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Precipitation of Clinical Infections in Chickens by Infectious Bursal Disease Virus Preserved under Different Storage Temperatures

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Abstract: Biological activities and precipitation of clinical infection potentials of Infectious Bursal Disease Virus (IBDV) stored under different storage conditions were assessed in cockerel chicks. Infective bursae were stored for between 10-20 months at different storage temperature (+4°C, -20°C and -196°C), homogenized and used to challenge IBD-antibody-naïve chickens at 21 days of age. Clinical infections were precipitated in varying degrees. The sample stored at -196°C produced the most marked effect (signs, symptoms and deaths) in susceptible chickens and had an index score of 8.700; followed by the bursae tissue stored at -20°C with an index score of 5.167. The tissue stored at +4°C produced barely insignificant symptomatic/clinical effects in the chickens. This affirms that biological activities of infectious organisms like IBDV is best maintained at deep freezing or ultra low temperature.

Key words: Infectious bursal disease, storage, infectivity

INTRODUCTION

Infectious bursitis also known as Infectious Bursal Disease (IBD) or Gumboro disease is a contagious viral disease of young chicken, which in its acute form, is characterized by sudden onset, short course with sharp peak in mortality and extensive destruction of lymphocytes in the bursa of Fabricius and other lymphoid tissues (Faragher *et al.*, 1974; Nwosuh *et al.*, 1989).

The clinical manifestations of the disease have been reported to occur as early as in 9 days old chicks (Onunkwo, 1975; Nwosuh *et al.*, 1989; OIE, 2008a) and in chickens up to 20 weeks of age (Okoye and Uzuokwu, 1982; Durojaiye *et al.*, 1984). However, birds of between ages 2-6 weeks are said to be the most susceptible (Faragher *et al.*, 1974; Lukert and Saif, 2003).

The Agar Gel Immunodiffusion (AGID) test is recognized as one of the most effective assay for the diagnosis of IBD (OIE, 2008a). This technique has been used for both antigen and antibody detection in IBDV infection. It employs the homogenization of portion of the infected bursa of Fabricius as antigen or known sera from clinically infected birds as positive sera (OIE, 2008a).

In this study, infective bursae of Fabricius were reactivated following periods of prolonged storage at different temperature and tested for their retention of antigenic and infective potentials.

MATERIALS AND METHODS

Chicks: One hundred and ten (110) day-old cockerel chicks were obtained from the Poultry Division of the National Veterinary Research Institute, Nigeria. The birds were housed in the Poultry Experimental Unit of the Viral Research Division of the Institute and raised under

a stable environmental condition. Brooding temperature were maintained from 37°C and gradually reduced to 32-33°C at 21 days. The birds were placed on commercially available chicks mash and were given water *ad-libitum*. At day 19, twenty-five chicks were bled randomly from the group and assessed for antibodies to IBD and Newcastle Disease (NDV) (OIE, 2008a; 2008b). Briefly described, the wing vein (brachial vein) of each bird was exposed, sterilized with 70% ethanol and a sterile 21-gauge needle inserted into the vein through the brachial muscle for the collection of approximately 0.5 ml of blood. The blood was dispensed into a sterile Bijou bottle and left in a slanted position until clotted. The separated sera were collected, inactivated at 56°C for 30 minutes and kept at -20°C until needed.

1.25% agarose gel was prepared by the addition of 1.25g of sterile agarose powder (Sigma Aldrich, Steinheim, Germany) and 8g of sodium chloride to 100ml of sterile distilled water and heating at 115°C in an autoclave at 15 pound pressure for 15 min until the agar was completely melted. The heated gel was allowed to cool and approximately 20 ml was dispensed into sterile Petri dishes and allowed to solidify. Six peripheral wells and a central well were cut into the solidified gel with the use of a template. All the plates were kept in sealed plastic container at 4°C until needed.

Test sera were dispensed in 4 adjacent wells while the control positive and negative sera were dispensed into the two remaining adjacent wells. Standardized IBDV antigen was placed in the central well. The plates were incubated at 37°C for 24 h and lines of identity were observed between the sera and the antigen. Results were taken as valid if the positive control sera produce

line of identity while the negative control sera did not produce any line of identity.

For the Newcastle disease antibody screening, haemagglutination inhibition test was carried out according to standard protocol (OIE, 2008b).

Following the above tests on the chicks sera, the chicks were randomly divided into five (5) groups as follows: Group A {+4°C (20% w/v tissue homogenate), 30 birds}; Group B {-20°C (20% w/v tissue homogenate), 30 birds}; Group C {-196°C (20% w/v tissue homogenate), 30 birds}; Group D {Penicillin-Streptomycin-Gentamicin-Amphotericin B (PSGA) enriched PBS solution challenged controls (10 birds)}; Group E {Unchallenged controls (10 birds)}.

Infective materials: Bursae of Fabricius of IBDV infected chickens previously stored at different temperature: +4°C (stored for 10 months); -20°C (stored for 12 months); and -196°C (stored for 20 months) were retrieved and prepared under a sterile environment in a biosafety cabinet. 1 g portion of the bursae was taken and macerated in sterile mortar with pestle. The macerates were dissolved with 4 ml of PSGA (PBS enriched with PSGA) to make a 20% w/v homogenates of the bursae stored at +4°C. This process was repeated for samples stored at -20°C and -196°C. The homogenates were allowed to stand for 1 h at +4°C after which they were centrifuged at 3000 rpm and clear supernatants were decanted. The supernatants were kept at +4°C until used same day.

Challenge experiment: Chickens in the Group A were inoculated intraocularly (eye drop) with 0.1 ml of the clarified homogenate. The birds were observed twice daily afterward for signs and symptoms indicating infection with IBDV. Chickens in group B and C were similarly treated using appropriate homogenates. Chickens in Group D were treated with intraocular inoculation of 0.1 ml each of the PSGA solution. No treatment was given to chickens in Groups E.

Clinical assessments: The symptoms and clinical signs observed include inappetence, ruffled feathers, whitish pasted vent, emaciation or complete prostration/moribund. Experimental animals were assigned index scores based on these signs as found below:

Apparently healthy = 0; mild-relatively severe sickness = 1, moribund/complete prostration = 2; death = 3. Summation of all scores was made after day 14 and a mean index score was calculated (Table 1a-c).

Post mortem assessment: At post mortem, classical signs of IBD were observed in all the infected groups including enlarged bursae and spleen and linear ecchymotic haemorrhages of the breast and thigh muscles. This signs seem more severe especially in

the groups treated with virus stored at -196°C. Sections of the bursae and visceral organs (spleens, livers, intestines and lungs) from each group were obtained and tested by AGID and virus isolation in embryonating eggs respectively to confirm whether the cause of death was due to IBD infection or other causes-specifically Newcastle disease (OIE, 2008b).

RESULTS

20% w/v homogenate of bursa materials from the +4°C seem to produce no clinical infection after prolong storage at that temperature. Only a mild ruffling of feathers and slight inappetence were observed in 2 chickens on 3 days post inoculation (dpi) and one of the bird recovered completely by 4 dpi. As at the 5th dpi onward, no sign of sickness was observed in the group till the end of 14 dpi (Table 1a).

The 20% w/v homogenate of bursa materials stored at -20°C produced clinical infections, severe symptoms and death in 3-5weeks old birds used for the experiment. Death and other signs/symptoms started at 3 dpi and continued till 10 dpi. The most significant numbers of death was observed in this group. A total sum of 27 chickens were sick, 22 moribund and 28 died from this group. Details of the results are available in Table 1b.

The 20% w/v homogenate of bursa materials stored at -196°C produced clinical infections, severe symptoms and death similar to the pattern observed in the -20°C group. However, signs and symptoms appeared 24 h post infection in this group but death only occurred from the 3 dpi and stopped at 8 dpi. Although, a lesser number of deaths were observed (19), a significantly higher numbers of summed moribund (57) and sick (90) chickens were recorded (Table 1c).

There were no observable signs, symptoms or lesions in chickens in Groups D and E.

All bursae recovered from 14 dpi chickens in Groups A, B and C were inflamed in varying degrees and were positive by AGID, although, the Group A bursa was weakly positive when compared with others. Bursae from the other groups (D and E) were of normal size and shape and did not produce any line of identity with the known positive sera when tested by AGID.

DISCUSSION

Our results indicated that IBD virus will survive and thrive better for relatively longer periods at temperatures of -20°C and -196°C, but its biological and antigenic activities will tend to decrease if stored for a long time at +4°C. The homogenates from the bursa kept at +4°C produced no apparent symptoms in this experiment. This may be due to loss of biological activities by the virus due to long period of storage in unsuitable temperature. The antigen recovered from the bursa of the 14 dpi chicken were however weakly antigenic by

Table 1a: Infection study with 20% w/v bursa of Fabricius materials stored at +4°C

Days post challenge	Total chicken	Total dead	Total moribund	Total sick	Apparently healthy	Index score
1	30	0	0	0	30	0
2	30	0	0	0	30	0
3	30	0	0	2	28	2
4	30	0	0	1	29	1
5	30	0	0	0	30	0
6	30	0	0	0	30	0
7	30	0	0	0	30	0
8	30	0	0	0	30	0
9	30	0	0	0	30	0
10	30	0	0	0	30	0
11	30	0	0	0	30	0
12	30	0	0	0	30	0
13	30	0	0	0	30	0
14	30	0	0	0	30	0
Total	-	0	0	3	-	3/30=0.100

Scoring for signs: Apparently healthy = 0; Sick = 1; Moribund = 2; Dead = 3

Table 1b: Infection study with 20% w/v bursa of Fabricius materials stored at -20°C

Days post challenge	Total chicken	Total dead	Total moribund	Total sick	Apparently healthy	Index score
1	30	0	0	0	30	0
2	30	0	0	0	30	0
3	30	7	4	6	13	35
4	23	2	3	7	11	19
5	21	3	4	4	10	21
6	18	0	5	5	8	15
7	18	7	4	3	4	32
8	11	6	1	2	2	22
9	5	2	1	0	2	8
10	3	1	0	0	2	3
11	2	0	0	0	2	0
12	2	0	0	0	2	0
13	2	0	0	0	2	0
14	2	0	0	0	2	0
Total	-	28	22	27	-	155/30=5.167

Scoring for signs: Apparently healthy = 0; Sick = 1; Moribund = 2; Dead = 3

Table 1c: Infection study with 20% w/v bursa of Fabricius materials stored at -196°C

Days post challenge	Total chicken	Total dead	Total moribund	Total sick	Apparently healthy	Index score
1	30	0	2	21	7	25
2	30	0	8	17	5	33
3	30	4	12	10	4	46
4	26	3	10	8	5	37
5	23	3	9	7	4	34
6	20	5	8	5	2	36
7	15	3	5	6	1	25
8	12	1	3	4	4	13
9	11	0	0	5	6	5
10	11	0	0	3	8	3
11	11	0	0	2	9	2
12	11	0	0	1	10	1
13	11	0	0	1	10	1
14	11	0	0	0	11	0
Total	-	19	57	90	-	261/30=8.700

Scoring for signs: Apparently healthy = 0; Sick = 1; Moribund = 2; Dead = 3

AGID test and this may be an indication of partial loss of antigenic properties. Standard protocols had emphasized the need to keep IBDV infective materials at lower temperatures than +4°C unless needed for immediate processing (OIE, 2008a).

The homogenates from the bursa kept at -20°C produced significant signs, symptoms and death in this experiment. This proved that the viral agent was active in the bursa homogenate and can cause active infection in the field and under laboratory experiments. The virus in

this homogenate produced the most significant death and this may be linked to the possible virulence of the IBDV used. Although, an assessment of the virulence of each of the IBDV agent present in the bursa sample used was not done, a trace-back study of the records of sample submission indicated that the all the bursa samples stored at the different temperature and used in this experiment caused between 18 and 37% mortality in the field.

The homogenates from the bursa kept at -196°C produced more significant signs and symptoms but lesser death when compared with the homogenate from -20°C. It will appear that the bursa samples store better and retain their original properties more when stored at ultra-low temperature -80 to -196°C as evidenced by index score of 8.700 which was much higher than those stored at other temperatures. Other authors had similarly confirmed that temperature of -40°C or lower is best suited for IBDV (OIE, 2008a).

The pattern of infection and death recorded in this experiment was higher than had been reported from the field (Durojaiye *et al.*, 1984; Nwosuh *et al.*, 1989; Lukert and Saif, 2003). It may be because the experiment was controlled and each chicken received the full viral dose rather than seeded infection which may occur under natural conditions and may allow uneven distribution of the virus. In this experiment, the viruses retained the ability to precipitate clinical infection comparable to the field situation with symptoms observed ranging from ruffled feathers, soiled vent and emaciation to being moribund and death. Similarly, at necropsy, the carcasses and organs produced classical signs associated with Gumboro disease which includes enlarged and hemorrhagic bursa and spleen, linear ecchymotic hemorrhages in breast and thigh muscles.

Infectious bursal disease represents a major limitation to the growth of the poultry industry in Nigeria and elsewhere (Durojaiye *et al.*, 1984; Makadiya, 2004). It will therefore be important to cautiously handle samples stored at -20°C or lower temperature to prevent viral escape from the laboratory to the field.

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