

## Histopathological and Molecular Detection of Turkey Pox in Chittagong, Bangladesh

Rana EA<sup>1</sup>, Rahman M<sup>2</sup>, Das T<sup>3</sup>, Haque MM<sup>4</sup>, Ahmad M<sup>5</sup> and Yadav SK<sup>6\*</sup>

<sup>1</sup>Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Bangladesh

<sup>2</sup>Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University, Bangladesh

<sup>3</sup>Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Bangladesh

<sup>4</sup>Department of Applied Food science and Nutrition, Chittagong Veterinary and Animal Sciences University, Bangladesh

<sup>5</sup>Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University, Bangladesh

<sup>6</sup>Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University, Bangladesh

### Abstract

**Aim:** The objective of the present study was molecular revealing of turkey pox virus as well as identification of associated histopathological changes that was vindicating in skin lesions of infected Turkey.

**Methods:** A presumptive diagnosis of turkey pox was done constructed on clinical signs and symptoms. A total of 12 cutaneous clinical specimens for histopathology and 40 nodular, as well as scab lesions, were collected from clinically suspected field cases for molecular detection of turkey pox. Histopathological examination and Polymerase chain reaction (PCR) was performed as described by Bancroft and Gamble and, Lee and Lee respectively.

**Results:** External gross lesions of infected turkey included nodular, papular, pustular, erosion and at recovery stages crust on around the eye, beak, head, snood, wattle, caruncles and neck. All histopathological observation includes marked thickening of epidermis layer and hemorrhage, necrosis as well as infiltration of inflammatory cells. The most pathognomonic histopathological lesions intracytoplasmic inclusions bodies (Bollinger bodies) were found in 75% samples. Out of 40 fields, clinical specimens only 32 (80%) samples gave positive PCR band during molecular detection of turkey pox virus.

**Conclusion:** On the basis of clinical signs and symptoms and histopathological findings like cutaneous lesions, intracytoplasmic inclusion (Bollinger) bodies and molecular findings of the present study confirmed that the disease which was occurred in the flock was turkey pox.

**Keywords:** Bangladesh; Bollinger bodies; Histopathology; Pox; Turkey

### Introduction

In a precise modern year, turkey farming in Bangladesh is being measured as an imperative income creating sources for middle-class poultry farmer entrepreneurship due to its faster growth rate as well as a prospective source of good quality lean meat [1]. But this advanced turkey farming is hindered by the concurrent infection of several viral diseases that causes high morbidity and mortality. Turkey pox is one of the highly contagious diseases that infects all age, sex and breed which is caused by double-stranded DNA virus under the family Pox viridae and sub-family Cordopoxvirinae within the genus Avipoxvirus having a 188.53 kb size genome [2-4]. Like another avian pox, turkey is more susceptible to turkey poxvirus. This disease is clinically characterized by the progressive development of visible papules, vesicles, pustules and finally crust formation. The most predominant necrotic nodular lesions are observed in head, neck, around the beak, eyelids, wattle, snood, wings, vent and sometimes in legs of the turkey [5]. Occasionally, diphtheritic form with fibro-necrotic lesions may develop in the mucous membrane of mouth, pharynx, and esophagus which is visible after postmortem of dead birds [6]. Usually, low mortality was recorded in cutaneous form, but high mortality was observed concurrent infection of diphtheritic or combined form as well as with secondary bacterial infections [7]. Although turkey pox is a contagious disease and the virus is slow spreading but sometimes mechanical vectors such as insects may increase the infection rate of disease [8]. However, it causes considerable economics loses due to retarded growth rates of young birds, loss of egg production, treatment cost as well as mortality of birds [9]. Turkey pox is an emerging disease in Bangladesh, but appropriate treatment protocol is not available in our country. Moreover, Confirmatory diagnosis is the first prerequisite for

the control and prevention of turkey pox. So, the present experiment was conducted to find out the histopathological changes and molecular detection of turkey pox using clinical specimen and PCR with specific primer.

### Materials and Methods

The present study was conducted in the Department of Microbiology and Veterinary Public Health and Department of Anatomy and Histology at Chittagong Veterinary and Animal Sciences University (CVASU). The necessary PCR was performed at the Poultry Research and Training Center (PRTC).

### Sample collection

A total of 40 turkey pox suspected clinical samples (nodular lesion) were collected from two different turkey farms in Chittagong district in the duration of December 2017 to January 2018. In this study, all large and small types of fresh and young nodular and crust type samples

**\*Corresponding author:** Dr. Saroj Kumar Yadav, MS fellow, Department of Medicine and Surgery, Chittagong Veterinary and Animal Sciences University, Bangladesh, Tel: 008801770307209; E-mail: [shirfraz@gmail.com](mailto:shirfraz@gmail.com)

**Received** August 13, 2018; **Accepted** August 28, 2018; **Published** August 31, 2018

**Citation:** Rana EA, Rahman M, Das T, Haque MM, Ahmad M, et al. (2018) Histopathological and Molecular Detection of Turkey Pox in Chittagong, Bangladesh. J Mol Genet Med 12: 360 doi:10.4172/1747-0862.1000360

**Copyright:** ©2018 Rana EA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

were collected from clinically suspected live and sick turkey. Collected clinical specimens were kept in  $-80^{\circ}\text{C}$  deep freezer until DNA extraction and molecular detection of the virus. And total 12 samples, 5 from one farm and remaining 7 samples from another farm (skin with nodular lesions) were collected from dead turkey and immediately preserved in 10% formalin for histopathological examination.

### Histopathological examination

After proper fixation of clinical specimens, 5 microns thick histopathological slides were prepared and stained with hematoxylin and eosin stain for microscopic detection of histological changes and intra-cytoplasmic inclusion bodies (Bollinger bodies) as described by Bancroft and Gamble [10]. Histopathological study was done in the Department of Anatomy and Histology of the Chittagong Veterinary and Animal Sciences University (CVASU).

### DNA extraction

DNA was directly extracted from the collected clinical specimens (Nodular and scraping) lesions by using a QIAamp DNA extraction kit (QIAGEN). Tissue materials were thoroughly ground, and liquid

nitrogen was added. Tissue powder (15 mg) was placed in a 2 ml microfuge tube, lysis buffer and proteinase K were also added in ground tissue sample. Finally, total cell lysate was incubated at  $56^{\circ}\text{C}$  in a hot water bath until complete lysis of the clinical specimens. Then, DNA was extracted according to the manufacturer's procedures, eluted with 100  $\mu\text{l}$  elution buffer and stored at  $-20^{\circ}\text{C}$  for molecular detection of turkey pox.

### Molecular detection

For molecular detection of pox virus, Polymerase chain reaction (PCR) was performed with selective primers that were previously described by Lee and Lee [11]. Avian pox virus *P4b* gene was amplified by using forward 5'-CAGCAGGTGCTAAACAACAA-3' (F) and reverse 5' CGGTAGCTTAACGCCGAATA-3' (R) primers for 578 bp amplicons of poxviruses. To performed PCR a total of 25-ml reaction mixture was prepared which was consisting of 1x PCR buffer (Invitrogen Vienna Austria) supplemented by 1.5 mM  $\text{MgCl}_2$  (Invitrogen), 0.2 mM dNTP mix (QIAGEN), 1.25 ml of each primer (primers were used in 10 pmol/ml concentration), 1.5 U of Taq DNA polymerase (Invitrogen) and 1.5 ml of prepared DNA as template.

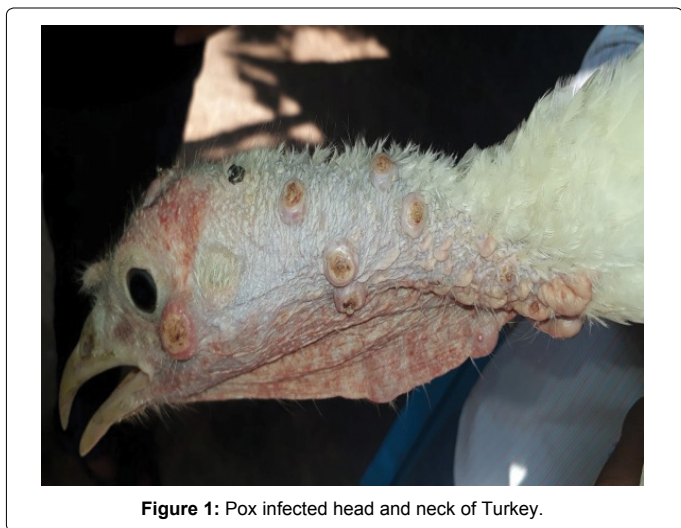


Figure 1: Pox infected head and neck of Turkey.



Figure 2: Nodular lesions in beak and eyelid of Turkey chick.

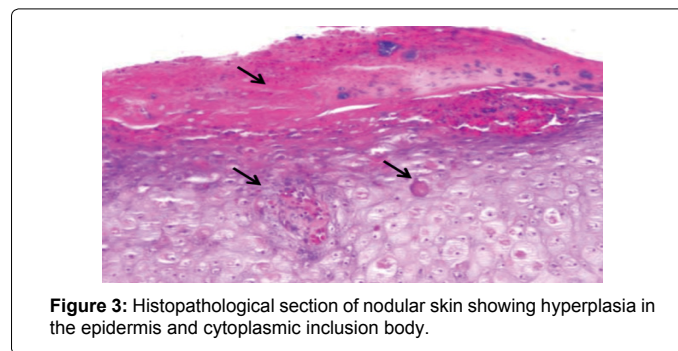


Figure 3: Histopathological section of nodular skin showing hyperplasia in the epidermis and cytoplasmic inclusion body.

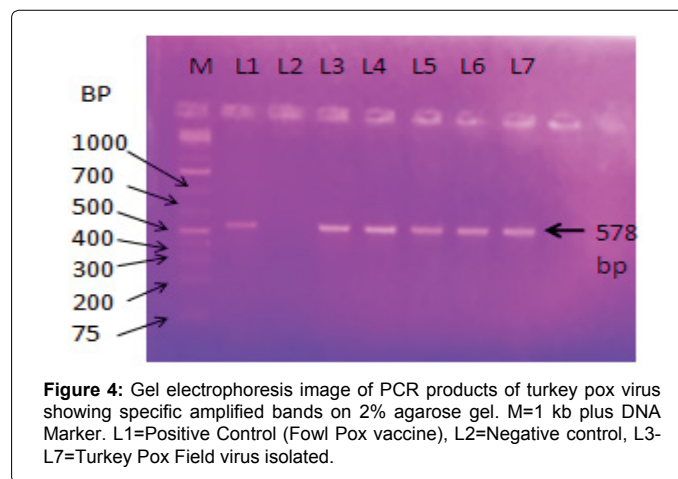


Figure 4: Gel electrophoresis image of PCR products of turkey pox virus showing specific amplified bands on 2% agarose gel. M=1 kb plus DNA Marker. L1=Positive Control (Fowl Pox vaccine), L2=Negative control, L3-L7=Turkey Pox Field virus isolated.

Farm no	For Molecular Detection		For Histopathology	
	Sample	PCR Positive	Sample	Bollinger Bodies Positive
1	20	17 (85%)	5	4 (80%)
2	20	15 (75%)	7	5 (71.42%)
Total	40	32 (80%)	12	9 (75%)

Table 1: Summary of molecular detection (PCR) and histopathology of turkey-pox viruses.

For amplification the initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycles of denaturation continued at 95°C for 45 seconds (s), annealing at 55°C for 30 s and elongation at 72°C for 30 s and final extension at 72°C for 5 minutes in a thermocycler (Applied Biosystem, 2720 thermal cycler, Singapore). Then PCR products were electrophoresed at 2% NA Agarose gel for 1 h at 100 V. For negative control, we used Nuclease-free water and a commercial fowlpox virus as used as positive controls in PCR. Finally, DNA was visualized under a U.V. transilluminator.

## Results and Discussion

All the turkey birds during diseased condition showed cutaneous lesions mainly nodular, papular, pustular, erosion and at recovery stages crust lesions mainly around the eye, beak, head, snood, wattle, caruncles and neck (Figures 1 and 2). The nodular lesion varies from birds to birds which were recorded up to 1 to 3mm in diameter mainly in head regions. This cutaneous form of turkey pox is closely similar with previous several works [12-14]. In histopathological examination, there was found marked thickening (hyperplasia) of the epidermis layer and epithelial cells become more eosinophilic. This observation and histo-morphological features agreed with the work of Jorge [13,15-17]. In the case of pustular nodular lesions, there was found of degenerated epithelial cells with the superficial crust. Hemorrhage, necrotic and inflammatory cells were found under the crust lesions. It was noted that marked thickening of the epidermis of skin due to proliferated fibrous tissue and infiltration of lymphocytes, histiocytes and other materials. This histopathological lesion closely similar to the previous study carried out by Yoshikawa and Alam [13]. It mainly found in the chronic case as well as recovery stages of turkey pox. The most pathognomic histopathological lesions intracytoplasmic inclusions bodies (Bollinger bodies) with different size were noted in infected skin lesions (Figure 3). Out of 12 samples from farm-1 and farm-2, it was revealed that about 80% and 71.42% samples have intra-cytoplasmic inclusions bodies (Table 1). These similar results were also reported by several studies [13,14,16-20].

As PCR is a highly sensitive molecular technique for detection of particular organism-specific gene, here all the clinical specimens were subjected to PCR for molecular detection and confirmation of turkey pox. The PCR was done based on *P4b* gene-specific products and amplification of 578 bp pox virus using a specific primer. This avipox virus specific primers have previously been used successfully in various studies for the detection of avipoxvirus gene by others [5,11]. Turkey pox was showed specific bands on 578 bp on 2% NA Agarose gel electrophoresis (Figure 4).

Out of 40 fields clinical specimens from Farm-1 and Farm-2 it was noted that both farms have 85% and 75% positive PCR result (Table 1). Average PCR positive result was 80% but the remaining 20% samples did not show any band during PCR. This molecular detection rate of turkey pox is strongly supported by Kabir et al. [5]. He described 80% samples of fowlpox were positive during molecular detection in his study. In the present study turkey pox virus, genome detection rate was higher than that of the previous study done by who reported that molecular detection of pigeon pox was only 62.5% by PCR. But, the present results slightly differ from the previous study which was done by Fahmy et al. and Roy et al. [21,22]. He defined that molecular detection of fowlpox virus was almost 100% by PCR. In our study, 20% PCR negative result may be a due collection of a sample from very early or very late stages of the disease and there was no culture of virus in embryonated hen eggs or tissue culture. Direct extraction of virus

nuclear materials from clinical specimens may be responsible for very minimal concentration viral DNA and which fail to amplify during PCR. Other factors such as samples size, types of sample, processing of samples transportation and storage of samples during the period of the collection which was considered as striking issues for negative PCR result in previous several studies.

## Conclusion

Fowl Pox is an endemic disease in Bangladesh and causes great economic losses for farmers. The gross and histopathological changes in pox affected turkey are revealed in this study. Among the histological findings, intracytoplasmic inclusions bodies (Bollinger bodies) were found more predominantly which was most pathognomic for pox virus. Although, molecular detection of pox virus was performed without gene sequencing and phylogenetic analysis, we are not sure about the origin and clades of circulating turkey-pox viruses in Bangladesh. However, it is timely demand for all the farmers as well as governments to take necessary steps to achieve the goal of pox-free Bangladesh.

## References

1. Samad MA (2013) Handbook of avian medicine. (2nd edn), LEP Publication, Dhaka, Bangladesh.
2. Weli SC, Tryland M (2011) Avipoxviruses: Infection biology and their use as vaccine vectors. *Virol J* 1: 49.
3. Tripathy DN, Reed WM (2008) Pox, In: A laboratory manual for the isolation, identification and characterization of avian pathogens. (5th edn), Am Assoc Avian pathol Ch 5: 116-119.
4. Haydar MR, Ara MS, Soma SS, Rahman MM, Rahman MB, et al. (2017) Isolation and molecular detection of turkeypox virus from turkey for the first time in Bangladesh. *Ban J Vet Med* 15:87-90.
5. Kabir ML, Haque ME, Borty SC, Mustafa K, Kamal MM, et al. (2015) Isolation and molecular detection of fowl pox and pigeon pox viruses from recent outbreak in Bangladesh. *Ind J Life Sci* 5: 1.
6. Joshi RK, Joshi N (2011) Growth and cytopathogenicity of a field isolate of fowl pox virus in BGM-70 cell line. *Ind J Com Microb Immunol Inf Dis* 32: 19-21.
7. Shukla SK, Rajesh C, Agrawal DK (2000) Fowlpox in the trachea of laying hens. *Ind J Ani Sci* 70: 1129-1130.
8. Tripathy DN (1991) Pox. In: Textbook of Diseases of Poultry, B. W. Calnek (Ed) Iowa State University Press, Ames, Iowa, USA.
9. El-Mahdy SS, Awaad MH, Soliman YA (2014) Molecular identification of local field isolated fowlpox virus strain from Giza governorate of Egypt. *Vet World* 7: 66.
10. Bancroft J, Marilyn-Gamble D (2002) Theory and practice of histological techniques. (5th edn), Churchill Livingstone, London, UK.
11. Lee LH, Lee KH (1997) Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. *J Virol Method* 63: 113-119.
12. Ideris A, Ibrahim AL (1986) Poxvirus infection in turkeys. *Kaj Vet* 18: 85-87.
13. Yoshikawa MG, Alam J (2002) Histopathological studies of fowl pox in Bantams. *Int J Poult Sci* 1: 197-199.
14. Prukner-Radovcic E, Lu'schow D, Grozdanic IC, Tisljar M, Mazija H, et al. (2006) Isolation and molecular biological investigation of avian poxvirus from chickens, a turkey, and a pigeon in Croatia. *Avi Dis* 50: 440-444.
15. Orós J, Rodríguez F, Rodríguez JL, Bravo C, Fernández A (1997) Debilitating cutaneous poxvirus infection in a Hodgson's grandala (*Grandala coelicolor*). *Avi Dis* 1: 481-483.
16. Haligür M, Özmen Ö, Vural SA, Berkin Ş (2009) Pathological, immunohistochemical and electron microscopical examinations on chorioallantoic membrane lesions in experimental fowl poxvirus infection. *Kafkas Univ Vet Fak Derg* 15: 345-350.
17. Hess C, Maegdefrau-Pollan B, Bilic I, Liebhart D, Richter S, et al. (2011)

- 
- Outbreak of cutaneous form of poxvirus on a commercial turkey farm caused by the species fowlpox. *Avi Dis* 55: 714-718.
18. Tripathy DN, Schnitzlein WM, Morris PJ, Janssen DL, Zuba JK, et al. (2000) Characterization of poxviruses from forest birds in Hawaii. *J Wild Dis* 36: 225-230.
19. Singh P, Kim TJ, Tripathy DN (2000) Re-emerging fowlpox: Evaluation of isolates from vaccinated flocks. *Avi Pathol* 29: 449-455.
20. Kikuyasu KN, Kazuo KW, Yamamoto YY, Yamada MM, Nakazawa MM, et al. (2006) Pathology of cutaneous fowl pox with amyloidosis in layer hens inoculated with fowl pox vaccine. *Avi Dis* 50: 152-156.
21. Fahmy HA, Arafa A, Kanawaty ZR, Mahmoud AH (2009) Molecular detection of pox virus in pigeon. *Vet Med J Giza* 57: 253-262.
22. Roy B, Joardar SN, Samanta I, Das PK, Halder A, et al. (2013) Molecular characterization of fowl pox virus isolates from backyard poultry. *Adv Anim Vet Sci* 1: 54-58.