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Studies on leucocytozoonosis of chickens in Honam districts.

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호남지방의 닭 Leucocytozoon증에 관한 연구

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초 록 : 1992-1993년 호남지방의 4개 양계장에서 처음으로 닭의 *Leucocytozoon caulleryi* 자연감염증을 보이는 87수를 검색하였는데, 임상증세와 혈액내 merozoite 및 gametocyte 검출 그리고 내장기관으로부터 schizont의 발견으로 본 질병을 확증할 수 있었다. 본 감염증은 일년 중 6월 하순부터 9월 중순까지에 발견되었으며, 자연감염된 혈액(merozoite포함)을 미감염된 닭에 접종하여 인공감염상을 볼 수 있었다. 자연감염후 회복된 닭중 1수는 12월에 간에서, 또 다른 1수는 다음해 2월에 심장 근육에서 schizont가 발견되어 본 감염증의 재발이나 동절기를 지나는 장기간 감염 가능성을 보여 주었다. 본 원충의 gametocyte 추출물에서 특이 항원 항체 반응을 보이는 polypeptide는 50.1kD이었다.

Key words : leucocytozoonosis, merozoites, gametocytes, schizonts, polypeptide 50.1 kD

Introduction

Leucocytozoon caulleryi, the causative agent of chicken leucocytozoonsis, was first described by Mathis and Leger(1909)[1] and in Korea by Akiba[2]. *Leucocytozoon caulleryi* infection begins when an infected culicoides biting midge takes a blood meal from a chicken, simultaneously injecting sporozoites into the blood stream of the chicken[3,4]. Leucocytozoonsis in the chicken affects the productivity of chicken through the reduction in egg production, weight loss and sometimes death[5]. The prevalence of this protozoan disease has been recognized in various districts in Korea recently, but the infection has not been reported in Honam. It has been suspected for a long time that the virulence of *L. caulleryi* might vary from place to place[6]. The present report deals with the first data on the chicken leucocytozoonsis in Honam districts, the development of gametocyte-derived antigenicity, and long term infection of the protozoa in such period as late autumn and winter.

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Materials and Methods

Natural infection with *L. caulleryi* : In order to detect the merozoite and gametocyte of *L. caulleryi*, blood smears were prepared from the suspected chickens from 4 chicken farms in Honam, fixed in methanol for about 10 minutes, stained with Giemsa stain, and observed microscopically during the period from June, 1992 to September, 1993. Each organ of the sacrificed chickens was observed at necropsy, and tissue samples were fixed in 10% buffered formalin. They were dehydrated, embedded in paraffin and sectioned at 5μm. Serial sections were stained with H& E and examined by light microscope.

Artificial infection test : To examine the appearance and infectivity of the blood merozoites from affected chickens, uninfected twenty 45-day-old chickens were inoculated IV with 2ml peripheral blood samples collected from infected chickens. The blood smears and the tissue preparations were prepared according to the procedures above and inspected microspically.

Long-term infection or relapse test : A total of 50 infected chickens from 4 poultry farms in Honam were kept in the ckicken cages in our institute. On every 10 days, blood samples were prepared and stained with Giemsa for determination of the recurrence of merozoites or gametocytes, and schizonts were also inspected from serial sections of each organ of sacrificed chickens prepared for light microscopy during the autumn and winter seasons.

Preparation of antigen : For gametocyte antigen, blood was collected from natually infected chickens into heparin solution at peak gametocytemia by microscopic inspection. It was centrifuged at 2, 000rpm for 5 minutes and washed in PBS thrice. The supernatant was discarded. The presence of gametocytes was confirmed by light microscope. The purified gametocytes were resuspended in PBS(1:5v/v) and sonicated at 20kilocycles at a flow rate of 40ml/ minute. The sonicate was centrifuged at 20,000g for 1 hour and the supernatant was collected according to the procedure of Baek[7]. The negative control antigen was obtained similarly from normal 12-day-old chickens

Partial characterization of the antigen : The antigen was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE) according to the procedure of Laemmli[8] and Western immunoblot[9]. The positive and negative sera used in Western immunoblot were obtained from natually infected chickens and normal 12-day-old chickens, respectively.

Results

Obsevation on natural infections : The most of infected chickens were anorexic, anemic, listless, diarrheal, had pallid combs and wattles, but some showed no clinical symptoms. Merozoites or gametocytes were detected in blood smears of 87 naturally infected chickens from June to September only(Table 1. Fig 1,2). At necropsy, they might show splenomegaly, hepatomegaly and hemorrhages in pericardium, spleen, liver, and intestines accompanying some evidences of anemia. Microscopically, schizonts were apparent in the lung, spleen, liver, heart, brain, and pancreas. They often occurred singly within the tissues. Schizonts were sperical or ovoid and were 30-240μm in diameter. They contained fine granular merozoites and were enclosed by well defined walls. The type of host cells for the schizonts could not be determined because infected cells were enlarged or destroyed.

Artificial infection : A few gametocytes were detected in the blood smears of inoculated chickens from the 5th day to the 14th day after inoculation. The same schizonts were sometimes found from serial tissue sections of some organs.

Long-term infection or relapse : Merozoites or gametocytes were observed in blood smears during summer season and the parasitemia rates were decreased in September. But no protozoa were detected in the blood smears of infected chickens which were prepared every 10 days during late autumn, winter and spring seasons. Only one schizont was found in the liver of an infected chicken in December and degenerated schizont cluster was detected in cardiac muscle of a different chicken in next February, even though all recovered chickens

Table 1. Detection of *Leucocytozoon caulleryi* in naturally infected chickens in Honam districts by blood smear examination.

Date of bleeding	Farm's place	Age of chickens (days)	Results of blood smear (No.positive/No.tested)
Jun 24, 92	Wanju(A)	90	41/67
Jul 11, 92	Kimje(B)	140	18/24
Aug 5, 92	Kwangju(C)	120	8/16
Sep 15, 92	Wanju(A)	150	4/18
Nov 10, 92	Kimje(B)	260	0/23
Dec 12, 92	Wanju(A)	240	0/31
Apr 5, 93	Wanju(A)	390	0/42
Jul 12, 93	Jungju(D)	150	57/73
Oct 7, 93	Jungju(D)	240	0/75

A, B,C, D : names of chicken farms

had no clinical symptoms(Fig. 3,4).

SDS-PAGE and Western immunoblot : The specific antigenic moiety of naturally infected chickens was the polypeptide comprised of 50.1kD elucidated by SDS-PAGE and Western immunoblot treated with the positive serum. However, the normal RBC antigen of 12-day-old chickens was not recognized by Western immunoblot. Also, the normal and infected antigens were not recognized in Westerin immunoblot treated with negative sera of non-infected 12-day-old chickens(Fig, 5,6).

Discussion

As shown in Table 1, the naturally infected protozoa were first detected from June 24 to September 15, 1992. They were again detected next summer, but the merozoites and gametocytes disappeared in late autumn, winter, and spring seasons.

The detection of some intact gametocytes and schizonts in the chickens which were inoculated with infected chicken blood suggested that the merozoite could serve as an experimental source for part of life cycle between schizonts and gametocytes.

The recurrence of gametocytemia which is known as spring relapse[10], occurred in various avian hosts recovered from the infection of some *Leucocytozoon* species. But very few informaton is available on the possibility of the latent state of *L. caulleryi* infection in such period as late autumn, winter, and early spring when transmission by *Culicoides arakawae* ceases[11-13]. A single schizont was observed in serial preparation of liver in December and clustered schizonts in heart in next February, from the naturally infected chickens killed at every 10 days throughout the winter period. It is speculated that chickens recovered from the infection may preserve the protozoa up to the next epidemic season.

Various investigators have reported that antigenic characters and immunity of *L. caulleryi* were significantly different according to applied methods and sources[14-19]. Antigen profile of blood gametocytes of *L. caulleryi* was analysed by SDS-PAGE and Western blotting techniques. Only one specific protein 50.1kD was revealed and no false positive reactions were observed. Therefore the relative importance of this polypeptide antigen 50.1kD is to be considered for developing immunity on *L. caulleryi* in Korean chickens.

Summary

In the year 1992/93 leucocytozoonosis could be first diagnbosed in 87 chickens of 4 chicken farms in Honam districts. The diagnosis was confirmed by detection of the blood merozoites or gametocytes and histological finding of the schizonts from various organs with some clinical signs. Cases of leucocytozoonosis only occurred from the end of June to the middle of September. Artificial infection could be observed by means of inoculation of infected blood merozoites.

The schizonts were found in the liver and cardiac muscle of the different chickens recovered from the natural infection, respectively, in September and next February. Thus the relapse or long-term infection in cold seasons might be possible. The unique gametocyte antigen polypeptide was 50.1kD.

Legends for figures

Fig 1. Endo-erythrocytic merozoites(arrow) in Giemsa-stained smear of peripheral blood of the naturally infected chicken. ×400.

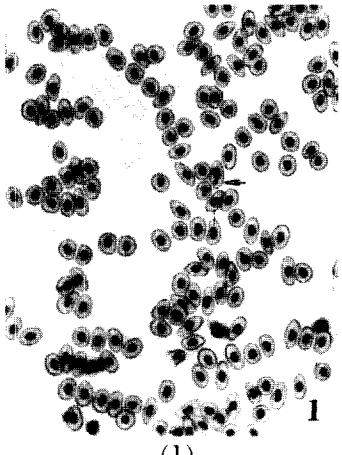
Fig 2. Macro-gametocytes(arrow) free from the host cells in peripheral blood of the naturally infected chicken. × 400.

Fig 3. A schizont in the liver of the naturally infected chicken in December 1992. H&E. ×200.

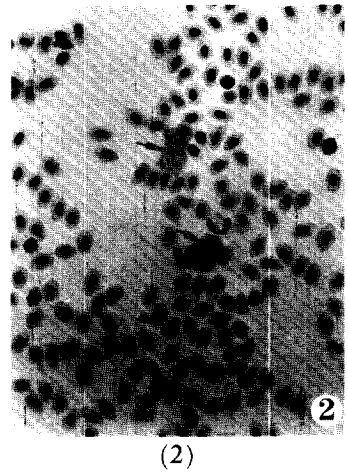
Fig 4. Clustered schizonts in the cardiac muscle of the naturally infected chicken in Fedruary 1993. H&E. ×200.

Fig 5. SDS-PAGE results on L.caulleryi gametocyte antigen
Key : KD=molecular weight, kilodaltons.
L=L.caulleryi gametocyte antigen.
N=normal chicken RBC extract antigen.

Fig 6. Western immunoblot analysis of L.caulleryi gametocyte antigen
Key : L=positive serum from naturally infected chicken.
N=negative serum from normal chicken.



(1)



(2)

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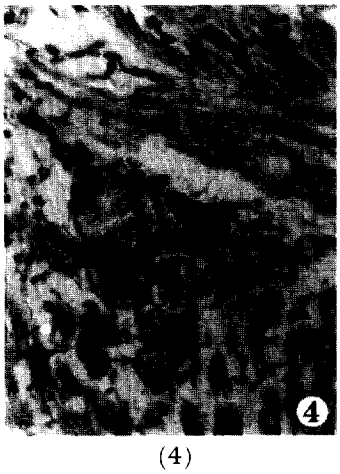

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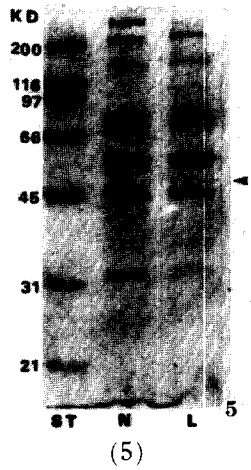

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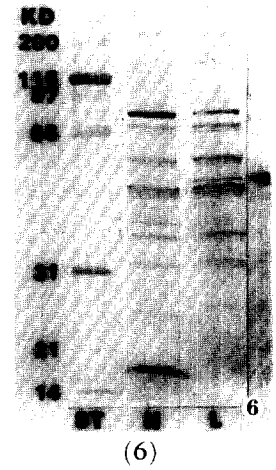

(6)

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