Indian Journal of Animal Sciences **92** (2): 183–186, February 2022/Short communication
https://doi.org/10.56093/ijans.v92i2.122078

Comparative adaptive immune response in Indian and exotic breeds of domestic chicken

RENUKA YADAV[1], RAJESH KUMAR[1]✉, GARIMA PANDEY[1], AAKANKSHA TIWARI[2], RITIKA BISHT[1], ANIL KUMAR[1], ANUPAMA MISHRA[3] and PRAKASH BHATT[1]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145 India

Receieved: 14 August 2021; Accepted: 11 November 2021

Keywords: Chicken, Immune response, Kadaknath, Rhode Island Red, Uttara fowl

Indian native breeds are considered to have comparatively better disease resistance than exotic breeds and the knowledge of this variation in immune response between different strains may be valuable in genetic selection programme. Uttara fowl is a native chicken breed of the Uttarakhand and considered to be resilient to cold winter stress and adverse environment conditions hence it is believed to be a favourable breed of chicken for high altitudes (Ansari 2015). Uttara fowl is reared in traditional rearing system and has popularity among marginal farmers, tribals, etc. in high altitude areas of Himalaya (Ansari 2015). Kadaknath is a native chicken breed located in Madhya Pradesh and in some regions of Gujarat and Rajasthan states of India (Rao and Thomas 1984). Kadaknath breed is known for its delicious meat and flesh and has many distinctive features, however it is being overlooked due to weak production potential (Haunshi *et al.* 2011). Even though indigenous or native stocks show a poor performance relative to highly selected commercial lines, they have better ability to survive in challenging environments. Rhode Island Red (RIR) is a domestic purpose chicken breed in the United States. Because of its ability to lay eggs and hardness, it is common for backyard flock (Dewna 2016).

The immune system is an important part of any living organism, which protects the host from microbial invaders present in the environment and from other non-infectious foreign substances such as protein and polysaccharides (Abbas *et al.* 2001). Although, very effective innate immunity is often not able to fight off the pathogen and thus fails to prevent infection. This requires adaptive immunity to specifically target pathogen resulting not only in the elimination of that pathogen but also protection from repeated encounter with the same pathogen (Abbas *et al.* 2001). Genetic differences in immune responses have been reported, for experimental random bred, inbred lines, commercial strains, and local indigenous birds. Improving genetic resistance to disease and immune capabilities of these birds is more important and desirable. Indian native breeds are considered to have comparatively better disease resistance than exotic breeds. However, data regarding immune status of indigenous and exotic poultry birds is scanty. Hence, there is a need for studying immune status of Indian and exotic breeds of domestic fowl, which will help greatly in developing poultry breeds having better immune status and environment adaptability for profitable poultry production.

To assess the humoral immune response of Uttara fowl, Kadaknath and RIR breeds against an immunogen, Newcastle Disease Virus (NDV) vaccine (Lasota strain) was administered in day old chicks in drinking water and Haemagglutination Inhibition (HI) was carried using β-technique (Allan and Gough 1974) at 7, 14, 21 days post immunization. Cell mediated immune response was assessed by skin test and Lymphocyte stimulation test.

Skin test: Cutaneous hypersensitivity testing was conducted as per Haribabu *et al.* (1993) with slight modifications. Interdigital skin space between the third and fourth digit as suggested by Corrier (1990) was chosen as the site for injecting 2,4-Dinitrochlorobenze (DNCB) at 56th day of age. Six birds were randomly selected from each group and sensitized on the foot web by using 0.1 ml of 1% DNCB injected into two birds in each replicate, intradermally at inter digital space between 3rd and 4th digits of right leg using tuberculin syringe and allowed to dry immediately by blowing air to prevent the solution running down the sides. The thickness of the foot web at that site was measured using digital Vernier Callipers at 0, 24, 48 h post inoculation.

Lymphocyte stimulation test: Blood was collected from wing vein of experimental birds in heparinised vial and Peripheral Blood Mononuclear Cells (PBMC) were separated by density gradient centrifugation (Boyum 1967) and Lymphocyte Transformation Testing was conducted (Okamura *et al.* 2004).

To assess the resistance to infection, experimental birds

Present address: [1]College of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. [2]ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. [3]Central Agricultural University, CCS, Tura, Meghalaya. ✉Corresponding author email: rajeshvet@rediffmail.com

were infected intramuscularly with $\log_{10}$ 6.2 $TCID_{50}$ virulent Fowl adenovirus (FAdV-2/11) isolate (Pantnagar/HA-14/R-21) at 30th day of age. Clinical signs, mortality, antibody titre and change in body weight were recorded. Dead birds were examined for post-mortem lesions and organs were evaluated for presence of any lesion and liver, bursa, spleen, and caecal tonsils were collected for histopathological examination.

All the collected tissue samples were processed for demonstration of virus antigens by agar gel immuno-diffusion (AGID) and antibody titre of all the experimental groups at 0, 5, 10, 14 days post infection was determined by single dilution ELISA. Briefly, ELISA plates (Grenier) were coated with 100 µl of CEL cell culture fluid containing FAdV-2/11 ($TCID_{50}$ $2\times10^{5.43}$/ml). Then, the plates were reacted with test sera from infected chickens. Commercial peroxidase-conjugated rabbit anti-chicken IgG (Sigma) was used as the secondary antibody and ortho-phenylenediamine (OPD) as substrate (Kumar *et al.* 2003).

Statistical analysis for the test of significance was done by analysis of variance (ANOVA) following Snedecor and Cochran (1994) based on computer programme OPSTAT (Sheoran 2010). The means were separated using Duncan Multiple Range Test (DMRT) (Duncan 1955) based on SPSS16 (Statistical Product and Service Solutions) computer programme.

Adaptive immune response is an important mechanism to fight invading pathogens. Chickens having capacity to mount robust immune response following microbial invasion are favoured for poultry production. Different antigens have been used for evaluation of immune response of chickens. Haemagglutination (HA) test was performed to titrate Newcastle disease (ND) virus suspension prior to perform the haemagglutination inhibition (HI) test as a measure of capability of experimental birds to mount antibody response. HA test revealed complete haemagglutination at 1:640 dilution of virus suspension, which indicated that 0.5 ml of a 1:640 dilution of virus contained 1HA unit. HI antibodies titers in sera of experimental birds of different breed against Newcastle disease vaccine at 7, 14, 21 days of post vaccination are presented in Table 1. HI titers at 7 days post vaccination in RIR, Uttara fowl, Kadaknath were significantly different by ANOVA (P<0.05). The highest value of HI titre at 7 days was recorded in RIR followed by Kadaknath and Uttara fowl and at 14 days, RIR and Kadaknath titers were significantly (P<0.05) different from Uttara fowl birds but difference between RIR and Kadaknath was non-significant. The highest value of HI titre at 14 days was recorded in RIR followed by Kadaknath and Uttara fowl. At 21 days of post vaccination, ANOVA revealed significant (P<0.05) difference in HI titre among RIR, Uttara fowl, and Kadaknath breeds. The highest value of HI at 21 days was recorded in RIR followed by Kadaknath and Uttara fowl. Measurement of HI antibodies is widely used method for assessment of humoral response in birds. In the present study, highest HI antibody titre was recorded for RIR chickens at different time intervals. Singh *et al.* (2009) evaluated the primary immune response to sheep red blood cells in three strains of White Leghorn (WHL), two strains of RIR and two cross breed groups and reported that the primary immune response was highest in WHL compared to RIR. The crosses between breeds or between strains did not exhibit significant difference in HI antibody titre.

The results of cell mediated immune response to DNCB were recorded at 0, 24, and 48 h (Table 2). Increase in skin thickness became evident at 24 h post injection. The foot web region was swollen, erythematous, hot, and edematous. ANOVA revealed that RIR and Kadaknath were significantly (P<0.05) different from Uttara fowl but difference between RIR and Kadaknath was non-significant. At 24 h, RIR had higher thickness than Kadaknath and Uttara fowl. Statistical analysis revealed significant variability of immune response to DNCB among various experimental breeds at 24 and 48 h post injection. RIR and Kadaknath showed significantly (P<0.05) higher cell mediated immune response when compared to Uttara fowl but non-significant in Kadaknath and RIR. The mean thickness of foot web at 48 h was highest in RIR breed followed by Kadaknath and Uttara fowl. Lymphocyte stimulation test was performed as an aid to cutaneous hypersensitivity test to measure cellular immune response in experimental birds. There was significant (P<0.05) difference among all three breeds studied. The highest value of control was observed in RIR followed by Kadaknath and Uttara fowl. Uttara fowl and Kadaknath differed significantly (P<0.05) from RIR but Uttara fowl and Kadaknath were non-significantly different compared to each other. The highest stimulation index of con A was observed in RIR followed by Kadaknath and Uttara fowl. Mean±SE values of vaccinated birds with antigen in RIR, Uttara fowl and Kadaknath breeds were 2.5±0.10, 2.2±0.04, 2.3±0.09, respectively. Uttara fowl and Kadaknath were significantly (P<0.05) different from RIR but Uttara fowl and Kadaknath were non-significant with each other. The highest stimulation of antigen was observed in RIR followed by Kadaknath and Uttara fowl. In poultry, as in humans and other mammals, the antigen-specific component of CMI

Table 1. Haemagglutination inhibition titer ($\log_2$) (Mean±SE) in different breeds of chicken

Breed	7 days	14 days	21 days
RIR	7.3333±0.2	8.0000±0.2	8.3333±0.2
Uttara fowl	5.3333±0.2	6.0000±0.2	6.3333±0.2
Kadaknath	6.3333±0.2	7.3333±0.2	7.5000±0.2

Table 2. Skin thickness (Mean±SE) in cutaneous hypersensitivity test in different breeds of chicken

Breed	0 h (mm)	24 h (mm)	48 h (mm)
RIR	2.83±0.0168	3.03±0.0324	3.08±0.0265
Uttara fowl	2.72±0.0091	2.84±0.0147	2.91±0.0380
Kadaknath	2.73±0.0091	2.95±0.0325	3.00±0.0353

is the T cell (Chen *et al.* 1991). 2, 4-Dinitrochlorobenze (DNCB), a chemical "contact sensitizer" has been used to assess cell mediated immune response. Both effector and regulatory cells develop after exposure to DNCB and such exposure does not induce antibody response. DNCB induces a DTH reaction in chickens (Awadhiya *et al.* 1982). Contact sensitivity to DNCB is therefore, a convenient model to investigate the basic mechanisms of CMI response and its regulation in chicken (Huynh and Chubb 1986). The higher and quicker the skin reaction to DNCB, the better the immune response. RIR and Kadaknath produced comparable reaction to DNCB indicating better CMI response than Uttara fowl. RIR exhibited highest response against Con A, a T-cell mitogen, corroborating the findings of DNCB response indicating best CMI among the breeds studied.

To compare infectious disease resistance, birds were inoculated with a virulent field strain of fowl adenovirus (Pantnagar/HA-14/R-21) (Trivedi *et al*. 2018) at 30 days of age and were observed for clinical signs and mortality. On 4th and 5th day post infection (DPI), whitish droppings were observed in Uttara fowl group. Dullness, ruffled feather, pasty diarrhoea was observed in Uttara fowl chicks after 8th DPI. Dullness, depression, and closed eyes were observed in Kadaknath. No clinical signs were observed in RIR group during the entire period of study. No clinical signs or mortality were observed in the control group during the entire experimental period. Mortality started on 8th DPI in Uttara fowl group and one RIR bird died on 12th DPI but after that no bird died in RIR group. At 14th day DPI, another bird from Uttara fowl group was found dead but after it no death was recorded in this group. Onset of mortality was late in Kadaknath group (15 DPI). Highest mortality was observed in Uttara fowl group. Highest survivability rate was observed in Kadaknath and RIR birds. Mortality was first observed at 9th DPC in challenge bird from Uttara fowl group in which PM lesion like muscles was haemorrhagic, kidneys were swollen, yellow necrotic foci were observed on surface of spleen, liver was pale with necrotic foci (Fig. 1), proventriculus and gizzard were normal, bursa and thymus were also normal. On 12th DPI, mortality in RIR group was observed in which PM lesions like swollen spleen, congested kidney, and normal liver, tonsil, and bursa was recorded. On 15 th DPI, mortality was observed in Kadaknath group in which spleen was haemorrhagic (pinpoint) and liver was necrotic (Fig. 2) kidneys were swollen, intestine was congested, bursa was swollen, and thymus, Proventriculus and gizzard were normal. One bird from each of the three groups were humanely euthanized on day 15th DPI but there were no typical PM lesions observed in any group of bird. During necropsy, the pericardium had a light-yellow jelly or water-like transparent exudate, and the liver was swollen and pale**.** Onset of mortality was quickest and highest in Uttara fowl followed by RIR and Kadaknath. Clinical signs like like depression, dullness and closed eyes were recorded in Uttara fowl and Kadaknath but were absent in RIR indicating better

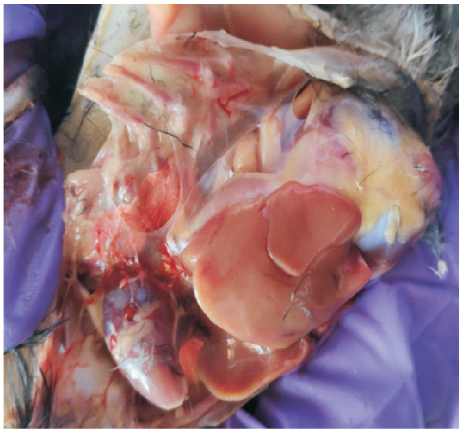

Fig. 1. Post-mortem lesion of FAdV infection like pale liver with necrosis, after challenge with virulent virus (Pantnagar/HA-14/R-21) in Uttara fowl breed of chicken.

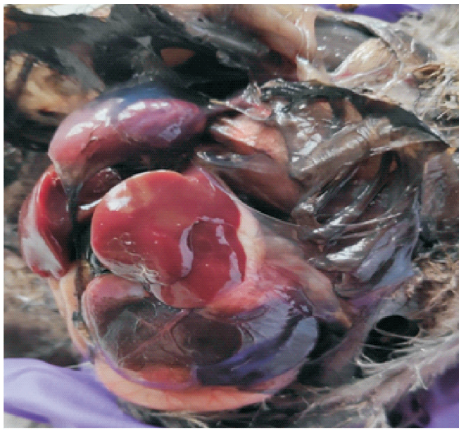

Fig. 2. Necrotic area on the liver parenchyma, after challenge with virulent FAdV in Kadaknath breed of chicken.

disease resistance. Body weight is an important parameter in poultry production and decreased weight gain leads to economic loss (Aral *et al.* 2014), Uttara fowl showed decreased body weight gain following virus infection, while rest two experimental breeds remained unaffected. Highest mortality following virulent FAdV challenge was observed in Uttara fowl. Mortality in FAdV infection may occur without clinical signs (Lim *et al.* 2011). PM lesions were observed in all breeds up to 15 days DPC. In RIR birds, liver, the target organ for FAdV, did not exhibit any change which indicates that these birds have capacity to protect the liver damage inflicted by the virus. Although, RIR and Kadaknath recorded same mortality but liver damage was observed in later.

AGID was performed using the liver antigens prepared from dead birds of Uttara fowl, Kadaknath, and RIR breed of chickens. Result showed that line of precipitation was seen in Uttara fowl and Kadaknath antigen in agarose gel precipitation test indicating the presence of IBH-HHS virus antigen in tissues from dead birds following challenge with virulent FAdV. But no precipitation line was observed for RIR liver antigen. Microscopic lesions were observed in liver, spleen, caecal tonsil of dead birds. Vascular congestion

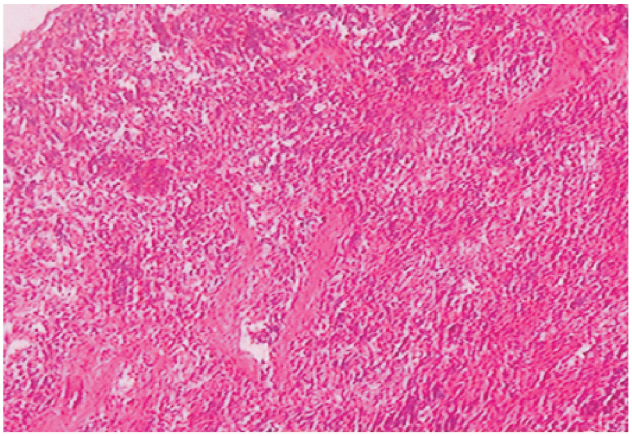

Fig. 3. Blood vascular changes with necrosis and depletion of lymphoid cells on the spleen of Uttara fowl breed of chicken (H&E 20×).

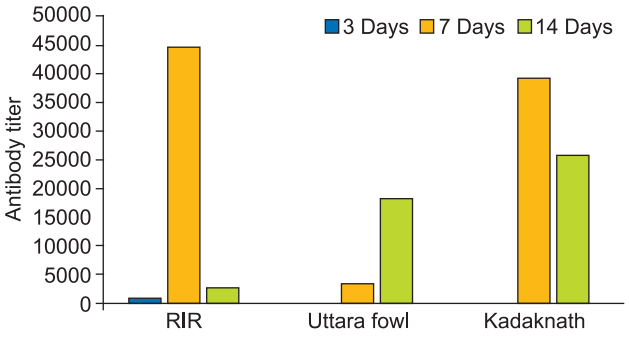

Fig. 4. Antibody titre in experimental chicks at 3, 7, and 14 days post FAdV challenge.

with degenerative and necrotic changes was seen in the liver parenchyma of Uttara fowl, congestion of central vein with dilatation of sinusoid and infiltration of leukocytes in the liver of Kadaknath was also observed. Necrosis of lymphoid cells with blood vascular changes and thickening of blood vessels was recorded in the spleen (Fig. 3). Highest antibody titre at 3rd, 7th DPI were recorded in RIR followed by Uttara fowl and Kadaknath. However, at 14th DPI highest antibody titre was exhibited by Kadaknath followed by Uttara and RIR (Fig. 4). Further, AGID failed to detect viral antigen in liver tissue which indicates very low concentration present (McFerran and Smyth 2000). Histopathological lesions were also mild in RIR birds. Humoral immune response following FAdV infection was highest in RIR again indicating birds' capability to mount robust immune response.

SUMMARY

RIR is best among three breeds studied in terms of adaptive immune response and disease resistance closely followed by Kadaknath. Uttara fowl ranked third hence, its resistance can be increased by incorporating traits from RIR or Kadaknath by employing appropriate genetic techniques. A new breed so developed is expected to exhibit better immune response and adaptability to high altitudes.

REFERENCES

Abbas A K, Lichtman A H and Pober J S. 2001. *Cellular and Molecular Immunology.* (Eds) Abbas A K, Lichtman and Pober J S. W.B. Saunders, Philadelphia, PA.

Allan W H and Gough R E. 1974. A standard Haemagglutination inhibition test for Newcastle disease. A comparision of macro and micro methods. *Veterinary Record* **95**:120–23.

Ansari V. 2015. 'Comparative study on productive performance of comb and crown ecotypes of Uttara fowl.' M.V.Sc. Thesis. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. P.3.

Awadhiya R P, Vegad J L and Kolte G N. 1982. Eosinophil leukocytic response in dinitrochlorobenzene skin hypersensitivity reaction in chicken. *Avian Pathology* **11**: 187–94.

Boyum A. 1967. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scandinavian Journal of Clinical Laboratory Investigation* **97**: 77–89.

Corrier D E and Deloah J R. 1990. Evaluation of cell mediated Cutaneous basophil hypersensitivity in young chickens by inter-digital skin test. *Poultry Science* **69**: 403–08.

Dewna A. 2016. 'Evaluation of performance of diallel cross of kadaknath, RIR and black australorp by griffing's and hayman's approach.' M.V.Sc. Thesis, Rajasthan University Of Veterinary and Animal Sciences, Bikaner. 3 p

Hari Babu Y, Bhaskar S K, Prabhakar Rao P and Krishna A. 1993. Evaluation of cell- mediated immune response in cattle affected with rinderpest using 2,4-dinitro- chlorobenzene. *Indian Journal of Comparative Microbiology, Immunology, and Infectious Diseases* **14**: 4–7.

Haunshi S, Niranjan M, Shanmugam M, Padhi M K, Reddy M R, Sunitha R, Rajkumar U and Panda A K. 2011. Characterization of two Indian native chicken breeds for production, egg and semen quality, and welfare traits. *Poultry Science* **90**(2): 314–20.

Kumar R, Chandra R and Shukla S K. 2003. Isolation of etiological agent of hydropericardium syndrome in chicken embryo liver cell culture and its serological characterization. *Indian Journal of Experimental Biology* **41**: 821–26.

McFerran J B and Smyth J A. 2000. Avian adenoviruses. *Revue Scientifique Et Technique* (*International Office of Epizootics*) **19**(2): 589–601.

Rao G V and Thomas P C. 1984. The breed characteristics of Kadaknath breed of indigenous (Desi) chicken. *Avian Research* **68**: 55–57.

Sheoran O P. 2010. Statistical Package for Agricultural Scientists (OPSTAT), CCS HAU. http://www. hau.ernet.in/about/opstat.php.

Singh S P, Brah G S, Chaudhary M L and Rajkumar G V P P S. 2009. Evaluation and comparison of immune response to sheep red blood cells and PHA-P inoculation in pure and crossbred chicken. *Indian Journal of Poultry Sciences* **44**(1): 225–32.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. Oxford and IBM Publications, New Delhi. Pp 265.

Trivedi R N, Kumar R, Metwal M, Khan A S, Tiwari A, Panday G and Kumar A. 2018. Epidemiological observations on some natural outbreaks of Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS) in domestic chicken. *International Journal of Current Microbiology and Applied Sciences* **7**(8): 3012–22.