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Epidemiological investigation and phylogenetic analyses of contagious ecthyma virus from goat in Bangladesh

Md. Thoufic Anam Azad^{1,2}, Sukumar Saha³, Md. Shahin Alam¹, Papia Monoura¹, Md. Giasuddin¹, Shake Mohammed Shariful Islam⁴, Mahbub Jang Fatey Ali Taimur¹ and Jahangir Alam^{1,5,*}

¹Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh

²Department of Animal Husbandry and Veterinary Science, Rajshahi University, Rajshahi, Bangladesh

³Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh

⁴Upazila Livestock Officer, Meherpur Sadar, Meherpur, Bangladesh

⁵National Institute of Biotechnology, Ganakbari, Savar, Dhaka-1349, Bangladesh

*Corresponding author: Dr. Jahangir Alam, Chief Scientific Officer, Animal Biotechnology Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349, Bangladesh. E-mail: alamjahan2003@yahoo.com

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Abstract: The aim of this study was to know the epidemiologic characteristics of contagious ecthyma and molecular characterization of the contagious ecthyma virus or ORF virus (ORFV). Sixty four households having 293 goats in Chuadanga district of Bangladesh were surveyed and 30 scab materials were collected from ORF affected goat. Virus detection by PCR was attempted. Sequencing was done for characterization. Goats (n=70) in 15 out of 64 (23.44%) households were found to be infected with ORF. The overall morbidity, mortality and case fatality was 23.89 (70/293), 1.02 (3/293) and 4.29% (3/70), respectively. A proliferative lesion in mouth, lips and gum was found in 100.00% affected goats. Besides, proliferative lesions were also found in legs (19.00%), body (13.00%), and ears (10.00%) of goats. Highest morbidity (29.00%) was found in goats of 7 months-1years aged group and lowest (2.05%) in goats of 2-3 years or more. About 79.00% goats were affected during January-March, 2011 and 21.00% during October-December, 2010. About 93.33% (28/30) scab samples were found positive by PCR. For molecular characterization PCR amplified viral interleukin-10 (vIL10) gene of 5 different ORFV were sequenced. One virus obtained from sheep and four from goat. Goat isolates were found very closely related (97.20-98.60%) to each other while sheep virus has 95.20-97.20% homology with goat viruses and clustered separately. Our isolates were closely related to Norwegian sheep isolates. It is concluded that ORFV is circulating in Bangladesh with high morbidity but low mortality and all age group of goats are susceptible to this disease. Circulating viruses are closely related to each other.

Keywords: contagious ecthyma; epidemiologic characteristics; molecular characterization and phylogenetic analysis

1. Introduction

Contagious ecthyma, also known as ORF is perhaps the most commonly encountered skin disease in domestic goats and sheep. ORF is one of the most widespread viral diseases worldwide, affecting mostly small ruminants and, sometimes, other species, including wild animals (Damon, 2007; Fairley *et al.*, 2008). Of late, there have been an increasing number of reports of new species being affected by the disease, implying a dynamic host-pathogen interaction (Fairley *et al.*, 2008). The causative agent, contagious ecthyma virus or ORF virus (ORFV), has been extensively investigated over recent years, owing to its zoonotic importance (Billinis *et al.*, 2012, Ozturk *et al.*, 2012, Nougairède *et al.*, 2013) and ability to cross-infect other species sporadically. Exposure of animals to stress or immunosuppression as a result of therapy or primary viral infection can

accentuate the severity of disease (Iketani *et al.*, 2002). Some infections may be confounded by similar clinical manifestations caused by other infections (Kottaridi *et al.*, 2006). In developed world, in addition to its economic impact, it is regarded as one of the most important disease factors affecting the welfare of farmed animals (Haig and McInes, 2002). Clinically, ORFV infection is characterized by proliferative and self-limiting lesions that are usually localized around the mouth, nostrils and lips. It also affects the gums and tongue, especially in young lambs. Occasionally, feet, teats and eyelids can be affected (Huixia *et al.*, 2013). The lesions may extend into the esophagus, stomach and intestines, and rarely, the respiratory tract (Zhao *et al.*, 2010).

Although morbidity in the affected flock is high, low-to-moderate mortality and fatality rates are reported. Secondary bacterial and fungal infections and maggot infestations are common sequelae to primary ORFV infection, often contributing to mortality (Musser *et al.*, 2008). Losses also occur owing to reduced growth and slaughter of the affected animals when the lesions become extensive and severe (Vikoren *et al.*, 2008). Disease thus impacts on the economic well being of farmers. In addition to its impact on young lambs and kids, the zoonotic nature of the infection and ability of the virus to crossinfect other species of animals besides sheep and goats has become increasingly evident (Hossamani *et al.*, 2009). Infection of camels (Housawi *et al.*, 2004), Japanese serows (Inoshima *et al.*, 2002), musk ox, reindeer (Kummeneje and Krogsrud, 1979) are some of the common examples.

The disease is prevalent in Bangladesh. Ershaduzzaman *et al.* (2010) reported the disease based on clinical signs and symptoms while Jahangir *et al.* (2011) performed PCR and gene sequencing in limited scale to confirm the existence of virus in the country. However, epidemiology of the disease remains unknown.

2. Materials and Methods

2.1. Survey, sample collection and inoculum preparation

Survey was conducted at a village in Chuadanga district of Bangladesh (23°38'N-88°41'E). A total of 64 households were surveyed by direct interviewing method. During survey 30 scab materials (samples) were collected from clinically infected and/or suspected goats with the history of papular lesions in different parts of the body. The samples were then transferred to the Virology laboratory of Bangladesh Livestock Research Institute (BLRI) and all the analysis are conducted there. About 20.00% suspension of sample was made with PBS containing 10X antibiotic and antimycotic solution and stored at -80°C until use.

2.2. DNA extraction, polymerase chain reaction and electrophoresis

DNA was extracted from inoculum using Genomic DNA Purification Kit-A1125 (Promega, USA) according to manufacturer's instruction. The PCR was carried out in a 25µl reaction volume using Qiagen kit (Qiagen, Germany). The reaction mixture consisted of 12.75µl of nuclease free water, 5µl of 5X/PCR buffer, 0.25µl of 25mM MgCl₂, 1µl of dNTP mix, 1µl of AmpliTaq DNA polymerase, 0.5µl of forward, 0.5µl of reverse primer (each 100 pmole) and 4µl of template DNA. Thermal cycling was performed as initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30s and extension at 72°C for 30s. Final extension was performed at 72°C for 10 min. Electrophoresis was done in 1.00% agarose gel containing ethidium bromide and visualized in UV transilluminator. A total of four sets of primers targeting four different genes of ORFV were used (Table 1).

2.3. Sequencing and phylogenetic analysis

PCR product was purified using Qiaquick gel extraction kit (Qiagen, Germany) according to manufacturer's instruction. For sequencing Dideoxy chain termination method (Sanger *et al.*, 1977) using Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used. Primer used for PCR was used from both forward and reverse direction. The product was purified by BigDye® XTerminator purification kit (Applied Biosystems, USA). Nucleotide sequences were determined using an automated DNA sequencer (ABI 3130 DNA Sequencer, Applied Biosystems, USA), edited and assembled with GENETYX (version 10.0; Software Development Corp., Tokyo, Japan). Multiple sequence alignment was performed with the Molecular Evolutionary Genetics Analysis (MEGA) version 4.1.0 software (Tamura *et al.*, 2007) with an engine based on the ClustalW algorithm. Blast searches were used to retrieve the sequences from the Gene Bank. Phylogenetic analysis was performed using neighbor joining tree inference analysis with the 1000 bootstrap replications. Nucleotide sequences of viral interleukin-10 (vIL10) gene were used in the phylogenetic analysis.

3. Results and Discussion

3.1. Overall survey

A total of 64 households were surveyed and total goat population in these households was 293 (male 122 and female 171) with an average 4.60 goat per household. All farmers rear goats in traditional management system without any vaccination against ORF. Clinical cases of ORF were found in about 23.44% (15) households. Overall morbidity, mortality and case fatality was 23.89 (70/293), 1.02 (3/293) and 4.29% (3/70), respectively (Table 2). However, the mortality in goats of up to 2 months of age was 4.76% (3/63) while the case fatality was 4.29%. The morbidity from ecthyma is generally high while the mortality can range from 0–93.00% depending on the age and physiological status of animals, generally being higher in young lambs or kids (Hosamani *et al.*, 2009; Mazur and Machado, 1989). Gumbrell and McGregor (1997) reported that in an outbreak the mortality in lamb may reach 10.00%. Our findings comply with the findings reported by Mazur and Machado (1989), Gumbrell and McGregor (1997), Vikoren *et al.* (2008), Billinis *et al.* (2012). High mortality in young animals is ascribed to the inability of the young animal to feed due to oral lesions and associated secondary infections leading to anorexia (Musser *et al.*, 2008). The route of transmission is not well understood but it may be due to use of common grazing land during winter season and gathering of the goat at same places.

3.2. Frequency of the disease occurrence according to age and sex

Out of 293 goats 70 were found clinically affected. Distribution of clinically affected animals is presented in Table 3. The morbidity in different age group of animals was found to range from 8.57 to 41.43%. Highest morbidity 41.43% (29/70) was found in goats of 7-12 months age. Young animals of <1 year old were found more susceptible to disease than animals of 2-3 years or more. Similar results, high frequency of infection in young animals were reported by other authors (Vikoren *et al.*, 2008; Billinis *et al.*, 2012). It was found that young animals are much more prone to infection and severity this might be due to their tender skin that can break its integrity easily. Higher infection rate 64.29% (45/70) was found in female than in male 35.71% (25/70) animal. In the present study some animals aged between 3-4 years were found to carry lesions in oral commissure for 3-4 months according to farmers' comments. Long time persistent might be due to immunocompromised goats or subsequent reinfection. Friebe *et al.* (2004) stated the evasive mechanisms that the virus has developed to adapt and grow in the presence of an active immune response helps to explain the ability of the virus to repeatedly re-infect the same host. The disease starts to occur from early winter and continued until end of summer. It means that there may be some seasonal and/environmental factors influence the occurrences of disease. The stress conditions from cold, scarcity of food during winter, tough-thorny grasses, low humidity leading to loss of integrity of lip epithelium etc. might contribute in the occurrences of the disease and yet to be enumerated. Loss of epithelial integrity is the important predisposing factor for initiation of infection (Robinson and Balassu, 1981).

3.3. Clinical profile of the affected goat

There were 70 clinically affected and/or suspected cases of CE in goat of different age groups. It was observed that the virus can affect different parts of the body and the degree of lesions differ among different animals. Different degrees of proliferative lesions were found in different parts of the body of affected animals. In 100% (n= 70) goats proliferative lesions were found in mouth, lips and gum (Figure 1). Lesions were also found in legs (19%), body (13%), and ears (10%) of affected goats. However, proliferative lesions were not found in genital organs, udder and anus. The rate of infection was higher in goats of up to 1 year age than those of 2-3 years and >3 years old. Of the affected goats (n=70) about 41.43% (29/70) goats were belong to age group 7-12 months followed by age group of up to 2 months (24.29%), 3-6 months (17.14%), 2-3 years (8.57%) and >3 years (8.57%) (Table 3). The recovery time from the disease reported by the farmers during survey was found to vary from 10-30 days or more. Majority of the animals recovered within 30-35 days. Some animals especially the animals aged > 3 years carried proliferative lesions in oral commissure for 3-4 months. The occurrence of the disease was found to depend on month of the year. In the present study 78.57% cases was found to occur in January-March whereas 21.43% in October-December.

3.4. Identification of ORFV in clinical samples by polymerase chain reaction (PCR)

During survey, 30 samples (scab materials) were collected. The samples were then processed in the laboratory for both virus detection and virus isolation. Out of 30 about 93.33% (28/30) samples were found positive for ORFV by PCR. Figure 2 represents the amplification of target DNA with two different sets of primers from clinical samples. Primer GIF5 and GIF6 targets the granulocyte-macrophage-colony-stimulating factor (GM-CSF) and interleukin-2 inhibition factor (GIF) while primer vIL10-3 and vIL10-4 amplifies viral interleukin-10

gene (Klein and Tryland, 2005). The OVA32LF1 and OVA32LR1 targets A32L which encodes ATPase of CEV (Chan *et al.*, 2009) while the VIR1 and VIR2 primer target the viral interferon resistance gene (Guo *et al.*, 2004). A comparative study of primers in detection of viruses from field samples were carried out using randomly selected 20 positive samples. GIF primer set was found more sensitive than others used. DNA was amplified from 60.00, 30.00 and 10.00% samples by all four sets, three sets and one set of primers, respectively (data not shown).

3.5. Gene sequencing and phylogenetic analysis

The phylogenetic indicates that the ORF virus isolated from sheep and goat are closely related to each other but clustered separately. Sheep virus was found to have 95.20-97.20% homology with goat viruses. On the other hand goat isolates were found very closely related to each other and the identity among these viruses ranges from 97.20-98.60%. Our isolates were closely related to Norwegian sheep isolates (Figure 3).

Table 1. Sequences and source of primers used in the study.

Sl No.	Sequence (5'-3')	Source
1.	GIF5 : GCT CTA GGA AAG ATG GCG TG GIF6: GTA CTC CTG GCT GAA GAG CG	Klein and Tryland, 2005
2.	vIL-10-3: ATG CTA CTC ACA CAG TCG CTC C vIL-10-4: TAT GTC GAA CTC GCT CAT GGC C	
3.	OVA32LF1: GAG GGC GCG AGC ACC ATT TA OVA32LR1: CGG AGC CGG TAA TTT AGT GAC AGT	Chan <i>et al.</i> , 2009
4.	VIR1: TTA GAA GCT GAT GCC GCA G VIR2: ACA ATG GCC TGC GAG TG	Guo <i>et al.</i> , 2004

Table 2. Distribution of goats surveyed in Digri, Chuadanga Sadar Upazila (n= 293).

Age group	Number of goat	No. affected	Morbidity (%)	Dead	Mortality (%)	Case fatality (%)
1- 2 months	63	17	27.00	3	4.76	4.29
3-6 months	79	12	15.00	-	-	-
7-12 months	72	29	40.00	-	-	-
24- 36 months	66	6	9.00	-	-	-
>36 months	13	6	46.00	-	-	-
Total	293	70	23.89	3	1.02	4.29

Table 3. Age and sex wise distribution of clinically affected goats (n= 70).

Age group	No. of male (%)	No. of female (%)	Total (%)
1- 2 months	8 (11.42)	9 (12.85)	17 (24.29)
3-6 months	2 (2.86)	10 (14.28)	12 (17.14)
7-12 months	12 (17.14)	17 (24.29)	29 (41.43)
24- 36 months	1 (1.42)	5 (7.14)	6 (8.57)
>36 months	2 (2.86)	4 (5.71)	6 (8.57)
Total	25 (35.71)	45 (64.29)	70 (100.00)

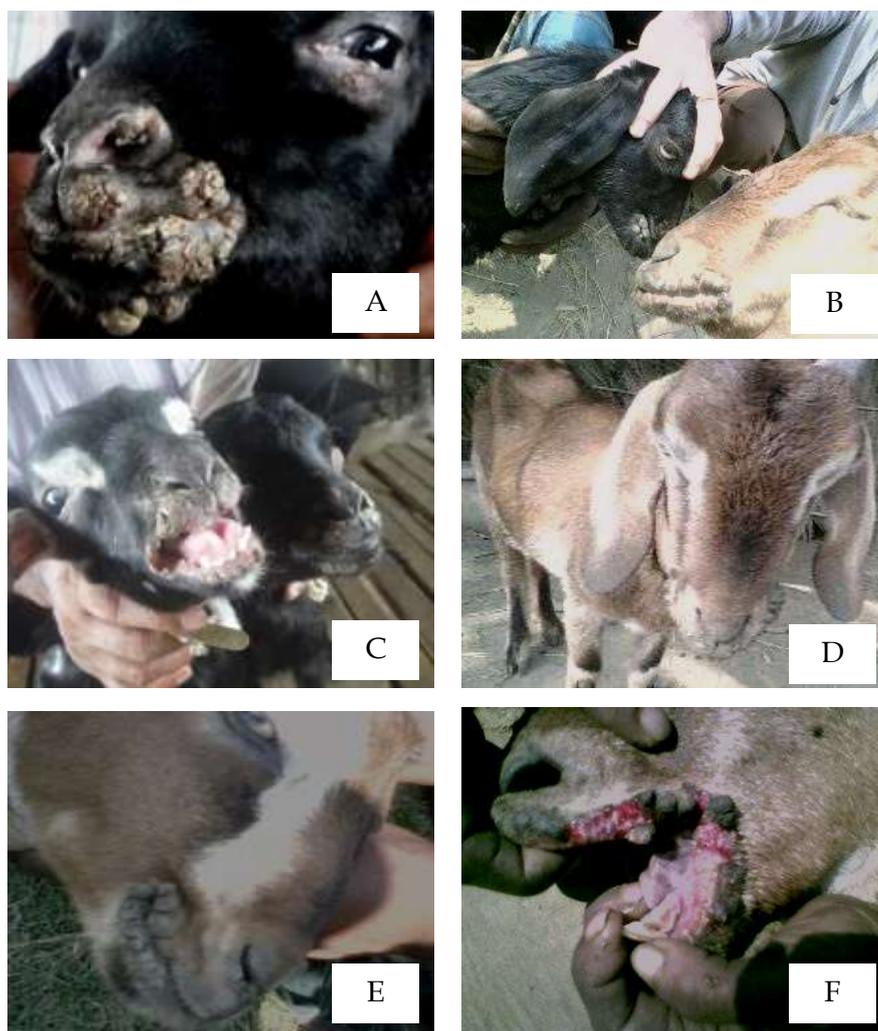


Figure 1. Clinically affected and/or suspected contagious ecthyma in goat. A. Lesions in lips, nostril and eyelid of a kid; B. Infected kid and dam; C. Two kids infected; D. and E. Adult animal infected; F. Lesions after taken sample.

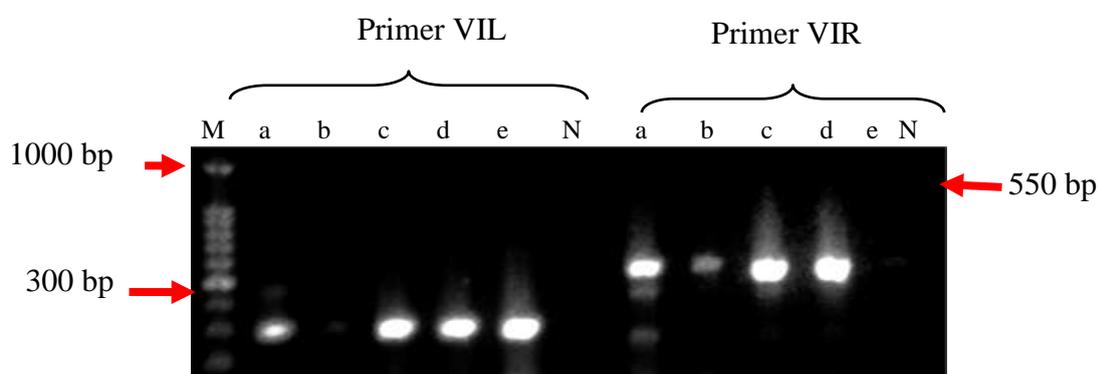


Figure 2. Detection of contagious ecthyma virus from field samples by PCR. Lane M= Marker, 100bp ladder; Lane N= Negative control, other lanes: represents field samples. VIL and VIR primer sets were used to amplify DNA. Positive samples marked by arrow. Primer name mentioned on the top of the panel.

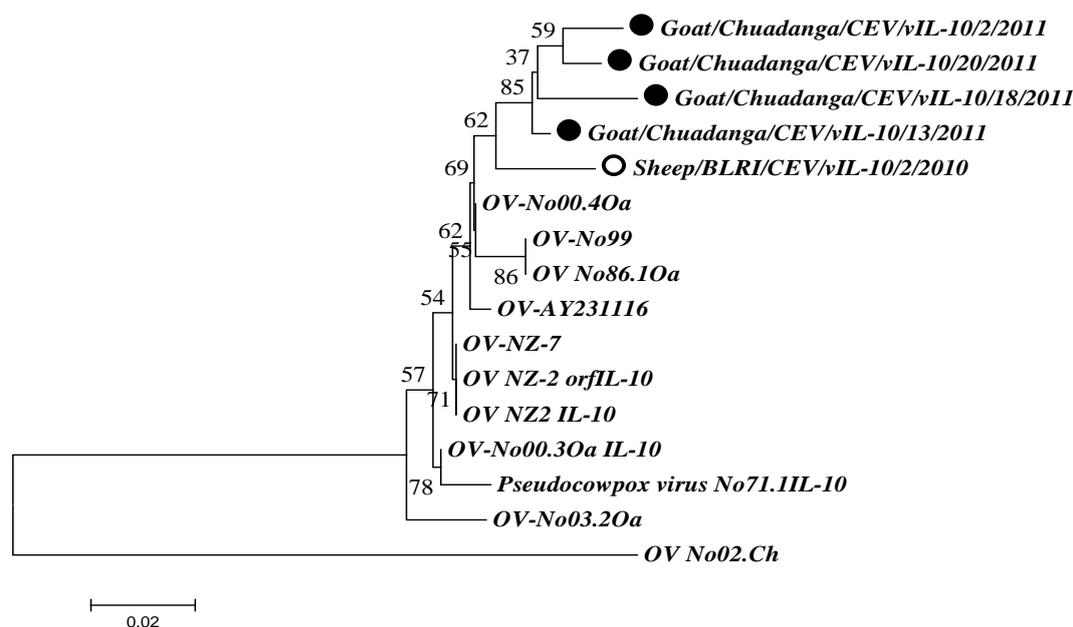


Figure 3. Phylogenetic analysis of ORFV isolated from goat and sheep in Bangladesh. The tree was generated using neighbor-joining method and 1,000 replications of bootstrap re-sampling. 235 bp from viral interleukin-10 (*vIL-10*) gene of ORFV was employed to generate phylogram. Bangladeshi isolates marked by black (Goat) and open (Sheep) circles in the tree.

4. Conclusions

It is observed that almost all age groups of animals are affected in the study area. It indicates that the virus prevalent in the environment and stress condition, repeated exposure to the virus laden areas during winter season due to limited grazing land most probably responsible for the spread of infection. Phylogenetically the viruses were found closely related to each other. The study provides some information about the circulating ORFV in Bangladesh.

Conflict of interest

None to declare.

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