Complete remission after treatment of *Capripoxvirus* infection in sheep using potassium arsenite 0.5% (Fowler's solution)

W. TARELLO[1*] AND J. KINNE[2]

[1] *C.P. 1644, 06129 PERUGIA, ITALY*
[2] *Central Veterinary Research Laboratory, P.O. Box 597, Dubai (UAE)*

** Corresponding author: E-mail:wtarello@yahoo.it*

SUMMARY

The aim of this study was to find an effective therapy against sheep pox during an outbreak affecting 145 sheep, 87 (60 percent) of which were pregnant. The disease occurred in December 2005 in a herd of 5500 non vaccinated animals, in the United Arab Emirates. Diagnosis was based on generalised skin pox lesions and isolation of the *Capripoxvirus* from a representative animal submitted for post-mortem examination. Collateral clinical signs, including weight loss, poor appetite, lethargy and dyspnoea, were present in 75 (51.7 percent) animals. Sheep were treated intramuscularly with potassium arsenite 0.5 percent at doses of 1 ml per 20 kg per day, thus 0.185 mg of As per Kg per day, for 5 consecutive days. Collateral signs disappeared within 24 hours, initial healing of skin pox lesions was evident within an average time of 36 hours and complete healing was obtained within an average recovery time of 3 days. No side effects were noticed. This study suggests that potassium arsenite can eliminate clinical signs and mortality associated with sheep pox in a rapid and effective way.

Keywords : ***Sheep pox, Capripoxvirus,*** Poxviridae, chemotherapy, potassium arsenite.

RÉSUMÉ

Le but de cette étude était celui de trouver une thérapie efficace contre la variole des brebis pendant une épidemie qui a touché 145 brebis, 87 (60 pour cent) d'entre elles étaient gestantes. La maladie a eu lieu en décembre 2005, dans un troupeau de 5500 animaux non vaccinés aux Emirats Arabes Unis. Un diagnostic de variole a été établi sur la base des lésions cutanées et sur l'isolement du *Capripoxvirus* des brebis chez un animal représentatif soumis a un examen postmortem. Soixante-quinze (51.7 pour cent) animaux présentaient des signes cliniques collatéraux, tels qu'une perte de poids, un manque d'appétit, une léthargie et des difficultés respiratoires. Les brebis ont reçu une administration intramusculaire d'arsenite potassique 0.5 pour cent à la dose de 1 ml pour 20 kg par jour, soit 0.185 mg d'arsenite par kg et par jour, pendant 5 jours.

Le signes collatéraux ont disparu au bout de 24 heures en moyenne, les signes initiaux de guérison de la variole ont débuté au bout de 36 heures et la complète guérison a eu lieu en moyenne au bout de 3 jours. Aucun effet secondaire n'a été observé. Cette étude suggère que l'arsenite potassique peut être un traitement valable contre la variole des brebis, en supprimant les signes cliniques et la mortalité de façon rapide et efficace.

Mots-clés : *Variole des brebis,* Capripoxvirus, Poxviridae, chimotherapie, arsenite potassique.

Introduction

Sheep pox is a contagious rapidly spreading infectious disease of sheep, caused by a DNA virus member of the family Poxviridae and of the genus *Capripoxvirus*, characterized by fever, generalised papules, vesicles (rarely), internal lesions and death [2, 4].

This is the most severe form of pox in domestic animals, partly due to high morbidity (up to 80%) and mortality (5-50%), but also because of significant economic losses of milk, meat and wool production [2, 3]. In most cases, natural infection occurs when the virus enters the body through inhalation of aerosols from infected animals or dust contaminated with pox scabs and colonise the upper respiratory tract, stimulating a local inflammation. Contamination through skin abrasions, insect transmission or direct contact with pox lesions is less common [4].

After the primary infection the virus may enter the bloodstream and replicate in different organs including the skin, producing fever and a generalised exanthema, constituted by round pink or red papules, 1-2 cm in diameter, which subsequently evolves into vesicles and crusts [2]. Sheep pox and goat pox (capripox) are endemic in North Africa, the Middle East and India [8].

There is no cure for *Capripoxvirus* infection. Supportive medication and prevention of secondary bacterial infections is suggested for diseased animals [4], but there is no prove that such treatments influence the duration and severity of clinical signs and mortality rate. The aim of this study was to provide a safe and effective treatment against the clinical manifestations of *Capripoxvirus* in sheep, in order to prevent the massive economic losses usually associated with such infection. For this purpose, chemotherapy was based on the use of potassium arsenite, formerly known as Fowler's solution. This is a solution of arsenic trioxide with potassium bicarbonate, where arsenic trioxide is the active molecule. The rational for the use of this medication is that it proved recently successful in inhibiting virus replication such as hepatitis C virus [6] and HTLV-1 and HTLV-2 viruses [7].

Materials and Methods

During the summer 2004 a *Capripoxvirus* outbreak in a sheep farm in Dubai killed approximately 400 animals. Fifteen months later, a new *Capripoxvirus* outbreak occurred in a sheep farm located 8 kilometers from the first one. In this case, therapy with potassium arsenite was tried.

Both farms were established during the '80 using local breeds. According to the owners, animals were not vaccinated against sheep pox and did not experienced *Capripoxvirus* infection during the last 25 years.

The second outbreak started on 27th November 2005, when 15 adult sheep in a herd of 5500 non vaccinated animals suddenly showed cutaneous and general clinical signs attributable to sheeppox virus infection. Generalized pox lesions were most easily observable on muzzle, neck, perineum, inguinal area and udder. One severely diseased sheep showing fever and papules on the whole body was sent to the Central Veterinary Research Laboratory (CVRL) (www.cvrl.co.ae) of Dubai for postmortem examination. Pathology showed pox dermatitis around the mouth and in the inguinal areas as well as numerous small grayish nodules (3 to 5mm) in the lung (Figure 1). Poxvirus was isolated from skin and lungs samples on Vero cells using routine virological methods [13].

A 5-day course of potassium arsenite 0.5% injected intramuscularly at a dose of 1 ml/20 kg, thus 0.185 mg of As/kg/day, was immediately started in the affected sheep, as soon as the skin lesions appeared. Infected animals were moved to quarantine premises for isolation. On 29th November there were 36 sheep with pox lesions and the next day a total number of 120 animals were found affected. Concurrently, vaccination with the Kenya O 180 sheeppox strain was administered to non infected non symptomatic animals in order to prevent the spreading of the disease. One sheep formerly showing severe clinical signs that was submitted to the full course of treatment suddenly died on the 7th December 2005 was subsequently sent for necropsy.

Results

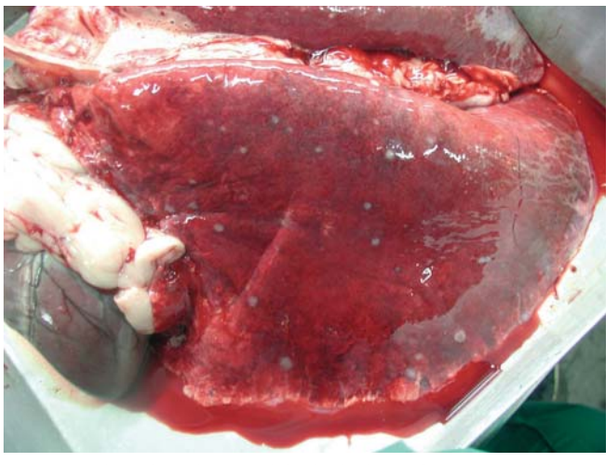

FIGURE 1: Disseminated pox nodules in the lung of an untreated sheep.

Therapy led to the disappearance of collateral clinical signs within 24 hours. Initial healing of skin pox lesions was evident within an average time of 36 hours and complete healing was obtained within an average recovery time of 3 days. All sheep experienced complete recovery from skin pox lesions and elimination of debilitating collateral clinical signs, within 3 days from the beginning of the therapy. No side effects were noticed.

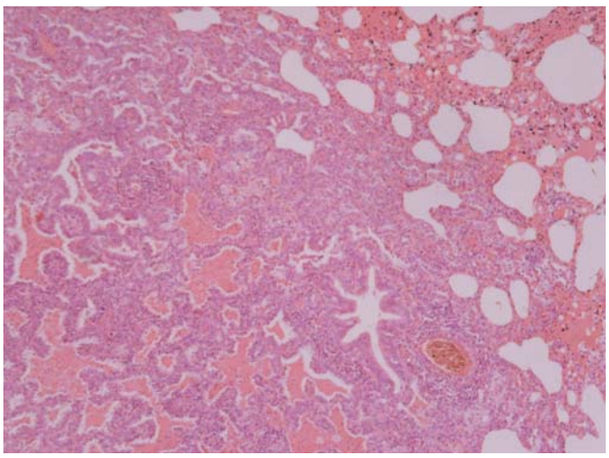

FIGURE 2: Focal catarrhal pneumonia with massive proliferation of type II-pneumocytes containing eosinophilic material of a treated sheep (H-E staining).

Eighty-seven (60%) sheep were pregnant females and delivered healthy lambs during or immediately after the end of the therapy. No fatalities and no abortions were observed in treated animals. None of the treated sheep received vaccination. One sheep formerly showing severe clinical signs that was submitted to the full course of treatment suddenly died on the 7th December 2005. Pathology showed pox dermatitis around the mouth and several small greyish nodules (3 to 5 mm) in the lung.

Histopathological examination showed the presence of pulmonary pox lesions (Fig. 2), similar like in the first case, but the sheep poxvirus could not be isolated and the death was attributed to rumen acidosis and clostridiosis.

By the 1st of December 2005 a total of 145 infected animals were counted and treated with potassium arsenite, and 87 (60%) of these were pregnant. Collateral clinical signs, including fever, weight loss, poor appetite/anorexia, lethargy and dyspnoea, were present in 75 (51.7%) animals. The epidemic was over by the 5th of December. No more cases of sheeppox occurred on the farm during the following 12 months. According to the owner, there was no evidence of economic losses as consequence of the epidemic.

Discussion

Potassium arsenite 0.5% was both fast and effective in eliminating clinical signs of sheep pox within 3 days from their onset in 145 infected animals, including 87 (60%) pregnant females that delivered healthy lambs during or immediately after the end of the therapy. This is of notice since recorded mortality rate is up to 95% in lambs under 1 month of age, and up to 50% in adults, particularly in pregnant sheep [3].

In this study, the isolation of a *Capripoxvirus* from a representative animal showing fever, generalized exanthema and characteristically severe lesions in the lungs, confirmed the cause of the epidemic and the aggressiveness of the strain involved. Spontaneous remission of sheep pox usually takes 1 month, from the appearance of the exanthema to the shedding of crusts [2].

In the treated animals, however, the disappearance of papules (without formation of vesicles and crusts), the elimination of debilitating collateral signs, and the complete absence of mortality observed within 3 days from the beginning of therapy, indicate that the strikingly rapid and complete cure obtained was strictly linked to the administration of potassium arsenite.

This is of significance since there is no medication currently available against sheep-pox [4]. It was interesting to note, that no poxvirus was isolated from pulmonary pox lesions from one sick sheep that was submitted to necropsy. Most probably the virus was eliminated due to the treatment but lesions had not receded.

The following conditions should be considered in the differential diagnosis of sheep pox: contagious echtyma, lumpy skin disease, bluetongue, peste des petits ruminants, photosensitization, psoroptic mange, dermatophitosis, insect bites, parasitic pneumonia and caseous lymphadenitis [3].

The capripox virion is different from other poxvirus commonly infecting sheep and goats, such as the *Parapoxvirus* that causes contagious ecthyma (contagious pustular dermatitis) characterized by the absence of fever and the presence of papules localized only around the mouth, nose and eyes [2].

Sheep pox is caused by strains of *Capripoxvirus* and produces a characteristic disease in fully susceptible breeds of sheep and goats that cannot be confused with any other disease [8]. In indigenous animals, generalised disease and mortality are less common, although severe outbreaks have been observed where disease has been absent from an area for a period of time or when it occurs with concurrent diseases, such as Peste des Petits Ruminants or Foot and Mouth Disease [8].

A *Capripoxvirus* outbreak in a sheep farm located 8 km away from the study farm killed approximately 400 non vaccinated animals a year earlier.

Indigenous breeds of sheep were present on both farms but both outbreaks were not benign as one might expect probably because these farms did not experienced *Capripoxvirus* infection since their establishment 25 years ago, and their sheep were not routinely vaccinated.

Although pathogenicity can vary from strain to strain, completely benign infections do not occur in non vaccinated herds. According to the OIE (www.oie.int), during the year 2003 in the UAE there have been 211 reported cases of sheep pox with 38 deaths (18%) (see:
http://www.oie.int/hs2/zi_pays_mald.asp?c_pays=annee=2003&c_mald=11).

Absence of mortality and rapid recovery in this study can only be attributed to the therapy with potassium arsenite. The active molecule of this solution, arsenic trioxide, has recently shown *in vivo* inhibitory effects against HTLV-1 virus in squirrel monkeys [5] and humans [7]. Therefore, it was expected to observe similar outcomes in sheep infected with *Capripoxvirus*. The exact reason for these excellent results is not known but current literature suggests that the mechanisms of action of arsenic derivatives are many and include the induction of apoptosis, partial cyto-differentiation, inhibition of proliferation and inhibition of angiogenesis [12]. At cellular and molecular level, one or more of these mechanisms may be involved in the therapeutic success in the *in vivo* treatment of *Capripoxvirus* infection. This clinical trial was also based on the assumption that the immune-stimulating effects observed using short-term therapies with potassium arsenite in human beings [10] and animals [11] might be of help even in controlling sheeppox virus and associated secondary bacterial infections. Additionally, arsenic trioxide is today successfully used for treating a variety of blood and solid cancers [12] and this excludes a potential carcinogenetic risk. In this study, the dosage of 0.185 mg of As/kg/day proved safe and efficacious, as confirmed by the observation that the treatment of squirrel monkeys with arsenic trioxide *in vivo* is highly toxic at 0.9 or 0.3 mg/day but not at 0.14 mg/day for up to 2 weeks [5]. Therefore, a short term (3-5 days) administration of 0.1-0.2 mg of As/kg/day should be considered therapeutically efficient and safe. Until recently, arsenic trioxide was recommended in the oral treatment of nutritional disturbances of small ruminants at doses of 0.2-0.5 mg/kg [9]. Doses of arsenic trioxide ≥1 mg/kg produce toxic effects and the lethal dose in sheep range from 60 to 200 mg/kg [1]. After multiple administrations arsenic can persists in the organism for up to 70 days [1], therefore an adequate withdrawal time should be observed. Sheep pox is considered today a possible biological weapon, because of the high morbidity and extremely high mortality in young animals, the significant economic losses and the capacity of spreading by many routes [3]. With this study an animal model was established and a safe and non expensive therapy in front of possible non conventional use of poxviruses.

In short, potassium arsenite should be regarded as a promising medication against sheeppox infections, since it can prevent fatal outcomes and massive economic losses by producing fast and complete remission within an average time of 3 days. However, in endemic areas, vaccination remains the best way to prevent the transmission between infected and non-infected animals.

Acknowledgements

The authors thank Dr. Dr. U. Wernery and Mrs. Sunitha Joseph of the Central Veterinary Research Laboratory (CVRL) (www.cvrl.co.ae), Dubai (UAE), for the virological examinations.

Sheeppox is a notifiable animal disease in the United Arab Emirates by Ministerial Decree n. 132 of 2004.

References

1.- BOTTARELLI F.: Arsenico. In: Manuale di Tossicologia Veterinaria (1st Italian ed.), TEP, Piacenza, 1993, 59-63.

2.- CASTRUCCI G.: Poxviridae. (2nd Italian ed.), Esculapio, Bologna, 1988, 47-76.

3.- CIDRAP (Centre for Infectious Disease Research and Policy): Sheep Pox and Goat Pox (SGP): University of Minnesota, 2003,

http://www.cidrap.umn.edu/cidrap/content/biosecurity/agbiosec/anim-disease/sgp.html (website accessed on 29/12/06).

4.- FARINA R., FLAMMINI C.F., PASCUCCI S., SIDOLI L.: Poxviridae. In: R. Farina, F. Scatozza (ed.): Trattato di malattie infettive degli animali. UTET, Torino, 2002, 453-492.

5.- HERAUD J.M., MORTREUX F., MERIEN F., CONTAMIN, H., MAHIEUX R., POULIQUEN J.F., WATTEL E., GESSAIN A., de THE H., BAZARBACHI A., HERMINE O., KAZANII M.: The efficacy of combined therapy of arsenic trioxide and alpha interferon in human T-cell leukemia virus type-1-infected squirrel monkeys *(Saimiri sciureus). Antiviral Res.* 2006, **70**, 132-139.

6.- HWANG D.R., TSAI Y.C., LEE J.C., HUANG K.K., LIN R.K., HO C.H., CHIOU J.M., LIN Y.T., HSU J.T., YEH C.T.: Inhibition of hepatitis C virus replication by arsenic trioxide. *Antimicrobial Agents and Chemotherapy*, 2004, **48**, 2876-82.

7.- MAHIEUX R., HERMINE O.: In vivo and in vitro treatment of HTLV-1 and HTLV-2 infected cells with arsenic trioxide and interferon-alpha. *Leukemia and Lymphoma*, 2005, **46**, 347-55.

8.- OIE (Office International des Epizooties): Sheep and goat pox. In: Manual of diagnostic tests and vaccines for terrestrial animals, 2004, Chap 2.1.10. (http://www.oie.int/eng/normes/mmanual/A_00033.htm website accessed on 29/12/06).

9.- SALI G.: Manuale di ricette veterinarie. Bayer (ed.), Milano, 1979, 16-17.

10.- TARELLO W.: Chronic Fatigue Syndrome (CFS) associated with *Staphylococcus* spp. bacteremia responsive to potassium arsenite 0.5% in a veterinary surgeon and his coworking wife, handling with CFS animal cases. *Comparative Immunology Microbiology & Infectious Diseases*, 2001, **24**, 233-46.

11.- TARELLO W.: Immunological anomalies and thrombocytopenia in 117 dogs and cats diagnosed with Chronic Fatigue Syndrome (CFS). *Acta Veterinaria Hungarica*, 2003, **51**, 61-72.

12.- WAXMAN S., ANDERSON K. C.: History and development of arsenic derivatives in cancer therapy. *Oncologist*, 2001, **6**, 3-10.

13.- WERNERY U., KINNE J., ZACHARIAH R.: Experimental camelpox infection in vaccinated and unvaccinated Guanacos. *Journal of Camel Practice and Research*, 2000, **7**, 153-157.