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Scanning electron microscopic studies on the bursa of Fabricius of local hill fowl of Uttarakhand

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Abstract
The present study was carried out on twelve birds of local hill fowl of Uttarakhand divided into two age groups viz. 0 day and 6 months of age with six birds in each group. The birds were procured from the Instructional Poultry Farm (IPF), Nagla, GBPUA&T, Pantnagar, Uttarakhand. The scanning electron microscopic (SEM) studies showed numerous plical folds lined by the surface epithelium in the local hill fowl. Further, the lymphocytes and other cellular elements were found to be protruded from the cut surface of plica of the bursa of Fabricius. The follicular associated epithelium and interfollicular epithelium were also observed.

Keywords: Scanning electron microscopic, bursa of Fabricius, local hill fowl of Uttarakhand

Introduction
The poultry production has taken a quantum leap in India since last four decades [18]. Similarly, the poultry population of Uttarakhand state is 26.01 lakh which has increased by 7.01% per annum, while the population of desi fowl in the state decreased 28.67% from 2003 to 2007 [5, 6]. There are many species of birds reared in Uttarakhand. One of the indigenous dual purpose breed of bird found in Uttarakhand is local hill fowl which is generally reared under the backyard system in Kumaon division of Uttarakhand that has evolved through natural selection [12, 13]. The climatic condition of this division during winter is quite harsh, where environmental temperature occasionally goes down below freezing point. The mortality rate of local hill fowl during this harsh climatic condition is very low. These birds have good resistance against the common diseases of poultry, bio-controller of insects and can easily survive on low quality fodder [8]. Meat of local hill fowl is very tasty and chewy, so it is very popular among the people of rural hilly areas. Due to low cholesterol level in the meat of local hill fowl it is considered appropriate for heart patients and obese people [10]. The major components of body defense mechanism are the innate and acquired or adaptive immunity. Innate immunity includes physical barrier like skin and mucous membrane, complement and cells like granulocytes, thrombocytes, macrophages and natural killer cells. On the other hand, acquired or adaptive immunity is mediated by immunocompetent cells viz., humoral response B-cells, cell mediated response T-cells and some other cells like phagocytic and adherent cells [4]. All these immunocompetent cells are located mostly in the lymphoid organs.
With the advancement of bird age, the distinction between the primary and secondary lymphoid organs becomes less apparent [15]. Typical fibro cellular architecture is present in case of lymphoid organs that undergoes structural development and maturation with the progressive aging and of course involution during later part of the life. The age of involution of the bursa of Fabricius is different in case of different breed of birds. Though some work on lymphoid system of broiler chicken [9], domestic chicken [7], Japanese quail [15], duck [17], turkey [2] has been done, but still the knowledge on the bursa of Fabricius of local hill fowl has not been explored. Keeping this fact in mind and viewing the increased popularity of the local hill fowl of Uttarakhand, the present study was undertaken to develop a data that will pave the path for the characterization of immunogenic potential of this bird.

Materials and methods
The present study was carried out on twelve birds of local hill fowl of Uttarakhand divided into two age groups viz. 0 day and 6 months of age with six birds in each group.

The birds were procured from the Instructional Poultry Farm (IPF), Nagla, GBPUA&T, Pantnagar, Uttarakhand. The electron microscopic study was performed according to standard method described by Electron microscopic manual of AIIMS, New Delhi [3, 11] with slight modifications. For ultra structural studies, approximately 2 mm thickness sections were collected and fixed with Karnovsky's fixative (glutaraldehyde + paraformaldehyde) for 24 hours at 4 degree Celsius. After fixation following steps was followed for preparation of samples:

Drying
The dehydrated sample was transferred from acetone to drying medium i.e. liq. CO_2 into a chamber which was cooled and put under pressure. The sample was then dried at 31.5-degree Celsius at 1100 p.s.i. (pressure).

Mounting
Specimen was then mounted on aluminum stubs with conductive paint or adhesive tape.

Coating
Specimen was coated by a thin layer (30-40nm) of metal conductor to increase its electrical conductivity. Sputter coating was done by using evaporating metal (gold) under vacuum in inert atmosphere. Coating of approximately 35 nm thick film was obtained within 3-5 minutes.

Viewing
The specimen was then observed under JEOL scanning electron microscope at 8 kV voltages and photography was performed.

Results and Discussion
The scanning electron microscopic studies (SEM) showed numerous plical folds (Fig. 1) in the luminal surface of bursa of Fabricius lined by the surface epithelium (Fig. 2 and Fig. 6). The lamina propria composed of numerous bursal follicles or lymphoid follicles in the local hill fowl (Fig. 3, Fig. 4 and Fig. 5).

1. Surface Epithelium
The surface epithelium of the plical folds of bursa of Fabricius was mostly of pseudo stratified columnar type. This surface epithelium further consisted of follicle associated epithelium (FAE) and interfollicular epithelium (IFE) (Fig. 3). The present finding was dissimilar to the findings of Tsuji and Miyoshi [20] where they found two types of epithelium i.e. follicle associated epithelium and marginal epithelium in bursa of Fabricius of chicken. The portion of the surface epithelium that lined the underlying follicles was termed as follicle associated epithelium (FAE). FAE cells are capable of incorporating intestinal substances or microorganisms via the cytoplasm into the intercellular spaces as reported by Schaffner *et al.* [14] and Toro *et al.* [19]. Further, the surface epithelium which lined the interfollicular area was termed as interfollicular epithelium. The surface epithelium of the bursal plicae had wavy margins with raised and depressed pitted area (Fig. 3). These pitted portion of the surface epithelium also lined by pseudo stratified columnar epithelium**.**

2. Bursal or Lymphoid follicle
The cut surface of the bursal plica showed elongated lymphoid follicles (Fig. 4 and Fig. 5) in a foliated arrangement that were lined by the follicle associated epithelium (Fig. 6). The surface of follicle associated epithelium showed polygonal epithelial cells (Fig. 7). The present findings were dissimilar to the observations of Tsuji and Miyoshi [20] who reported that the apical dome of bursal follicle was covered by follicle associated epithelial cells and the lateral surface of the dome was lined by marginal epithelial cells. The bursal follicles comprised of various cellular elements (Fig. 8). These bursal follicles or lymphoid follicles presented a centrally placed massive medulla surrounded by a layer of cortex. The cortex was composed of relatively compact zone of cellular elements. Ackerman and Knouef [1] reported that the cortex of bursal follicle was an intramural accumulation of cells which were emigrated from the medulla.

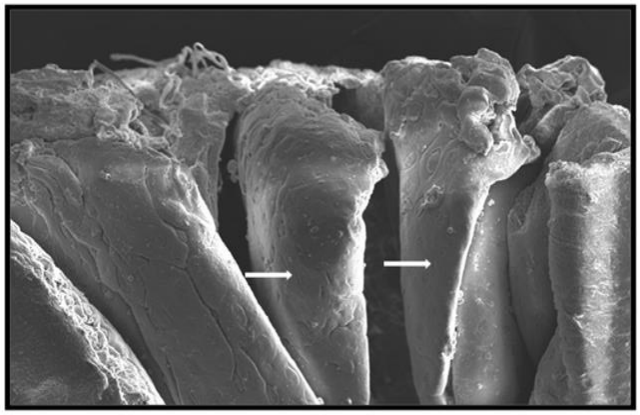

Fig 1: Scanning electron micrograph of large plical fold (arrows) in the bursa of Fabricius of day old Local hill fowl (20 kV×156)

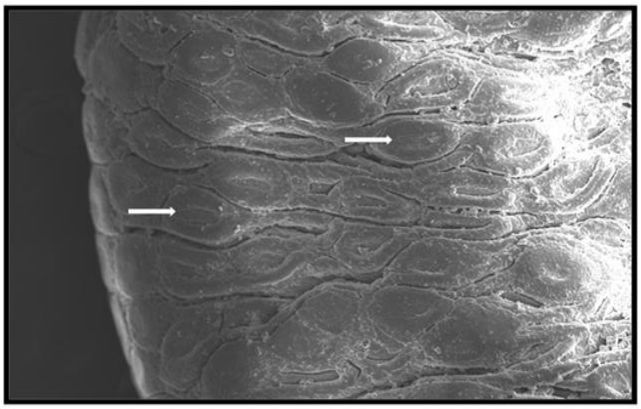

Fig 2: Scanning electron micrograph showing follicle associated epithelium (FAE) (arrows) in the luminal surface of plical fold in adult Local hill fowl (20 kV×156)\

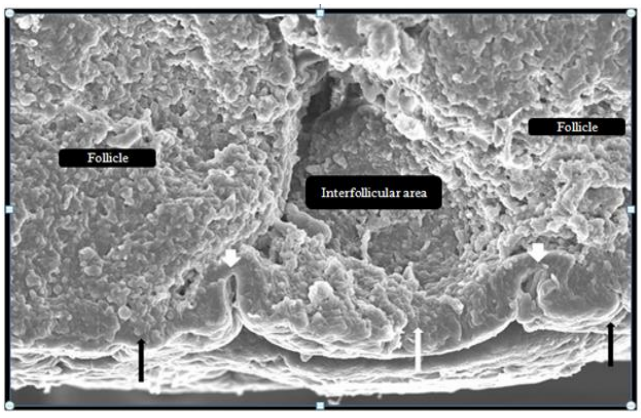

Fig 3: Scanning electron micrograph showing follicle associated epithelium (black arrows), interfollicular epithelium (white thin arrow) and pits of the surface epithelium (thick white arrows) the cut section of bursal plica in adult Local hill fowl (20 kV×716)

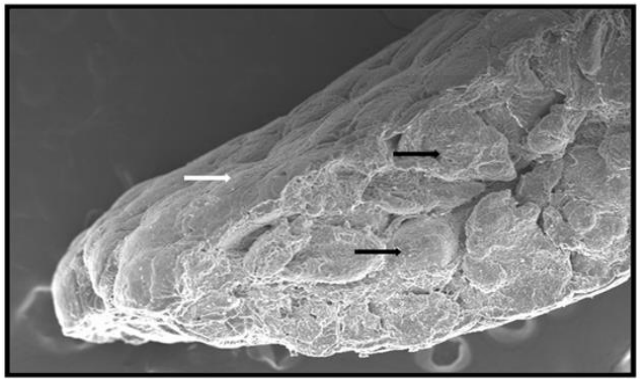

Fig 4: Scanning electron micrograph showing epithelial surface (white arrows) and bursal follicles (black arrows) in the cut section of bursal plica in day old Local hill fowl (20 kV×97)

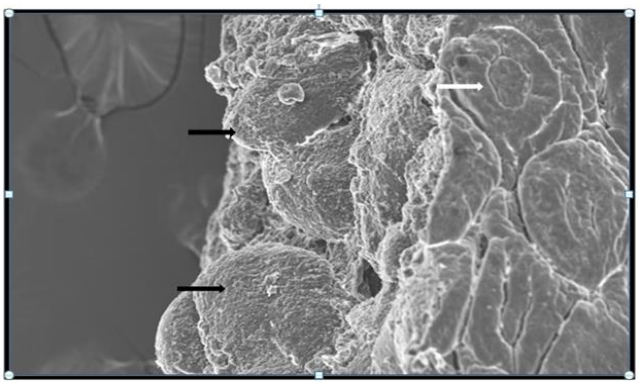

Fig 5: Scanning electron micrograph showing follicle associated epithelium (white arrow) and bursal follicles (black arrows) in the cut section of bursal plica in adult Local hill fowl (20 kV×275)

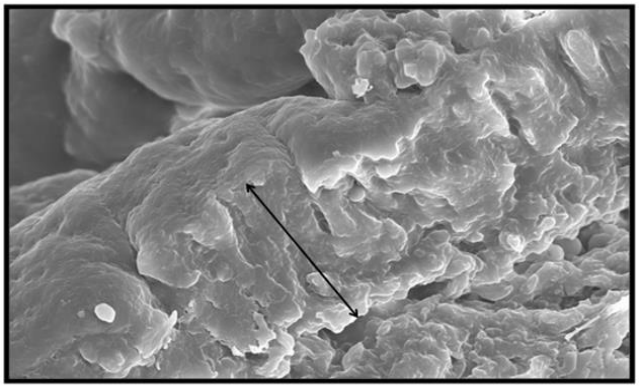

Fig 6: Scanning electron micrograph showing follicle associated epithelium (black line) in the cut section of bursal plica in adult Local hill fowl (20 kV×2390)

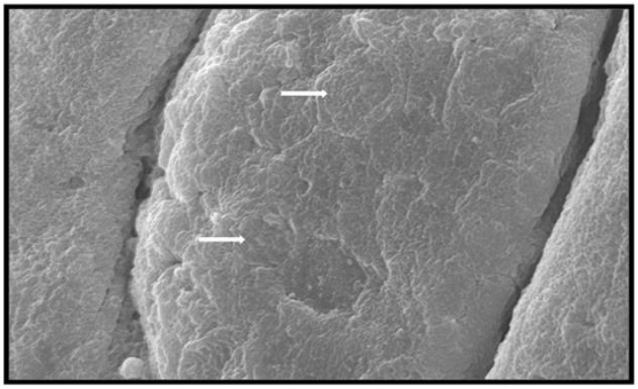

Fig 7: Scanning electron micrograph showing polygonal epithelial cells (arrows) in the surface of follicle associated epithelium (FAE) (arrows) of bursa of Fabricius in adult Local hill fowl (20 kV×5840)

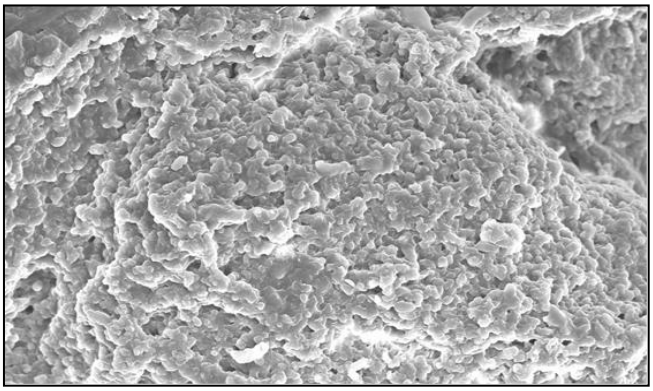

Fig 8: Scanning electron micrograph showing cellular elements of a bursal follicle in adult Local hill fowl (20 kV×1000)

Conclusion

The scanning electron microscopic structure of the bursa of Fabricius of local hill fowl of Uttarakhand was successfully studied in this case. The present work revealed the presence of numerous plical folds lined by the surface epithelium in the bursa of Fabricius of local hill fowl. The surface epithelium further consisted of follicle associated epithelium and interfollicular epithelium. The epithelial surface was disrupted by pitted areas. The cut surface of the bursal wall plica showed elongated lymphoid follicles that were lined by the follicle associated epithelium. The surface of follicle associated epithelium showed polygonal epithelial cells. The bursal follicles comprised of various cellular elements.

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