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AGE RESISTANCE IN CHICKENS AGAINST INFECTIOUS BURSAL DISEASE

By

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PANISUP, A. S., K. C. VERMA and G. C. MOHANTY *Age resistance in chickens against infectious bursal disease.* Acta vet. scand. 1984, *25*, 561—566. — The day old broiler chickens possessing IBD precipitating maternal antibody when exposed either to IBD contaminated environment or challenged intrabursally with virulent virus at weekly intervals indicated 100 % susceptibility around 4—5 weeks of age. However, chickens lacking maternal antibody upon intrabursal challenge were found susceptible by 2 weeks of age.

maternal antibody; agar gel precipitation test; fluorescent antibody test; Gumboro disease; IBD.

The infectious bursal (Gumboro) disease (IBD) is a highly contagious viral infection of young chickens. The maternal immunity to this infection gradually declines with advancement of age (*Winterfield & Hitchner* 1964, *Hanson* 1967) and affords protection to chickens during embryonic (*Hitchner* 1970) and early post-embryonic periods (*Faragher* 1972, *Lucio & Hitchner* 1979 and 1980). The present study was undertaken to determine the period of protection afforded by the maternal antibodies (MA) and the age at which the chickens become most susceptible to IBD infection. This would help in institution of a proper vaccinationn programme against IBD.

MATERIALS AND METHODS

The day-old broiler chickens (received from Central Avian Research Institute, Izatnagar, India) were divided into 2 groups. The chickens of group 1 (140 chickens) free from MA were placed in IBD contaminated environment shortly after hatching.

Group II was further divided into subgroup A (80 chickens) possessing precipitating MA and subgroup B (50 chickens) lacking such antibody. The chickens of group II were kept in an isolation room which was sterilized by formaldehyde gas prior to the placement of the chickens. These were given sterilized feed to placement ed with Nuvimin forte® 2.5 kg/ton feed.

The chickens of group II were challenged with virulent IBD virus at weekly intervals up to 8 weeks of age. Each bird was inoculated intrabursally with 0.2 ml of 20 % infected bursal suspension in phosphate buffer solution (PBS) pH 7.2. These birds were sacrificed at 48 h post-challenge. Bursae of Fabricius (BF) were collected and processed for detecting antigen by Agar gel precipitation test (AGPT), fluorescent antibody test (FAT) and histopathological examination. For FAT the frozen sections were cut at 3—4 μm thickness, fixed in chilled acetone and stained with specific chicken anti IBD globulins conjugated with fluorescence isothiocynate (FITC) following the method of *Purchase* (1973) with slight modification. This in brief was as follows: Acetone fixed frozen sections were washed thoroughly in PBS pH 7.3 then flooded with anti IBD conjugated globulins and kept over night in refrigerator at 4—5°C instead of at 37°C. This gave better stainability results. The sections were washed thoroughly in chilled PBS pH 7.3, mounted in 50 % glycerine saline and examined under Zeiss fluorescence microscope.

The IBD serum or tissue antibody were detected by AGPT following the methods of *Hirai et al.* (1972). The standard IBD antigen was the same as used for challenge studies and antisera as described earlier (*Verma et al.* 1981). Histopathological examination was done on paraffin embedded tissue sections of BF stained with heamatoxyline and eosin.

The chickens which upon challenge revealed IBD antigens and/or characteristic lesions of IBD in BF were taken as susceptible, where as susceptible age for the chickens under group I was taken approximately 1 week prior to the appearence of precipitating antibody (considering the incubation period of the virus and appearence of serum antibody) in hen.

RESULTS AND DISCUSSION

The results of sera collected at weekly intervals from chickens of group I (lacking MA) which experienced field IBD exposure

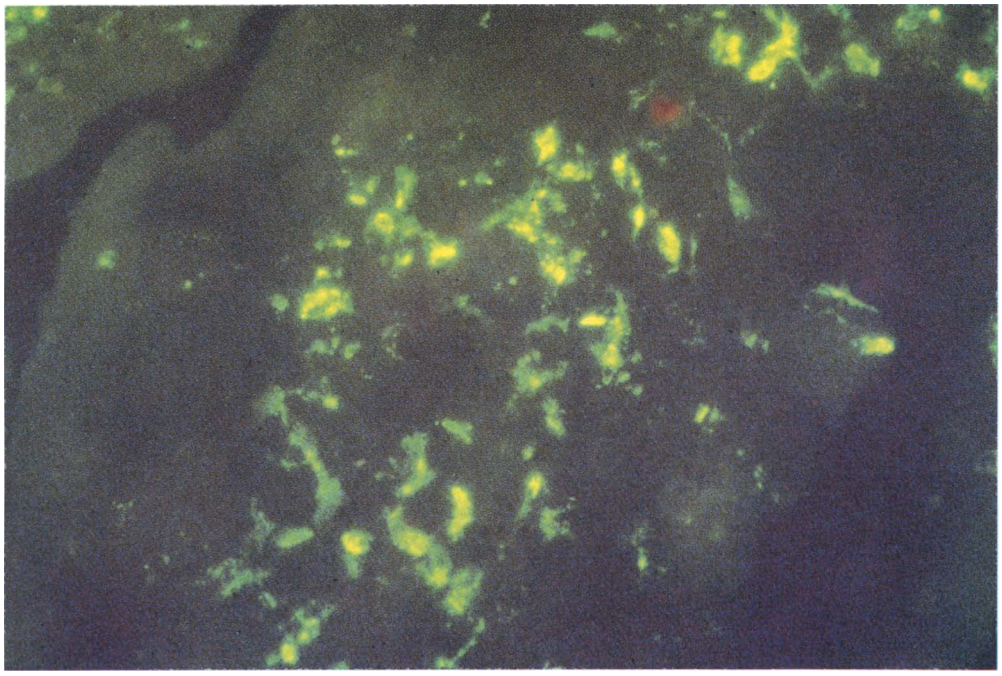

Figure 1. Infectious bursal disease fluoresent antigen in the bursal follicles, FITC staining, 400 ×.

Table 1. The serological and challenge results of IBD virus in chicken based on agar gel precipitation test (AGPT).

Age	Group I	Group II			
	Positive sera No. tested	Sub-group A		Subgroup B	
		Positive sera No. tested	Positive bursae No. challenged	Positive sera No. tested	Positive bursae No. challenged
0 day	0/15	13/16	ND	0/20	ND
1st week	0/15	9/10	2/5	0/20	0/5
2nd week	0/15	6/15	3/5	0/20	6/6
3rd week	0/25	3/15	4/6	0/20	6/6
4th week	0/25	0/15	6/6	0/20	6/6
5th week	25/25	0/15	6/6	0/20	6/6
6th week	25/25	0/15	6/6	0/20	6/6
7th week	30/30	0/15	6/6	0/20	6/6
8th week	30/30	0/15	6/6	0/20	6/6

ND Not done.

Group I — lacking MA and placed in IBD contaminated environment.
Subgroup A — Possessing maternal antibody. Challenged intrabursally with virulent IBD virus.
Subgroup B — Lacking maternal antibody. Challenged intrabursally with virulent IBD virus.

are given in Table 1. It is evident that these chicks continued to remain free from precipitating antibodies till 4 weeks of age. This indicates that the chicks did not allow the replication of IBD virus till this age while remaining continuously in the contaminated environment. It was only during the 5th week of age when precipitating antibodies appeared in the serum of these chicks. The IBD virus is known to be antigenically very potent and incites an early antibody response which can be detected by AGPT within a week after natural route of infection (*Becht* 1980, *Panisup et al.* 1982). The IBD virus in the present experiment might have been either neutralised or not allowed to multiply in the system of the chickens and thus keeping the virus out of access to the immunological system till 4 weeks of age.

The disease does not clinically affect neonatal chickens. The most common natural outbreaks of IBD are recorded during 3 to 9 weeks of age (*Cosgrove* 1962, *Hanson* 1967, *Lüthgen* 1969, *Hirai et al.* 1974, *Verma et al.* 1981, *Mohanty et al.* 1981). This age is considered to be the period of maximal bursal activity (*Hirai et al.* 1974, *Becht* 1980).

Serological and challenge results of the chicks of group II are presented in Table 1. It is seen that MA as well as resistance to IBD challenge in chickens of subgroup A (possessing MA) started declining gradually with the advancement of age. By 4th week the chicks became almost free from MA and showed IBD bursal antigen in all the challenged birds thus indicating 100 % susceptibility to IBD infection at this age. The chicks of subgroup B (lacking MA) on the other hand became fully susceptible to IBD challenge by the second week and remained so till 8 weeks, as revealed by the detection of antigen in all the BF examined. The bursae which by AGPT were found negative for IBD antigen could reveal minute foci of the fluorescent antigen indicating the failure of viral replication in the BF. The bursae, which were positive for IBD antigen by AGPT revealed a very diffuse and characteristic fluorescent antigen (Fig. 1).

This indicates that MA played significant role in affording resistance to IBD infection in chickens. Other workers (*Hitchner* 1970, *Yamaguchi & Kawamura* 1974, *Wyeth & Cullen* 1976, and *Lucio & Hitchner* 1979) also opined and supported our observations.

This study also showed that chicks under field exposure became fully susceptible comparatively 1 week later than the chicks challenged experimentally. This difference in time of susceptibility can be explained by the fact that the virus upon intrabursal inoculation might have by-passed the natural barrier of the gastrointestinal tract or primary multiplication site other then BF resulting into earlier multiplication of the virus into the target organ as compared to the natural route of infection (*Kaufer & Weiss* 1976). Other possible factors might be that the artificial challenge given to the chickens might be much higher than what the chicks might have received under field exposure. The histopathological examination of the bursae having detectable IBD antigen (by AGPT) in them revealed acute form of the disease and the lesions consisted of oedema, lymphocytic necrosis in the bursal follicles, inter- and-intra-follicular heterophilic and few mononuclear cell infiltrations. The bursae showing minute foci of fluorescence but no pricipitating antigens had a very mild reaction without any significant heterophilic infiltration. *Becht* (1980) has also described that antibody blocks the replication and spread of virus and thus possible explains, why the few necrotic foci which had formed into the BF did not expand further

in the organ and were fully regenerated within a short time. However, *Fadly & Nazerian* (1983) observed that the age resistance to clinical manifestation of IBD is probably independent of the ability of the virus to replicate and induce lesions in the host.

The study thus concluded that MA lacking chickens on placement in an IBD contaminated environment became susceptible and reactive to infections at about 4 weeks of age. Upon intrabursal challenge this period is reduced to 2 weeks. The MA harbouring chicks upon intrabursal challenge became 100 % susceptible to IBD infection at 4—5 weeks of age.

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SAMMANDRAG

Åldersresistens hos höns mot den infektiösa bursiten (IBD).

Efter att daggamla broilerkycklingar med IBD-precipiterande maternala antikroppar exponerats för IBD-kontaminerad miljö eller utsatts för intrabursal challenge med virulent virus, upprepat en gång i veckan, visade de 100-%ig mottaglighet vid 4—5 veckors ålder. Kycklingar utan maternala antikroppar visade sig mottagliga för intrabursal challenge vid 2 veckors ålder.

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