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A Sheeppox Virus Outbreak in Central Turkey in 2003: Isolation and Identification of Capripoxvirus Ovis

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ABSTRACT

Poxvirus epidemics occur almost every year and cause significant economic losses for small-scale animal producers in Turkey. In this study, the causative agent of the most recent epidemic in Central Anatolia was detected in clinical samples using electron microscopy (EM) and amplified using an in house polymerase chain reaction procedure for the first time. Additionally, the aetiological agent was isolated from a sheep and identified using EM and PCR.

Keywords: sheeppox virus (capripoxvirus ovis), cultural isolation, electron microscopy, polymerase chain reaction

Abbreviations: AGID, agar gel immunodiffussion; CPE, cytopathic effect; DIFA, direct immunofluorescence assay; ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy; HE, haematoxylin–eosin; OIE, Office International des Epizooties; PBS, phosphate-buffered aliae; PCR, polymerase chain reaction; PTA, phosphotungstic acid; SFT-R, sheep fetal thymus cells; $TCID_{50}$, tissue culture infective dose 50%

Capripoxviruses belonging to the subfamily *Chordopoxvirinae* of the family *Poxviridae* cause worldwide important and serious diseases that are known as sheeppox, goatpox and lumpy skin disease in sheep, goat and cattle, respectively (Esposito and Fenner, 2001).

Transmissible diseases from poxvirus infections that tend to spread rapidly and cause pandemics have great importance for the public health and socio-economic status of countries. These diseases cause great economic damage to the international trade in animals and animal products.

Capripoxvirus infections tend to spread enzootically, especially in North Africa and some Asian countries as well as in Turkey and Greece (Gülbahar *et al*., 2000; Markoulatos *et al*., 2000). The diseases cause economic losses as a result of deaths seen via generalization, and are in the A list of the Office International des Epizooties (OIE) (World Organization for Animal Health, 1996–1997).

The capripox virion is a brick-shaped particle with a linear double-stranded DNA and 270–290 nm in size. Several laboratory confirmation techniques are based on electron microscopy (EM) and virus isolation in cell cultures. Conventional seromonitoring techniques such as immunoflourescence, immunoprecipitation, virus neutralization and ELISA

have been reported to be widely used for routine diagnosis of the disease. Formerly, a polymerase chain reaction (PCR) method based on fusion and attachment protein genes has been described for the detection of capripoxvirus and has been reported to be more sensitive than antigen trapping ELISA (Ireland and Binepal, 1998).

Since clinical signs of sheeppox and goatpox virus infections show similarity within parapoxviridae infections, differential diagnosis is of great importance. During the epizootics in Turkey between September to October 2003, 148 outbreaks were officially declared (World Organization for Animal Health, 2004). In these outbreaks 1396 animals were detected as to clinically affected, of which 8 died due to pyrexia, cutaneous lesions and pneumonia.

In this study, isolation and identification procedures of a capripoxvirus from an outbreak in a province (Cankiri) of central Turkey were described.

MATERIALS AND METHODS

Clinical materials

For the diagnosis of the clinically suspected outbreak of capripoxvirus infections in Cankiri province in October 2003, clinical samples were obtained from 7 sheep housed in three different private flocks. Clinical samples including the skin and crusted scabs in the form of papules collected from sheep were transferred to the laboratory in transport medium (PBS) containing antibiotics and under chilled conditions (Table I). The samples were processed for electron microscopy. Additionally, scab samples from animal no. 7 were used for virus isolation and PCR amplification using specific primers for capripox attachment proteins.

Cells and virus

Sheep fetal thymus cells (SFT-R) (catalogue no. 43 from BFAV-Insel Riems, Germany) were used for isolation of the causative agent from a clinical sample (no. 7). Attenuated live capripox vaccine produced in Turkey (Pendik Research Institute, Istanbul, Turkey), used a positive control in PCR, was propagated in Vero cells.

TABLE I
Results of EM, virus isolation and PCR techniques

		Results		
Animal no.	Material	EM	Virus isolation	PCR
1–6	Scabs	+	ND	ND
7	Scab/isolate	+/+	+	+/+

ND, not done

Electron microscopy (EM)

Direct EM was used to visualize viral morphology. Briefly, skin and scab materials in the form of papules and crusts were dissected from the surface of the skin with a scalpel and transferred into sterile tubes. Homogenization of samples with 300 µl sterile distilled water was followed by centrifugation for 10 min at 900 *g* and 4°C. A drop of supernatant was placed on the surface of individual grids coated with poly-L-lysine. After 2 min the surface was washed with distilled water and the excess fluid was removed with filter paper. Electron-microscopic examination was performed after staining with 1% phosphotungstic acid (PTA). Isolates were also examined by EM.

Virus isolation procedure

The clinical sample (no. 7) was prepared according to Mangana-Vougiouka and collleagues (2000). The processed materials were inoculated in 0.5 ml volume into SFT-R cells grown to confluency on a 25 cm^2 tissue cell culture flask. After 1 h incubation at 37°C, the monolayer culture was washed and refreshed with maintenance medium. The culture was further incubated in a stationary position and monitored daily for the appearance of cytopathic effect (CPE). CPE was visualized by staining with haemotoxylen–eosin (HE) for the detection of eosinophilic inclusion bodies.

The infectivity titration of the Cankiri isolate was carried out as described by Frey and Liess (1971) and calculated as $TCID_{50}$ per ml (Kärber, 1931).

DNA extraction and PCR

Viral DNA was extracted from the clinical sample and the isolate from no. 7 by alkaline phenol chloroform–isoamylalcohol (24:1) technique (Sambrook *et al.*, 1989). Capripoxvirus vaccine strain and non-infected SFT-R cell culture were used as positive and negative controls. A primer set directed to capripoxvirus attachment protein was used (Ireland and Binepal, 1998). The PCR had an initial cycle of 94°C for 4 min, followed by 35 cycles for 47°C for 1 min, 72°C for 1 min, 95°C for 45 s and final extension at 72°C for 10 min. The reaction products were separated on a 1% agarose gel containing ethidium bromide and visualized by fluorescence in UV light, and the results were recorded on Kodak 1D film.

RESULTS

Electron microscopy

Particles showing typical poxvirus morphology were detected in all of the clinical samples. Also, the isolates were identified as pox virus by EM (Figure 1A, B).

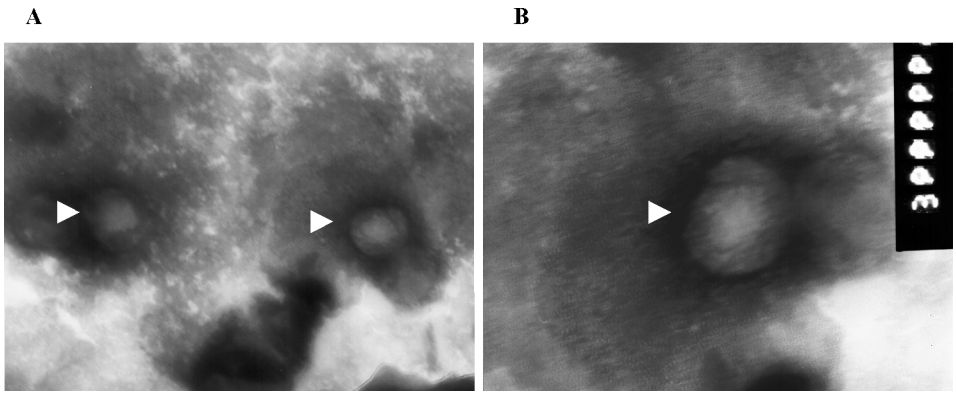

Figure 1. Electron microscopic appearances of capripoxvirus particles prepared from tissue culture fluid after isolation. (A) Original magnification X22 500; (B) Original magnification X45 000

Virus isolation

The massive cellular damage (CPE) caused by virus propagation was detected on the fourth day of the second serial blind passage of the isolation sample from no. 7. CPE, characterized by rounding of the cells, vacuoles and cytolysis was observed during daily microscopic examinations (Figure 2). Poxvirus-specific eosinophilic intracytoplasmic inclusion bodies and multiple vacuoles in cultured cells were also detected after staining with HE (Figure 3A, B).

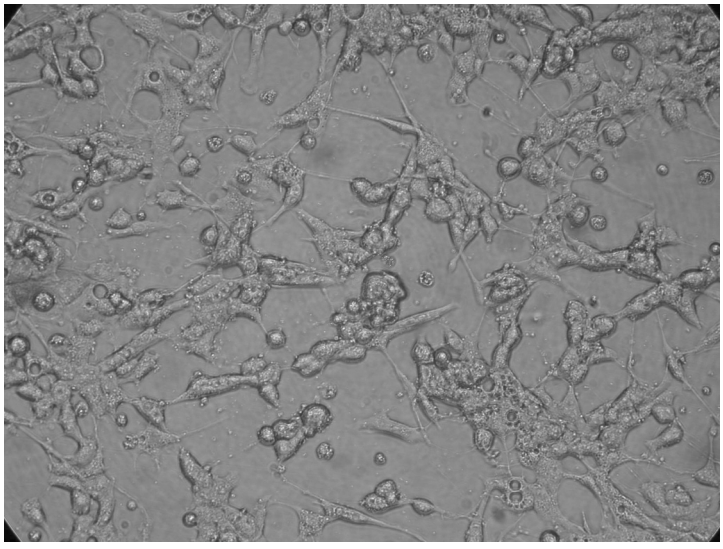

Figure 2. Capripoxvirus-induced cytopathic effect detected in STF-R cells on day 4 post infection

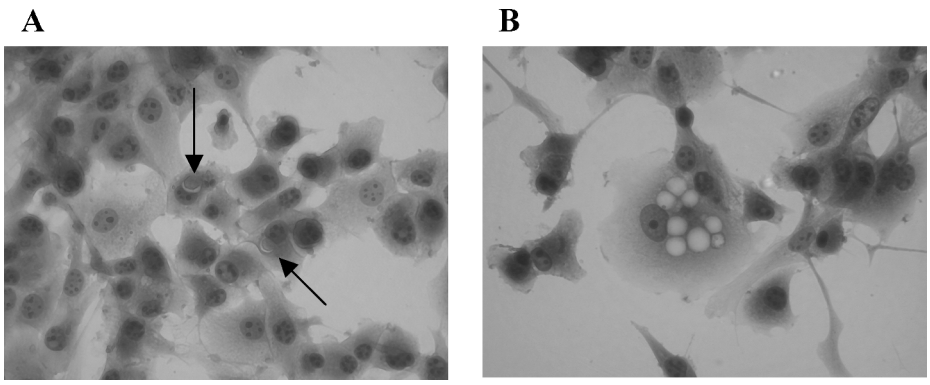

Figure 3. Capripoxvirus-induced cellular damage in STF-R cells after staining with HE. (A) Intracytoplasmic eosinophilic inclusion bodies (arrows); (B) virus-induced vacuolysis in a cell

The infectivity titre of the isolate was calculated as $TCID_{50} = 10^{4.5}$/ 0.1 ml at the end of second passage.

PCR

The DNA product of the expected size (192 kb) was detected in a clinical sample and the isolate from no. 7, and also the capripox vaccine strain used positive control, after single round of PCR (Figure 4).

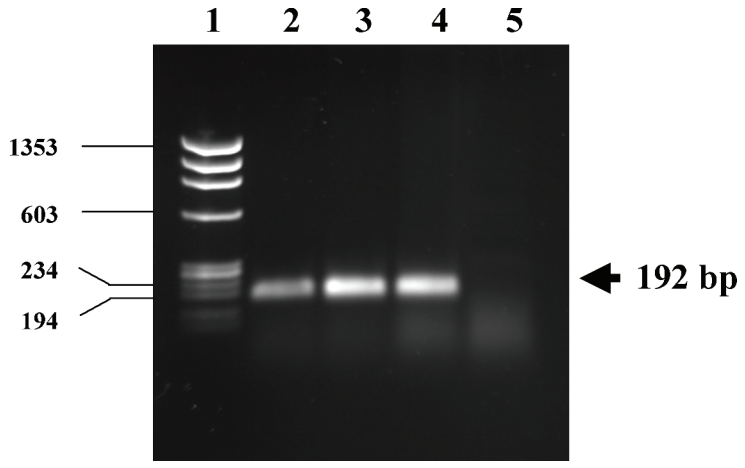

Figure 4. Detection of capripoxvirus DNA after amplification by PCR. Lane 1, Phi X DNA/*Hae*III digest; lane 2, capripoxvirus DNA prepared from infected cell culture fluid of the vaccine virus; lane 3, capripoxvirus DNA prepared from skin lesion; lane 4, capripoxvirus DNA prepared from infected cell culture fluid of the sample; lane 5, DNA from uninfected STF-R cells

DISCUSSION

Clinical sheeppox virus infections were diagnosed using EM, virus isolation and PCR techniques. The diagnosis of the capripoxvirus infection was successfully achieved using both conventional (EM and cultural isolation) and newer techniques (PCR). There is no doubt that EM has the advantage of speed in the diagnosis. Several workers have shown that scab materials, preferably from more than one individual case, are useful for the EM technique (Harkness *et al*., 1977; Gibbs *et al*., 1980). In the present study, poxviruses with the development of an oval- or rectangular-shaped particle containing a central core were detected by electron-microscopic examination in all scab samples from sheep suspected of capripoxvirus infection in the order of a few hours. However, it is known that the electron microscope is not a commonly used in all routine diagnostic laboratories and the technique needs experienced eyes for critical judgements at the diagnostic step.

A well-known conventional virus detection techniques is virus isolation, which is accepted as a 'gold standard' for numerous viruses. The most important disadvantages of this technique are that it is more time-consuming than other techniques and it needs at least 2–3 mandatory cell culture passages. Moreover, following these procedures, viruses isolated have to be identified by virus neutralization or immunfluorescence techniques using anti-capripoxvirus hyperimmune serum. The average time for complete diagnosis using cell culture-based techniques may be at least 10 days in a routine cultural isolation procedure (Plowright and Ferris, 1958).

Similarly, the isolation of the causative agent from a clinical sample was done at the second blind passage on the 15th day after the sampling in this study. Subsequently the virus was identified as capripoxvirus by electron-microscopic examination and PCR.

An in-house PCR system was established for the first time for detecting capripoxvirus infection. On the basis of previous knowledge, the virus was identified within 24 h after completing the set-up procedures. The PCR technique clearly has advantages for the diagnosis of the virus. Mangana-Vougiouka and colleagues (2000) reported that PCR was found to be more sensitive than DIFA and AGID techniques for the identification of sheeppox virus.

In this study, the purpose was the diagnosis of clinical infections. Additionally, the PCR primer for amplification of capripox virus was used in an in-house-PCR system. EM, virus isolation and PCR techniques were used for only one sample and this sample was found to be positive using these three methods; however, evaluation of the diagnostic value of these techniques obviously requires greater number of positive samples.

Sheeppox virus epidemics occur almost every year in Turkey (World Organization for Animal Health, 2004). The exact level of economic losses is not known but, from our observation of the epizootic seen in Cankiri and the owners' statements, it may be said that the economic losses due to capripoxvirus infection are fairly high.

The occurrence of sheeppox virus infection is affected by many factors. Long infection period is an important factor in the spread of the virus after infections. The virus is resistant to adverse environmental conditions for long periods and can thus persist in nature for a long time. In addition to this feature, factors such as the level of contact between flocks, insufficient cleaning of transport vehicles, and are so on important in the transmission. The use of common pastures is accepted as the most important source of virus in Turkey.

As well known, vaccination is the major strategy to prevent capripoxvirus infections in affected countries, especially in enzootic and outbreak areas (Kitching *et al.*, 1987; Carn, 1993; Carn *et al.*, 1994). Live-attenuated vaccine is still in use in Turkey. However, flock type and the owners' approaches (reporting of the disease to the official sections and removal of sick animals from the flocks, etc.) in particular play an effective role in maintenance of the disease in Turkey. Additionally, it must be remembered that vaccine-induced disease, vaccine failure and restrictions on the use of live virus vaccines in non-enzootic areas are important problems in the control strategy of capripoxvirus infections (Carn, 1993).

In this study capripoxvirus infection was diagnosed in sheep in the outbreak area and a field virus was isolated and identified. The resuts will provide information for future investigations into the characterization of the isolate by nucleotid sequencing and its comparative genome analysis with other field isolates and the vaccine virus produced in Turkey.

REFERENCES

Carn, V.M., 1993. Control of capripoxvirus infections. *Vaccine*, **11**, 1275–1279

Carn, V.M., Timms, C.P., Chand, P., Black, D.N. and Kitching, R.P., 1994. Protection of goats against capripox using a subunit vaccine. *Veterinary Record*, **135**, 434–436

Esposito, J.J. and Fenner, F., 2001. Poxviruses. In: B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monathy, B. Roizman and S.E. Straus (eds.), *Fields Virology*, 4th edn, (Lippinchott, Williams and Wilkins, Philadelphia), 2885–2921

Frey, H.-R. and Liess, B. 1971. Vermehrungskinetik und Verwendbarkeit eines stark zytopathogenen BVD-MD-Virusstammes für diagnostische Untersuchungen mit der Mikrotitermethode. *Zentralblat für Veterinärmedizin B*, **18**, 61–71

Gibbs, E.P.J., Smale, C.J. and Voyle, C.A., 1980. Electron microscopy as an aid to the rapid diagnosis of virus diseases of veterinary importance. *Veterinary Record*, **106**, 451–458

Gülbahar, M.Y., Cabalar, M., Gul, Y. and Icen, H., 2000. Immunohistochemical detection of antigen in lamb tissues naturally infected with sheeppox virus. *Journal of Veterinary Medicine Series B. Infection Diseases Veterinary Public Health*, **47**(3), 173–181

Harkness, J.W., Scott, A.C. and Hebert, C.N., 1977. Electron microscopy in the rapid diagnosis of orf. *British Veterinary Journal*, **133**(1), 81–87

Ireland, D.C. and Binepal, Y.S., 1998. Improved detection of capripoxvirus in biopsy samples by PCR. *Journal of Virological Methods*, **74**, 1–7

Kärber, G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Archives of experimental Pathology and Pharmacology*, **162**, 480

Kitching, R.P., Hammond, J.M. and Taylor, W.P., 1987. A single vaccine for the control of capripox infection in sheep and goats. *Research in Veterinary Science*, **42**, 53–60

Mangana-Vougiouka, O., Markoulatos, P., Koptopoulos, G., Nomikou, K., Bakandritsos, N. and Papadopoulos, O., 2000. Sheep poxvirus identification from clinical specimens by PCR, cell culture, immunofluorescence and agar gel immunoprecipitation assay. *Molecular and Cellular Probes*, **14**(5), 305–310

Markoulatos, P., Mangana-Vougiouka, O., Koptopoulos, G., Nomikou, K. and Papadopoulos, O., 2000. Detection of sheeppox virus in skin biopsy samples by a multiplex polymerase chain reaction. *Journal of Virological Methods*, **84**, 161–167

Plowright, W. and Ferris, P.D., 1958. The growth and cytopathogenicity of sheeppox virus in tissue culture. *British Journal of Experimentall Pathology*, **39**, 424–435

Sambrook, J., Fritsch, E. and Maniatis, T., 1989. Molecular Cloning, (Cold Spring Harbor Laboratory, NY)

World Organization for Animal Health, 1996–1997. Reports on the Animal Health Status and disease control methods and List A disease outbreaks, Statistics OIE. *Manual of Standards for Diagnostic Tests and Vaccines*, (OIE, Paris), 4

World Organization for Animal Health, 2004. In OIE Classification of Diseases. *Annual Animal Health Information*, Handistatus II, Last update 16 Jan. 2004

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