoms and the mortality in chickens rose with an increase in the number of sporozoites inoculated. The pattern of parasitemia in chickens was determined by the time and dose of the first inoculation when the birds were inoculated with different doses of sporozoites repeatedly two or more times at 1 or 2 days' intervals. Clinical symptoms were first observed on day 12 after sporozoite inoculation. Chickens harbouring heavy infections were listless, discharged green feces, lost their appetite, and often died of hemorrhage. Survivors suffered from severe anemia due to hemorrhage and erythrocyte destruction. Petechial hemorrhages and edema were observed in chickens with innumerable second-generation schizonts which formed thrombi in the capillaries and blood vessels of most of the organs and tissues.

IMMUNE RESPONSES

Some of the chickens primarily infected with sporozoites of *L. caulleryi* within 14 days of age were reinfected with sporozoites, but almost all of the chickens challenged with sporozoites after a primary infection at various ages showed complete resistance to reinfection and the acquired immunity to this parasite was expressed against second-generation schizogony (Morii and Kitaoka 1970; Morii et al., 1986, 1989).

Morii (1972) found soluble antigens in the sera of chickens on days 10-15 after sporozoite inoculation. The highest titer of serum-soluble antigens was recognized 2 days prior to the peak of parasitemia. The titer of these antigens increased in proportion to the number of sporozoites inoculated and second-generation schizonts formed (Morii 1977). The serum-soluble antigens originated from the schizonts and were of a proteinaceous nature (Morii 1974). Precipitating antibodies against these antigens and the antigens prepared from second-generation schizonts began to be demonstrated in the sera of chickens infected with *L. caulleryi* on the 17th day, and against antigens from second-generation merozoites and gametocytes on the 21st day after sporozoite inoculation. The antibodies reacted specifically with antigens prepared from *L. caulleryi*. After that, the agar gel precipitation test has been used for the seroimmunological diagnosis and seroepizootiological surveys

of *L.caulleryi* infections in chickens (Fujisaki et al., 1979; Morii et al., 1981).

Fluorescent antibody techniques (Fujisaki et al., 1981; Isobe and Akiba 1982), counterimmunoelectrophoresis (Fujisaki et al., 1980), and enzyme-linked immunosorbent assay (Isobe and Suzuki 1986, 1987a) for detection of antibodies to *L.caulleryi* in chickens have been reported.

The long-term antibody responses to *L.caulleryi* were recognized in chickens that had recovered from a primary infection (Isobe and Suzuki 1987b), but a relapse or recurrence phenomenon in these chickens has not yet been observed.

The immunogenicity of serum-soluble antigens and second-generation schizonts of *L.caulleryi* to chickens has been recognized (Morii 1978; Isobe and Suzuki 1988; Morii et al., 1989). Effective immunization using a killed vaccine prepared from chicken organs containing second-generation schizonts and serum-soluble antigens of *L.caulleryi* was achieved experimentally in chickens (Morii et al., 1990). A proportion of the chickens that had been immunized with the vaccine survived the lethal sporozoite challenge. This protection was attributed to partial suppression of the development of second-generation schizonts in vaccinated chickens after sporozoite challenge.

Further research is needed to elucidate the immunogenic components of this vaccine and immunodominant epitopes of each developmental stage of *L.caulleryi*.

Chicken leucocytozoonosis caused by infection with *L.caulleryi* is an economically important disease that is presently controlled by medication. Recently, remains of chemotherapeutic agents in meat and eggs of chickens have led to a renewed interest in the development of effective vaccines against *L.caulleryi*.

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