INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2003/05–3–292–294
http://www.ijab.org

Indirect Hemagglutination Test Based Sero–prevalence of Hydropericardium Syndrome in Commercial Broilers

HUSSAIN, I., M.H. RASOOL, M.A. ZAHOOR AND M.K. MANSOOR
Department of Veterinary Microbiology, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

A total of 380 serum samples collected from different commercial broiler farms and slaughter shops were subjected to indirect hemagglutination (IHA) test for the detection of antibodies against Hydropericardium syndrome (HPS) virus. These samples were divided into three groups according to the age of the birds as chicks 1-7 days (n=54), growers 1-4 weeks (n=45) and adults 5-8 weeks (n=281). In broiler growers out of 45 samples, 13 samples were taken from clinically positive cases which did not show any antibody titer. The positive samples on the basis of age groups were broiler chicks (14/54), growers (12/45) and adults (218/281) with the positive percentages 25.93, 26.67 and 77.58%, respectively. The GMT of chicks, growers and adults were 1.5, 1.6 and 7.1, respectively. The broiler chicks of 1-7 days of age as well as the broiler growers of 1-4 weeks of age had lower levels of IHA antibody titers than the adults. Therefore, the latter could be comparatively resistant.

Key Words: Sero–prevalence; Hydropericardium syndrome; Indirect hemagglutination test; Broiler

INTRODUCTION

During the previous few years in Pakistan, poultry industry has developed very rapidly. However, further expansion is confronted with the prevalence of various infectious and non-infectious diseases (Siddique & Javed, 1989). Among the infectious diseases, now-a-days Hydropericardium syndrome (HPS) is posing a serious threat to the poultry industry. It has caused huge economic losses to the broiler industry in Pakistan, since September 1987, when it was first reported at Angara Goth, an exclusively broiler growing area in Karachi (Jaffery, 1988). Initially, it was believed that HPS was a nutritional disorder (Qureshi, 1989). But the reproduction of the disease by inoculation of the infected liver homogenate proved that the disease was infectious in nature (Anonymous, 1988). Several studies have been conducted on the aetiology of HPS agent (Afzal *et al*., 1991; Khawaja *et al*., 1988). It is a non-enveloped, DNA virus with eight polypeptides and nuclear mass of 23 KDa (Haq *et al*., 1997). The virus has been isolated in highest concentrations from liver of the affected birds, where it produces eosinophilic or basophilic intranuclear inclusion bodies (Ahmad *et al*., 1990). The syndrome is typically seen in 3-5 weeks old growing broilers with a mortality of 30-60%. Disease is characterized by the accumulation of a clear straw-colored fluid in the pericardium, swollen, discolored and friable liver, pale and enlarged kidneys (Anonymous, 1988; Anjum *et al*., 1989; Cheema *et al*., 1989). The HPS virus does not hemagglutinate directly chicken erythrocytes, therefore, indirect hemagglutination (IHA) test is performed by using either human "O" erythrocytes or sensitized sheep erythrocytes for monitoring antibodies.

The incidence and prevalence of HPS in Pakistan has been reported by many workers. The present study was conducted to measure the antibodies in the serum of commercial broilers using IHA test and to understand the prevalence of antibodies in different age groups.

MATERIALS AND METHODS

Isolation of the virus. Liver samples were collected from HPS infected birds from different outbreaks. These samples were processed for isolation of the HPS virus according to the method described by Rahman *et al.* (1997). Briefly, about 15 g liver samples were triturated in a sterilized pestle and mortar having sterilized sand and phosphate buffered saline (PBS) containing antibiotics (penicillin 1000 μg mL^{-1} and streptomycin 1000 μg mL^{-1}). The homogenized suspension was subjected to centrifugation at 1500 rpm for 15 minutes. The supernatant was filtered through 0.2 μm APD filter.

Purification of the virus. The above supernatant was mixed with chloroform (1:1) in centrifuge tube and centrifuged at 5000 rpm for 20 minutes (Reddy *et al*., 1997). The middle layer having liver proteins, cell debris and the bottom layer of chloroform was discarded. The clear supernatant was collected in sterilized screw capped test tube and stored at -20ºC for further use.

Identification of the virus. a) Raising of hyperimmune serum: hyperimmune serum was raised in rabbits against HPS vaccine (Bioangara Plus, Sana Labs) according to the method described by Barnes *et al.* (1982), b) Agar gel precipitation test: the hyperimmune serum was used to identify and confirm the HPS virus in chloroform treated purified supernatant fluid by using AGPT. The test was carried out according to the method described by Cullen and Wyeth (1975).

Collection of serum samples. A total of 380 blood samples were collected from commercial broiler farms and slaughter

shops from in and around Faisalabad. These samples were divided into three groups according to the age of the birds as 54 samples from chicks (1-7 days), 45 samples from growers (1-4 weeks) and 281 samples from adults (5-8 weeks). Serum was separated, heat inactivated and stored in separate plastic bottles at -20^{0}C.

Indirect hemagglutination test. IHA test was performed for measuring the antibodies in serum samples against HPS according to the method described by Rahman *et al*. (1997). After making two fold serial dilutions of test serum samples, equal quantity of sensitized human "O" erythrocytes (1%) were added in each well along with a negative control. The plates were gently tapped and incubated at 37^{0}C for 30 minutes. The highest dilutions of serum showing agglutination were taken as end point and its reciprocal was recorded as IHA antibody titer. The percentage of positive samples and GMT of each group was calculated.

RESULTS AND DISCUSSION

Out of total 380 serum samples collected from different commercial farms and shops, 54 serum samples belonged to 1-7 days old broiler chicks. Out of these, 14 samples were positive and the remaining 40 were negative when subjected IHA test. The IHA antibody titer varied from 1:4 to 1:8. Eight samples had antibody titer 1:4 while other six had 1:8. The geometric mean titer (GMT) of this group was 1.5 (Table I). The positive percentage of broiler chicks was 25.93% as shown in Table II. These results showed that the broiler chicks of 1-7 days old are susceptible to Hydropericardium syndrome because of low antibody titer. This finding is supported with the observation recorded by various workers who reported the occurrence of HPS immediately after the age of 2-3 weeks (Irfan, 1988; Jaffery, 1988, 1989; Jalalee, 1988; Khan *et al*., 1988; Khawaja *et al*., 1988; Solmontos, 1988; Anjum *et al*., 1989; Shane 1989).

The total number of serum samples was 45 in case of broiler growers. The 12 samples were positive and rest of the 33 samples including 13 samples taken from clinically positive cases were negative. The IHA antibody titer varied from 1:4 to 1:16. The antibody titer noted were such that 8, 3 and 1 serum samples had antibody titer 1:4, 1:8 and 1:16, respectively. The GMT for growers was 1.6 as detailed in Table I. The positive percentage of broiler growers was 26.67 as shown in the Table II. The negative results of 13 serum samples collected from clinically positive cases supported the fact that during disease, the infectious agent is present and the antibody formation starts after 10-14 days. The antibodies become detectable about one week after first injection, and the amount of antibodies present in serum then climbs to reach its highest level by 10-14 days before declining rapidly (Tizzard, 1992). The overall percentage of the positive sera in this group was 26.67, which was higher than the previous group of broiler chicks. This indicates induction of an antigenic response in chicks. It also explains that this group might have experienced the infection during the life. The results can be supported partially with Rahman *et al*. (1997), who titrated the IHA antibodies in the convalescent sera of chickens in the same age group.

Out of 281 serum samples, collected from adult broilers of 5-7 weeks of age, 218 serum samples were positive and remaining 63 were negative. The IHA antibody titer in positive samples varied from 1:4 to 1:256. The serum samples 49, 66, 45, 40, 7, 9, and 2 had IHA antibody titer 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256, respectively with overall GMT of this group as 7.1 (Table I). The positive percentage of sera in adult broilers was 77.58 as shown in table II. Adult broilers showed still higher IHA antibody titer than chicks and growers. This group of birds is less susceptible to disease and it may be due to higher antibody titers.

The broiler chicks of 1-7 days of age as well as the broiler growers of 1-4 weeks of age had lower levels of IHA antibodies than adults so it may be concluded that the chicks of 1-7 days are also susceptible to HPS infection, but the most susceptible period of infection is from 1-4 weeks of age i.e. the broiler growers are more likely to be infected as 13 samples collected from clinically positive cases also belonged to the same age group. Moreover, the antibodies become detectable after 7-10 days of infection and then their level increases up to 6th or 7th weeks of age and then again started to decrease. That is why the adult broilers of 5-8 weeks of age are comparatively resistant to HPS infection.

Table I. IHA antibody titers and GMT in three age groups of broilers

Groups	No. of Samples	IHA antibody Titer									GMT
		Negative	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Chicks (1-7 days)	54	40	-	8	6	-	-	-	-	-	1.5
Growers (1-4 weeks)	32+13*=45	33	-	8	3	1	-	-	-	-	1.6
Adults (5-8 weeks)	281	63	-	49	66	45	40	7	9	2	7.1

*Clinical cases

Table II. Age wise Comparison in three groups of broilers using indirect hemagglutination (IHA) test

Groups	Total Samples	No. of Positive samples	No. of Negative Samples	Positive Percentage
Chicks (1-7 days)	54	14	40	25.93%
Growers (1-4 weeks)	32+13*= 45	12	33	26.67%
Adult (5-8 weeks)	281	218	63	77.58%

*Clinical cases

Acknowledgement. The funds for this project were provided by the Ministry of Science and Technology (MoST) Islamabad, Pakistan under the consultancy programme for strengthening of research faculty in S & T/ R & D Organizations and Universities.

REFERENCES

Afzal, M., R. Muneer and G. Stein, 1991. Studies on aetiological agent of Hydropericardium syndrome (Angara disease) in broilers. *Vet. Rec.,* 128: 591–3

Ahmad, I., K. Ahmad, K.I. Iqbal and S. Naz, 1990. Efficacy of Formalinized liver organ vaccine against Angara disease in broiler. *Vet. Archiv,* 60: 131–8

Anjum, A.D., M.A. Sabri and Z. Iqbal, 1989. Hydropericardium syndrome in broiler chickens in Pakistan. *Vet. Rec.*, 124: 247–8

Anonymous, 1988. Synopsis of the Proceedings of 1st National Seminar on Hydropericardium-pulmonary odema cum-hepatonephritis syndrome. *Pakistan Vet. Med. Assoc.*, pp. 1–10

Barnes, H.J., J. Wheeler and D. Reed, 1982. Serological evidence of infectious bursal disease virus infection in Iowa turkeys. *Avian Dis.,* 26: 560–5

Cheema, A.H., J. Ahmad and M. Afzal, 1989. An adenovirus infection of poultry in Pakistan. *Rev. Sci. Tech. Off. Intl. Epiz.*, 8: 789–95

Cullen, G.A. and P.J. Wyeth, 1975. Quantitation of the antibodies to infectious bursal disease. *Vet. Rec.,* 93: 315

Haq, I., I. Hussain and A.A. Anjum, 1997. Polypeptides and nucleic acid identification of Hydropericardium syndrome agent. *Pakistan Vet. J.,* 17: 21–3

Irfan, M., 1988. Some observations on Hydropericardium syndrome. pp: 38–48. *In: Proc. Natl. Sem. on HPS*, held on July 4, 1988 under Auspices of Poul. Res. Inst. Rawalpindi, Pakistan

Jaffery, M.S., 1988. A Treatise on Angara disease in chicken. *Pakistan Vet. Med. Assoc.,* pp. 1–33

Jaffery M.S., 1989. Angara disease and adenovirus. pp: 70–2. *In: Int. Conf. and Trade Show*, 1989 held on Feb. 27-Mar. 2, Karachi organized by Poult. Prod and Dev. Soc. Pakistan

Jalalee, M.A., 1988. Hydropericardium syndrome in Chickens. pp: 83–95. *In: Proc. Natl. Sem. on HPS*, held on July 4, 1988 under Auspices of Poul. Res. Inst. Rawalpindi, Pakistan

Khan, M.Z., M. Siddique, M.A. Sabri and M.A. Majeed, 1988. Prevalence and Pathology of Hydropericardium Syndrome in Broiler chicks. pp: 105–10. *In: Proc. Natl. Sem. on HPS,* held on July 4, 1988 under Auspices of Poul. Res. Inst. Rawalpindi, Pakistan

Khawaja, D.A., S. Ahmed, A.M. Rauf, M. Zulfiqar, S.M.I. Mehmood and M. Hussain, 1988. Isolation of an adenovirus from Hydropericardium syndrome in broiler chicks. *Pakistan J. Vet. Res.,* 1: 2–17

Qureshi, A.A., 1989. Hydropericardium and kidney lesions. *Poult. Int*., 27: 48–9

Reddy, Y.K., A. Koteeswaren and N. Doairajan, 1997. Pattern of infectious bursal disease in commercial white leghorn chickens. *Indian Vet. J.,* 74: 1019–21

Rahman, S.U., M. Ashfaque, A.D. Anjum and T.A. Sindhu, 1997. Indirect hemagglutination test for detecting angara disease (hydropericardium syndrome) agent antibodies. *Pakistan J. Livstock and Poult.,* 3: 176–8

Shane, I.S.M., 1989. Emergence of new disease affecting broilers in Pakistan. *Zootechnica Int.,* (2): 24–5

Siddique, M. and T. Javed, 1989. Prevalence, diagnosis and control of common poultry diseases. *J. Anim. Hlth. Prod.,* 9: 18–27

Solmontos, P., 1988. A new challenge for the Pakistan Poultry Industry. *In: Proc. Sem., Organized by Hubbard Poultry U. K. Ltd.*, at Lahore on Oct. 1

Tizzard, I., 1992. *An Introduction to Veterinary Immunology,* 5nd Ed., pp: 119–53. W.B. Sounders Company, London

(Received 04 April 2003; Accepted 22 May 2003)