RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 2: 239-242 (February 2022)

Differential Disease Resistance of Indian Native Chicken Breeds to Experimental *P. multocida* Infection

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10.18805/IJAR.B-4536

ABSTRACT

Background: Native chicken breeds are considered more disease tolerant than exotic chicken breeds especially for the bacterial diseases. Aseel, Ghagus and Vanaraja chicken breeds/variety were evaluated for the disease tolerance/susceptibility pattern after experimental infection with *P. multocida* A:1 isolate.

Methods: A total of 72 birds of three breeds *viz*., Aseel, Ghagus and Vanaraja (n=24 each) were divided into three groups. The birds were inoculated with 2.5×10^6 CFU/ml of virulent *Pasteurella multocida* A:1 isolate through intraperitoneal (I/P) and intranasal (I/N) routes at 12 weeks of age. Clinical signs, morbidity, mortality rates and lesions were observed in the infected birds.

Result: The mortality rates were 83.3% in Assel breed against 100% in both Ghagus and Vanaraja breed in intraperitoneally infected groups. Upon intranasal infection, the mortality was 83.3% in Assel and Vanaraja breed against 100% in Ghagus breed. Aseel birds showed significantly better survivability and longer death time than Ghagus breed upon experimental infection with *Pasteureall multocida* A:1 isolate. Vanaraja breed showed tolerance comparable to Aseel in experimental infection via intranasal route.

Key words: Aseel, Disease resistance, Fowl cholera, Ghagus.

INTRODUCTION

Differences in disease susceptibility/resistance exists among different chicken breeds and strains (Zekarias *et al*., 2002). Native chicken breeds are considered genetically distinct and more disease tolerant than exotic chicken breeds that are under huge selection pressure for production traits (Rout *et al*., 1992; Wimmers *et al*., 2000). Better immune competence in Indian native chicken breeds has been indicated earlier by higher complement activity, higher serum lysozyme level and antibody response (Haunshi and Sharma, 2002; Baelmans *et al*., 2005). Aseel and Ghagus are native chicken breeds from the native tract of Andhra Pradesh and Karnataka, respectively. *Vanaraja* is a dual-purpose variety developed at ICAR-DPR from exotic breeds adapted to Indian environment for rearing in low input backyard system and most popular, adapted to different regions of India (Ayyagari, 2001). Fowl Cholera (FC), caused by *Pasteurella multocida* A:1 serotype is one of the important diseases especially in the free-range backyard varieties (Rhodes and Rimler, 1989). Exploration of native chicken germplasm for their disease resistance traits will emphasis the importance of incorporating them in breeding program for development of better varieties with more fitness traits. Although, native chicken breeds are observed as sturdy and resistant to many diseases at field conditions, their specific disease resistance/ tolerance traits are yet to be explored. Hence, in the present study, we investigated the response of native and backyard chicken breeds to *Pasteurella multocida* A:1 through experimental infection model.

MATERIALS AND METHODS

The study was carried out at ICAR-Directorate of Poultry Research (ICAR-DPR), Hyderabad, India during 2018. The study was carried out with the approval of IAEC (IAEC/DPR/2017/7) and by following the guidelines of CPCSEA.

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How to cite this article: Kannaki, T.R., Priyanka, E., Abhilash, M. and Haunshi, S. (2022). Differential Disease Resistance of Indian Native Chicken Breeds to Experimental *P. multocida* Infection. Indian Journal of Animal Research. 56(2): 239-242. DOI: 10.18805/IJAR.B-4536.

Submitted: 21-05-2021 **Accepted:** 08-09-2021 **Online:** 28-09-2021

Experimental birds

Aseel, Ghagus and Vanaraja chicks hatched at hatchery and reared at farm of ICAR-DPR were used in this study. Birds were reared in battery brooders until 12 weeks with standard diet and *ad libitum* water. All the birds were vaccinated against Marek's disease, Newcastle disease, infectious bursal disease, infectious bronchitis and fowl pox by following regular vaccination protocol. No fowl cholera vaccine was done for the breeder or for chicks and were seronegative for *Pasteurella multocida* (PM) specific antibodies confirmed by indirect ELISA with commercially available kit.

Pasteurella multocida A:1 isolate

The *Pasteurella multocida* A:1 isolate maintained at avian health lab was used in the study. It was originally isolated from the fowl Cholera (FC) outbreak from broiler breeder flock. The isolate was confirmed by colony morphology,

Gram's staining and by PCR. The virulence was confirmed by inoculating in day-old chicks. The isolate induced death within 24 hrs and the organism was re-isolated from heart blood swab collected from chicks upon necropsy in brain heart infusion (BHI) agar (HiMedia labs, Mumbai, India; Cat # M210). The inoculum for the experiment was prepared as per the method of Petersen *et al*. (2001). Briefly, single colony of virulent culture from BHI agar plate was inoculated into BHI broth and was incubated aerobically at 37°C for 24 hrs. The size of the inoculum was determined by plate-spread method to contain approximately 2.5×10^6 CFU/ml.

Experimental infection of chicken

A total of 72 birds, 24 from each breed (Aseel, Ghagus and Vanaraja) at the age of 12 weeks were divided into three groups for each breed (n=6/ group). One group of each breed was inoculated with 1 ml of BHI broth containing 2.5×10^6 CFU/ml through intraperitoneal route (I/P) and the other group was inoculated with same dose through intranasal route (I/N). The third group of each breed was kept as uninoculated control. The birds were kept in chicken isolators during the entire inoculation experiment following the institute bio-security guidelines. Birds were given standard diet and water *ad libitum*.

Morbidity and mortality pattern

The inoculated birds were observed for any clinical signs, morbidity and mortality for 7 days. The dead birds were removed from the isolators and necropsy was performed (Bermudez and Stewart-Brown, 2003). PM specific lesions in different organs including liver, spleen, heart, lungs and wattles were examined. Swabs from liver and heart were taken for re-isolation of *P. multocida* by standard procedure.

Histopathology

The organs showing PM specific lesions collected during necropsy were fixed in 10% buffered formal saline, processed and embedded in paraffin wax. The sections were made and stained with Hematoxylin and Eosin. The slides were analysed under light microscopy under high magnification (40x and 100x) for pathological changes.

RESULTS AND DISCUSSION

The mortality pattern, percentage and mean death time for each breed are presented in Table 1. Difference among the breeds were observed upon experimental infection. During the experiment, the infected birds manifested depression, fever, mild rales, cough and predominantly white diarrhoea in most of them (Fig 1). The mortality rates were 83.3% in Assel breed against 100% in both Ghagus and Vanaraja breed in intraperitoneally infected groups. Upon intranasal infection, the mortality was 83.3% in Assel and Vanaraja breed against 100% in Ghagus breed. Aseel birds showed significantly better survivability and longer death time than Ghagus breed. Vanaraja breed showed tolerance

Table 1: Mortality pattern, percentage and mean death time of Aseel, Ghagus and Vanaraja chicken with experimental *P. multocida* A:1 infection.

Category	Aseel		Ghagus		Vanaraja	
	Infected	Control	Infected	Control	Infected	Control
Intraperitoneal route						
Mortality (dead/total)	5/6	0/6	6/6	0/6	6/6	0/6
Mortality %	83.3%	0%	100%	0%	100%	0%
Mean death time	24 hrs	-	24 hrs	-	28 hrs	-
Intranasal route						
Mortality (dead/total)	5/6	0/6	6/6	0/6	5/6	0/6
Mortality %	83.3%	0%	100%	0%	83.3%	-
Mean death time	43.2 hrs	-	52.33 hrs	-	38.4 hrs	-

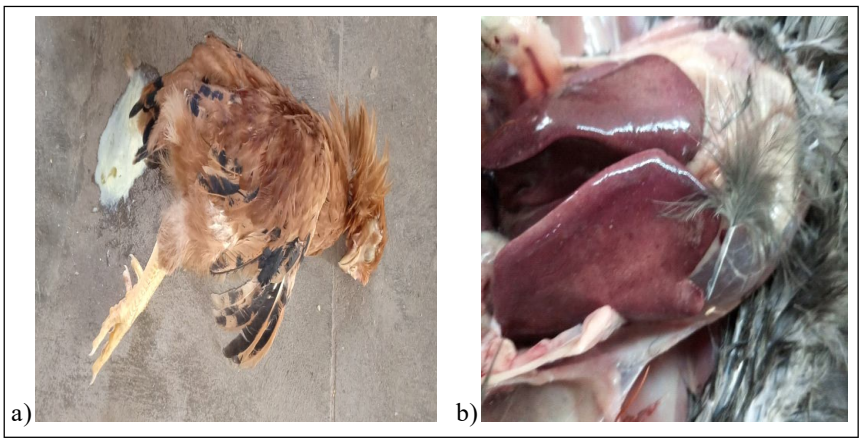

Fig 1: Clinical signs and lesions in experimentally infected chicken.
a) Dead Vanaraja chicken showing diarrhoea; b) Liver showing white necrotic foci.

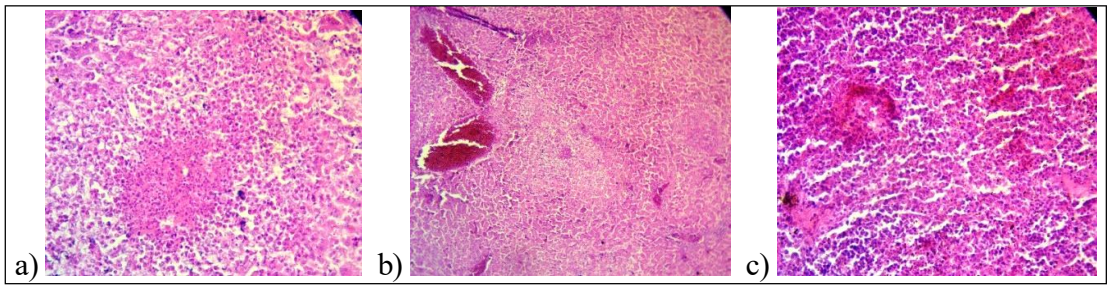

Fig 2: Histopathological changes in the organs of experimentally infected chicken.
a) Necrotic foci in liver (40x); b) Microabscess, necrotic foci in liver (10x);
c) Necrosis in spleen and lymphocytic infiltration (40x).

comparable to Aseel in intranasal experimental infection. No mortality was observed in control birds. On necropsy, lesions like congestion of liver and intestines, necrotic foci in liver, spleen, haemorrhages in epicardial fat and heart were observed in varying degree. Histopathological changes included lymphocytic infiltration, micro-abscesses and haemorrhages in liver and spleen of infected birds (Fig 2).

The degree of susceptibility to *P. multocida* infection is variable among different and types of birds and different age groups. Serotype A of *Pasteurella* in chicken induce acute form of Fowl Cholera (Rhodes and Rimler, 1989). Generally, younger birds are less susceptible to FC than older birds (Heddleston, 1962). The clinical signs observed in infected birds were like earlier reports (Christensen and Bisgaard, 2000; Mbuthia *et al*., 2008).

Genetic basis of disease resistance in chicken was demonstrated by several studies. Some of the genes and gene clusters namely, MHC (major histocompatibility complex) genes, the Nramp1 (Natural resistance-associated macrophage protein 1) gene, IFN (Interferon) genes, Mx (Myxovirus-resistance) genes, anti-ALV (Avian leucosis virus) genes and the Zyxin gene were demonstrated to contribute for disease resistance in chicken (Briles *et al*., 1983, Bacon, 1987, Lamont *et al*., 1987, Caron *et al*., 1997; Jie and Liu, 2011). Previous studies demonstrated that resistance to Marek's disease, fowl cholera and coccidiosis was associated with B21, B1 and B3 haplotypes of the histocompatibility (B) complex of the White Leghorns chickens, respectively (Lamont *et al*., 1987). Vietnamese native chicken breed *Ri* was shown to be more susceptible to experimental Pasteurella infection than commercial *Luong Phuong* chicken breed (Schou *et al*., 2010). The observed relative tolerance/ resistance of Aseel chicken may be attributed to MHC haplotype, NRAMP1 or PAMPs *etc*.

CONCLUSION

Native chicken breeds showed difference in susceptibility/ tolerance to experimental *Pasteurella multocida* A:1 infection. Aseel breed showed significant tolerance than Ghagus and Vanaraja breeds in terms of morbidity, mortality percentage and mean death time. The underlying immune factor for this differential response needs to be explored.

ACKNOWLEDGEMENT

The authors sincerely thank the Director, ICAR-Directorate of Poultry Research, Hyderabad for providing necessary facilities for the research work.

Conflict of interest

Authors declare that they have no conflict of interest.

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