Natural Bluetongue virus infection in alpacas in South Africa

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Summary
Bluetongue virus (BTV) was sporadically isolated over a four year period (2010-2014) from several alpaca carcasses that were presented for necropsy at the Western Cape Provincial Veterinary Laboratory, South Africa. Typically, the affected animals had a history of acute dyspnoea and progressive weakness before death. Consistent hydrothorax and severe lung oedema in all lead to a preliminary diagnosis of Bluetongue, despite the absence of ulceration and hyperaemia of the oral mucosa which is characteristic of this viral infection in sheep. The diagnosis was confirmed by virus isolation in embryonated eggs and subsequent sequencing of the extracted RNA. Assembled sequences were subjected to Blast analysis and two of the isolates could be verified as BTV 3. These cases, originating from the Western Cape Province of South Africa, represents the first official report of BTV infection in alpacas in Africa and demonstrates the susceptibility of the species to this disease when maintained in BTV endemic areas.

Infezioni naturali da virus della Bluetongue in alpaca del Sudafrica

Parole chiave
Alpaca,
Patologia,
Virus della Bluetongue.

Riassunto
Il virus della Bluetongue (BTV) è stato isolato sporadicamente, nell'arco di 4 anni, in diverse carcasse di alpaca sottoposte ad esame necroscopico al Western Cape Provincial Veterinary Laboratory in Sudafrica. Gli animali esaminati hanno mostrato dispnea e crescente debilitazione prima della morte. La Bluetongue è stata diagnosticata in tutti gli animali esaminati vista la presenza di consistente idrotorace e grave edema polmonare, nonostante l'assenza di ulcere e iperemia della mucosa orale segni caratteristici dell'infezione negli ovini. Questa diagnosi è stata confermata dal successivo isolamento del virus in uova embrionate e dal sequenziamento dell'RNA virale. La Blast (*Basic Local Alignment Search Tool*) analisi condotta sulle sequenze assemblate ha permesso di identificare il sierotipo 3 del BTV. Questi casi circoscritti alla provincia di Western Cape in Sudafrica rappresentano il primo rinvenimento ufficiale di infezioni da BTV in alpaca in Africa e dimostrano la suscettibilità di questa specie in aree in cui il virus è endemico.

In South Africa (SA) Bluetongue virus (BTV) infection is primarily a disease of sheep with a seasonal occurrence. Unvaccinated (or susceptible) sheep frequently develop disease towards the end of Summer or during Autumn. Other ruminants, including exotic species such as alpaca, may also sporadically contract the disease. Since 2010, 4 BTV isolates (of which 2 could by typed as BTV-3) were found in sub-adult to mature alpacas that had been presented for necropsy examination at the Western Cape Provincial Veterinary Laboratory, SA.

The clinical and pathological phenotype observed in the affected animals included acute onset of respiratory distress, which rapidly progressed within hours to weakness and terminal dyspnoea.

On presentation cyanotic mucus membranes were noted and foamy bloodstained fluid was oozing from their nostrils. All cases revealed a marked hydrothorax with congested, firm rubbery and heavy lungs that did not collapse when opening the thorax. Copious amounts of foam in the trachea and larger bronchioles (noted on cut sections of the lungs) confirmed severe pulmonary oedema.

None of the other classic lesions associated with BTV infection in sheep, such as ulceration and hyperaemia of the oral mucosa, oedema of the subcutis of the head, neck musculature, around the *ligamentum nuchae*, and sub-intimal haemorrhages of the pulmonary artery were detected.

Histological examination of the pulmonary tissue confirmed the presence of extensive protein-rich oedema that widened the interstitium and flooded the alveoli. Diffuse vascular congestion and multifocal, scant perivascular accumulates of lymphocytes and plasma cells were noted.

Bluetongue virus was successfully isolated from splenic tissue homogenates using intravenously inoculated, embryonated chicken eggs. Cherry-red and oedematous embryos were observed, which died from days 3-5 post inoculation. The presence of BTV was confirmed by real time reverse transcriptase polymerase chain reaction (RT-PCR). Further characterization of the specific isolates was performed by means of molecular serotyping and analysis of sequencing data. Briefly, the following approach was taken: embryo material was passaged in BHK-21 cells grown in Eagle's minimum essential medium containing 1% fetal bovine serum (FBS).

Tissue culture material was harvested following complete destruction of the monolayer. After centrifugation, total RNA was extracted from the cell pellet using TRI-REAGENT (Molecular Research Center, Cincinnati, USA, Catalogue number TS-120) according to the manufacturers' instructions. Single-stranded RNA was removed by precipitation with 2 Molar lithium chloride and double stranded RNA was purified using Qiagen silica-based technology (Whitehead Scientific, Qiagen, Hilden, Germany). The BTV RNA was sequenced using the Roche 454 GS FLX technology (Roche, Connecticut, USA) at Inqaba Biotec in Pretoria South Africa. Genomes were assembled using Lasergene 8 software package from DNAstar (Wisconsin, USA). Assembled sequences were subjected to Blast analysis using BlastN for nucleotide and BlastP for protein sequences. Two of the isolates could be verified as BTV-3.

Bluetongue in alpacas has previously been reported from other countries; including UK (Heinrich *et al.* 2007) (London), USA (Ortega *et al.* 2010) (California) and Llamas (Meyer *et al.* 2009) from France. The first SA case of BTV infection in an alpaca was reported and confirmed from the Montagu district at the Western Cape (Wright 2014).

Although vaccination of alpacas against BTV is not routinely recommended, this report demonstrates susceptibility of the species when maintained in BTV endemic areas, and suggests that further research regarding immunoprophylaxis should be warranted.

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