FACULTY OF VETERINARY MEDICINE

IMMUNOGENICITY ANALYSIS OF BPV-1 POSITIVE EQUINE SARCOID-DERIVED CULTURED FIBROBLASTS

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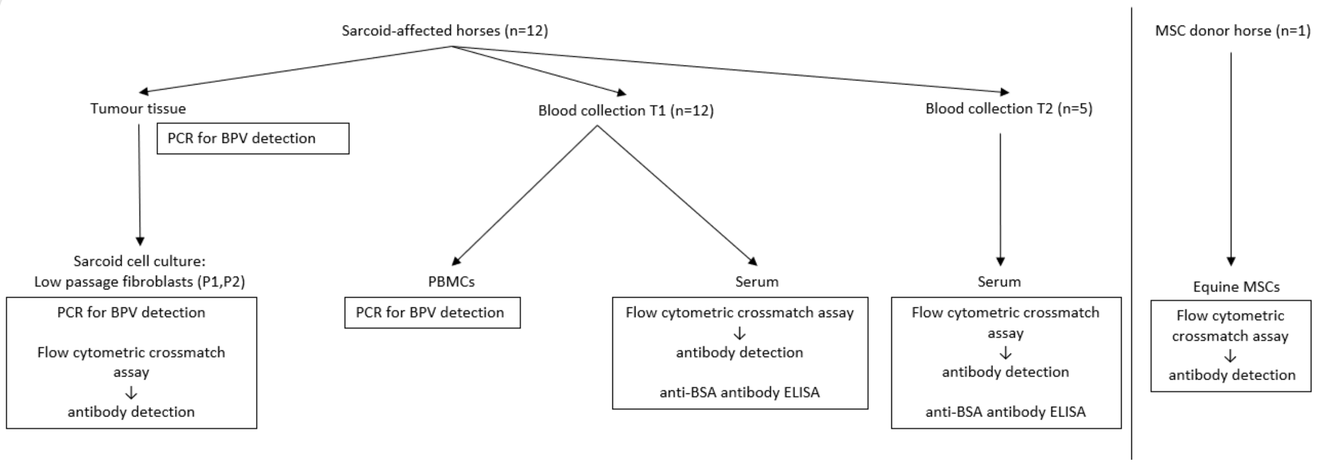
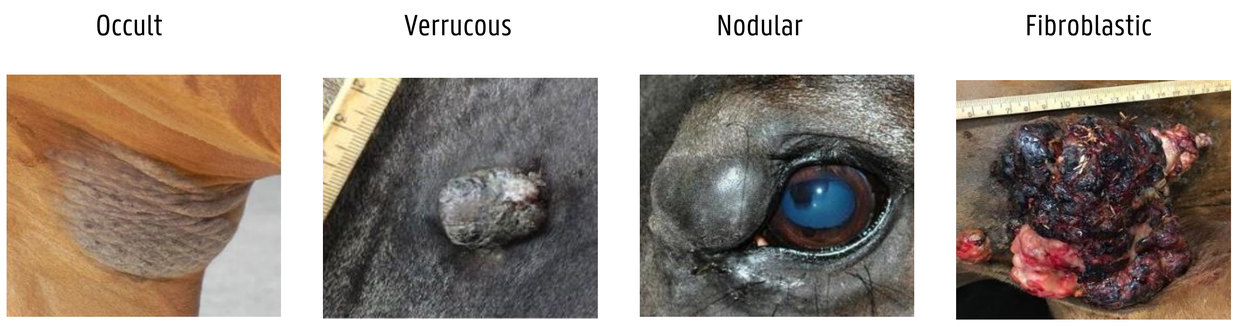
Background

Sarcoids are the most common **equine skin tumours**. Although they do not metastasize, they can be locally aggressive and cause significant clinical symptoms in affected horses. Despite being common, very little is known about the host immune response and the biological mechanisms underlying persistence and recurrence of equine sarcoids. The latter reflects the need for further research in this field. This **in-vitro** study used sarcoid explants from horses with naturally occurring sarcoids (n = 12) with the **aim** to evaluate the induction of a **humoral immune response** directed against equine sarcoid-derived bovine papilloma-virus **(BPV) type 1** infected fibroblasts using a flow cytometric crossmatch assay. The presence of antibodies against exogenous bovine serum albumin (BSA) and fibroblast-like mesenchymal stromal cells (MSCs) was also evaluated by **ELISA** and **flow cytometry**, respectively. The viral load in the sarcoid explants, the corresponding cultured sarcoid fibroblasts, and matched peripheral blood mononuclear cells (PBMCs) from affected horses were determined by quantitative BPV type 1/ type 2 **PCR analysis.**

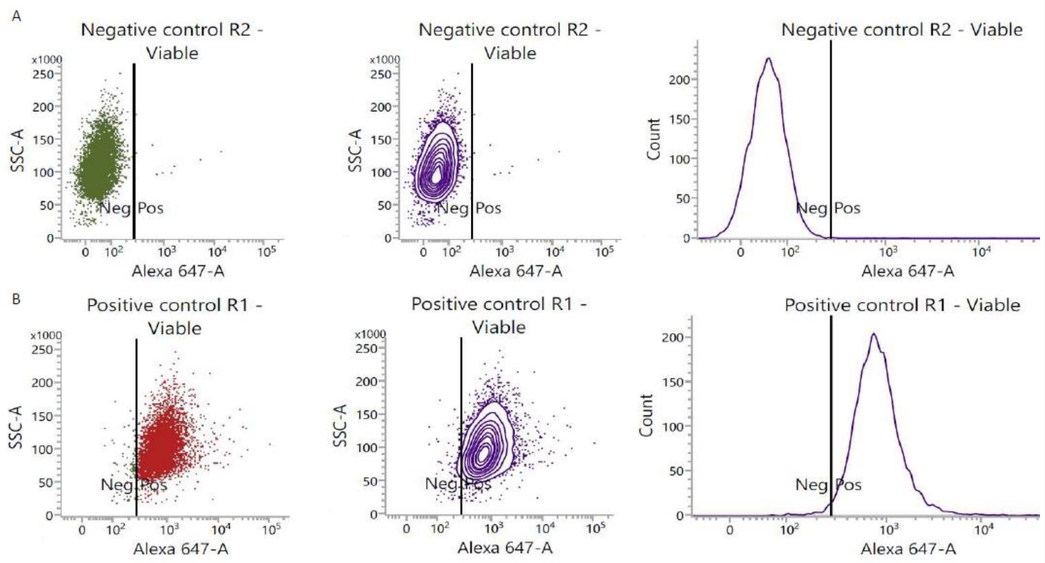
Material and Methods

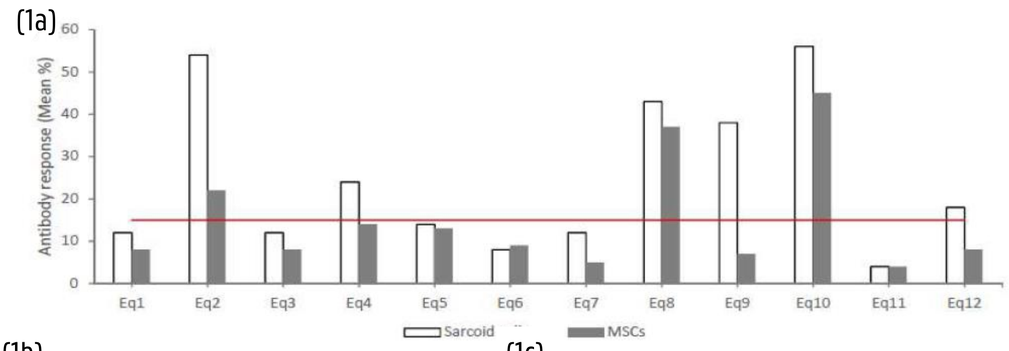


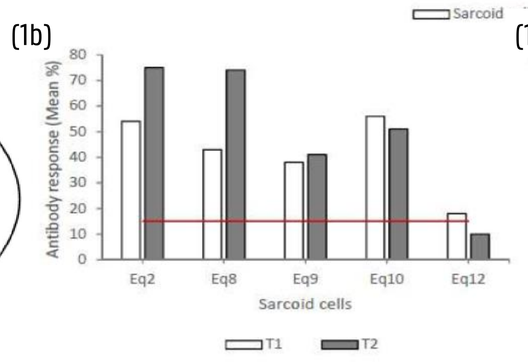
Samples

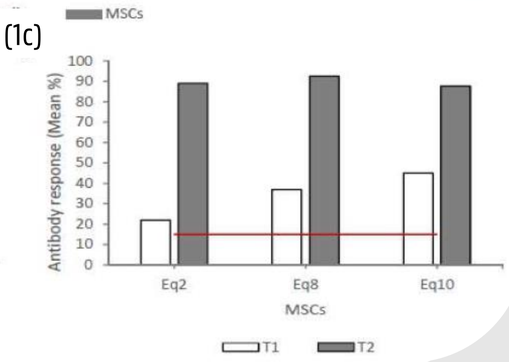


Flow cytometry

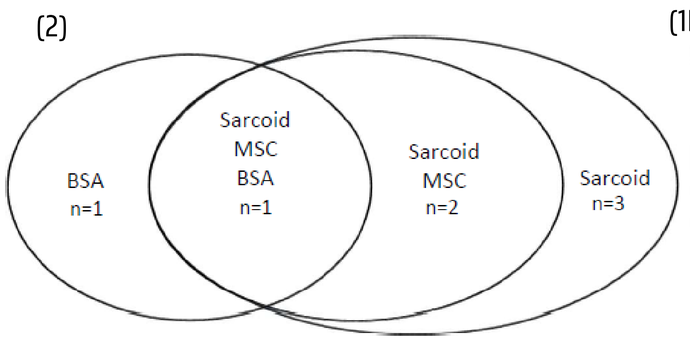




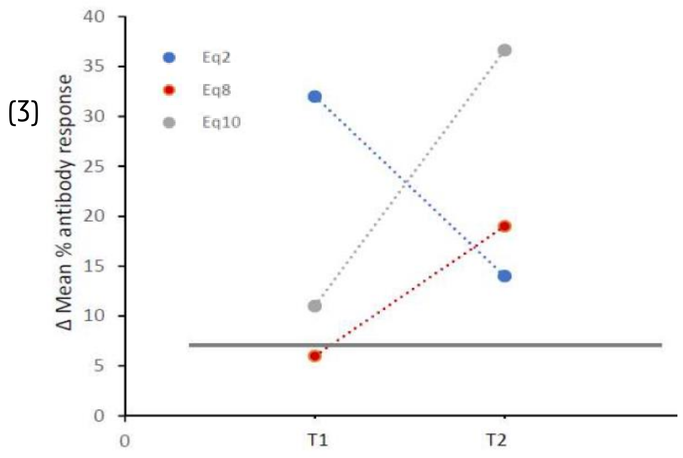




ELISA



Crossmatch



(A) Tumour **cells** originating from sarcoid-bearing horses without humoral response against autologous sarcoid cells.

Sera from horses with a positive humoral response against autologous sarcoid cells.

(B) Tumour **cells** originating from horses with humoral response against autologous sarcoid cells.

Results

Discussion

(1a) Six out of twelve sarcoid-affected horses revealed the presence of autologous anti-tumour antibody development, with three of them (Eq 2, 8 and 10) also reacting to the allogeneic MSCs exhibiting fibroblast-like morphology **(2)**.

(1b,c) In one of these horses (Eq 2), anti-BSA antibodies were detected. However, comparison between the antibody responses towards autologous sarcoid cells and allogeneic MSC at the time of surgical excision (T1) and one year later (T2), given that the anti-BSA antibodies remained at a constant level, indicates that the anti-sarcoid antibody population in Eq2 does not appear to be exclusively directed towards BSA.

(3) For Eq 2, 8 and 10, the antibody response to sarcoid cells was measurably higher compared to the response to MSCs, on at least one of both timepoints.

(4) The flow cytometric crossmatch assay identified cross-reaction between serum containing autologous anti-sarcoid antibodies and allogeneic sarcoid cells derived from horses with and without an antibody response to autologous sarcoid cells.

In **conclusion**, this study adds to the evidence that horses with sarcoids develop specific anti-tumour antibodies. All parental sarcoid tissue explants and matched cells scored BPV-1 positive in PCR analysis with the latter exhibiting a lower viral load.
In contrast, it was not possible to detect BPV nucleotides in equine PBMCs from horses with sarcoids.

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