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A Deka
Assistant Professor, Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

DJ Kalita
Professor Cum Head, Department of Biochemistry, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

BN Bhattacharyya
Professor Cum Deputy Director, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

J Kachari
Assistant Professor, Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

U Barman
Assistant Professor, Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

DK Sarma
Assistant Professor, Animal Reproduction Gynecology and Obstetrics, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

R Saikia
Assistant Professor, Department of Poultry Science, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Abhijit Deka
Assistant Professor (Veterinary Pathology), Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

R Deka
Assistant Professor, Department of Livestock Production and Technology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Corresponding Author:
A Deka
Assistant Professor, Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Chemical characterization of B and T lymphocytes of certain lymphoid organs of Pati duck (*Anas platyrhynchos domesticus*) of Assam

A Deka, DJ Kalita, BN Bhattacharyya, J Kachari, U Barman, DK Sarma, R Saikia, Abhijit Deka and R Deka

Abstract
Purpose: The study of chemical characterization of B and T lymphocytes of certain lymphoid organs of Pati duck (*Anas platyrhynchos domesticus*) of Assam is great value in regards to the immunity of duck. The aim of this study was to know the immune status of duck.
Materials and Methods: The current research was carried out on 45 Pati ducks from Assam. The ducks were procured from Pathsala and nearby areas of Bajali district, Assam. The Pati duck were brought to the Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22, where they were sacrificed using Gracy's procedure (1986). The birds were placed on a clean dissecting table after slaughter, and the skin and fascia were gently reflected without disturbing the organs. By making a ventro-median incision, the abdominal cavity of each Pati duck was revealed, and the abdominal muscle layers, peritoneum, and air sacs of the abdominal region were properly reflected. Then collected the parts of lymph node, Fabricius' bursa, and gut with lymphoid tissue or Peyer's patches. After that, the samples were frozen in liquid nitrogen (-196 °C). Cryosections were cut at a thickness of 10 µm and stored at -20 °C for a period of time at -20 °C. The following processes were used to prepare them for histochemical staining:
a. Lead method for ATPase (Bancroft, 2008)
b. 1 Naphthyl acetate method for non-specific esterase (Bancroft, 2008)
Results: The lymphatic nodule of the lymph node had a significant reaction, while the interfollicular area had a moderate reaction. B-lymphocytes were found in greater numbers in the lymphatic nodule of the lymph node than in the interfollicular area and capsule. T-lymphocytes were found in lower numbers in lymphatic nodules and were completely missing in lymph node capsules. In the follicular related epithelium and undifferentiated epithelial cell of the lymphoid follicle of Fabricius' bursa, there was a robust reactivity to ATPase. In Pati ducks, the lamina propria of Fabricius' bursa and the cortex of lymphoid follicle showed no reactivity to non-specific esterase, while the medulla of lymphoid follicle showed no sensitivity to enzyme. The reaction revealed that B-lymphocytes were more abundant in the cortex than in the medulla, and that T-lymphocytes were absent. The lymphoid follicles of both Peyer's patches and solitary lymphoid nodules revealed high adenosine triphosphatase activity. The lymphoid nodules of the gut in Pati ducks showed a high sensitivity to non-specific esterase activity. The presence of B-lymphocytes and T-lymphocytes was higher in lymphoid follicles of the gut in both reactions.
Conclusion: This study will helpful to the duck farmer, poultry scientist, physiologist and microbiologist for vaccine production and duck meat, egg production and diseases control regime.

Keywords: B-lymphocytes, characterization, lymphoid organs, Pati duck, T-lymphocytes

Introduction
The poultry business is now India's fastest-growing industry. Duck is also a crucial species in the poultry business and expanding farming. Duck may further contribute to growth of the poultry. Duck husbandry play a significant role in the uplift of rural economy. The unique agro-climatic conditions of Assam like marshy as well as waterlogged areas throughout the state, make for an ideal habitat for duck farming. Ducks are one of the most versatile and useful of all domesticated animals and birds, and their popularity is growing in many parts of the world. In the state of Assam, the Pati duck population is indigenous duck breed. The annual egg production of a Pati duck ranges from 70 to 95 eggs (Kalita *et al.*, 2009) [4]. In terms of conventional academic and biomedical research, the study of the GALT of the Pati duck of Assam is quite valuable. It is also required for accurate detection and treatment of diseases caused by various pathogens, such as duck virus enteritis, duck cholera, aflatoxicosis, botulism, and others, as well as food poisoning and food allergies. The failure of vaccination

and control of enteric disease is a cause of concern for disadvantaged farming communities since it leads in massive and irreversible economic losses. Because there is a paucity of literature on chemical characterization of B and T cells from various lymphoid organs in Pati duck, Assam, the current study was conducted.

Aim and Objective

The study of chemical characterization of B and T lymphocytes of certain lymphoid organs of Pati duck (*Anas platyrhynchos domesticus*) of Assam is great value in regards to the immunity of duck. The aim of this study was to know the immune status of duck.

Materials and Methods

The current research was carried out on 45 Pati ducks from Assam. The ducks were procured from Pathsala and nearby areas of Bajali district, Assam. The Pati duck were brought to the Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22, where they were sacrificed using Gracy's procedure (1986). The birds were placed on a clean dissecting table after slaughter, and the skin and fascia were gently reflected without disturbing the organs. By making a ventro-median incision, the abdominal cavity of each Pati duck was revealed, and the abdominal muscle layers, peritoneum, and air sacs of the abdominal region were properly reflected. Then collected the parts of lymph node, Fabricius' bursa, and gut with lymphoid tissue or Peyer's patches. After that, the samples were frozen in liquid nitrogen (-196 °C). Cryosections were cut at a thickness of 10µm and stored at -20 °C for a period of time at -20 °C. The following processes were used to prepare them for histochemical staining:

c. Lead method for ATPase (Bancroft, 2008)
d. 1 Naphthyl acetate method for non-specific esterase (Bancroft, 2008)

Results

The lymphatic nodule of the lymph node showed a significant reaction, while the interfollicular area showed a moderate reaction (Fig.1). In Pati ducks, the lymph node capsule showed a mild sensitivity to ATPase (Table.1). However, due to a lack of literature, it was difficult to compare. In the lymphatic nodule of the lymph node, there was a moderate reaction and a weak reaction in the interfollicular area (Fig.2). In all age groups of ducks, the lymph node capsule revealed a negative reactivity to non-specific esterase (Table.1). B-lymphocytes were abundant in the lymphatic nodule of the lymph node than in the interfollicular area or capsule. T-lymphocytes were found in fewer in numbers in lymphatic nodules and were completely absent in lymph node capsules. In the follicular associated epithelium and undifferentiated epithelial cell of the lymphoid follicle of Fabricius' bursa, there was a significant reactivity to ATPase (Fig.3). In Pati ducks, the lamina propria of Fabricius' bursa as well as the cortex of lymphoid follicle showed no ATPase reaction, whereas the medulla of lymphoid follicle showed a weak reaction (Table.1). For non-specific esterase activity, there was a mild reactivity for follicle related epithelium and undifferentiated epithelial cell of lymphoid follicle of Fabricius' bursa. (Fig.4). In Pati ducks, the lamina propria of Fabricius' bursa and the cortex of lymphoid follicle showed no reactivity to non-specific esterase, while the medulla of lymphoid follicle showed no sensitivity to enzyme (Table.1). The reaction revealed that B-lymphocytes were more abundant in the cortex than in the medulla, and that T-lymphocytes were absent. The presence of ATPase was determined in representative samples from the intestines of Pati ducks. In adult Pati ducks from Assam, the lymphoid follicles of both Peyer's patches and solitary lymphoid nodules revealed high adenosine triphosphatase activity (Fig.5 & Table 1). In Pati ducks, lymphoid nodules in the intestine showed a high sensitivity to non-specific esterase activity (Fig. 6 & Table 1). However, due to a lack of literature, it was difficult to establish compare. The presence of B-lymphocytes and T-lymphocytes was higher in lymphoid follicles of the gut in both reactions.

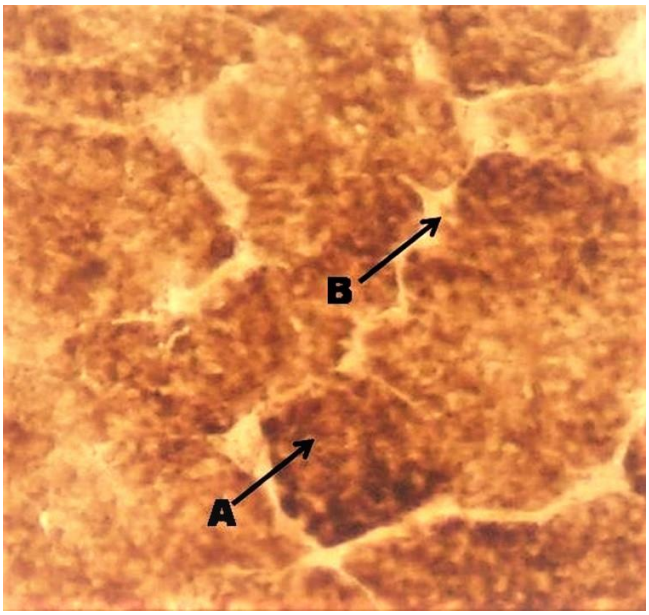

Fig 1: Photomicrograph showing the Adenosine Triphosphatase activity in lymphatic nodule (A) and inter follicular area (B) of Lymph node of Pati Duck (*Anasplatyrhynchosdomesticus*). Lead method, 10X.

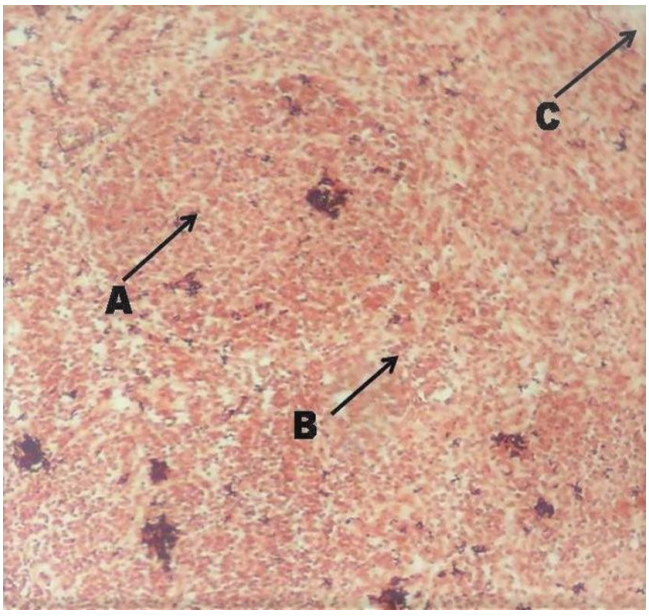

Fig 2: Photomicrograph showing the Non-Specific Esterase activity in lymphatic nodule (A), inter follicular area (B) and capsule (C) of lymph node of Pati duck. 1 Naphthyl Acetate Method, 10X.

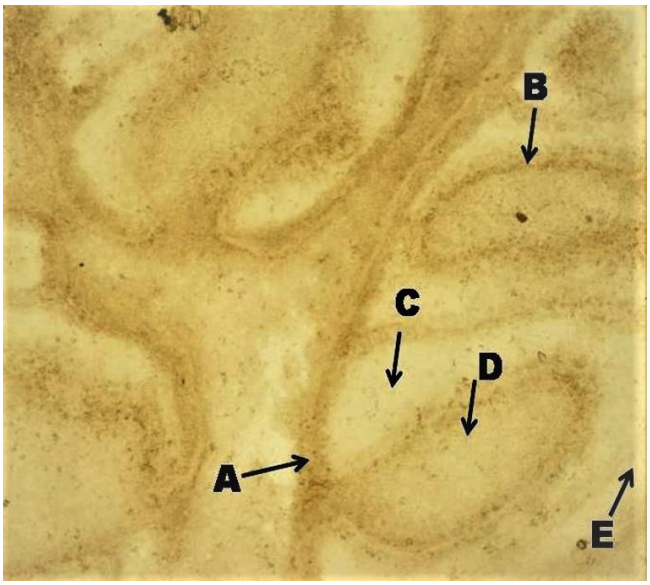

Fig 3: Photomicrograph showing the Adenosine Tri Phosphatase activity in follicle associated epithelium (A), undifferentiated epithelial cells (B), medulla (D), cortex (C) and lamina propria (e) of bursa of Fabricius of Pati duck. Lead Method, 10X.

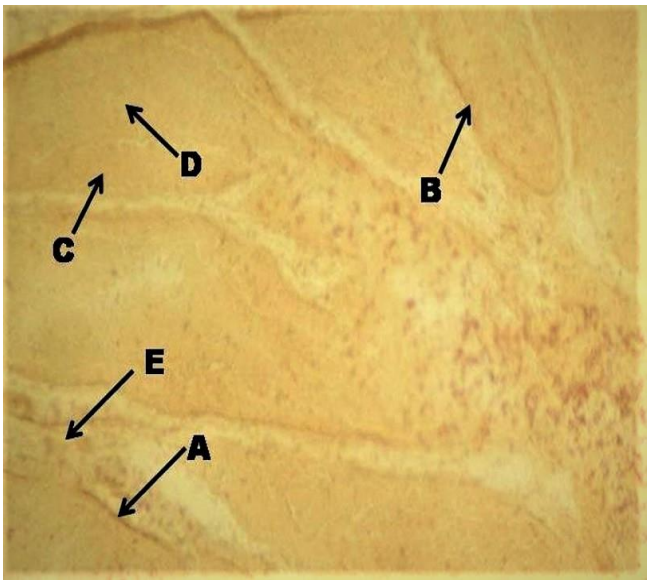

Fig 4: Photomicrograph showing the Non-Specific Esterase activity in follicle associated epitheliums (A), undifferentiated epithelial cells (B), medulla (D), cortex (C) and lamina propria (E) of bursa of Fabricius of Pati duck. 1 Naphthyl Acetate Method, 10X.

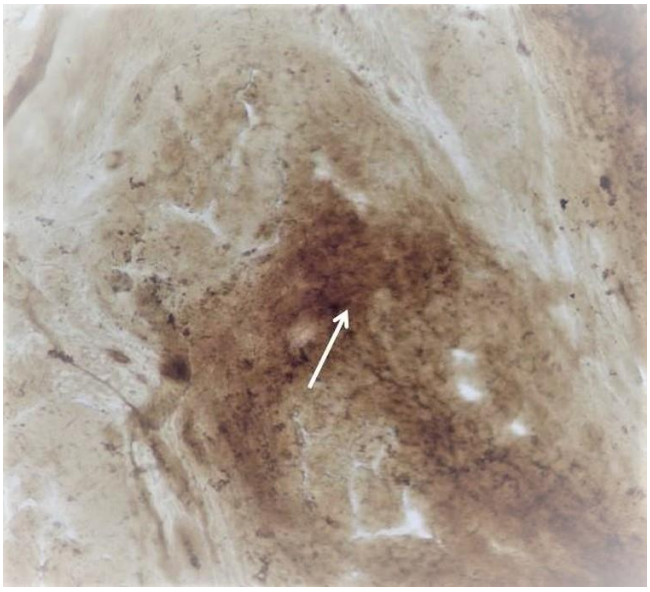

Fig 5: Photomicrograph showing the ATPase activity in lymphatic nodule (White arrow) of intestine of Pati Duck. Lead Method, 10X.

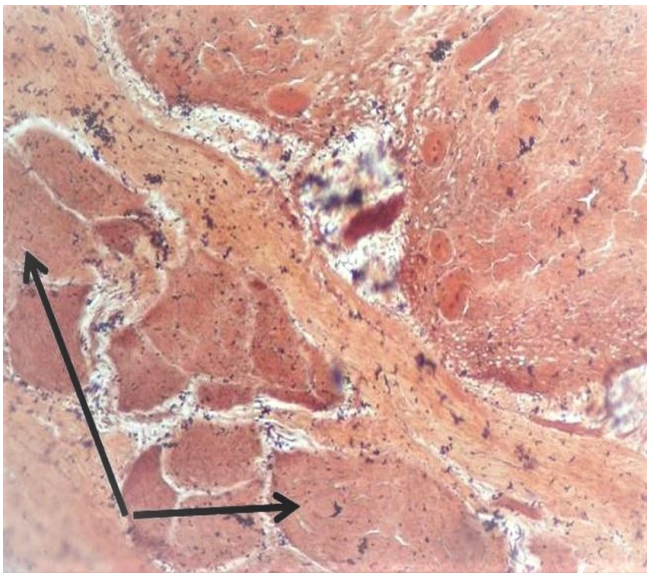

Fig 6: Photomicrograph showing the non-Specific Esterase activity in lymphatic nodule (Black arrow) of intestine of Pati Duck.1-Naphthyl Acetate Method, 10X

Table 1: Histochemical Characterization of B-Lymphocytes and T-Lymphocytes of Lymph Node, Bursa of Fabricius and Lymphatic Nodule (Ln) of Intestine of Pati Duck.

Histoenzymes	Lymph node			Bursa of Fabricius					Lymphatic nodule of Intestine
	Lymphatic nodule	Inter follicular area	Capsule	Follicle associated epithelium	Undifferentiated epithelial cells	Cortex	Medulla	Lamina Propria	
Adenosine triphosphatase	+++	+	+	+++	+++	-	+	-	+++
Non specific esterase	++	+	-	+	+	-	-	-	+++

Gradation for intensity of histochemical reaction

- = Negative
+ = Weak
++ = Moderate
+++ = Strong
++++ = Intense

Discussion

The lymphatic nodule of the lymph node showed a significant reaction, while the interfollicular area showed a modest reaction. In Pati ducks, the lymph node capsule showed a mild reaction to ATPase. However, due to a lack of literature, it was difficult to compare. In the lymphatic nodule of the lymph node, there was a moderate reaction and a weak reaction in the interfollicular area. In all age groups of ducks, the lymph node capsule revealed a negative reaction to non-specific esterase. B-lymphocytes were abundant in the lymphatic nodule of the lymph node than in the interfollicular area or capsule. T-lymphocytes were found in fewer in numbers in lymphatic nodules and were completely absent in lymph node capsules. In the follicular associated epithelium and undifferentiated epithelial cell of the lymphoid follicle of Fabricius' bursa, there was a significant reaction to ATPase. In Pati ducks, the lamina propria of Fabricius' bursa as well as the cortex of lymphoid follicle showed no ATPase reaction, whereas the medulla of lymphoid follicle showed a weak reaction. Mazzone *et al.* (2003) [6] found that the epithelial covering of bursal plicae and the muscular layer reacted positively to ATPase activity, whereas the cytoplasm of cortical and medullary lymphocytes reacted negatively. For non-specific esterase activity, there was a mild reactivity for follicle related epithelium and undifferentiated epithelial cell of lymphoid follicle of Fabricius' bursa. In Pati ducks, the lamina propria of Fabricius' bursa and the cortex of lymphoid follicle showed no reactivity to non-specific esterase, while the medulla of lymphoid follicle showed no sensitivity to enzyme. These results were similar to those of Kempashi *et al.* (2017) [5] in Chicken and Ebru *et al.* (2015) [2] in Long-Legged Buzzard. The reaction revealed that B-lymphocytes were more abundant in the cortex than in the medulla, and that T-lymphocytes were absent. The presence of ATPase was determined in representative samples from the intestines of Pati ducks. In adult Pati ducks from Assam, the lymphoid follicles of both Peyer's patches and solitary lymphoid nodules revealed high adenosine triphosphatase activity. In Pati ducks, lymphoid nodules in the intestine showed a high sensitivity to non-specific esterase activity. However, due to a lack of literature, it was difficult to establish compare. The presence of B-lymphocytes and T-lymphocytes was higher in lymphoid follicles of the gut in both reactions.

Conclusion

The presences of B-lymphocytes were more in lymphatic nodule of lymph node compared to the interfollicular area and capsule. The reaction showed that the presences of B-lymphocytes were more in cortex compared to the medulla and absence of T-lymphocytes in bursa of Fabricius. The lymphoid follicles of both Peyer’s patches and solitary lymphoid nodules were showed strong activity for adenosine tri-phosphatase activity in adult Pati duck of Assam. The lymphoid nodules of intestine showed strong reaction for non-specific esterase activity in Pati duck. Both the reaction revealed that the presence of B-lymphocytes and T-lymphocytes were more in lymphoid follicle of intestine. These would help physiologist, pathologist and poultry scientists for effective disease control regime.

Acknowledgement

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