

Diagnosis of Chicken Anemia Agent Infection

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Abstract

Chicken anemia (CAA) is a novel avian virus which recently been identified. It produces aplastic anemia in young chicks on an experimental basis. It may presumably be a major causative agent of anemic diseases in field chickens. CAA fails to grow under a standard avian cell culture. It propagates, however, in some lymphoblastoid cell lines established from lymphomas of chickens. These cell lines are used to isolate and titrate CAA, and also to detect the antibody to CAA. Owing to the development of an assay system in vitro, knowledge on CAA has been accumulated rapidly.

Discipline: Animal health

Additional key words: Aplastic anemia, chicken anemia virus (CAV)

Chicken anemia agent (CAA) is a small DNA virus which was first isolated from field chickens in 1979 by Yuasa et al.¹¹⁾ It was named CAA because it produced anemia in chicks experimentally and could not be classified into one of the existing virus groups at that time. Until now, CAA has been isolated in many countries^{1,2,3,5)}. Recently, CAA was well characterized virologically and proposed to term chicken anemia virus (CAV) instead of CAA by Gelderblom et al.⁴⁾ However, the term of CAA is used in this report.

Anemic diseases of chickens accompanied by aplasia of the bone marrow, hemorrhages and atrophy of the lymphoid organs have been reported by a number of researchers⁷⁾. They are recognized to have multiple etiologies. However, those etiologies have not been well identified so far. The recent results indicate that CAA plays a major role in inducing anemic diseases^{2,6,10,17)}. So anemic diseases in the field chickens have to be diagnosed, taking into consideration CAA infection.

Isolation of CAA from diseased chickens is indispensable to diagnose anemia caused by CAA infection. Since the standard tissue culture cells and embryonating chicken eggs could not be used for assay of CAA, specific pathogen free (SPF) chicks or lymphoblastoid cell lines are used for that purpose¹³⁾. This report describes clinical features of

CAA infection and some assay methods of CAA by using chicks or cell culture.

Clinical features of experimental chicks inoculated with CAA^{8,9,11)}

Chicks inoculated with CAA at one day of age show anorexia, lethargy, drooping of the neck and anemia in approximately ten days after inoculation and some of them die during the period 12 to 21 days. The incidence of anemia in inoculated chicks takes place by 100%, but the mortality of the affected chicks is generally less than 50%. The hematocrit values of the affected chicks start declining from 8 days and reach a minimum level in about 16 days after inoculation. Cell number in all kinds of cell series such as red and white blood cells and thrombocyte decreases at the same time. Anemia in the CAA infection is pancytopenia. Chicks die at this stage. In the surviving chicks, these peripheral blood changes return to almost the same level as those in normal chicks.

Macroscopically, discoloration of the bone marrow to yellow and atrophy of the thymus are characteristic changes always taking place in the chicks affected with CAA. Atrophic changes of the bursa of Fabricius are also observed. Hemorrhages appear occasionally in the skin, skeletal muscle, and proven-

tricular mucus membrane of the affected chicks.

Histopathological changes are observed in parallel with macroscopical changes, at the same time, in frequency and severity. In the bone marrow, all the cells known as the erythrocytic, thrombocytic and granulocytic series are reduced remarkably in number and disappear with a displacement by adipose cells. Lymphocytes decrease in number and disappear in the lymphoid organs throughout the body. In the chicks which survive infection, these clinical and pathological changes disappear gradually and the chicks are restored to almost the same condition as normal chickens.

Frequency of the incidences and severity of anemia by CAA infection depend on various factors such as age, presence or absence of maternal antibody, pathogenicity among strains, genetic difference in the susceptibility of chickens, and dose and route of infection. They are influenced particularly by the age at which birds are exposed to infection. With an advanced age, the birds rapidly acquire resistance to CAA. Chicks inoculated with some isolates of CAA at over 2 weeks of age generally show no clinical signs. Chicks with maternal antibody are refractory to infection with CAA.

Field cases of CAA infection

Recently, some field cases of anemia have been reported to be CAA infections^{2,6,10,17}. However, it is not so easy to diagnose CAA infection. Since CAA exists commonly among commercial chicken flocks and field chickens are inapparently infected with it, even when CAA is isolated from diseased chickens, it could not be simply deduced that CAA has caused the diseases. Considerations have to be made on the following two points in order to confirm the diagnosis of CAA infection: (1) CAA is isolated from almost all the affected chickens; and (2) the parent flock from which the diseased chicks have derived have no antibody to CAA.

A diagnosis was made on a field case of anemia accompanied with aplasia of the bone marrow and hemorrhages in the body as CAA infection¹⁷. That incidence took place among 16- to 26-day-old broiler chicks. The losses reached 9.3% of 6,374 birds at the age of 26 days. Hemorrhages of the muscles, aplastic bone marrow and atrophy of the lymphoid organs were commonly observed in the affected

chicks. The results of post mortem and virological examinations are summarized in Table 1. The disease closely resembled an experimental CAA infection from the viewpoints of clinical and pathological findings, and the age of chicks at which it took place. CAA was isolated from all the liver materials examined shown in Table 1.

From the result of antibody test to CAA in the parent flock from which the diseased chicks were derived, it was evident that some of them lacked a maternal antibody. CAA might have infected them immediately after hatching or possibly via the egg, and induced the disease.

Methods for isolation of CAA from field materials^{13,14}

Since CAA can be recovered from any organ of chickens inoculated with it, any material from the affected chickens may be available for isolation of CAA. In the studies undertaken by the author, the livers from the affected chickens were used for isolation of CAA. A 20% liver homogenate is made with a cell culture medium. It was frozen and thawed three times and centrifuged at 2,500 rpm for 10 min: the resulting supernatant fluid was used as the material for isolating CAA. In some cases, it was heated at 70°C for 5 min and centrifuged again to eliminate large tissue debris.

1) Isolation of CAA by chick inoculation

Chicks free from CAA infection were inoculated with 0.1 ml of the material at one day of age. Their hematocrit values were determined 14 days after inoculation and the chicks were subjected to investigation of their femoral bone marrow. The material was recognized to be CAA-positive when the inoculated chicks showed lower hematocrit values than the control chicks as well as aplastic bone marrow.

2) Isolation of CAA by cell cultures

MDCC-MSBI cells of an established cell line derived from Marek's disease lymphomas were used for CAA isolation. They were cultured in culture tubes, with a size of 10 × 110 mm covered by an aluminum cap, which contained a growth medium, i.e. RPMI 1640 supplemented by 5% fetal bovine serum. The cells were cultured in an incubator containing 5% CO₂ at 39°C.

Table 1. Clinical, post mortem and virological examinations of CAA infections under field conditions

Chick no.	Age (days)	Red blood cells ($\times 10^4/\text{mm}^3$)	Hematocrit value (%)	Macroscopic lesion ^{a)}			Isolation of virus	
				Bone marrow	Hemorrhages	Lymphoid organs	CAA	Others ^{b)}
1	17	ne	ne	+	-	+	+	-
2		ne	ne	+	+	+	+	-
3		ne	ne	+	-	+	+	-
4		ne	ne	+	+	+	+	-
5		ne	ne	+	+	+	+	-
6		ne	ne	+	+	+	+	-
7	18	ne	ne	+	+	+	+	-
8		ne	ne	-	+	+	+	-
9		ne	ne	+	+	+	+	-
10		ne	ne	-	+	+	+	-
11	20	23	15	+	+	+	+	-
12		71	6	+	+	+	+	-
13		101	28	-	+	-	+	-
14		24	10	+	+	+	+	-
15		ne	ne	-	+	+	+	-
16		100	13	+	+	+	+	-
17		111	13	+	+	+	+	-
18		87	32	-	+	+	+	-
19		48	11	+	+	+	+	-
20		14	5	+	+	+	+	-
21		ne	ne	+	+	+	+	-

a): Discoloration of the bone marrow. Hemorrhages of breast or thigh muscle. Atrophy of thymus, bursa of Fabricius or spleen.

b): Viruses producing cytopathic effect (CPE) in chicken kidney cell culture.

ne: Not examined.

An MSB1 cell suspension had a concentration of 2×10^5 cells/ml. One ml of the suspension was dispensed into each tube. One-tenth ml of the material was inoculated into the tube. The inoculated cells were cultured, and 0.2 ml of the cell suspension was transferred every other day to a new tube containing 1 ml of the fresh growth medium. A success of the transfer was confirmed on the basis of coloring of the culture medium. The medium that showed a red color was recognized to be CAA-positive. The outcome of isolation was finally evaluated after the 7th transfer.

Detection of antibody against CAA^{15,16)}

1) Neutralization test

The serum, which was inactivated at 56°C for 30 min and followed by a series of four-fold dilution starting with a 1 : 5 dilution, was mixed with an equal volume of CAA containing 200 TCID₅₀/ml.

The mixture was incubated over night at 4°C. The infectivity of the mixture was assayed by MDCC-MSB1 cells according to the procedure as described above. The reciprocal of the highest dilution of the serum that perfectly inhibited the infectivity of CAA was applied as a neutralizing titer.

2) Indirect immunofluorescent antibody (IFA) method

Since the IFA test has the same sensitivity as the neutralization test and can be completed more quickly and easily, the IFA test was adopted for detecting CAA antibody. MDCC-MSB1 cells infected with CAA were used as a source of antigen. The MSB1 cells which were inoculated by a possible highest titer of CAA were collected 24 hr after cultivation. Those cells were centrifuged and smeared on to a glass microscope slide, dried and fixed with acetone for 10 min. Fifteen spots of the smears were made with a micropipette on the slide (76 × 26 mm) to examine

15 samples. The smears were stained in a conventional way, using at first a primary serum and then with fluorescence isothiocyanate-cojugated rabbit anti-chicken IgG, at 37°C for 30 min in each treatment. The CAA positive antigens were observed mainly in the nuclei of infected cells. The MSBI cells were specifically stained with an anti-Marek's disease virus (MDV) serum or an anti-turkey herpesvirus (HVT) serum under the IFA test. This was because the cells possessed MDV-induced intracellular antigens. It was, however, possible to differentiate MDV-antigens from CAA-antigens since the MDV-positive cells were proportionally smaller in number than the CAA-positive cells when the MSBI cells heavily infected with CAA were used. Furthermore, the MDV-positive cells were generally stained more intensely than the CAA-positive cells.

A method for diagnosis of CAA infection in chickens is briefly explained hereafter. It seems that aplastic anemia similar to CAA infection might also be induced by some other causes such as sulfonamide intoxication and some viral infections. Differential diagnosis from these diseases should be acquired. From the above-mentioned results obtained from the experimental infections with CAA, the diagnostic points of CAA infections are summarized as follows:

- (1) Epidemiology
 - (a) The maternal antibody of the affected chicks are negative.
 - (b) The disease occurs in chicks of less than 6 weeks of age in general, and 1-4 weeks of age in particular.
- (2) Pathology
 - (a) Pancytopenia.
 - (b) Aplastic bone marrow and atrophic thymus are always observed in the affected chicks.
- (3) Etiology

CAA can be isolated from almost all the affected chicks.

It is proved that CAA infection is enhanced by other factors¹²⁾. In these cases, diagnosis may probably be more complicated. There might be other clinical and pathological features that are not mentioned above.

At present, it is considered that the outbreaks of CAA infections are not very frequent throughout the world. Therefore, they are not regarded as a major economic threat to the poultry industry. However, the presence of CAA should not be overlooked

because of its so-called 'complicated infections' or its aggravating effects on the efficacy of vaccines. The SPF flocks from which biological products are produced should be checked carefully regarding their CAA infections.

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