

Characterization of a North American orf virus isolated from a goat with persistent, proliferative dermatitis

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Abstract

The characterization of an orf virus (OV) isolated from skin lesions of a goat kid with severe, persistent, proliferative dermatitis, and designated orf virus-San Angelo 2000 (OV-SA00) strain, is described. The identity of OV-SA00 was confirmed by a combination of methods, including electron microscopy, amplification of specific fragments of viral DNA by polymerase chain reaction, restriction enzyme analysis of viral DNA and gene sequencing. Restriction endonuclease analyses of viral DNA and the protein profile studied by Western blot revealed differences between OV-SA00 strain and the profiles of other OV strains that have been published. The restriction enzyme profile of OV-SA00 was also different from the orf virus vaccine (OV-V) strain used to vaccinate this kid. Comparison of the nucleotide and deduced amino acid sequences indicated that OV-SA00 is closely related to OV-V strain, the Scottish OV strains orf11 and MRI Scab, and the human OV-CE/Shoe strain and more distant to bovine papular stomatitis virus (BPSV) reference strain and the pseudocowpox virus (PCPV)-MNV/Till strain. These results indicate that OV-SA00 is a strain of OV rather than a different parapoxvirus. Further studies are necessary to determine if the severity of orf-induced lesions in this goat kid was the result of individual host susceptibility factors.

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1. Introduction

Parapoxviruses of veterinary importance include orf virus (OV), bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), parapox virus of deer and sealpox virus (de la Concha-Bermejillo, 1995). Members of the *Parapoxvirus* genera are genetically and antigenically related and have a similar morphology, genomic organization and virulence mechanisms (Fleming et al., 1993). OV, also called contagious ecthyma or 'soremouth' virus, is an epitheliotropic parapoxvirus that induces proliferative lesions in the skin of sheep,

goats, wild ruminants, humans, and very rarely, dogs (Bassioukas et al., 1993; Haig and Mercer, 1998). The precise geographic distribution of the disease is not known, but it is thought to be present in any part of the world where sheep and goats are raised (de la Concha-Bermejillo, 1995).

In sheep and goats, orf is recognized by the appearance of vesicles, papules and crusty, rapidly growing scabs in the skin of lips and nose of affected animals. In more severe cases, the skin of other areas, such as the eyes, feet, vulva, or udder, may be affected (de la Concha-Bermejillo et al., 1999). The disease usually runs its course in 3–4 weeks. However, prolonged infections and secondary bacterial infections or myiasis of affected areas may occur (Abu and Housawi, 1997; Greig et al., 1984; Zamri-Saad et al., 1993). Occasionally, mortality as high as 10% have been reported (Gumbrell and McGregor, 1997).

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Clinical and epidemiologic evidence indicates that in most cases, animals that have had a bout of orf or have been vaccinated are resistant to natural reinfection for several years. However, outbreaks of orf disease in orf-vaccinated animals have been reported (Buddle et al., 1984a; de la Concha-Bermejillo et al., 1999; Pye, 1990). Therefore, continuous reinfection with OV is possible. This may occur as a result of evolutionary genetic mechanisms that poxviruses have developed to evade the immune response of the host (Deane et al., 2000; Haig et al., 1996, 1997).

Considerable heterogeneity has been observed between different sheep OV field strains when restriction endonuclease digests of viral DNA were compared (Robinson et al., 1982). However, there is little information on the characterization of goat OV strains that are endemic in North America. Furthermore, the relationship between the severity of disease and the molecular profile of OV strains has not been fully elucidated. The objective of the present research was to characterize an OV strain isolated from skin lesions during an epidemic of atypical, multifocal, persistent, severe, proliferative dermatitis in young goats.

2. Material and methods

2.1. Clinical case

During an outbreak of severe disseminated, proliferative dermatitis in young goats, a 6-month-old, male Boer goat was presented for evaluation. The kid and his twin had been born clinically normal from a Boer-cross nanny in a 200-goat Texas flock. The ranch where the affected kid originated raises meat and hair goats and sheep in a mixed management system that includes range pastures and shaded pens.

2.2. Necropsy and microscopic analyses of tissues

After clinical evaluation, the kid was euthanized with an overdose of pentobarbital sodium (Beuthanasia-D Special; Schering Plough Animal Health Corp. Union, NJ). A complete necropsy was performed and macroscopic changes recorded. Tissues for histology were fixed in 10% buffered neutral formalin solution, sectioned at 5µm, stained with hematoxylin and eosin and examined under a light microscope. Expression of actin, cytokeratin, factor VIII and vimentin in the proliferative skin lesions was assessed by immunohistochemistry using primary monoclonal antibodies (MAbs)(DAKO Corp. Carpinteria, CA) specific for these proteins and an immunoperoxidase staining system (Hsu et al., 1981). Positive controls consisting of tissues known to express these proteins, and negative controls consisting of

sections of normal tissue and sections from which the primary antibody was omitted were included.

2.3. Virus isolation

Virus isolation was achieved from scab material collected at necropsy, and from the orf virus vaccine (OV-V) (Soremouth virus vaccine. Texas Agricultural Experiment Station, Sonora, TX) lot used to vaccinate the kid, as described with some modifications (Hussain and Burger, 1989). Briefly, 50% w/v scab suspensions in Hanks' Balanced Salt Solution were homogenized in tissue mortars. The suspensions were centrifuged at 2000 × g for 15 min, and the supernatants inoculated into Madin-Darby ovine kidney (MDOK) cells. Cell cultures were examined daily for the presence of cytopathic effect (CPE), and passed blindly once a week. When 50% CPE was observed, cells and media were frozen at −70 °C. The specificity of the CPE was confirmed by the polymerase chain reaction (PCR).

2.4. Polymerase chain reaction

To confirm the specificity of the CPE in cell culture, fragments of viral DNA were amplified by semi-nested polymerase chain reaction (snPCR) (Inoshima et al., 2000). DNA was extracted from infected cell culture material or virus purified from scabs (Esposito et al., 1981), by adding 100 µl of 10% SDS and 20 µl of proteinase K (10 mg/ml. Promega Co. Madison, WI) to 900 µl of resuspended MDOK cells or purified virus suspension. The mixtures were incubated at 37 °C for 2 h, and DNA was extracted once each with an equal volume of phenol; phenol:chloroform and chloroform. One tenth volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol were added to the final aqueous phases to precipitate the DNA. The DNA pellets were washed once in 70% ethanol, air dried, and suspended in TE buffer.

The first round of PCR was carried out in a 50µl reaction volume containing 5µl of 10 × PCR buffer (10 mM Tris-HCl and 50 mM KCl), 5 µl of DNA template, 200 µM dATP, dTTP, dCTP, dGTP, 0.4 µM of each primer, 25 µM MgCl₂ and 0.5 µl of *Taq* polymerase (Promega Co.). PCR was performed in a thermocycler (GeneAmp PCR 2400, Perkin Elmer, Shelton, CT) for 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 30s and extension at 72 °C for 30s. The PCR was ended at 72 °C for 7 min. Five microliters of a 1:1000 dilution of the first PCR round were subjected to a second round of amplification as above but substituting the forward primer by the internal primer. The amplified DNA products were resolved by agarose gel electrophoresis and analyzed with an IS-1000 Digital Imaging System (Alpha Innotech Corp. San Leandro, CA).

To rule out that border disease virus (BDV) was associated to skin lesions in the goat kid, a RT-PCR assay was used to amplify BDV nucleic acid from RNA extracted from skin lesions and cells culture material (Vilcek and Paton, 2000). PCR products were analyzed as above.

2.5. Virus purification

Virus was purified from scab material by ultracentrifugation (Robinson et al., 1982). Briefly, scabs were ground with sterile sea sand in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Subsequently, the mixture was centrifuged at $3000 \times g$ for 15 min. The resulting supernatant was layered onto a 36% sucrose cushion and centrifuged at $65000 \times g$ for 1 h. The pellet was resuspended in TE buffer, layered onto a 30–50% sodium diatrizoate gradient and centrifuged at $65000 \times g$ for 75 min. The visible virus band was aspirated and washed once with TE buffer. The virus was recovered by centrifugation at $65000 \times g$ for 45 min. All the centrifugation steps were carried out at 4°C .

2.6. Purification of viral DNA from infected cell cultures

Cell cultures infected with orf virus-San Angelo 2000 (OV-SA00) or OV-V strain were harvested by trypsinization 5 days after infection, as described with some modifications (Esposito et al., 1981). Briefly, trypsinized cells were washed twice in NTE buffer (10 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA), resuspended in KTE buffer (10 mM Tris pH 8.0, 10 mM KCl, 5 mM EDTA) and kept on ice for 10 min. Then, 10% Triton X-100 and 0.25% mercaptoethanol were added to the cell suspensions and kept on ice for 10 additional min. This was followed by a 10 min centrifugation step at 3000 rpm. To purify the viral cores, supernatants were collected, layered onto 30% sucrose and centrifuged at 14000 rpm for 1 h. Pellets were incubated at 37°C for 16 h in 2 ml total volume of TE buffer containing 1% SDS and 0.5 mg/ml proteinase K. Viral DNA was extracted with phenol:chloroform and chloroform, ethanol precipitated and resuspended in TE buffer.

2.7. Electron microscopy

The morphology of the virions was studied using the negative staining technique (Watson, 1962), and photographed with a Phillips electron microscope model 301.

2.8. Western blot analysis

Proteins from purified virus preparations were resolved on a 12% SDS-PAGE gel in a vertical mini-gel electrophoresis system (Bio-Rad, Hercules, CA) and

transferred to a nitrocellulose membrane (Harlow and Lane, 1988). The membrane was rinsed in PBS and blocked with 5% horse serum for 1 h at room temperature (RT). Subsequently, the membrane was incubated overnight at 4°C with sheep primary anti-OV polyclonal antibody (APHIS, USDA, Ames, IO), washed three times in 0.05% Tween-20/PBS (T-PBS) and incubated with horseradish peroxidase-conjugated monkey anti-sheep IgG (Sigma Chemical Co. St. Louis, MO) for 1 h at RT. The membrane was washed three more times in T-PBS and incubated at RT in fresh substrate solution (50 mM Tris-HCl, pH 7.5, and 30 μl of 30% hydrogen peroxide mixed with 0.03 g 4-chlorol-1-naphthol dissolved in 10 ml of methanol). The reaction was stopped by rinsing with distilled water.

2.9. DNA extraction from purified virions

Virions purified from scab material were treated with guanidinium isothiocyanate buffer (4 M guanidinium isothiocyanate; 5 mM Tris-HCl pH 7.5; 1 mM EDTA; 2% lauryl sarcosiae; 1% β -mercaptoethanol), layered on a 40% (w/w) cesium chloride/TE buffer cushion and centrifuged at $150000 \times g$ for 16 hrs at 20°C (Gilray et al., 1998). The pellet was resuspended in TE buffer and treated with 100 $\mu\text{g/ml}$ RNase A for 30 min. Subsequently, the suspension was digested for 1 h with proteinase K (10 mg/ml) and 10% SDS, and extracted with phenol:chloroform (1:1). DNA was precipitated with ethanol and 3 M ammonium acetate at -20°C , washed two times with 70% ethanol and resuspended in 50 μl of TE buffer.

2.10. Restriction endonuclease digestion and agarose gel electrophoresis

DNA (0.5 μg) extracted from virions purified from scab material was digested with BamHI, EcoRI or HindIII restriction enzymes (Promega Co.) in separate reactions following procedures recommended by the manufacturer. In addition, DNA extracted from cell cultures infected with OV-SA00 or OV-V strain was digested with BamHI. Digested DNA was resolved by gel electrophoresis at 1 V/cm in a 0.7% agarose and visualized by ethidium bromide staining (Mazur et al., 1991; Robinson et al., 1982). The size of DNA bands was estimated with the help of an IS-1000 Digital Imaging System.

2.11. Cloning and sequencing of the virus interferon resistant gene and the envelope gene

The complete virus interferon resistance (VIR) gene from OV-SA00 purified from scab material, OV-V strain (used to vaccinate the goat kid described in this report), OV-CE/Shoe (a human OV) strain (Hessami et al., 1979;

Lard et al., 1991), BPSV-reference strain (Lard et al., 1991), and PCPV-MNV/Till strain (Hessami et al., 1979; Lard et al., 1991) was amplified by PCR using primers (forward primer 5' aag ctt aga agc tga tgc cgc ag 3'; reverse primer 5' gga tcc aca atg gcc tgc gag tg 3') that were designed based on a previously published sequence of the OV-NZ2 strain VIR gene (McInnes et al., 1998). The envelope gene from OV-SA00 and from OV-V strain was amplified using two sets of primers (set 1: forward primer 5' att tat tgg ctt gca gaa ctc cga gc 3'; reverse primer 5' tac gtg gga agc gcc tgc ct 3'; set 2: forward primer 5' gcg agt ccg aga aga ata cg 3'; reverse primer 5' atg tgg ccg ttc tcc tcc atc 3'), and the nucleotide and deduced amino acid sequences compared to partial sequences of OV-NZ2 strain, OV-S-1 strain, OV-Iwate strain, BPSV-V660 and PCPV-VR634 (GenBank accession numbers AB044794, AB044796, AB044795, AB044793 and AB044792). Characteristics of these strains have been previously described (Friedman-Kien et al., 1963; Inoshima et al., 2001; Kumagai et al., 1976; Menna et al., 1979; Robinson et al., 1982; Suzuki et al., 1993).

The PCR products were cloned into pGEM-T easy Vector (pGEM-T vector system, Promega Co.) following instructions by the manufacturer. Nucleotide sequencing was performed in both orientations by automated sequencing at Gene Technologies Laboratory (Texas A&M University, College Station, Texas). Sequences were read on an automated sequencer (Applied Biosystems DNA Sequencer 373A, Norfolk, CT) and then edited using Sequencer version 3.0 (Gene Codes Corp., Ann Arbor, MI). Sequences obtained in this study have been submitted to GenBank. Accession numbers are: AY278208 (OV-SA00 env gene); AY278209 (OV-V env gene); AY278210 (OV-V VIR gene); AY278211 (OV-CE/Shoe VIR gene); AY278212 (PCPV-MNV/Till VIR gene); AY278213 (BPSV-reference VIR gene); AF380126 (OV-SA00 VIR gene).

3. Results

3.1. Clinical case

A 6-month-old, male Boer goat with severe multifocal, proliferative dermatitis was presented for evaluation. All kids in the flock had been vaccinated with a live OV-V (vaccine strain) a few days after birth. When weaned, roughly at $2\frac{1}{2}$ months of age, approximately 10% of 220 goat kids presented typical signs of orf, characterized by mild proliferative lesions mainly in the skin of lips and nose. Similar skin lesions appeared in the lips of the kid goat presented in this report. While most of the kids with orf recovered spontaneously within 3–4 weeks, lesions in 2% of them, including the

one reported here, continued to grow for about 3 months and disseminated to the skin of face, ears, feet, flanks, and scrotum. Orf lesions were not observed in this kid's sibling during the outbreak. None of the sheep or hair-breed goats raised in the same premises presented clinical signs of orf.

3.2. Necropsy and microscopic analyses of tissues

Macroscopic lesions consisted of irregular, multifocal areas of papillomatous, crusty, fissured, horny proliferation in the skin of the face, ear, feet, flanks, and scrotum that ranged in size from a few millimeters to 30 cm in diameter (Fig. 1, panels A and B).

Histologically, skin lesions were characterized by severe, papillated, hyperplastic dermatitis with a mixture of epidermal and dermal proliferation (Fig. 2). Hyperkeratosis, mild parakeratosis and some elongation of the rete ridges were found in the epidermis. Clusters of epidermal cells with vacuolated cytoplasm or cells with intracytoplasmic viral inclusions were found in the stratum spongiosum. Subcorneal vesicles and pustules were present throughout the epidermis. The dermal component consisted of proliferating connective tissue with various degrees of lymphocyte and plasma cell infiltration. The proliferating cells in the dermis were vimentin and smooth muscle actin-positive and cytokeratin and factor VIII negative, indicating a fibroblast rather than endothelial or epithelial origin of these cells.

3.3. Virus isolation and purification

CPE, consisting of cell rounding, pyknosis and cell detachment, was observed in MDOK cell cultures infected with scab or vaccine suspensions, but not in mock-infected cells. CPE was evident only after 3 days of the third blind passage.

A major virus band was obtained after centrifugation of scab material in the 30–50% sodium diatrizoate gradient. Negative staining electron microscopy revealed that this band consisted almost exclusively of complete, ovoid-shaped, characteristic parapoxvirus virions having an outer membrane of a single, long tubule that appeared to swirl around a homogeneous electron-dense core (Fig. 2, insert). No other viruses were detected by this technique.

3.4. Polymerase chain reaction

DNA template for PCR was prepared both from scab-purified virus and from infected MDOK cells. DNA from non-infected MDOK cells was used as negative control. DNA fragments of 235 and 594 bp were amplified by snPCR from DNA extracted from virions purified from scab material and from MDOK cells infected with scab or OV-V strain suspensions, but

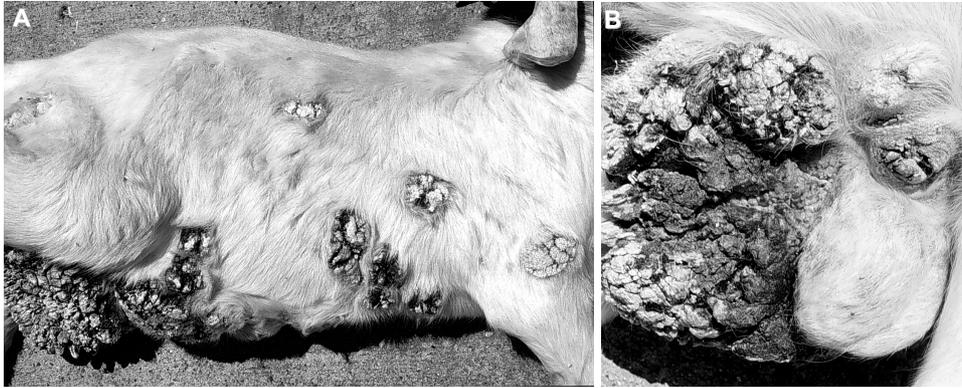


Fig. 1. Goat kid showing skin lesions characterized by multifocal areas of severe proliferative dermatitis (1a). Close up (1b) of skin lesion illustrating large papillomatous, crusty, fissured, horny proliferation in the skin caudal to the scrotum.

not from non-infected controls (Fig. 3). BDV was not detected by RT-PCR in skin lesions or cell culture material used to isolate OV.

3.5. Western blot analysis

Immunoblot analysis of proteins from virions purified from scab material was carried out using an OV-positive antiserum (vaccine strain EOK/4)(Fig. 4). The strongest OV reactive protein was 49.62 kDa, but 223.43, 118.36, 111.99, 67.00, 59.46, 42.39, 39.99, 33.77,

31.85, 30.05, 28.18, 25.22 and 22.71 kDa bands were also detected.

3.6. Restriction endonuclease digestion and agarose gel electrophoresis

The electrophoretic migration patterns of OV-SA00 DNA fragments generated by digestion of viral DNA with three separate restriction endonucleases are shown in Fig. 5, panel A. Each enzyme had a different restriction pattern. HindIII gave the most complex DNA cleavage pattern, followed by BamHI and then

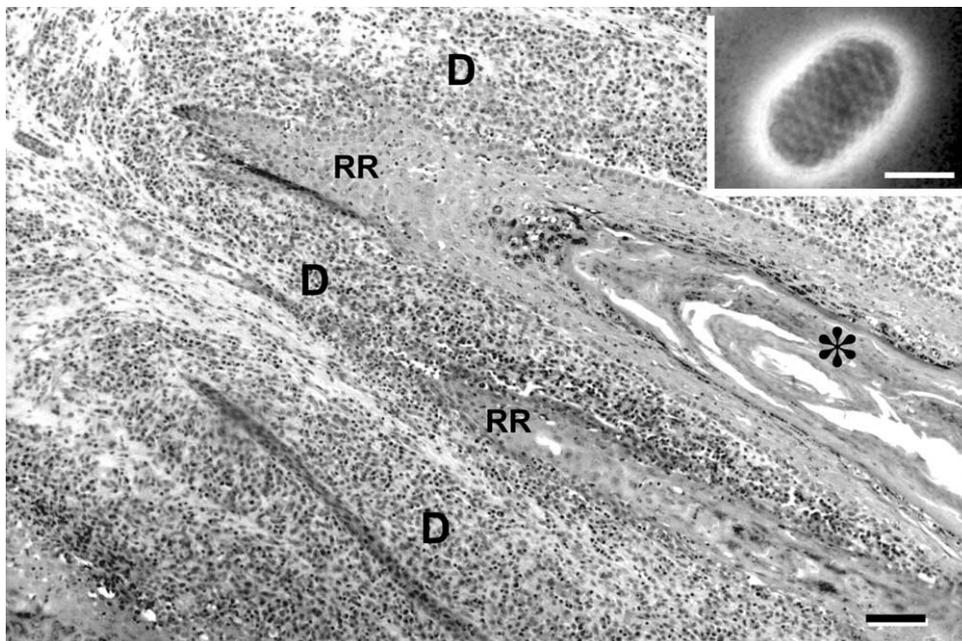


Fig. 2. Histology of orf skin lesion of goat kid showing hyperkeratosis (*), elongation of the rete ridges (RR) of the epidermis, and proliferating connective tissue with various degrees of lymphocyte and plasma cell infiltration in dermis (D) (Bar = 150 μ m). The electron microscopy morphology of a purified virion is shown in the insert (top right)(Bar = 130 nm).

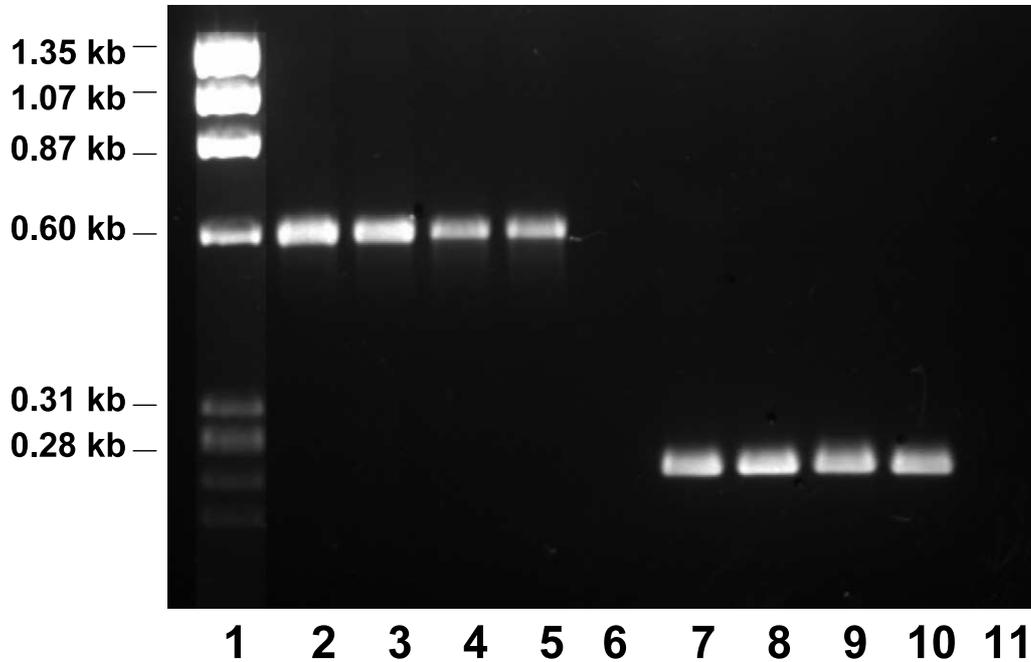


Fig. 3. Amplification of parapoxvirus specific DNA fragments by snPCR. DNA molecular weight markers (lane 1), amplification of viral DNA from purified virions from skin lesions (lane 2), from orf virus vaccine (OV-V) strain (lane 3), from OV-SA00-infected Mardin-Darby ovine kidney (MDOK) cells (lane 4), from OV-V-infected MDOK cells (lane 5), using flanking primers. Lanes 7–10 correspond to DNA extracted from purified virions and infected MDOK cells, amplified during the second round of PCR using semi-nested primers. Lanes 6 and 11 are negative controls.

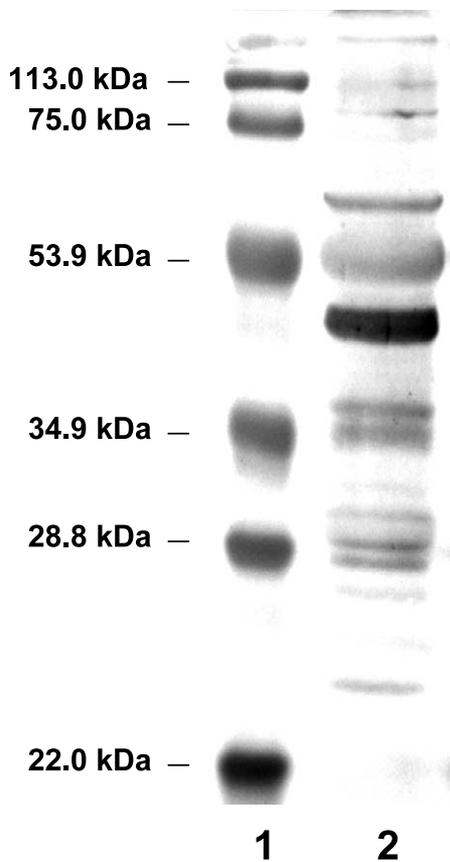


Fig. 4. Western blot analysis of proteins from virions purified from scab material. Protein molecular weight markers (lane 1) and viral proteins (lane 2).

EcoRI. The BamHI restriction patterns of OV-SA00 and OV-V strain were clearly different (Fig. 5, panel B).

3.7. Cloning and sequencing of the virus interferon resistant gene and the envelope gene

After PCR amplification and cloning into a TA vector, the entire VIR gene of OV-SA00, OV-V strain, OV-CE/Shoe strain, PCPV-MNV/Till strain and BPSV-reference strain was sequenced bidirectionally. The sequences were edited, aligned and compared to two previously reported sequences of the VIR gene of OV Scottish strains orf-11 and MRI Scab (McInnes et al., 1998). Results showed that the sequence of the OV-SA00 VIR gene was identical in size (552 bp) to the OV-V, OV-orf11, OV-MRI Scab and OV-CE/Shoe strains. The VIR gene of the PCPV-MNV/Till strain and of BPSV-reference strain were 543 and 549 bp, respectively. This was the result of deletions at nucleotides 221–223 and 225–230 in the PCPV-MNV/Till and nucleotides 221–223 in the BPSV-reference strain. The OV-SA00 sequence shared 94–95% nucleotide and 92–94% deduced amino acid identities with OV-V, OV-orf11, OV-MRI Scab and OV-CE/Shoe strains (Fig. 6). The identities between OV-SA00 and PCPV-MNV/Till and BPSV-reference strain were 77 and 78% at the nucleotide level, and 73 and 75% at the deduced amino acid level, respectively. The PCPV-MNV/Till and BPSV-reference strain shared 94% nucleotide and 88% deduced amino acid identity.

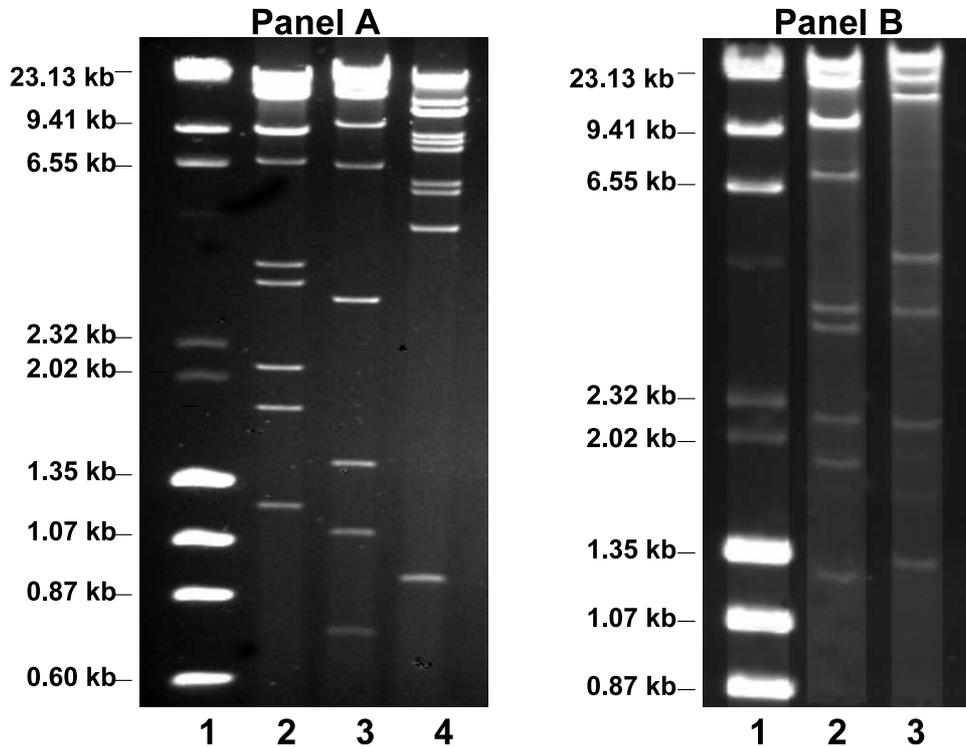


Fig. 5. Restriction enzyme profiles of OV DNA. *Panel A*: OV-SA00 strain. DNA molecular weight markers (lane 1), viral DNA BamHI (lane 2), EcoRI (lane 3) and HindIII (lane 4) digestions. *Panel B*: Comparison between restriction enzyme profiles of DNA from OV-SA00 and OV-V strains. DNA molecular weight markers (lane 1), OV-SA00 (lane 2) and OV-V (lane 3) strains.

The partial sequence of OV-SA00 virus envelope gene was compared to OV-V strain, OV-NZ2, OV-S-1, OV-Iwate, PCPV-VR634 and BPSV-V660. At the nucleotide level, a 98, 99, 98, 98, 94 and 83% identity was found

between OV-SA00 and OV-V, OV-NZ2, OV-S-1, OV-Iwate, PCPV-VR634 and BPSV-V660, respectively. The percent identities at the deduced amino acid level were 98, 99, 97, 97, 94 and 82% (Fig. 7).

						60
OV-SA00	MACECASLIL	ELLRKSDDKL	PAKQIAKELG	ISKHEANRQL	YRMLELDDVC	CEDGNPPRWF
OV-V	*****	*****	*****	*****	**L*DSN**	*****
OV-Orf11	*****	*****	*****	*****	**L*DS*E**	*****
OV-MRI Scab	*****	*****	*****	*****	**L*DS*E**	*****
OV-CE/Shoe	*****	*****	*****	*****	**L*DS*E**	*****
PCPV-MNV/Till	***W*****	D**KTCG**	**N*****	*****Y*	**L*DK*E*R	V*E*H**H*Y
BPSV-reference	*****	D**K*CGV*	**D*****	*****Y*	**L*DT**R	V**H*****Y
						120
OV-SA00	VECAPSAPTE	EDENSDETEPM	ETEAGCDTLF	GGDIDIMTQS	AVMRLKSLNP	VSAVNEFCMM
OV-V	****A****	*****	*****	*****	*****	*****
OV-Orf11	*****	*****	*****	*****	*****	**P*****
OV-MRI Scab	*****	*****	*****	*****	**I*****	*****
OV-CE/Shoe	*****	*****	*****	*****	**I*****	*****
PCPV-MNV/Till	***E*ATCV*	**--*-----	*****I*	*****VLSKT	C*****T***	*****I
BPSV-reference	A**E*ATSV*	**D-T*****	*****I*	**E**VL**	C*V**T***	*****I
						180
OV-SA00	THRSLEFCET	RSGGKDHCP	FTCTITIFGK	VVAAADGASK	KLARHTACAS	ALTILINCG
OV-V	*R*P*****	***E*****R	*****S**	**SV*****	*****S*	*****
OV-Orf11	*R*****	***E*****R	*****S**	**SV*****	*****S*	*****
OV-MRI Scab	*R*****	***E*****R	*****S**	*****S**	*****S*	*****
OV-CE/Shoe	*R*P*****	***E*****R	*****SE*	*****	*****S*	*****V
PCPV-MNV/Till	***P*****	***Q*****N	*****VVS**	LI*T*T*T*R	*****A**T	**I**D***
BPSV-reference	***P*****	***Q*****N	*****MVS**	*I*T**T**	*M**A**T	**I**D***
OV-SA00	ISF					
OV-V	***					
OV-Orf11	*T*					
OV-MRI Scab	***					
OV-CE/Shoe	***					
PCPV-MNV/Till	***					
BPSV-reference	***					

Fig. 6. Deduced amino acid alignment of the VIR gene of OV-SA00, OV-V, OV-orf-11, OV-MRI Scab, OV-CE/Shoe, PCPV-MNV/Till and BPSV-reference strain. Areas of identity are indicated by asterisks. Dashes represent amino acid deletions.

						60
OV-SA00	TGGSVSTIKN	LGLYSTNKHL	AWDLMNRYNT	FYSMIVEPKV	PFTRLCCAIV	TPTATNFHLD
OV-V	*****	*****	*****	*****	*****V*	*****
OV-NZ2	*****	*****	*****	*****	*****	*****
OV-S-1	*****	*****	*****	*****	*****V*	*****
OV-Iwate	*****	*****	*****	*****	*****V*	*****
BPSV V660	***I*N**	**V**R**	*T*****	*****	**S**MI	***D**
PCPV-VR634	*****	*****	*A*****	*****	*****	***D**N
						120
OV-SA00	HSGGGVFFSD	SPERFLGFYR	TLDEDLVLHR	IENAKNSIDL	SLLSMVPVIK	HASAVEYWPQ
OV-V	*****	*****	*****	*****	*****	*****
OV-NZ2	*****	*****	*****	*****	*****	*****
OV-S-1	*****	*****	*****	*****	*****	*****R
OV-Iwate	*****	*****	*****	*****	*****	*****R
BPSV-V660	*A*****	A**K*****	*****	*DS*****	***L****R	**DR****R
PCPV-VR634	*****	*****	*****	***N*****	*****	*****
						180
OV-SA00	IIDALLRAAI	NRGVRVRII	TEWKNADPLS	VSAARSLDDF	GVGSVDM SVR	KFVVPGGDDA
OV-V	*****	D*****	*****	*****	*****	*****R**
OV-NZ2	*****	*****	*****	*****	*****	*****R**
OV-S-1	*****	D*****	*****	*****	*****	*****R**
OV-Iwate	*****	D*****	*****	*****	*****	*****R**
BPSV-V660	*M*****	D*S*****V	*****	****T*N**	***I*I*T*	L*S*I**R**
PCPV-VR634	*****	*****I*T	*****	L*****N**	*****	***I**R**
OV-SA00	ANNT					
OV-V	****					
OV-NZ2	****					
OV-S-1	****					
OV-Iwate	****					
BPSV-V660	****					
PCPV-VR634	****					

Fig. 7. Deduced amino acid alignment of the envelop gene of OV-SA00, OV-V, OV-NZ2, OV-S-1, OV-Iwate, BPSV-V660, and PCPV-VR634. Areas of identity are indicated by asterisks.

4. Discussion

Occasionally, OV is presumed to cause severe proliferative dermatitis in goats. However, virus isolation and characterization is seldom achieved (Abu and Housawi, 1997). In the case reported here, the identity of OV-SA00 strain was confirmed by a combination of methods, including electron microscopy, amplification of specific fragments of viral DNA by PCR, restriction enzyme analysis of viral DNA and gene sequencing. Primer pairs used in the snPCR resulted in amplification of specific parapoxvirus DNA fragments of 594 and 235 bp in the first and second rounds of amplification, respectively. The primers used in the snPCR have been shown to be pan-parapoxvirus specific and effective in amplifying viral DNA from OV, BPSV, PCPV, and parapoxvirus of red deer, but do not amplify DNA from vaccinia or fowlpox virus (Inoshima et al., 2000). Negative staining electron microscopic examination of virus purified from scab material revealed typical parapoxvirus virions. These two features distinguish parapoxviruses from other poxviruses (Fenner, 1999; Inoshima et al., 2000; Nagington et al., 1964). The nucleotide and deduced amino acid sequences of OV-SA00 VIR gene had higher identity with the VIR gene of other OV strains than with BPSV-reference strain and PCPV-MNV/Till strain. Similarly, the partial sequence of the OV-SA00 envelope gene shared a higher nucleotide and deduced amino acid sequence identity with the envelope gene of other OV strains than with BPSV-V660. Therefore, based on the host species, the typical skin lesions, the PCR results, the morphology of the

virus and the results of the VIR and envelope gene sequences, we can conclude that the virus isolated from the case reported here is a strain of OV. Caprine papillomatosis may result in similar macroscopic lesions. However, a viral etiology of this condition has not been clearly established. Viruses have not been isolated from goat papilloma lesions and DNA hybridization studies have given conflicting results (Manni et al., 1998; Theilen et al., 1985). Orf and papillomatosis share some of the epidermal proliferative microscopic lesions, but the latter condition does not result in subcorneal vesicle and pustule formation as the ones seen in this case.

Typically, OV infections of sheep and goats result in mild crusty or papillomatous lesions in the skin of lips and nose that regress spontaneously within 3–4 weeks (de la Concha-Bermejillo et al., 1999). In the case reported here, the lesions continued to disseminate and persisted until the kid was euthanized 3 months after the initial clinical signs appeared. Orf lesions spontaneously regressed in all other affected kids in this flock within 4 weeks. In a previous report, OV isolated from a goat with severe, long-lasting orf did not result in severe disease when passed into susceptible kids (Abu and Housawi, 1997). Although the OV isolated from the goat kid reported here has not been passed into other goats, other isolates obtained in subsequent years from goats with severe persistent orf at the same location were used to inoculate orf naive goats. Only mild, transient skin lesions were induced (unpublished observation). This suggests that host factors may play an important role in the susceptibility of some goats to orf.

Because there is no information on the molecular features of goat OV strains endemic in North America, we characterized the protein profile and the DNA restriction pattern of OV-SA00 strain. Up to 35 polypeptides have been detected in SDS-polyacrylamide gels of purified virions disrupted in SDS/2-ME (Balassu and Robinson, 1987). Structural analysis of polypeptides from 11 isolates of OV by SDS-PAGE indicate that the profiles obtained are similar to one another, except for differences in the molecular weight region of 37–44 kDa (Buddle et al., 1984b). By Western blot analysis, we showed that the strongest immunoreacting OV-SA00 protein was 49.62 kDa. Other six viral proteins, including 67.00, 59.46, 42.39, 39.99, 31.85, and 30.05 kDa also were strongly recognized by the sheep OV antiserum. OV proteins have been studied by Western blot using MABs, and rabbit polyclonal monospecific antibodies, as well as serum from hyperimmunized sheep (Chand et al., 1994; Czerny et al., 1997). A 39 kDa protein, that most likely corresponds to the 39.99 kDa protein identified in the OV-SA00 strain, has been detected in the envelope fraction of OV using a MAB and shown to be homologous to the envelope protein encoded by the H3L gene of vaccinia virus (Housawi et al., 1998). A 32 kDa protein, that could correspond to the 31.85 kDa protein in this report, has also been detected in ovine OV using sheep hyperimmune sera (Chand et al., 1994). Because of different experimental conditions, full comparisons of other parapoxvirus proteins described in previous studies and those of OV-SA00 could not be made.

Considerable heterogeneity has been observed between different orf field isolates when restriction endonuclease digests of orf DNA were compared by gel electrophoresis (Gilray et al., 1998; Mazur et al., 2000; McKeever et al., 1987; Robinson et al., 1982). The restriction enzyme profile of OV-SA00 strain was different from the profiles of previously reported sheep isolates (Gilray et al., 1998; Robinson et al., 1982). A common feature of 11 of 12 New Zealand isolates and some Scottish isolates was the presence of a 2.2 kDa (3.33 kb) DNA band in EcoRI digests. A band of similar molecular weight was not found in OV-SA00 when viral DNA was digested with EcoRI. Furthermore, in EcoRI digests two bands of molecular weight 1.87 kDa (2.83 kb) and 1.37 kDa (2.07 kb) were common to 32 of the New Zealand isolates. The restriction enzyme profile of OV-SA00 contained a 2.87 kb band that may be the same as the 2.83 kb band present in the New Zealand isolates, but the profile did not have the 2.07 kb band. Profiles of Scottish isolates had two other bands of 1.95 kDa (2.95 kb) and 2.88 kDa (4.36 kb) molecular weight in the EcoRI restriction endonuclease profiles that were not found in profiles of OV-SA00. The OV-SA00 restriction enzyme pattern was also different from that of two Brazilian goats and one sheep OV isolates

(Mazur et al., 2000). These differences in restriction enzyme patterns indicate that there are substantial genetic differences between the New Zealand, Scottish, and Brazilian isolates and OV-SA00. We also compared the BamHI restriction pattern of OV-SA00 and OV-V strain, both grown in cell culture. The BamHI restriction pattern of OV-SA00 was different from the OV-V strain used to vaccinate this goat kid. This indicates that the proliferative dermatitis observed in the goat kid reported here was not the result of OV from vaccine origin. The BamHI restriction pattern of OV-SA00 grown in cell culture was the same as OV-SA00 purified from skin lesions, indicating that OV-SA00 restriction enzyme pattern remained stable in cell culture. One study found that some cell culture-grown OV did not lose restriction endonuclease cleavage sites after 22 passages in cell culture, while others did (Robinson et al., 1982). The reasons for this are not clear, but it may be related to intrinsic factors of particular strains, the cell line used for cultivation or the number of cell passages.

Poxviruses have developed evolutionary genetic mechanisms to evade the immune response of the host (Buddle et al., 1984a; de la Concha-Bermejillo et al., 1999; Haig et al., 1997; Pye, 1990). An interferon-resistance gene has been discovered in the left terminal region of the OV genome (McInnes et al., 1998). This gene, referred to as the VIR gene, encodes the information for a dsRNA-binding protein that inhibits the antiviral activity of interferons, and may play a role in virus persistence and reinfection (Haig and Mercer, 1998). For this reason, we compared the full sequence of this gene in OV-SA00 and those of OV-V strain, two Scottish OV strains (orf-11 and MRI Scab), OV-CE/Shoe strain, PCPV-MNV/Till strain and BPSV-reference strain, some of which have been reported to have different pathogenicity (McInnes et al., 1998). Some differences in nucleotide and amino acid sequences of the VIR gene were found among all OV strains described here. The OV-SA00 nucleotide sequence shared 95, 94, 95 and 94% identity with the sequences of OV-V, OV-orf-11, OV-MRI Scab and OV-CE/Shoe strains, respectively. Further comparisons of the predicted amino acid sequences revealed 92, 92, 94 and 92% identities, indicating that overall the VIR gene is conserved among OV strains.

OV envelope gene encodes a 42 kDa protein which shows a 42% amino acid sequence identity to the vaccinia virus major envelope 37 kDa protein (Sullivan et al., 1994). Comparison of the nucleotide and deduced amino acid sequences of this gene allows for a clear differentiation of OV, BPSV, and PCPV (Inoshima et al., 2001). A high degree of identity at the nucleotide (98–99%) and amino acid (94–95%) level were found between OV-SA00 and the other OV strains, in spite of differences in type of host species, geographic region of

origin and date of characterization among them. However, OV-SA00 differed from BPSV-V600 by 17 and 18% at the nucleotide and amino acid levels, respectively. As indicated above, OV-SA00 shared 94% identity at the nucleotide and amino acid levels with PCPV-VR634.

OV infections are common in sheep and goats throughout the world. Sheep and goats are often raised together and OV infections are endemic in West Texas. Furthermore, the same live virus vaccine is used in both species, and when orf outbreaks occur in previously vaccinated animals, scabs from the outbreak are incorporated into the vaccine (de la Concha-Bermejillo, 1993; de la Concha-Bermejillo et al., 1999). Nevertheless, the orf vaccines produced with OV obtained from sheep do not protect goats against challenge with OV obtained from goats (de la Concha-Bermejillo et al., 1999). Experimentally, OV strains from sheep are infectious to goats and vice versa, but during orf outbreaks is not uncommon to observe that while clinical disease is observed in one species, the other remains clinically normal in spite of both being raised in close proximity. This suggests that some OV strains may have a greater ability to infect sheep and others to infect goats. As full parapoxvirus genome sequences become available, more light will be shed on the genetic diversity and epidemiology of this complex group of viruses.

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