幼雛における鶏貧血因子に起因した貧血症の発生

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Outbreak of Anemia Associated with Chicken Anemia Agent in Young Chicks

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ABSTRACT. An outbreak of anemia caused by chicken anemia agent (CAA) infection was observed in the progeny chicks derived from the breeder flocks imported on a poultry farm. The mortality rate of female and male chicks between 12 to 25 days of age was about 2.4% and 20.9%, respectively. Macroscopically, the affected chicks revealed yellowish bone marrow, marked atrophy of the thymus and bursa of Fabricius, discoloration and swelling of the liver and consolidation of the lung. Histologically, marked aplasia or hypoplasia of the bone marrow and severe depletion of lymphocytes in the lymphoid organs were observed in most birds examined. CAA was isolated in MDCC-MSB1 cells from livers of the affected chicks at 17 days of age. Pulmonary aspergillosis and bacterial infections were high in incidence and an important cause of loss in the present chick flocks. One-day-old progeny chicks derived from the same breeder flock as that of the naturally affected birds showed low susceptibility to the agent isolated.—KEY WORDS: anemia, aplasia, CAA, young chick.

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Chicken anemia agent (CAA) is a transmissible agent that produces marked anemia with aplasia of the bone marrow and atrophy of the lymphoid organs in susceptible chickens [7, 8, 10]. CAA is widespread in commercial chicken flocks in Japan, since it can be isolated frequently [5, 10, 13] and the neutralizing antibody is detectable at a high rate [14]. However, spontaneous occurrence of anemia caused by CAA has not been documented in commercial young chicks less than 3 weeks of age. This paper describes an outbreak of anemia in young chicks associated with CAA and pathological changes of the affected chicks.

MATERIALS AND METHODS

Naturally occurring disease: One-day-old chicks for the breeder were imported from a foreign country in September, 1985, divided into two flocks (A and B), and reared in separated condition. The disease occurred in the progeny flocks of about 4,200 female chicks derived from the breeder flock (A) and 680 males derived from the breeder flock (B) in a poultry farm of Tottori Prefecture in May, 1986. They had been reared in 6 brooders (5 groups of females and one group of males) on a floor.

Forty-two moribund or 16 dead birds of both sexes at 16, 17, 20, 22 and 25 days of age from the farm were submitted to pathological examination. Blood sample was taken from the wing vein into a heparinized capillary tube from the moribund chicks, and then the hematocrit value (%) was read after centrifugation at 12,000 rpm for 5 min. Following post-mortem examination, tissue samples were collected, fixed in 10% buffered formalin solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin (HE). Parts of livers from the moribund chicks at 17 days of age were collected for examination of pathogens and stored in a freezer at −80°C.

Preparation of inoculum and isolation of CAA: The livers from the affected chicks

were homogenized and prepared as 20% suspensions in RPMI1640 medium with 100 μg/m*l* kanamycin sulfate and 1 μg/m*l* Fungizone. The supernatants resulting from the homogenate after centrifugation at 3,000 rpm for 10 min were used as an inoculum. The isolation of CAA was performed from the inoculum using MDCC-MSB1 cells [1] by the method reported previously [3, 9].

Table 1. Number of dead or moribund chicks in naturally occurring disease

Age (days)	Female		Male	
	Dead	Moribund	Dead	Moribund
12	–	–	–	–
13	1	–	3	–
14	–	–	9	–
15	7	8	11	8
16[a)]	9	–	10	20
17[a)]	4	42	2	14
18	3	12	4	12
19	1	3	2	3
20[a)]	1	4	4	7
21	1	1	–	2
22[a)]	–	2	1	7
23	–	2	–	4
24	–	2	1	3
25[a)]	–	–	1	10
Total	27	80	48	90

a) Submitted to laboratory investigation.

Table 2. Mean body weight and hematocrit value of naturally occurring cases

Age (days)	Body weight (g) Mean±s.d. (range)	Hematocrit value (%) Mean±s.d. (range)
16	91±10(12)[a)] (70–120)	19.5±5.7(4)[a)] (16–24)
17	108±12(16) (80–160)	17.0±4.9(13) (6–30)
20	108±18(9) (70–150)	16.0±8.8(6) (5–28)
22	145± 9(10) (130–170)	26.1±5.2(9) (18–36)
25	196±24(11) (120–250)	18.2±5.7(10) (5–32)

a) Number of chicks examined.

Serial 10-fold dilutions of the inoculum were inoculated into each of two culture tubes containing 5×10^5 cells/m*l* and titrated by a modification of the method reported previously [6].

Experimental reproduction of the disease: Twenty one-day-old chicks hatched from fertile eggs of the breeder flock (B) were used. Ten chicks were inoculated intramuscularly with 0.1 m*l* of the inoculum from the affected livers, and others were kept as the uninfected control. They were reared separately and fed a commercial feed and tap water *ad libitum*. At 14 days postinoculation, hematocrit values of all chicks were determined and they were sacrificed for pathological examination.

RESULTS

The affected chicks showed depression with anemia, and the mortality was increased between 2 to 3 weeks of age as shown in Table 1. Retardation of body weight gain and decrease of hematocrit value were marked in most affected birds (Table 2). Male chicks derived from the breeder flock (B) showed a relatively high mortality (20.9%), while females derived from the breeder flock (A) were low in mortality (2.4%).

Main macroscopical lesions of the birds examined are summarized in Table 3. The bone marrow was fatty and yellowish, and

Table 3. Main macroscopical lesions of naturally occurring cases

Age (days)	16	17	20	22	25
Number of chicks examined	12	16	9	10	11
Yellowish bone marrow	10[a)]	16	9	5	10
Atrophy of thymus	11	16	9	10	9
Atrophy of bursa	8	12	6	9	7
Swelling of liver	7	15	5	1	9
Consolidation of lung	4	6	3	3	4
Swelling of eye-lid	5	1	0	0	0

a) Number of affected birds.

Table 4. Main histological lesions of naturally occurring cases

Age (days)		16	17	20	22	25
Number of chicks examined		12	16	9	10	11
Bone marrow	Aplasia or hypoplasia	12[d)]	15	8	6	8
	Hyperplasia	3	7	7	10	8
Thymus	Depletion of lymphocytes	12	15	8	7	8
	Repopulation of lymphocytes	1	5	4	6	6
Bursa	Depletion of lymphocytes	12	3	9	2	3
	Repopulation of lymphocytes	0	1	2	8	10
	Intranuclear inclusion	0	1	0	0	1
Spleen	Depletion of lymphocytes	12	13	7	5	5
	Bacterial clumps	7	1	1	0	1
	Fibrinoid substances	12	7	5	1	5
Liver	Swelling of RES[a)] cells	12	14	8	9	11
	Fibrinoid substances	7	4	3	2	0
	Aggregation of lymphocytes	1	12	5	10	10
Lung	Aspergillosis	8	5	5	6	6
	Bacterial clumps	1	4	1	1	1
Intestinal coccidiosis		0	0	3	7	10
Perivascular cuffs in CNS[b)]		0	3	0	4	2
Colibacillosis		1	1	1	0	1
Intranuclear inclusion in FFE[c)]		0	0	0	2	0

a) Reticular endothelial system.
b) Central nervous system.
c) Feather follicle epithelium.
d) Number of affected birds.

the thymus and bursa were markedly atrophic in most affected chicks. Discoloration and swelling of the liver were seen in about half of the birds. Consolidation of the lung was noted in one third of the birds. *Staphylococcus aureus* was isolated from the swollen eye-lid seen in some birds.

Main histological lesions of the affected chicks are summarized in Table 4. Severe aplasia or hypoplasia of the bone marrow was noted in most birds. In markedly involved areas, hematopoietic cells of both erythrocytic and granulocytic series from the intravascular and extravascular spaces disappeared, and only mature erythrocytes were spared (Fig. 1). Hyperplastic foci consisting of hematopoietic cells were scattered within the aplastic areas, which were in the convalescent stage, and extended gradually with increasing age (Fig. 2).

Lymphocytic depletion with hyperplasia of reticular cells occurred in the thymus (Fig. 3), bursa (Fig. 4) and spleen (Fig. 5). The population of lymphocytes in these lymphoid tissues recovered gradually with increasing age, which was in the convalescent stage. Reticular cells of the bursas in two chicks had amphophilic or eosinophilic intranuclear inclusion bodies. On electron microscopical examination, the inclusions contained some herpesvirus particles having hexagonal nucleocapsids approximately 100 nm in diameter.

The hepatic sinusoids were dilated, fre-

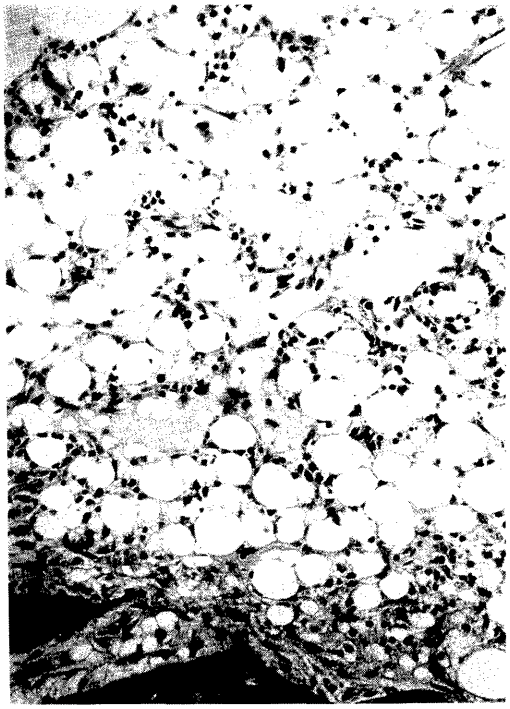

Fig. 1. Bone marrow of a 17-day-old chick. Aplasia showing severe depletion of erythrocytic and granulocytic series. Mature erythrocytes are sparsely observed in intravascular spaces. HE. ×230.

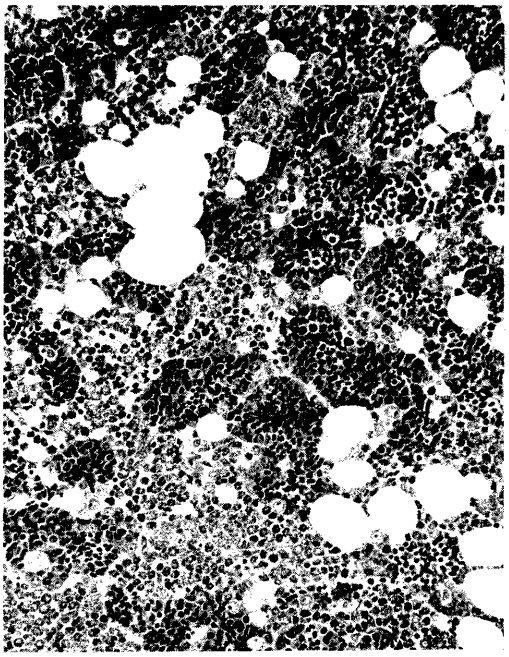

Fig. 2. Bone marrow of a 22-day-old chick. Hyperplasia of hematopoietic cells in intravascular and extravascular spaces. HE. ×230.

quently with serous fluid, fibrinoid thrombi and swollen endothelial cells. There were aggregations of lymphocytes in the interlobular connective tissue of some birds.

Aspergillosis in the lung was observed in about half of the affected chicks. The pneumonic foci were composed of fibrinous or catarrhal pneumonia with branching septate hyphae. Foreign body giant cells against the hyphae were frequent with increasing age. Bacterial clumps without inflammatory reaction were seen occasionally in blood vessels of the lung as well as in the spleen. Various life cycle phases of *Eimeria acervulina* were observed within the epithelial cells of the small intestine, and the infection became gradually higher in incidence with increasing age. Perivascular cuffs in the central nervous system were seen in 9 affected chicks. There were colibacillosis

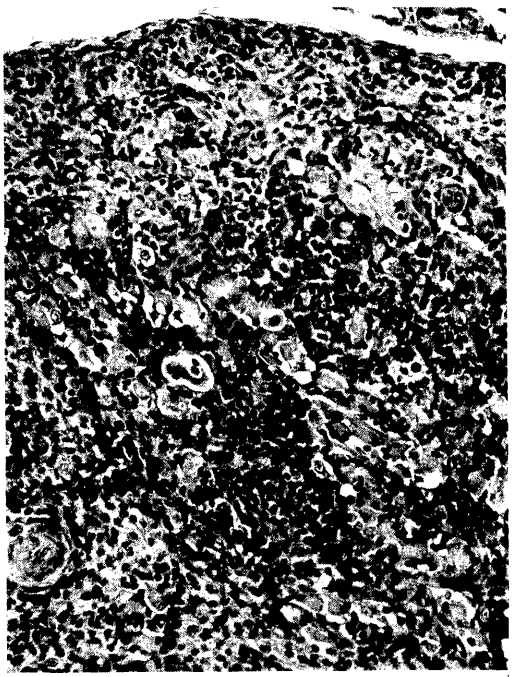

Fig. 3. Thymus of a 17-day-old chick. Severe atrophy of cortex with lymphocytic depletion and hyperplasia of reticular cells. Myoid cells are prominent in medulla. HE. ×230.

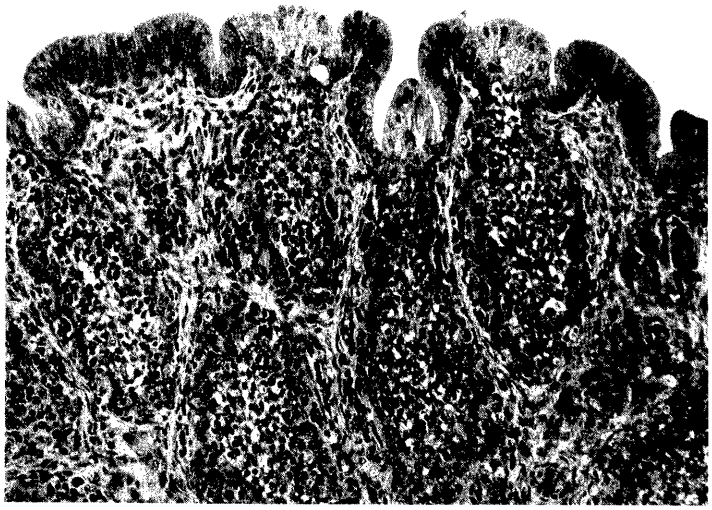

Fig. 4. Bursa of a 17-day-old chick. Severe atrophy showing lymphocytic depletion with hyperplasia of reticular cells in medulla of follicles. HE. ×230.

Fig. 5. Spleen of a 25-day-old chick. Lymphocytic depletion from white pulps. Severe exudation of fibrinoid substances around the sheathed arteries. HE. ×110.

consisting of fibrinopurulent pericarditis and airsacculitis with granulomatous lesions in 4 birds and intranuclear inclusion bodies of Marek's disease (MD) in the feather follicle epithelium of 2 birds.

Suspension of MDCC-MSB1 cells infected with the inoculum from the affected livers stopped growing of the cells after a few passages. An example of titration of CAA is shown in Table 5. The titer of the inoculum was $10^{5.5}$ $TCID_{50}$/0.1 m*l*.

All 1-day-old chicks infected with the inoculum survived without any signs for 14 days postinoculation. There were no differences in mean hematocrit value between control and inoculated chicks, although one bird decreased moderately in hematocrit value (16%). Macroscopically, the chick with decreased hematocrit value showed yellowish bone marrow and severe atrophy of the thymus and bursa. Histologically, aplasia of bone marrow and depletion of lymphocytes in the thymus and bursa were observed in the chick.

DISCUSSION

The characteristic lesions in the present disease, anemia with aplasia or hypoplasia of the bone marrow and depletion of lymphocytes in the lymphoid organs, were similar to those seen in susceptible chicks

Table 5. Titration of CAA in MDCC-MSB1 cells

Number of passages	Dilution of inoculum										Control
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	
1	−[a]	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−
2	+[b]	−	−	−	−	−	−	−	−	−	−
	+	−	−	−	−	−	−	−	−	−	−
3	+	−	−	−	−	−	−	−	−	−	−
	+	−	−	−	−	−	−	−	−	−	−
4	+	+	−	−	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−	−	−
5	+	+	+	−	−	−	−	−	−	−	−
	+	+	+	−	−	−	−	−	−	−	−
6	+	+	+	+	+	−	−	−	−	−	−
	+	+	+	+	+	+	−	−	−	−	−
7	+	+	+	+	+	+	−	−	−	−	−
	+	+	+	+	+	+	−	−	−	−	−

a) Yellow color of culture medium.
b) Red color of culture medium.

inoculated with CAA [5, 7, 8, 10]. CAA is widespread in commercial chicken flocks in Japan [10, 13]. It can be isolated readily not only from the chickens suffering from anemia but also from healthy ones. However, spontaneous occurrences of this disease had been confined to chickens older than 4 weeks of age and associated with complications such as inclusion body hepatitis, MD, respiratory diseases and colibacillosis [5, 10, 13]. The present outbreak is a first clinical disease in field young chicks less than 3 weeks of age.

A peak of anemia and a development of characteristic lesions in susceptible chicks appear between 2 and 3 weeks after the inoculation of CAA at one day old [8]. On the other hand, CAA produces no clinical disease when inoculated into chicks more than 2 weeks of age [10]. When the present chick flocks were invaded with CAA is unknown, but from the progression of the disease it was considered that they have been infected with CAA within at least 7 days after hatching.

The susceptibility of chicks to CAA is varied with rearing status of the parent flock and closely related to the presence of maternal antibody [11]. In the present study CAA was easily isolated from the affected chicks and one of the chicks experimentally inoculated with CAA had characteristic lesions of the disease. Therefore, a few chickens of the parental flocks might have had low titer or no antibody to CAA.

There was no apparent relationship between the infection of CAA and the concurrent lesions observed in the present cases. The pathogenicity of CAA is enhanced by concurrent infection with MD virus [2, 4] or infectious bursal disease virus (IBDV) [4, 12]. The incidence of the lesions related to MD or IBDV was low in the present affected chicks. Pulmonary aspergillosis and bacterial infections were relatively high in incidence and an important cause of loss in the present chick flocks. These concurrent infections would have been serious resulting from the immunosuppression induced by CAA affection.

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REFERENCES

1. Akiyama, Y., and Kato, S. 1974. Two cell lines from Marek's disease. *Biken J.* 17: 105–117.
2. Bülow, V. v., Fuchs, B., Vielitz, E., and Landgraf, H. 1983. Früsterblichkeitssyndrom bei Küken nach Doperinfektion mit dem Virus der Marekschen Krankheit (MDV) und einem Anämie-Erreger (CAA). *Zbl. Vet. Med. B,* 30: 742–750.
3. Bülow, V. v., Fuchs, B., and Bertram, M. 1985. Untersuchungen über den Erreger der infektiösen Anämie bei Hühnerküken (CAA) *in vitro*: Vermehrung, Titration, Serumneutralisationstest und indirekter Immun-fluoreszenztest. *Zbl. Vet. Med. B,* 32: 679–693.
4. Bülow, V. v., Rudolph, R., and Fuchs, B. 1986. Erhöhte Pathogenität des Erregers der aviären infektiösen Anämie bei Hühnerküken (CAA) bei simultaner Infektion mit virus der Marekschen Krankheit (MDV), Bursitisvirus (IBDV) oder Reticuloendotheliosevirus (REV). *J. Vet. Med. B.* 33: 93–116.
5. Goryo, M., Sugimura, H., Umemura, T., and Itakura, C. 1985. Isolation of an agent inducing chicken anaemia. *Avian Pathol.* 14: 483–496.
6. Goryo, M., Suwa, T., Matsumoto, S., Umemura, T., and Itakura, C. 1987. Serial propagation and purification of chicken anaemia agent in MDCC-MSB1 cell line. *Avian Pathol.* 16: 149–163.
7. Taniguchi, T., Yuasa, N., Maeda, M., and Horiuchi, T. 1982. Hematopathological changes in dead and moribund chicks induced by chicken anemia agent. *Natl. Inst. Anim. Health Q. (Jpn.)* 22: 61–69.
8. Taniguchi, T., Yuasa, N., Maeda, M., and Horiuchi, T. 1983. Chronogical observation in hemato-pathological changes in chicks inoculated with chicken anemia agent. *Natl. Inst. Anim. Health Q. (Jpn.)* 23: 1–12.
9. Yuasa, N. 1983. Propagation and infectivity titration of the Gifu-1 strain of chicken anemia agent in a cell line (MDCC-MSB1) derived from Marek's disease lymphoma. *Natl. Inst. Anim. Health Q. (Jpn.)* 23: 13–20.
10. Yuasa, N., Taniguchi, T., and Yoshida, I. 1979. Isolation and some characteristics of an agent inducing anemia in chicks. *Avian Dis.* 23: 366–385.
11. Yuasa, N., Noguchi, T., Furuta, K., and Yoshida, I. 1980. Maternal antibody and its effect on the susceptibility of chicks to chicken anemia agent. *Avian Dis.* 24: 197–201.
12. Yuasa, N., Taniguchi, T., Noguchi, T., and Yoshida, I. 1980. Effect of infectious bursal disease virus infection on incidence of anemia by chicken anemia agent. *Avian Dis.* 24: 202–209.
13. Yuasa, N., Taniguchi, T., Goda, M., Shibatani, M., Imada, T., and Hihara, H. 1983. Isolation of chicken anemia agent with MDCC-MSB1 cells from chickens in the field. *Natl. Inst. Anim. Health Q. (Jpn)* 23: 75–77.
14. Yuasa, N., Imai, K., and Tezuka, H. 1985. Survey of antibody against chicken anaemia agent (CAA) by an indirect immunofluorescent antibody technique in breeder flocks in Japan. *Avian Pathol.* 14: 521–530.

要　約

幼雛における鶏貧血因子に起因した貧血症の発生：御領政信・柴田良久・諏訪隆彦・梅村孝司・板倉智敏[1)]（鳥取大学農学部家畜病理学教室，[1)]北海道大学獣医学部比較病理学講座）——外国より導入された種鶏からふ化した幼雛において，鶏貧血因子に起因した貧血症が認められ，12日から25日齢までの死亡率は，雌で約2.4%，雄で20.9%であった。肉眼的には，骨髄の黄色化，胸腺及びファブリキウス嚢の萎縮，肝臓の退色・腫大及び肺の硬化が認められた。組織学的には，骨髄低形成及びリンパ性器官におけるリンパ球の消失がかなりの発症雛で見られた。17日齢の発症雛の肝臓から，MDCC-MSB1細胞により，chicken anemia agent（CAA）が分離され，自然感染例と同一の種鶏群由来の1日齢雛は，CAAに対し低感受性であった。肺アスペルギルス症及び細菌感染症が多くの例に合併しており，これらも死因として重要と考えられた。