DOI: 10.1111/j.1365-3164.2008.00698.x

Sarcoids in two captive tapirs (*Tapirus bairdii)*: clinical, pathological and molecular study

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Sources of Funding
This study is self-funded.
Conflict of Interest
No conflict of interest has been declared.

Abstract

This case report describes for the first time sarcoids in tapirs (*Tapirus bairdii*), namely, a 2-year-old male and a 3.6-year-old female born and housed at the same facility. The male presented with a 3-cm nodular, red, pedunculated, hairless, ulcerated mass on the inner surface of the left pinna. No recurrence or additional growths were present during the 3 years following surgical excision of the mass. The female presented with a similar 2-cm mass on the inner surface of the right pinna, which recurred 2 months following surgical excision, but was subsequently successfully treated locally with liquid nitrogen with no further recurrence during a 2-year follow-up period. Histologically, these two masses closely resembled equine sarcoids. Similarly, an association with bovine papillomavirus 1 was demonstrated using polymerase chain reaction and *in situ* hybridization.

Accepted 7 August 2008

Introduction

The order *Perissodactyla* (odd-toed ungulates) consists of three families: Equidae (horses, zebras and donkeys), Rhinocerotoidea (rhinoceros) and Tapiridae *(Tapirus bairdii*, *Tapirus terrestris*, *Tapirus indicus* and *Tapirus pinchaque*).[1] Tapirs became extinct across most of North America and *T. bairdii* (Baird's tapir) is found only in Central America and is the only member of the *Tapiridae* family found in Costa Rica.[2] Tapirs are primarily forest animals that usually inhabit wet locations and swamps and feed on soft vegetation. They typically weigh 250–300 kg and are approximately 1.5 m in height. One of the most recognizable features of the tapir is the short prehensile trunk that looks and functions like a shortened version of that of the elephant.

The term 'sarcoid' was introduced in 1936 to describe a unique locally invasive fibroblastic nonmetastatic cutaneous neoplasm. Equine sarcoids have been reported in horses, donkeys, mules and zebras and are the most common skin neoplasm in horses accounting for 35 to 90% of cutaneous neoplasms.[3] In a study including donkeys and mules, 86% of cutaneous neoplasms were sarcoids, compared to 51% of horse cutaneous neoplasms.[4] Sarcoids have also been reported in nonequine species, including felids,[5–7] cervids[8] and camelids.[9] A causal relationship between equine sarcoids and bovine papillomavirus-1 and -2 (BPV1 and BPV2) has been confirmed by DNA *in situ* hybridization (ISH) that indicates a nonproductive infection in nonbovine species.[10,11] In the literature, reports of neoplastic processes in tapirs are uncommon.

This report describes, for the first time, clinical and histopathological findings of neoplasms resembling equine sarcoids in two captive tapirs from Costa Rica. Molecular studies to investigate an association with BPV type 1 or 2 infection were performed.

Case reports

Case histories and clinical findings

The first case was a 2-year-old male tapir that had for 7 months an actively growing mass on the inner surface of the left pinna that had reached a size of 3 cm in diameter at the time of presentation. The mass was nodular, red, pedunculated, hairless and ulcerated (Fig. 1). The second

Figure 1. Skin; tapir, case 1. Nodular, 3 cm diameter, pedunculated, alopecic, ulcerated mass on the left pinna.

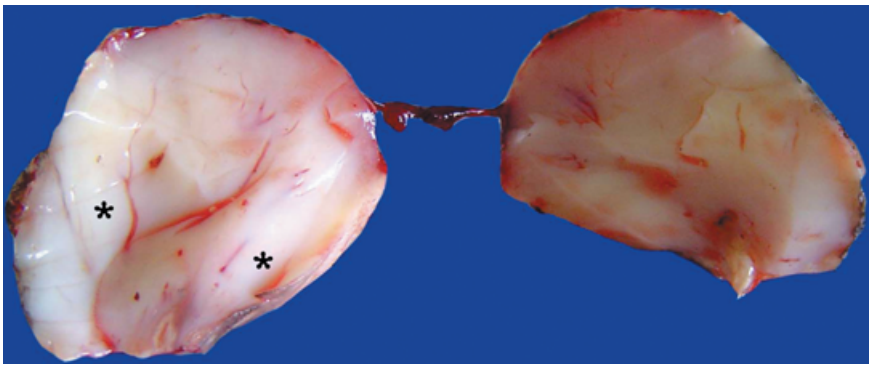

Figure 2. Skin; tapir, case 1. The mass on the left pinna sliced open to demonstrate interweaving bands of white tissue (*).

case was a 3.6-year-old female tapir with a similar nodular, red, pedunculated, hairless, ulcerated mass on the inner surface of the right pinna that had been growing slowly over the previous 8 months to a size of 2 cm in diameter at the time of presentation. Physical examination of both tapirs at the time the masses were noticed did not reveal any other abnormalities. Both tapirs were born in captivity at the La Marina Rescue Center, Costa Rica, and were closely related sharing a common grandfather. In addition, six other tapirs of different ages, 40 cows, 10 horses, eight pigs and one goat were at the rescue centre. The tapirs had never been in direct contact with one another and were housed in separate natural field enclosures, separated by a distance of about 150 m, with free running water and swamps within the enclosures. The rescue centre was separated by a fence from a dairy farm. Ten months elapsed between the onset of the sarcoids in the two tapirs. A sarcoid was diagnosed by biopsy from a horse housed at the same facility, approximately 6 months after receiving the biopsy from the first tapir sarcoid.

The masses from the tapirs were surgically excised, fixed in 10% neutral buffered formalin and then processed routinely through graded ethanols and xylene to paraffin wax. Tissue sections (3–4 μm) were cut and stained with haematoxylin and eosin. Case one was followed for 3 years and no recurrence or new growth formation was observed. Recurrence was, however, evident in case two, 2 months following excision of the mass, which was then treated locally with liquid nitrogen. No further recurrence was observed during a 2-year follow-up period.

Histopathological findings

The masses from both tapirs had similar gross and histopathological findings. Both nodules were firm and when sliced the interior revealed interweaving bands of white tissue (Fig. 2).

Histologically, the nodular masses were nonencapsulated containing a dermal proliferation of neoplastic cells that were confluent with the epidermis and extended into the subcutis. The epidermis showed areas of ulceration associated with numerous bacteria and cellular debris. The nonulcerated areas demonstrated epidermal hyperplasia with prominent rete pegs extending into the dermis (Fig. 3). Both masses were composed of densely packed spindle and stellate cells, haphazardly arranged in fascicles

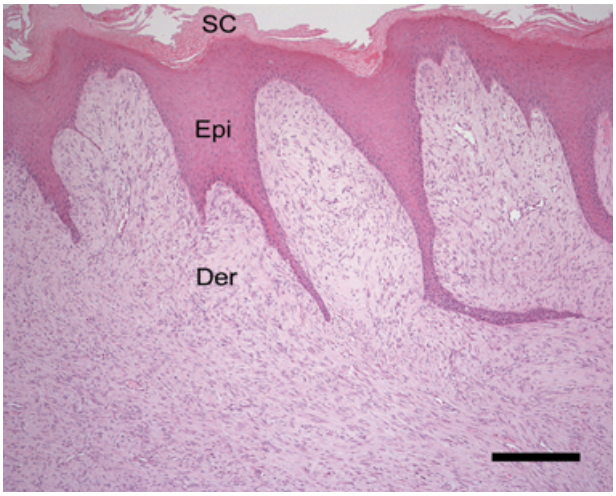

Figure 3. Skin; tapir, case 1. Epidermal hyperplasia (Epi = epidermis) with prominent rete pegs extending into the dermis (Der). SC, stratum corneum. Haematoxylin and eosin. Bar = 250 μm.

and interlacing bundles separated by small amounts of collagenous nonvascularized stromal tissue. The neoplastic cells had indistinct cellular borders, scant eosinophilic cytoplasm, oval to elongated nuclei showing moderate anisokaryosis and single, centrally located nucleoli. Occasional mitotic figures were present. The histopathologic findings of a thickened epidermis with prominent rete pegs extending into a dermal proliferation of fibroblasts were similar to those of sarcoids observed in horses.

Molecular analysis

Polymerase chain reaction assay and nucleotide sequencing

A tissue block from neoplastic sarcoid tissue from each of the tapirs was utilized for the polymerase chain reaction (PCR) assay. A primer set referred to as E5+ and E5– was used to amplify a 244-base pair (bp) or a 248-bp fragment of the BPV1 or BPV2 E5 gene, respectively.[11] PCR, nucleotide sequencing and analysis of sequence data were performed as previously described,[12] and the latter were compared with nucleotide sequences from other papillomaviruses using GenBank's automated alignment-search programme,

BLAST (www.ncbi.nlm.nih/gov/BLAST). Bovine papillomavirus DNA was detected in the sarcoid tissue from both tapirs and a 244-bp PCR product was visualized on agarose gel; the nucleotide sequences were identical in both instances. The sequences demonstrated 99% identity to BPV1 (GenBank accession number gi 60965) with the exception of two nucleotides. In tissues from both tapirs, a nucleotide substitution of guanosine for adenosine was found at site 3937 and there was one deleted nucleotide at site 3769.

Nonradioactive in situ *hybridization*

The BPV probe was prepared as previously described[11] using the same PCR procedure as described above, except for inclusion of digoxigenin-11-dUTP (Boehringer Mannheim, Laval, Quebec, Canada). The probe was purified by washing in lithium chloride and ethanol according to standard molecular techniques. A MicroProbe work station (Fisher Scientific, Ottawa, Ontario, Canada) was used to accommodate handling of tissue sections and to provide controlled reaction temperatures. The procedure used was similar to a previously published method[13] with some modifications as follows. The tissue sections were deparaffinized in xylene and rehydrated by passage through graded alcohols to 1× Automation buffer (a rinsing buffer for use in autostainers employing capillary action technology), pH 7.4 (Biomedia Corp., Foster City, CA, USA). To enhance probe penetration, the tissue sections were digested in 0.25% pepsin in 1× Automation buffer, pH 2.0, for 10 min at 37 °C, followed by incubation at 105 °C for 8 min. The tissue sections were washed by dipping five times in 1× Automation buffer, pH 7.4, prehybridized using 100% formamide treatment for 5 min at 105 °C, then hybridized to the digoxigenin-labelled BPV probe [1 : 5 dilution in hybridization solution consisting of 22.5% deionized formamide, 7.5% chondroitin sulphate, ×5 saline sodium citrate (SSC), and 50 mM sodium phosphate] for 5 min at 105 °C and then overnight at 33 °C. The sections were subjected to three stringent washes in ×0.5 SSC followed by three stringent washes in 0.2× SSC, both at room temperature, then incubated in 0.2× SSC 5 min at 37 °C. The hybridized probes were visualized immunohistologically by an alkaline phosphatase-conjugated antibody against digoxigenin according to the manufacturer's recommendations (Boehringer Mannheim). Nitroblue tetrazolium dye served as the chromogen and the presence of blue-black pigment corresponded to the presence of BPV nucleic acid. Tissue sections were rinsed in distilled water and subsequently counterstained with 1% fast green to which glacial acetic acid has been added, then rinsed in distilled water, cover slipped using Crystal mount and examined using a light microscope. Tissue from a bovine papilloma served as a positive control and hepatic tissue from a cat as a negative control. Tapir sarcoid tissue subjected to the above procedure except using an unlabelled BPV probe provided an additional negative control. ISH demonstrated positive staining in the nuclei of a few neoplastic cells in the dermis (Fig. 4), indicating the presence of BPV nucleic acid in both tapirs, but no staining was evident in the epidermis. The positive control sections had staining in epidermal cells and the negative controls were negative.

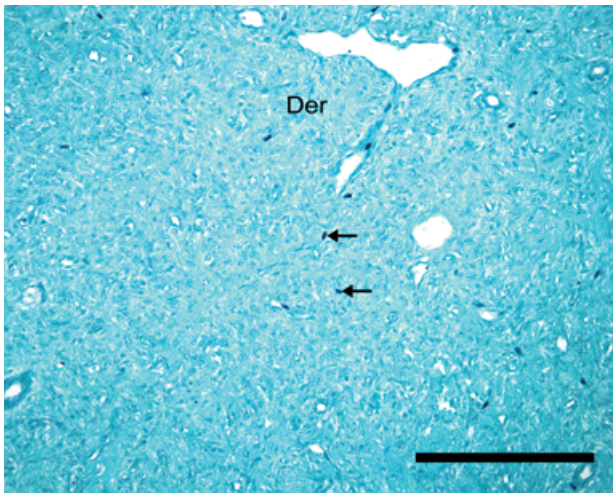

Figure 4. Skin; tapir, case 1. *In situ* hybridization with bovine papillomavirus-specific probe demonstrating positive staining in nuclei (arrows) of a few neoplastic cells in the dermis (Der). Fast green (1%) counterstain. Bar = 250 μm.

Discussion

The cause of equine sarcoids is believed to be viral, a belief based on the common finding of BPV type 1 or 2 DNA within them using PCR or ISH.[11,14] BPV viral particles are not found within these tumours and antigen cannot be demonstrated using immunohistochemistry (IHC); the virus apparently cannot replicate in the fibroblasts that it infects and hence the infection is nonproductive. In both tapirs in this report, the BPV DNA detected using PCR was located within the neoplastic mesenchymal cells using ISH, a finding similar to that reported in sarcoids of other equine species including horses[11,14] and zebras.[10] Moreover, the finding of the same BPV1 nucleotide substitutions in both tapir sarcoids suggests inherited substitutions, and possibly descent of these organisms from a common ancestor. It is interesting to note that the nucleotide substitution at 3937 is the same as the Swiss I E5 open reading frame sequence variation previously reported in a high proportion of equine sarcoids.[15]

Although equine sarcoids may occur anywhere on the body, the majority are found on the head (especially on the pinnae, commissures of the lips and periocularly), neck, legs and ventral body surface.[3] These locations are areas of either thin skin or skin susceptible to trauma, allowing infection of the skin by the virus. In one study involving an outbreak in a population of Cape mountain zebra in the Gariep Nature Reserve, a difference in location was noted between males (55% in inguinal area) and females (41% on the head and neck).[16] In the two tapirs described in this report, the tumour was located on the pinnae. This anatomic location has been associated with biting, rubbing, fomites, and insects; factors considered as possible mechanisms of viral spread to other areas on the same horse and possibly to other horses. The precise mode of transmission of BPV in equine sarcoids remains unclear. Hypotheses for infection of horses include direct or indirect contact with other infected horses and cattle; transmission by insects may also play a role.[17] Circumstantial evidence suggests that flies are involved in

the pathogenesis and epidemiology of equine sarcoids.[18] There may be a predilection for sarcoid development at wound sites and it has been proposed that this may be due to flies acting as a vector as they move between wound sites on different horses or BPV infection may be transmitted via stable management practices, such as sharing contaminated tack.[19] The source of BPV infection in the tapirs in this report has not been elucidated however, cattle may have played a role. As the San Carlos region in which the centre is located is a major milk production region of Costa Rica, there is a high density of dairy cows in this area; 40 dairy cows housed adjacent to the rescue centre and separated by only a fence from animals in the facility may have provided a possible source of BPV for the tapirs. Since a sarcoid was diagnosed by biopsy from a horse housed at the same facility during the same time period during which the tapir sarcoids were diagnosed, all three animals may have contracted the BPV infection from the same source.

Another factor that may be involved in equine sarcoid pathogenesis is viral latency. BPV DNA has been detected on the normal skin of horses, mainly sarcoid affected horses and horses living in contact with cattle having recently suffered a papillomavirus infection, but also in 50% of horses living in contact with sarcoid-affected horses and in 30% of healthy control horses.[17,20]

Genetic predisposition may also play a role in sarcoid development; the incidence of sarcoids is reportly higher in certain breeds of horses.[19] The two tapirs in this report were closely related and shared a common grandfather, therefore genetic factors may have contributed to the development of sarcoids in these tapirs.

The clinical aspect of both neoplasms was very similar to nodular sarcoids described in horses.[21] Furthermore, the histopathological findings observed in these two cases were very similar to those reported for equine sarcoids, including fibroblastic proliferation with associated epidermal hyperplasia. The tapir sarcoids did not metastasize and this is similar to their counterparts in other species. Recurrence after surgical excision occurred only in case two. Recurrence following wide surgical excision of sarcoids in horses has been reported to occur in 50–72% of cases within 6 months,[3] although lower recurrence rates were reported when a wide margin of normal skin was excised and rigorous measures taken to avoid autoinoculation.[22]

In summary, this report documents the first described cases of sarcoids in tapirs and an association with BPV was documented using PCR and ISH.

Acknowledgement

The authors thank Christian Gonzalez, veterinarian (La Marina Rescue Center, Costa Rica) for assistance with these cases.

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Résumé Cet article rapporte pour la première fois des sarcoides chez des tapirs (*Tapirus bairdii*), un mâle de deux ans et une femelle de 3.6 ans nés dans le même endroit. Le mâle présentait une masse nodulaire, rouge, pédonculée, alopécique et ulcérée de 3 cm sur la face interne du pavillon auriculaire gauche. Aucune rechute ou récidive n'a été notée trois ans après l'éxérèse chirurgicale de la masse. La femelle présentait une masse similaire de 2 cm sur la face interne du pavillon auriculaire droit, qui a récidivé duex mois après exérèse chirurgicale mais qui a été ensuite traitée efficacement par l'application locale de nitrogène liquide sans récidive après un suivi de deux ans. Histologiquement, ces masses ressemblaient fortement à des sarcoides équins. Une association avec le papillomavirus bovin 1 a été démontrée en utilisant une réaction de polymérase en chaîne et une hybridization in situ.

Resumen Este reporte de caso describe por primera vez la presencia de sarcoides en tapires (*Tapirus bairdii*) en un macho de dos años y una hembra de 3.6 años nacidos y mantenidos en las mismas premisas. El macho presentó un nódulo de 3 cm, rojo, pedunculado, alopécico y ulcerado en la superficie interna de la oreja izquierda. No se observó recidiva or crecimientos adicionales durante los tres años que siguieron a la cirugía. La hembra presentó un nódulo similar en la superficie interna de la oreja derecha, que recurrió dos meses tras la cirugía, pero después fue tratado con éxito mediante nitrógeno líquido tópico sin nuevas recurrencias durante los dos años de seguimiento. Histológicamente estas masas semejaban sarcoides equinos. También se demostró una asociación con papilomavirus bovino 1 mediante técnica de polimerasa en cadena e hibridación *in situ*.

Zusammenfassung Dieser Fallbericht beschreibt erstmals Sarkoide bei Tapiren (*Tapirus bairdii*) und zwar bei einem zwei Jahre alten männlichen und einem 3.6 Jahre alten weiblichen Tier, welche in derselben Anlage geboren und gehalten wurden. Das männliche Tier wurde mit einer 3 cm großen nodulären, roten, gestielten, haarlosen, ulzerierten Masse an der inneren Oberfläche der linken Ohrmuschel präsentiert. Drei Jahre nach der chirurgischen Entfernung der Umfangsvermehrung gab es weder ein erneutes Auftreten noch ein zusätzliches Wachstum. Das weibliche Tier wurde mit einer ähnlichen 2 cm großen Umfangsvermehrung an der inneren Oberfläche der rechten Ohrmuschel präsentiert, welche 2 Monate nach der chirurgischen Entfernung wiederkehrte, aber daraufhin lokal mit Flüssigstickstoff erfolgreich behandelt wurde und in einem weiteren 2 jährigen Beobachtungszeitraum nicht mehr auftrat. Histologisch waren beide Massen den equinen Sarkoiden sehr ähnlich. In gleicher Weise wurde ein Zusammenhang mit dem bovinen Papillomavirus 1 mittels Polymerasekettenreaktion und *in situ* Hybridisierung demonstriert.