Detection of Serum Antibody to *Leucocytozoon caulleryi* in Naturally Infected Chickens by Enzyme-Linked Immunosorbent Assay

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Leucocytozoon caulleryi, a causative agent of chicken leucocytozoonosis, was first described by Mathis and Leger [9] and in Japan by Akiba *et al.* [1]. *L. caulleryi* infection in chickens has been recognized in various Asian countries and affects the productivity of chickens through a reduction in egg production, weight loss and sometimes death. To date, antibody to *L. caulleryi* has been detected by the agar gel precipitation (AGP) test [11, 12], the counterimmunoelectrophoresis [2] and the indirect immunofluorescent antibody test [6]. Recently, an enzyme-linked immunosorbent assay (ELISA) has been reported to be useful as a sensitive and high specific serological test for *L. caulleryi* infection in chickens [7]. However, in the study, the sera collected from experimentally infected chickens were used. In the present study, we applied the ELISA for detecting antibodies in the sera collected from naturally infected chickens with *L. caulleryi*, and the results were compared with those of AGP test as well as the detection of protozoa in peripheral blood.

Ten 40-week-old specific-pathogen-free chickens [4] were kept in the chicken house and exposed to natural infection with *L. caulleryi* for 13 months, from June 1982 to June 1983 in our laboratory. The serum samples were collected from them once a month at a fixed time. The total 132 serum samples were used for the assay. Conjugate, antigen, cutoff point and ELISA procedures were the same as the previous report [7]. To detect merozoites and gametocytes, blood smears were prepared from them at the same time, fixed in methanol for about 10 minutes at room temperature, stained with Giemsa stain and observed microscopically. AGP test was carried out in the same manner described previously [6].

All chickens were naturally infected with *L. caulleryi*. ELISA antibodies were detected with/after detection of merozoites and/or gameto-

cytes in blood smears. After that, they persisted for nearly one year showing various levels (Fig. 1). On the other hand, AGP antibodies were detected with/after detection of merozoites and/or gametocytes in blood smears and sometimes could not be detected although ELISA antibodies were detected. Merozoites and/or gametocytes in blood smears were detected once in each bird from June to August only. In other period, any protozoa could not be found in blood smears (Fig. 1).

Table 1 shows the high sensitivity of ELISA compared with AGP test. Detection rate of antibodies by ELISA was significantly higher than that of AGP test.

ELISA antibodies to *L. caulleryi* persisted for nearly one year. The long-term antibody response to *L. caulleryi* in natural infection has been recognized by AGP test [8]. Recently, schizonts have been detected in some chickens recovered from infection with *L. caulleryi* [3, 5]. At present, it is not clear whether or not these remaining schizonts cause the antibody response observed in the present study. Further studies are necessary to investigate this phenomenon.

In the present study, ELISA antibodies were detected every time after their arising although the levels were variable. On the other hand, AGP antibodies were not detected consistently. Chickens recovered from sporozoite infection

Table 1. Detection of antibody to *Leucocytozoon caulleryi* in naturally infected chicken sera by ELISA compared with the agar gel precipitation test

AGP ^{a)}	ELISA		Total (%)
	+	-	
+	70	0	70 (53.8)
-	58	2	60 (46.2)
Total	128 (98.5)	2 (1.5)	130 (100)

a) The agar gel precipitation test.

Fig. 1. Antibody response to *Leucocytozoon caulleryi* in naturally infected chickens by ELISA and the agar gel precipitation test, and the detection of protozoa in blood smears. *: Chicken No., OV.: Over, P: Detection of protozoa in blood smears, AGP: The agar gel precipitation test, M: Merozoites, G: Gametocytes, +: Precipitin lines were observed, -: not detected.

with *L. caulleryi* show a strong resistance to reinfection with sporozoite [10]. From these results, the infected chicken with *L. caulleryi* could be differentiated from the non-infected chicken by detection of antibodies to *L. caulleryi* using ELISA. Thus, it would be easy to plan the preventive method for leucocytozoonosis in the flock against next epizootics.

Detection rate of antibodies to *L. caulleryi* by ELISA was significantly higher than that of AGP test. It was the same result as mentioned in the previous report using experimentally infected

chicken sera [7]. This time, the cutoff point of 0.20 was used when determined using 0- to 40-week-old specific-pathogen-free chicken sera, and then some falsepositive samples were come out. Therefore, in the present study, cutoff point should be set at 0.50 because OD values of the negative sera ranged from 0.13 to 0.40. In future, if this ELISA will be applied for routine sero-diagnosis in field case, the procedure conditions such as antigen preparation, conjugate and serum concentration, and cutoff point have to be standardized accurately.

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要 約

Leucocytozoon caulleryi 自然感染鶏血清での ELISA (短報): 磯部 尚・鈴木 恭 (農林水産省家畜衛生試験場鶏病支場)——*Leucocytozoon caulleryi* 自然感染鶏血清において, ELISA は寒天ゲル内沈降反応に比べ, 非常に感度が高いことが示された。また, ELISA 抗体価は変動するものの, 翌年の流行期まで陰転することはない。