

**Short Communication**

**ISOLATION AND MOLECULAR DETECTION OF TURKEYPOX VIRUS FROM TURKEY FOR THE FIRST TIME IN BANGLADESH**

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**ABSTRACT**

Turkey rearing is getting popularity day by day in Bangladesh. Turkeypox is an important viral disease of turkey with great economic importance. In this study, turkeypox virus was isolated from a clinical case in Mymensingh, Bangladesh. Virus isolation was carried out through serial passage of viral inoculum into developing chicken embryo through chorioallantoic membrane (CAM) route. The virus isolate was confirmed by PCR targeting pox virus *P4b* gene. This is the first report of the isolation and PCR based molecular detection of turkeypox virus from a clinical case in Bangladesh.

**Keywords:** Turkeypox virus, turkey (*Meleagris gallopavo*), isolation, molecular detection, PCR.

**INTRODUCTION**

Turkey farming has gained attention of poultry farmer particularly the entrepreneurs of Bangladesh because they grow faster than chicken (Samad, 2013). It has a great potential in income generation and poverty alleviation. Turkey meat is a good quality lean meat having 6.6% fat in 100gm meat (Samad, 2013).

Avian pox is a common viral disease of chickens, pigeons, ostriches, quails, pheasants, and canaries as well as other pet and wild birds (Tripathy and Reed, 2003). Approximately 9000 bird's species, about 232 in 23 orders have been reported to have acquired a natural poxvirus infection (Bolte *et al.*, 1999). Although contagious, it is a slow spreading viral infection. Like many other birds turkey is susceptible to turkey poxvirus. It is one of such important viral diseases affect in gall ages of turkeys except recently hatched ones; however, the turkey aging more than 4 weeks of age are mostly affected. The disease is caused by turkeypox virus of the Genus Avipox virus, under *Poxviridae* family having a genome of 188.53 kb size compared to 288.54 kb genome of fowlpox virus contain. Pox virus infection in turkeys tends to be more chronic in nature with longer duration than fowl pox virus (Wakenell, 2001).

The disease in turkeys has drawn attention across the globe. The very first time turkeypox virus was reported by Brunett (1934) in a turkey flock in New York, Veterinary College. The disease has been reported in India as well as in other countries, resulted into severe economic losses in spite of proper management and health care (Singh *et al.*, 2003; Metz *et al.*, 1985). Mortality in the cutaneous form is usually low, contrary to the diphtheritic or combined form; but is under the influence of virulence of the agent, host susceptibility and environment factors. In Bangladesh, most of the turkey farmers are entrepreneur. However, no work has yet been carried out in Bangladesh on turkey pox. In the present study we isolated and detected turkeypox virus from field case samples.

**MATERIALS AND METHODS**

**Sample collection and inoculum preparation**

A suspected field sample of turkeypox (nodular tissue) was collected from a turkey arrived at the Veterinary Teaching Hospital at Bangladesh Agricultural University, Mymensingh-2202, in October, 2016. Collected tissue materials were grinded immediately and a 20% suspension of inoculum was prepared as described by Kabir *et al.* (2015).

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### **Virus isolation**

Turkeypox virus was isolated using 10-day-old embryonated hen eggs through chorio-allantoic membrane (CAM) route of inoculation. Initially eggs having live embryos were marked with a pencil at the centre of the air cell followed by creation of artificial air cell over the CAM by applying suction with the help of small rubber bulb. 0.2 ml of sterile inoculum was inoculated onto the CAM. Once inoculated, the eggs were sealed with melted wax and incubated at 37°C for five to six days. Embryos died within 24 hours of inoculation were discarded. After 5 to 6 days of inoculation, the embryos which either died or remained alive were chilled in refrigerator at 4°C to 8°C for 1-2 hour. The CAM that was found thickened was harvested for inoculum preparation. Using the same procedure five serial passages were done to increase the concentration of virus for DNA extraction and PCR.

### **Molecular detection**

DNA was extracted from CAM materials using Wizard® Genomic DNA Purification Kit (Promega Corporation) as described by manufacturers. PCR based amplification of avian pox virus *P4b* gene was carried out using forward 5'-CAGCAGGTGCTAAACAACAA-3' (F) and reverse 5'-CGGTAGCTTAACGCCGAATA-3' (R) primers developed by Lee and Lee (1997) to amplify 578 bp amplicons. The thermal profile used to amplify pox virus gene was as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing 48°C 1.5 minutes, elongation at 60°C for 2 minutes, and a final extension at 60°C for 10 minutes. At the end of the PCR, amplified PCR products were separated in 2.0% agarose and DNA was visualized in UVsolo TS Imaging System (Biometra, Germany).

## **RESULTS AND DISCUSSION**

The turkey is a large bird in the genus *Meleagris*. These are the native birds of Americas. Domestic turkey or wild turkey is scientifically known as *Meleagris gallopavo*. Turkey is excellent source of high quality meat and egg. Although not common, turkey farming is getting popular among the people involved in poultry production in Bangladesh now these days.

Despite management and nutritional improvement, infectious disease are a major constrain in the development of poultry industries. Avian pox (both fowl pox and pigeon pox) is enzootic in Bangladesh affecting any age of birds (Siddique *et al.*, 1997). Avian pox is economically important in commercial poultry farming (chicken and pigeons) since it may cause decline in egg production, mortality and lower growth rate (Isa *et al.*, 2002; Arivshi *et al.*, 2003). Turkey pox is an important and common viral disease of turkey (Wobeser, 1997). The virus is transmitted either through skin contact or by arthropods (typically mosquitos) acting as mechanical vectors. In Bangladesh turkey farmer are incapable to maintain good bio-security. So it is easy to affect other farm that is near to the infected one. The disease also causes severe economic loss in turkey farming industry.

Several studies have been carried out in Bangladesh where fowl pox and pigeon pox viruses have been successfully isolated from clinical cases and confirmed by PCR (Siddique, 1998; Kabir *et al.*, 2015). No work has so far been reported on the isolation and detection of pox virus from turkey in Bangladesh. In this study, we isolated turkey pox virus from a suspected clinical case of turkey pox. Pox virus is able to produce both nodular and diphtheric lesion on the skin. The affected turkey examined in this study had nodular pox lesions at the neck region (Figure 1). Developing chicken embryos were successfully used for the isolation of the virus. CAM materials of the inoculated embryos were found thickened and oedematous as compared to control suggesting growth of virus (Figure 2). Infected embryos were found to have retarded growth as compared to control (Figure 3).

PCR is a highly specific molecular technique for specific gene detection. The presence of turkey pox virus in the CAM materials was confirmed by PCR with the amplification of 578 bp pox virus *P4b* gene specific products (Figure 4). These avian pox virus specific primers have earlier been used successfully in various studies for the detection of avian pox virus by others (Lee and Lee, 1997; Kabir *et al.*, 2015). No literature is available on the detection of turkey pox virus in Bangladesh. This is the first report on the isolation and PCR based molecular detection of turkey pox virus from Bangladesh. The isolated turkey pox virus provide candidate virus for further molecular and biological characterization to identify their origin and closeness to other avian pox virus circulating in Bangladesh.



Figure 1. Suspected clinical cases of turkeypox (nodular pox lesions in the neck)



Figure 2. Thickening of the CAM and oedema following inoculation of chicken embryos with turkeypox virus



Figure 3. Retarded growth of chicken embryos following turkeypox virus inoculation

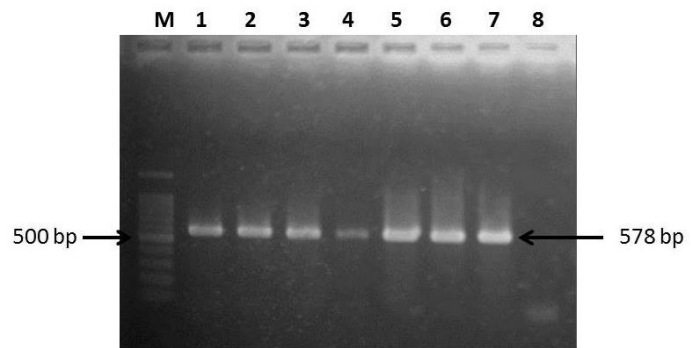


Figure 4. Electrophoresis image of PCR products of turkeypox virus isolates showing specific amplified bands on 2% agarose gel. M = 100 bp DNA Marker. L1-L2 = 1<sup>st</sup> passage (CAM), L3-L4 = 3<sup>rd</sup> passage (CAM), L5-L6 = 5<sup>th</sup> passaged CAM, 7 Positive control (Fowl Pox), L8 = Negative control

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