

## Assessment of innate immunity by phagocytic activity in non-descript and Sahiwal cattle in Chhattisgarh

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### Abstract

**Aim:** The objective of the research was to assess the innate immunity status of non-descript (ND) cattle in comparison to Sahiwal cattle in Chhattisgarh by the determination of the phagocytosis activity (PA) and opsonocytaphagic index (OI).

**Materials and Methods:** *In-vitro* phagocytic activity of neutrophil was studied in ND and Sahiwal (S) cattle after dividing them in groups *viz.* ND-I, ND-II, S-I and S-II. Neutrophils were separated from the blood and activated with *Staphylococcus aureus*. PA and OI were observed under high power of a microscope at  $\times 1000$  magnification.

**Results:** PA (percent) in groups ND-I, ND-II, S-I and S-II was  $73.00 \pm 1.03$ ,  $82.25 \pm 1.51$ ,  $80.75 \pm 1.36$  and  $82.37 \pm 1.27$ , respectively which vary significantly ( $p < 0.01$ ), with respect to age. The observations regarding the OI (bacteria per neutrophil) in groups ND-I, ND-II, S-I and S-II was  $7.17 \pm 0.04$ ,  $7.76 \pm 0.09$ ,  $7.02 \pm 0.04$  and  $7.79 \pm 0.06$ , respectively. Age difference was evident, i.e., OI increases as age increases.

**Conclusion:** Innate immunity plays a vital role in host defense against diseases. Neutrophil kills the invading pathogen by phagocytic action. PA and OI is an important tool for the assessing innate immune status. After coincubation of neutrophil with bacterial cells, PA was significantly lower ( $p < 0.01$ ) in ND-I, calves group then other groups. Further, the result of OI clearly indicates that OI has increased as age increases in both breeds. The study will, therefore, generate first hand information on ND cattle along with their relative statistical differences, if any, when compared with Sahiwal.

**Keywords:** innate immunity, non-descript cattle, opsonocytaphagic index, phagocytosis activity.

### Introduction

Most of the cattle population in India is of non-descript (ND) types that is difficult to categorize as any specific breed and hence broadly termed as indigenous local breed. While the research was conducted on production hike, the potential of indigenous breeds for disease resistance received less attention. India has 199 million cattle and 105.3 million buffalo population (9.49 million cattle and 1.6 million buffalo population present in Chhattisgarh [C.G.]) as per livestock census, [1]. Only 20% of these animals have been classified as recognized breeds and rest 80% is ND [2]. Major population of ND cattle remained undocumented although play an important role in the upliftment of small regional farmers [3].

In fact, ND and Sahiwal both are indigenous cattle breeds for C.G. later being a recognized breed. The Sahiwal is one such breed which is an established breed in C.G. and probably in whole India. Sahiwal is tick-resistant, heat-tolerant and noted for its high resistance to parasites, both internal and external [4]. In fact,

these unique characters are reflections of strong genetic make-up that govern innate immunity and its lineage with adaptive/acquired immunity. Fortunately, in perspectives of C.G. State, Sahiwal can be considered as useful candidate animal for comparison with ND cattle about genetic and phenotypic capabilities are concerned.

Innate immunity in cattle varies between breeds and also with in breeds as per age, sex, strain, season, and more importantly the physiological status which decides its adaptability to the environment and available feed resources. Peak production potential of local cattle is less, but their relative cost of milk production is very low as compared to the dairy cattle reared in well developed dairy farming system [5]. Reduced production cost is attributed to the unique feature of their survival in the minimum quantities of feed and has innate potential to adapt to diverse climatic factors, diseases and susceptibility to parasites of geographic importance.

Innate immune system represents a highly conserved strategy against a wide array of bacterial, fungal, and viral pathogens. Activation of innate immune system results in an inflammatory response, which is essential to control infections rapidly before the infections spread to other parts of the body. It is now clear those cells of innate immune system contribute

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towards initiation and subsequently focus on ensuing adaptive immune responses [6]. Phagocytes are critical to the acute inflammatory response because they can efficiently engulf and destroy a variety of pathogens. Phagocytes comprised of neutrophils, monocytes, macrophages, and eosinophils, and they are also given the name of professional phagocytes. Phagocytosis of microorganisms is a key element in host defences against bacterial infections [7]. Phagocytic activity embraces the phagocytosis activity (PA) and opsonocytophagic index (OI) of neutrophils.

Neutrophils are classified as polymorphonuclear cells (PMN) which serve as the first line of cellular defense [8]. Rapid recruitment of sufficient numbers of PMN and increased phagocytosis in the beginning prevents establishment of any infection. Neutrophils exhibit a highly ruffled surface by exhibiting pseudopods on the surface. This increases the surface area of neutrophils and helps in augmenting phagocytosis by increasing the formation of phagosomes [9].

Although a lot of work has been done on milk and blood PMN, there is no such available information about ND cattle. The objective of the research was to assess the immunological status of ND cattle in comparison to Sahiwal cattle by the determination of the phagocytic activity.

## Materials and Methods

### Ethical approval

The M. V. Sc. research study was conducted after the approval of the Institutional Animal Ethics Committee, College of Veterinary Science and Animal Husbandry, Anjora, Durg.

### Selection of animals

The study was planned to adjudge extent of innate immune response of village level local ND cattle and findings were compared with Sahiwal reared in an organized farm. ND cattle belonging to a Gaushala situated in Chhatagarh, Mohli, Durg (C.G.) and Sahiwal cows belonging to Bull Mother Experimental Farm, Anjora, Durg (C.G.) were selected to study innate immune response of local ND cattle and Sahiwal cows. 16 apparently and clinically healthy local ND cows (Group A), and 16 Sahiwal cows (Group B) were selected for study. The animals in each group were further divided into two subgroups viz. ND-I (6 months-2 years age), ND-II (above 2 years), S-I (6 months-2 years age) and S-II (above 2 years) with eight animals in each subgroup. The animals were apparently free from ectoparasite infestation. The faecal sample examination for parasitic eggs did not reveal the presence of gastrointestinal parasites. All animals under study were also administered preventive dose of oral antiparasitic medicines as regular deworming programme. The animals were offered ad lib feed and fresh water at all the time of day.

### Blood sampling

Blood samples were collected as per the protocols approved by the animal Ethics Committee of the

Institute. While collecting blood samples, adequate precautionary measures were taken to minimize pain and/or discomfort to the animals. 5 ml of blood was collected aseptically by external jugular vein puncture from each cattle brought under the study in heparinized vials and used to perform phagocytic activity.

### Preparation of bacterial suspension

*Staphylococcus aureus* was used to assess the phagocytic activity. Bacterial suspension was prepared as per the protocol followed by Samtani *et al.* [10]. Pure culture of *S. aureus* that maintained at department was used. 18 h old culture of *S. aureus* in nutrient broth was taken and centrifuged at 2000 rpm for 10 min, washed with physiological saline and opsonised at 37°C for 30 min, with 10% pooled normal human serum. The suspension was again centrifuged at 2000 rpm washed in PBS and diluted to give a final concentration of  $2 \times 10^8$  bacteria/ml.

### Isolation of blood PMN cells

Isolation of PMN from peripheral blood was performed using hypotonic lysis of erythrocytes as described by Ordonez *et al.* [11] with slight modification. Blood was centrifuged at 1000 g for 20 min at 4°C in propylene conical tubes to obtain PMN cells. Plasma and buffy coat were discarded, but the compact pellet layer was preserved. From the pellet erythrocytes were lysed by addition of 4 ml of blood to a sample tube containing 8 ml of cold 4°C saline A solution (NaCl, 0.020 M), after 30 s 2 ml of cold saline B solution (NaCl, 0.164 M) were added to restore isotonicity. The lysate was centrifuged 3 min at 300 g, and the recovered cell pellet was washed twice in Hank's balanced solution (0.1% of bovine serum albumin  $Ca^{2+}$  and  $Mg^{2+}$  free). RPMI 1640 cell culture media supplement (15% v/v fetal calf serum inactivated at 56°C, penicillin 100 UI/ml, streptomycin 100 µg/ml) was used for resuspension of pellet. PMN package was adjusted to  $5 \times 10^6$  viable cells/ml. Viability checked by mixing 100 µl of pellet and 0.4% trypan blue solution which reveal over 90% viable cells.

### Procedure of *in vitro* phagocytic activity

The PA and OI were estimated by the method given by Malik [12] with slight modification as suggested by Ordonez *et al.* [11]. *In vitro* phagocytosis assay was performed by mixing 0.2 ml of bacterial suspension and 0.2 ml neutrophil suspension in the ratio of 40:1 (bacteria:neutrophil) in polyethylene microtubes and incubated at 37°C for 30 min. Smears were prepared and stained with Leishman stain. Then slides were examined under high power microscope at  $\times 1000$  magnification (Trinocular, H600L, DS Fi1-Nikon Eclipse50i, Japan).

a. PA (phagocytosis percentage) was estimated as a percentage of cells taking part in phagocytosis

$$\text{Phagocytosis activity} = \frac{\text{Number of PMN that have engulfed bacteria}}{\text{Number of PMN counted}} \times 100$$

b. OI was estimated as the mean amount of bacteria phagocytosed per the neutrophil.

$$\text{Opsonocytaphagic index (OI)} = \frac{\text{Total bacteria in N neutrophil}}{N}$$

Here, N=25.

The result of PA was reported as percent (%) phagocytosing neutrophils counting 100 PMN cells in different microscope fields and the result of OI was reported as average number of bacteria per neutrophil (bacteria per neutrophil).

**Statistical analysis**

The data were analyzed statistically by independent mean t-test to see the effect of age within a breed, if any, and to see the effect of breeds within the same age group, if any, following the standard procedure as outlined by Snedecor and Cochran [13].

**Results**

The purpose of the present study was to compare local ND cows with Sahiwal with respect to an important immune parameter that is phagocytic activity of PMN cells. The study will, therefore, generate first hand information on ND cattle along with their relative statistical differences, if any, when compared with Sahiwal.

**PA (phagocytosis percentage)**

The co-incubation of bacterial cells with neutrophils results in phagocytosis of bacteria. Percentage phagocytosis was calculated by noting the number of PMN that had engulfed bacteria, and a 100 PMN were counted. The result of PA is reported as percent and shown in Table-1 and Figure-1. PA was significantly low ( $p < 0.01$ ) in ND calves with the values  $73.00 \pm 1.03$ . Another group of animal viz. ND-II, S-I and S-II had PA values rendered as  $82.25 \pm 1.51$ ,  $80.75 \pm 1.36$  and  $82.37 \pm 1.2$ , respectively.

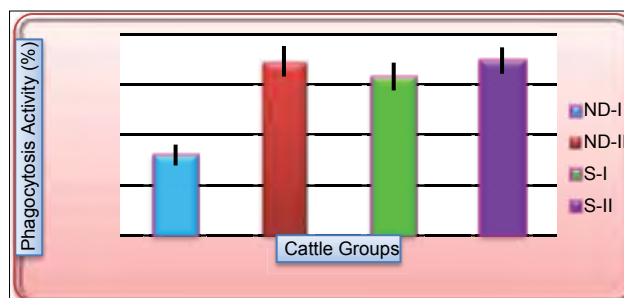
**OI (bacteria per neutrophil)**

The observations regarding the OI are shown in Table-1 and Figures-2 and 3. The analyzed data clearly indicate that OI has increased as age increases. The OI of ND calves was  $7.17 \pm 0.04$  which increased to  $7.76 \pm 0.09$  in adult group. Sahiwal group of animals showed a similar trend of increasing OI value with age, i.e.,  $7.02 \pm 0.04$  in S-I and  $7.79 \pm 0.06$  in S-II.

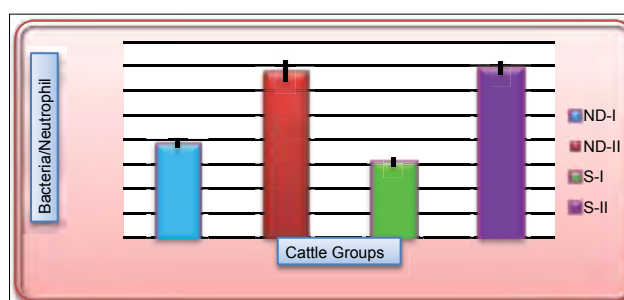
**Discussion**

In this study, there is a comparison of phagocytic activity between ND and Sahiwal based on breed and

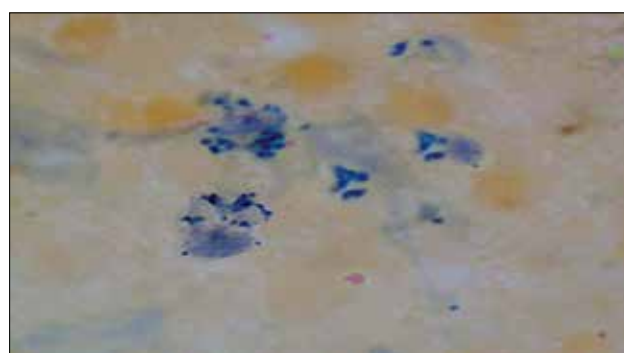
age specificity. A handful work had been carried out on different breed of well known significance at different physiological stages and different clinical condition, where as there was no information regarding phagocytic activity of local ND cattle. Blood neutrophils can be used as an efficient measure to assess the



**Figure-1:** Mean ( $\pm$ standard error) values of phagocytosis activity of non-descript (ND) and Sahiwal cattle, ND-I: ND cattle (6 months-2 years age), ND-II: ND cattle (above 2 years age), S-I: Sahiwal cattle (6 months-2 years age), S-II: Sahiwal cattle (above 2 years age).



**Figure-2:** Mean ( $\pm$ standard error) values of opsonocytaphagic index bacteria/neutrophil of non-descript (ND) and Sahiwal cattle. ND-I: ND cattle (6 months 2 years age), ND-II: ND cattle (above 2 years age), S-I: Sahiwal cattle (6 months 2 years age), S-II: Sahiwal cattle (above 2 years age).



**Figure-3:** Bacteria phagocytosed by neutrophil showing opsonocytaphagic index ( $\times 1000$  magnification; leishman stain).

**Table-1:** Phagocytosis in ND and Sahiwal cattle according to breed and age.

Group	ND		Sahiwal (S)		Level of significance
	ND-I	ND-II	S-I	S-II	
PA (%)	$73.00 \pm 1.03^{b**}$	$82.25 \pm 1.51^a$	$80.75 \pm 1.36^a$	$82.37 \pm 1.27^a$	**
OI (bacteria/neutrophil)	$7.17 \pm 0.04^b$	$7.76 \pm 0.09^a$	$7.02 \pm 0.04^b$	$7.79 \pm 0.06^a$	**

a, b, \*\*Highly significant at 1% level ( $p < 0.01$ ), \*Significant at 5% level ( $p < 0.05$ ). OI=Opsonocytaphagic index, PA=Phagocytosis activity, ND=Non-descript

innate immunity of cattle. The estimation of *in-vitro* activity of neutrophils in cattle can be effectively used for studying natural resistance [14]. They have reported that this technique can be further exploited to study the effect of antioxidants or specific drugs on neutrophils that may help to boost immunity in animals without having to affect their health. In present study PA of adult ND and Sahiwal cattle was more than that reported by Vishnoi *et al.* [14] who found 73 PA in neutrophils for yeast cells from blood of Murrah buffaloes with clinical mastitis. Other workers found 100 PA while incubating whole blood with yeast cells [15]. Present *in-vitro* studies of PA of neutrophils were in agreement to the report of Dosogne *et al.* [16] when neutrophils were incubated with *S. aureus*, blood PMN phagocytosed about 82% *S. aureus* and found no significant difference between different stages of lactation.

Many diseases can affect numbers of white blood cells (leukocytes) in peripheral blood, and several assays are available to determine total leukocyte number. Traditionally, peripheral blood mononuclear cells are separated from red blood cells using density-gradient centrifugation [17]. Neutrophils (PMN leukocytes) are important members of professional phagocytes. They are most numerous in circulation, and these are usually the first cells to arrive at the site of inflammation. It is possibly the most significant cellular component of the innate response during an acute infection [18]. Weak phagocytosis is better detected by phagocytosis percentage, and high PA is best shown by calculating phagocytosis index [19]. Further, the surface architecture demonstrated by scanning electron microscope provides insight for activity of phagocytic cells. Diminished phagocytic activity of milk neutrophils revealed a smooth membrane of milk neutrophils where as blood neutrophils exhibited more ruffled membrane and enhanced phagocytic activity [8]. To predict the resistance and susceptibility of animal depending on the PA and OI; concentration of neutrophils, various parameters such as activation state of PMN, their intrinsic bactericidal activity, the presence of antibodies opsonizing the bacteria and neutralizing their toxins have to be taken into account [20]. Ordonez *et al.* [11] assessed the *in-vitro* differential neutrophil PA on *S. aureus* obtained from blood and milk of Holstein dairy cows in early lactation period and observed a greater *in-vitro* mean PA (86.5±9.6%) and phagocytosis index (7.34±1.55) in blood PMN neutrophil cells in comparison to milk cells. They concluded that PMN cells obtained by erythrocyte lysis and lypopolysaccharide udder stimulation are useful for *in-vitro* reproducible phagocytosis assay.

### Conclusion

The result of the present study indicated that although, the PA and OI were less in ND animals of below 2 years of age there was a significant increase up to 2 years of age. Resultant optimal levels attained

are similar to with those of Sahiwal calves as well as adult animals. PA and OI in ND calves attained optimal levels *at par* with those of Sahiwal animals. This shows that the gain in natural resistance of ND animal though appear slow in early age, they make-up their innate immune status as age advances.

### Authors' Contributions

GD and SDH planned and designed the experimental study. GD performed the study under the supervision and technical support of SDH. GD, DKK and DKG performed the collection and processing of the sample. GD, DKG and DKK drafted and reviewed the manuscript. All authors read and approved the final manuscript.

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### Competing Interests

The authors declare that they have no competing interests.

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