Department of Companion Animals

University of Veterinary Medicine Vienna

Clinical Unit of Equine Internal Medicine

Head: Prof. Dr. Jessika-Maximiliane V. Cavalleri

Equine papillomavirus type 2-associated, carcinomatous lesions of the penis and laryngopharynx of an elderly Icelandic horse gelding

Equine Papillomvirus Typ 2 – assoziierte, karzinomatöse Läsionen am Penis und Laryngopharynx eines älteren isländischen Wallachs

Diploma thesis submitted for the fulfilment of the requirements for the degree of

Magistra Medicinae Veterinariae

University of Veterinary Medicine Vienna

submitted by

Dilara Lale

Vienna, Dezember 2022

First Supervisor: Prof Dr. Jessika-Maximiliane V. Cavalleri
Clinical Unit of Equine Internal Medicine
Department of Companion Animals
University of Veterinary Medicine Vienna

Second Supervisor: Dr. A. Sophie Ramsauer
Clinical Unit of Equine Internal Medicine
Department of Companion Animals
University of Veterinary Medicine Vienna

CONTENT

ABBREVIATIONS

BLAST	Basic local alignment search tool
DNA	Desoxyribonucleinacid
EcPV2	Equus caballus papillomavirus type 2
e.g.	Exempli gratia
HPF	High power field
HPV	Human Papilloma Virus
HSCC	Head squamous cell carcinoma
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase-chain-reaction
PVs	Papillomaviruses
SCC	Squamous cell carcinoma
TNM	Tumour classification system

1. INTRODUCTION

Data on equine papillomavirus type 2 (EcPV2) infection in association with equine head squamous cell carcinomas (SCCs) - located in the oral cavity, nasal cavity, sinuses, pharynx, and larynx - are limited. In equids, the infection routes of EcPV2 and the affecting factors for the development to a neoplastic lesion have not been elucidated and are still under investigation. Initial detection and early treatment of equine HSCCs (head squamous cell carcinoma) can be challenging. This manuscript briefly summarizes the recent literature on the EcPV2-associated penile and HSCCs in horses and papillomavirus (PV) associated SCCs in humans.

According to the context, the manuscript presents a case report describing a gelding that developed a carcinoma in situ in the genital region following a SCC in the laryngopharyngeal region that was probably related to the same EcPV2 infection. In this manner, possible EcPV2 infection pathways are discussed and the consequence of disease progression, if not treated and recognized early, is explained.

1.1 Squamous cell carcinoma in horses

Horses are known to have squamous cell carcinoma (SCC) as one of the most commonly seen neoplasms of the upper respiratory and gastrointestinal tract (Jones 1994). When considering the genital tract in male horses, in the penile region SCC is the most frequently detected tumour type (ibid.; Brinsko 1998). Further on, as the second most common neoplasm of the cutaneous region in equids, SCC is detected in the skin and mucous membranes of the urogenital tract, perianal area, ocular region, ear canal, and hoof (Durham 1997; Valentine 2006; van den Top et al. 2008; Scott 2011; Taylor et al 2013).

Specific breeds like Appaloosas, Pinto, and American Paint Horses that have unpigmented hair or skin adjacent to their mucocutaneous junctions are at increased risk to develop SCC. The Haflingers, although they have pigmented skin and hair, are also at increased risk for the development of SCC, especially in the ocular region (Reed et al. 2018). Increased occurrence of ocular SCC has been reported in draft breeds and genetic factors are discussed among the associated risk factors (King et al. 1991; Knickelbein et al. 2020).

SCC starts to differentiate at the keratinocyte level. Histology reveals irregular or cord-like cell formation of keratinocytes and infiltration into the dermis. Common findings are atypia, keratin formation, horn pearls, intracellular bridges, and mitoses (Scott 2011).

The initial clinical signs of SCC include changes in keratinocytes, which may manifest as mild desquamation, thickening, and ulceration. The lesion may become erosive or productive (Reed et al. 2018). The appearance of ulcerative lesions might be mistaken by the clinician for non-healing wounds or chronic granulation tissue, which could lead to incorrect or delayed treatment of the lesions (Auer 2012). Benign hyperplasia, papilloma and carcinoma in situ can be the initial lesion types occurring that are also known as precursor lesions in the early disease progression. The SCCs can develop from those precursor lesions (Bogaert et al. 2012; Ramsauer et al. 2019b).

The aetiopathogenesis of SCC is still unclear but multiple risk factors are reported and discussed. Depending on the region of occurrence, a history of sunburn, ultraviolet radiation, and lack of pigmentation is reported. Papillomavirus infections have also been discussed as an initial cause of SSCs among risk factors, especially since the detection of the equine papillomavirus type 2 (EcPV2) and its association with SCCs (Scase et al. 2010; Scott and Hughes 2017; Sykora et al. 2017).

Treatment options might be limited due to inaccessible location, as is the case with HSCCs on the head (Jones 1994). Although, there are a variety of treatment options (e.g. topical treatment or/and even extensive surgical excision), equids that are affected by SCC might have a poor life quality, and even fatal outcomes are reported. In most cases, this depends on early detection and progression of the lesion (van den Top et al. 2010).

Metastasis to the regional lymph nodes and/or recurrences have been described even after surgical removal (Mair et al. 2000; van den Top et al. 2008). In general, it takes a long time for SCC to metastasize, but in reported cases, metastasis rates range from 2% to 18.6% (Reed et al. 2018).

1.1.1 Equine Papilloma Virus Type 2 and the association with squamous cell carcinoma

Papillomaviruses (PVs) are known to infect humans and animals. They are considered a risk factor for the development of numerous benign and malignant tumours of the skin and mucous membranes (Doorbar et al. 2012). The EqPV2, which belongs to the family of PVs, was first detected in a genital lesion in 2010, and evidence for an association of genital SCC with EqPV2 infection increased with ongoing publications (Scase et al. 2010; Lange et al. 2013; Sykora et al. 2017). In general, the target cell type of PVs and EqPV2 are keratinocytes and they usually infect their specific hosts. The infection begins when micro-abrasions of the skin occur and the virus particles (virions) reach access to the basal keratinocytes (Doorbar 2006; Sykora et al. 2017). The infected cells of the basal layer can harbour the genome of the virus as episomes. The infection might be productive, when new infectious particles are produced and shed via desquamation. The viral gene expression is strictly controlled as the infected cells replicate in the direction of the superficial epithelial layers (Doorbar 2006). An abortive infection can occur in cancerous lesions, when the gene expression of the virus might get deregulated, and the life cycle of the virus cannot be completed. This is frequently caused by carcinogenic PVs since they possess the capability to transmit their functional DNA into the genome of the organism they invade. This is seen as a hallmark of transformation to malignity (Chambers et al. 2003; Doorbar et al. 2012; Sykora and Brandt 2017). Integration is described for the high-risk human PVs (human PV types 16 and 18) that are associated with carcinogenesis and metastasis in cervical cancer (Doorbar 2006), as well as for EcPV2 (Sykora and Brandt 2017; Ramsauer et al. 2019a). Clinically visible lesions develop upon excessive cell proliferation, induced by overexpression of the PV oncoproteins E6, E7, and for certain types of E5 (Doorbar et al. 2012).

For PVs, ubiquitous occurrence in healthy skin of humans and other vertebrates without clinical signs are also reported (Antonsson and Hansson 2002; Antonsson and McMillan 2006). For this reason, the presence of papillomavirus DNA alone is not sufficient to cause disease. Subclinical EcPV2 infection rate in equines ranges between 6.6 % and 29 % (Bogaert et al. 2012; Knight et al. 2013; Fischer et al. 2014; Greenwood et al. 2020). However, since its identification in 2010, EcPV2 is strongly suspected to play a key role in the aetiopathogenesis of genital precursor lesions and SCC (Scase et al. 2010; Knight et al. 2011; Bogaert et al. 2012; Zhu et al. 2015). Additionally, in some cases, EcPV2 infection was associated with the development of

SCC on the head and stomach area (Kainzbauer et al. 2012; Knight et al. 2013; Hibi et al. 2019; Alloway et al. 2020). However, investigations are still ongoing to establish the causative role of EcPV2-associated SCCs at sites other than the genital region of male equids.

1.1.2 Penile and preputial squamous cell carcinoma in horses

The most common neoplasia in the external genitalia of geldings aged between 12.4 – 21 years is SCC (Brinsko 1998). Poor genital hygiene and the presence of smegma accumulation is reported to be a predisposing factor (Sykora and Brandt 2017). In equids, there is increasing evidence of a viral aetiology, as EcPV2 has been detected for the first time in both lesions, CIS and SCC of the genital region (Scase et al. 2010; Bogaert et al. 2012). In genital precursor lesions, SCCs and smegma of affected horses, up to 100 % EcPV2 DNA was detected, which supports an aetiological link between EcPV2 infection in the progression of SCCs in this region suggesting an aetiological link between EcPV2 infection and progression of SCC in this region (Scase et al. 2010; Sykora and Brandt 2017).

SCC of the genital region can impair a horse's life and can cause discomfort; the consequences can even be fatal. It is known that penile SCCs can metastasize through the lymphatic system to the lymph nodes close to the primary tumour side and occasionally to other organs (e.g Lungs). When SCC invades the corpus cavernosum, corpus spongiosum of the penis or urethra, there is a higher risk of metastases (van den Top et al. 2011). Distant metastases to other organs or tissues are less common, and the most frequently reported sites include the lungs and liver (Brinsko 1998). Other reported sites of metastases are in the abdomen and the thoracic spine (Patterson et al. 1990; Mair et al. 2000). Other reported metastases are in the abdomen and thoracic spine.

Therefore, these tumours pose a challenge for diagnosis, correct therapy, prognosis and prevention. For this reason, early detection, correct histologic classification and proper treatment might increase the patients welfare and positive outcome. To improve diagnosis, a histological classification system for EcPV2-associated genital lesions was developed (Table 1) (Ramsauer et al. 2019b).

Table 1: Histological classification of EcPV-2-associated genital lesions.

Type of lesion	***Histological changes***
Bening hyperplasia	"Thickening of the epithelium with orderly maturation and formation of broad rete ridges."
Papilloma	"Finger-like projection of moderately hyperplastic, mildly hyperkeratotic stratified squamous epithelium with normal differentiation and thin central cores of connective tissue."
Carcinoma in situ	"Transepithelial and abnormal epithelial maturation with increased variability of keratinocytes and mitotic figures in the suprabasal region. Intra-epithelial keratin pearl formation might be present."
Squamous cell carcinoma	"Infiltrative islands and trabeculae of squamous epithelial cells with or without keratinization originating from the epithelium and infiltrating the underlying dermal tissue. The keratinocytes are highly variable and might show numerous mitotic figures."

Modified from Ramsauer AS, Wachoski-Dark GL, Fraefel C, Tobler K, Brandt S, Knight CG, Favrot C, Grest P, 2019b. Paving the way for a more precise diagnosis of EcPV2-associated equine penile lesions.

Chaux et al. also introduced a histological grading system for penile SCC in men, especially involving heterogeneous tumours (tumours harbouring more than 1 histologic grade), where grading is very challenging. In this system, the areas of the presented tumour are investigated for differentiation of the tumour cells (for anaplasia). According to this, a 3-stage grading system was designed. Grade 1 tumours show minimal basal/parabasal cell atypia and consist of well-differentiated cells which are nearly not distinguishable from normal squamous cells. Tumours mainly consisting anaplastic cells were categorized as grade 3. All tumours which did not fit in to the description of grade 1 or 3 were categorized as grade 2. The presence of a grade 3 in any area of the tumour was associated with significant risk for metastasis (Chaux et al. 2009).

Tumour behaviour might be predicted by tumour grading and staging. Recently, guidelines for diagnosis and treatment of SCC in horses have been developed following human medicine. As described previously, EcPV2-associated genital lesions can be examined histologically and classified according to the cellular differentiations. The most commonly used tumour classification system (TNM) for malignant neoplasms in humans is shown in table 2 (Sobin and Fleming 1997).

Table 2: “Classification of the TNM system and staging in humans”

T - Tumour	“Describes the size of the primary tumour and the invasion into adjacent tissues. T0 indicates that no evidence of tumour is present, while T1-T4 is used to identify the size and extension of the tumour, with progressive enlargement and invasiveness from T1 to T4. Tis indicates carcinoma in situ.”
N - Node	“Describes regional lymph node involvement of the tumour. N0 indicates no regional nodal spread, while N1-N3 indicates some degree of nodal spread, with a progressively distal spread from N1 to N3.”
M - Metastasis	“Identifies the presence of distant metastases of the primary tumour. A tumour is classified as M0 if no distant metastasis is present and M1 if there is evidence of distant metastasis.”
“Stage 0 -	Indicates carcinoma in situ. Tis, N0, M0.
Stage I	Localized cancer. T1-T2, N0, M0
Stage II	Locally advanced cancer, early stages. T2-T4, N0, M0.
Stage III	Locally advanced cancer, late stages. T1-T4, N1-N3, M0.
Stage IV	Metastatic cancer. T1-T4, N1-N3, M1.”

Modified from Ryan D. Rosen, Amit Sapra ., 2022. TNM Classification. StatPearls, Bookshelf ID: NBK553187 PMID: 31985980

This system is used to ensure a consistent clinical approach, favourable prognostic outcome, and the right treatment options. Categorization is made by the malignancy and anatomical extent of a lesion (Ryan D. Rosen and Amit Sapra 2020). Similar to humans, there is an introduction of a TNM classification system for equine SCCs exists as shown in table 3 (van den Top et al. 2011; Van den Top et al. 2014). This evidence-based categorisation can improve

the application of a universal approach and help the clinician in treatment choice and reassessment after treatment.

Table 3: Proposal for a TNM classification system for penile SCCs in the horse

TNM clinical classification	
T	Primary tumour (physical examination, imaging, biopsy)
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ (PIN)
Ta	Noninvasive verrucous carcinoma
T1	T1a: Tumour invades subepithelial connective tissue of the penis or prepuce without lymphovascular invasion
	T1b: Tumour invades subepithelial connective tissue of the penis or prepuce with lymphovascular invasion
	T1c: Tumour invades subepithelial connective tissue of the penis and prepuces with or without lymphovascular invasion
T2	Tumour invades corpus spongiosum or corpus cavernosum penis
T3	Tumour invades urethra
T4	Tumour invades other adjacent structures
N	Regional lymph nodes (physical examination, imaging, biopsy)
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N2	Metastasis in inguinal lymph node(s) fixed to surrounding tissue
N3	Metastasis in pelvic lymph node(s)
M	Distant metastasis (physical examination and imaging)
M0	No distant metastasis
M1	Distant metastasis

Stage grouping

Stage 0	Tis	N0	M0
	Ta	N0	M0
Stage I	T1a	N0	M0
	T1b	N0	M0
Stage II	T1c	N0	M0
	T2	N0	M0
	T3	N0	M0
Stage IIIA	T1, T2, T3	N1	M0
Stage IIIB	T1, T2, T3	N2	M0
Stage IV	T4	Any N	M0
	Any T	N3	M0
	Any T	Any N	M1

Taken from van den Top JGB, Ensink JM, Gröne A, Klein WR, Barneveld A, van Weeren PR, 2010. Penile and preputial tumors in the horse: literature review and proposal of a standardized approach.

1.1.3 Squamous cell carcinoma of the head in horses

SCC of the oronasal, pharyngeal and laryngeal regions are less frequently reported but are the most commonly reported tumours of this anatomical region (Jones, 1994). There is no breed predilection known to this region (Reed at al, 2018; Sykora et al, 2017). Clinical signs associated with HSCC are varying; dysphagia, ptyalism, cough, choking dyspnoea, aspiration pneumonia, anorexia, weight loss, haemoptysis, nasal discharge, or fetid breath. Some cases have been be treated symptomatically without any diagnostics carried out (Auer et al, 2012). One explanation could be that access to this area could be difficult and special instruments, e.g. endoscopy, are needed, which not every clinician has. Also, the beginning of clinical signs might be too mild and the initial signs may be considered not severe enough to investigate further and undertake additional examinations. This gives the tumour time to expand, which can delay proper treatment (Schuh 1986; Jones 1994).

As the behavioral risk factors described for HSCC in humans (excessive tobacco and alcohol use or/and sexual contact) are not present in equines, the development of HSCC in equines could be influenced by other factors. In humans, a correlation is found between vitamin A intake and the reduction of SCC (Kim et al. 2019). The same mechanism in horses is questionable as there are no reports, but horses have a very high store of vitamin A in the liver, but there are deficiencies known to occur (Jones 1994). Another possibility could be that mucous membrane irritation and SCC development are related. As in humans, in horses, approximately 25 % of HSCCs are related to EqPV2 infection (zur Hausen 2009; Kobayashi et al. 2018; Canete-Portillo et al. 2019). Although HSCC is common in canine and feline species, the mechanisms underlying tumour development are not yet clear, and PVs are reported to be a rare cause of it (Munday et al. 2015).

Reports on HSCCs in horses are rare and of those cases, approximately 30 % revealed lymph node metastases. In one case, metastasis to the retropharyngeal and cervical lymph nodes occurred. Pulmonary metastases and infiltration of the vagal nerve are described, where the primary SCC spread from the tongue (Schuh 1986; Jones 1994). According to this, multiple regions in the head of the horse can be affected and metastasis can occur in the regions close and/or far to the primary tumour.

1.2 Squamous cell carcinoma in human medicine

In humans, SCC is known to be the second most common non-melanotic skin neoplasm, and approximately 20 % of presented cases are reported to be cutaneous SCCs. Risk factors include advancing age, but SCC has also been found in younger men (Motaparthi et al. 2017). UV-light exposure is frequently associated with SCC and cutaneous SCC affects mainly sun-exposed areas such as the uncovered regions of the skin (Rowe et al. 1992; Gray 1997). However, other factors are known such as prolonged inflammation, contact with carcinogenic chemicals, and additional/other radiation than UV-light exposure. Organ transplant recipients also suffer from immunosuppression, which is reported to be a risk factor as well. Organ transplant recipients also suffer from immunosuppression, which is also reported to be a risk factor (Weinstock 1989). Human papillomavirus infection is also considered to be an etiologic agent of cutaneous SCC and association exits between the different regions like oral, perianal, cervical, and external genital areas (Marks 1996; Chaux et al. 2012; Doorbar et al. 2012).

1.2.1 Human Papilloma Virus associated squamous cell carcinoma in humans

HPV, like most PVS, is known to be epitheliotropic. Skin and mucous membranes can be infected when virus particles reach and infect the basal epithelial cells of stratified squamous epithelium. After infection precursor lesions like warts and plaques or in the further stage an SCC can develop (Doorbar 2005). Approximately 450 types of HPV exist (McBride 2022)Klicken oder tippen Sie hier, um Text einzugeben.. According to their oncogenic potential HPV has been categorized as either low-risk types or high-risk types (hr-HPV) (Muñoz et al. 2006; Doorbar et al. 2012). To date, it is known that hr-HPVs have an important aetiological role in cervical cancer (Doorbar 2006). HPV-associated SCC in other locations, like the urogenital region (anus, vagina, vulva, and penis), upper and lower respiratory tract, parts of the gastrointestinal tract, and the ungual and periungual regions have been reported (Astori et al. 2001; Kreimer 2004; Varnai et al. 2006; Petersen and Klein 2008; Riddel et al. 2011). This points out the importance of HPV infection-associated SCC development in other regions than the cervix. HPV has also been etiologically associated with a subset of HSCCs (Dayyani et al. 2010). Although the association of HPV infection in cervical SCC is well known and established, the etiologic association with other regions needs further investigations (Downes 2015; Hakenberg et al. 2018; Lafaurie et al. 2018).

1.2.2 Penile and preputial squamous cell carcinoma in human medicine

The occurrence of penile tumours in humans are rare, but of those presented, approximately 95% are reported to be SCCs. The regions of glans penis and the prepuce are locations where SCC might occur. Early metastatic spread to lymph nodes and invasive growth are characteristics of this type of SCC (Hakenberg et al. 2018). Demographic status, poor personal hygiene, tobacco consumption, lack of circumcision, and sexual behaviour is associated with SCC. Approximately one-third of the cases are associated with HPV infections (Downes 2015).

1.2.3 Squamous cell carcinoma of the head in human medicine

The incidence of HSCC around the world is varying (Kreimer 2004; Dayyani et al. 2010). Two types of HSCC are described. Firstly, it can be of the keratinized type, and secondly, it can be the non-keratinized type. The keratinized type occurs in association with tobacco and alcohol consumption and has nothing to do with HPV infection. Secondly, the non-keratinizing type occurs with little exposure to tobacco and alcohol with HPV DNA being the most characteristic feature (Kobayashi et al. 2018). HPV-associated HSCCs are reported to have a better clinical outcome than non-HPV-associated HSCCs (Dalianis 2014). In contrast, some reports discuss that only in tonsillar SCC, HPV can be used as a prognostic factor and not in other regions of HSCCs (Brandwein et al. 1994; Mellin et al. 2000; Gillison et al. 2012; Lai et al. 2017). Tumours lacking HPV infection might have a large number of gene mutations that are responsible for the cell cycle regulating proteins. This could result in therapy resistance and might explain the prognostic difference of consisting HPV infection or not (Mellin et al. 2000). Involvement of HPV is reported about 25 % in HSCCs (Parkin et al. 2006; Dayyani et al. 2010).

2. PUBLICATION

Dilara Lale, Antonia Geyer, Christoph Jindra, Jessika-Maximiliane V. Cavalleri, Anna Sophie Ramsauer

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Vetrecords 2022, e508; https://doi.org/10.1002/vrc2.508

Received: 2 August 2022 | Revised: 12 September 2022 | Accepted: 14 September 2022

DOI: 10.1002/vrc2.508

Vet Record CaseReports

CASE REPORT

Horses and other equids

Equine papillomavirus type 2-associated, carcinomatous lesions of the penis and laryngopharynx of an elderly Icelandic horse gelding

Dilara Lale[1] | **Antonia Geyer**[2] | **Christoph Jindra**[3] | **Jessika-Maximiliane V. Cavalleri**[1] | **Anna Sophie Ramsauer**[1]

[1]Clinical Unit of Equine Internal Medicine, University Equine Hospital, Vetmeduni Vienna, Vienna, Austria

[2]Clinical Unit of Veterinary Pathology, Vetmeduni Vienna, Vienna, Austria

[3]Clinical Unit of Equine Surgery, University Equine Hospital, Vetmeduni Vienna, Vienna, Austria

Correspondence
Jessika-Maximiliane V. Cavalleri, Clinical Unit of Equine Internal Medicine, University Equine Hospital, Vetmeduni Vienna, Vienna, Austria.
Email: Jessika.Cavalleri@vetmeduni.ac.at

Abstract

A 28-year-old Icelandic horse gelding was presented with a laryngopharyngeal squamous cell carcinoma. The gelding had been treated for penile carcinoma in situ with a partial phallectomy 2 years earlier. Polymerase chain reaction of tumour DNA and subsequent amplicon sequencing revealed that the equine papillomavirus type 2 E6 oncogene sequences of both lesions were identical. There is strong evidence that equine papillomavirus type 2 is causally associated with genital squamous cell carcinomas and precancerous lesions. Recent reports indicate that equine papillomavirus type 2 might also play an active role in the pathogenesis of approximately 20% of equine squamous cell carcinomas in the oronasal, pharyngeal and laryngeal regions. To the authors' knowledge, this is the first report of a horse consecutively developing a penile carcinoma in situ and a laryngopharyngeal squamous cell carcinoma that were apparently induced by the same equine papillomavirus type 2 variant. Possible equine papillomavirus type 2 infection pathways in this horse and the importance of early detection of lesions are discussed in this context.

BACKGROUND

Squamous cell carcinoma (SCC) is one of the most common tumour types in horses and arises from keratinocytes.[1,2] Reported locations of SCC in the horse are the skin and mucous membranes of the ocular region, external genitalia, urogenital tract, oesophagus, stomach and head and neck.[2–4] It is the most common neoplasm of the equine penile region and upper respiratory or gastrointestinal tract.[5,6] Horses affected by SCC may experience impaired quality of life, with a potentially fatal outcome, depending on progression and therapy.[7] Treatment usually consists of extensive surgical excision. Tumour recurrence and/or metastasis to regional lymph nodes are described even after excision.[3,8] Although SCCs are frequently diagnosed, the pathogenesis is still not fully understood. Precursor lesions such as wart-like lesions (papillomas) or plaques (representing benign hyperplasia or carcinoma in situ) can develop initially, and subsequently progress to SCC.[9,10] Multiple risk factors, such as ultraviolet radiation and a history of sunburns, lack of pigmentation and papillomavirus (PV) infection—particularly equine papillomavirus type 2 (EcPV2)—are discussed in the literature, depending on the location of the lesions.[2,11,12] Since it was first reported in 2010, there is increasing evidence that EcPV2 plays an active role in the development of equine genital SCC and precursor lesions such as plaques and papillomas.[9,12–14] The type of EcPV2-associated genital lesions can be accurately determined by histological classification as benign hyperplasia, papilloma, carcinoma in situ (CIS) and SCC.[10] Although EcPV2 has also been detected in a subset of gastric SCCs and head SCCs (HSCCs) located in the oral cavity, nasal cavity, sinuses or pharynx, a causative role of the virus has not been established for SCCs in these locations.[15–18]

EcPV2 belongs to the family of PVs, which are usually host specific and have a pronounced tropism for cutaneous and mucosal keratinocytes.[11] PV infections are known to induce benign or malignant epithelial lesions in humans and various animal species.[2,19] An almost ubiquitous presence of PV DNA on the skin of humans and other vertebrates in the absence of clinical signs has also been reported for various PV types.[21,22,40] Thus, detection of PV DNA alone is not enough to deduce a causal association of infection and disease. Subclinical EcPV2 infection in horses ranges from 6.6% to 29%.[9,15,23,24] As EcPV2 was detected in up to 100% of genital SCCs and precursor lesions, and approximately 20% of HSCC, there is evidence of disease association in genital SCCs, while the causality of EcPV2 in HSCCs warrants further investigation.[9,11,12,15,20]

This case report describes a patient with potentially EcPV2-associated tumour development, initially in the genital region and later in the laryngopharyngeal region. Data about EcPV2 infection in association with equine HSCCs are limited. The early detection and treatment of equine HSCCs can be challenging. The possible pathways of EcPV2 infection and transmission within a horse, leading to tumorigenesis, are not fully understood. It should be considered that horses with SCC or any precursor lesions could harbour an EcPV2 infection, which could lead to neoplastic progression.

LEARNING POINTS/TAKE-HOME MESSAGES

- Equine papillomavirus type 2 is associated with genital squamous cell carcinomas, precursor lesions and a subset of pharyngeal squamous cell carcinomas in horses.
- In affected horses, polymerase chain reaction testing for equine papillomavirus type 2 is recommended.
- Considering the viral aetiology of the disease, risk of transmission to in-contact-horses cannot be excluded.
- Other predilection sites, including the pharynx, should be examined when a genital lesion is present.
- After complete surgical resection of lesions, routine monitoring for tumour recurrence and for further lesions is recommended.

CASE PRESENTATION

In December 2019, a 28-year-old Icelandic horse gelding was presented to the University Equine Hospital of the University of Veterinary Medicine, Vienna, with complaints of intermittent colic, anorexia, weight loss and bilateral purulent nasal discharge.

The horse had previously been presented to the University Equine Hospital in 2017 with complaints of weight loss, cachexia, and a 5 × 5 × 5 cm friable tissue lesion on the glans penis. The owners had indicated that the lesion had been present for 5 years, but that a significant increase in size had been noted during the preceding 6 months. Other horses in the herd had not shown any comparable abnormalities. The lesion was subsequently biopsied and a histopathologic diagnosis of CIS was made. The tissue sample revealed exophytic growth, but no evidence of invasion of neoplastic cells into the adjacent stroma (Figure 1a). A partial phallectomy by en bloc resection, but without penile retroversion, was performed as described elsewhere.[25] Further investigations had been carried out because of the reported weight loss. The gelding had weighed 292 kg at the time. Plasma biochemistry had revealed a mild increase of the globulin fraction (1.3 g/dl, reference range: 0.4–0.9 g/dl). No abnormalities were detected upon transrectal palpation and transcutaneous ultrasound examination of the abdomen. No indications of metastases or other causative pathology were noted on thoracic radiographs or during the oral examination. Mild elevation of the soft palate and epiglottis were detected on endoscopic examination of the upper respiratory tract, but no distinct lesions were observed and the mucous membranes were intact. The horse was discharged 28 days after surgery.

Upon presentation in 2019, the gelding had weighed 280 kg and was lethargic. A bilateral, malodorous nasal discharge was observed. Seven percent dehydration, a high-normal heart rate (44 beats per minute), tachypnoea (28 breaths per minute), bilaterally increased, harsh lung sounds upon auscultation and a normal rectal temperature (37.8 C) were recorded during clinical examination. No lesions or signs of tumour recurrence were detected in the genital region.

INVESTIGATIONS

Initial haematology and plasma biochemistry revealed no abnormalities, apart from a moderate to severe increased plasma creatinine concentration (3.1 mg/dl, reference range: 0.8–2.21 mg/dl). Endoscopic examinations of the upper airways, oesophagus and stomach were performed under sedation with xylazine (0.9 mg/kg intravenously) and butorphanol (0.01 mg/kg intravenously). In the laryngopharyngeal region, starting from the arytenoid cartilage on the right side, adherent to the lateral pharyngeal wall and the soft palate, ending approximately at the tip of the epiglottis, a friable, exophytic lesion was observed. The lesion contained multifocal erosions and ulcerations (Figure 2).

Superficial swabs of the laryngopharyngeal area were collected manually in the sedated horse, and smears were submitted for cytological examination. Biopsy samples of the lesion were taken under endoscopic guidance with a biopsy clamp through the endoscopic biopsy channel. Cytological smear examination revealed the occasional presence of dysplastic squamous cells. Histopathological examination of the biopsy revealed islands and lobules of stratified epithelial cells, located in a hyperaemic, fibrovascular stroma that matured into stratified squamous epithelium. These findings led to the diagnosis of laryngopharyngeal SCC.

No additional abnormalities were detected during further endoscopic examination of the gastrointestinal tract and rectal palpation. An increased amount of anechoic peritoneal fluid was observed during transcutaneous ultrasound examination of the abdomen. Apart from the increased volume of free peritoneal fluid, no abnormalities were detected on abdominocentesis and abdominal fluid analysis.

DIFFERENTIAL DIAGNOSIS

In the pharynx of horses, one must distinguish between pharyngeal neoplasms (such as papilloma, SCC, adenocarcinoma, fibroma and lymphosarcoma), subepiglottic cysts, abscess formation, granulomatous tissue or penetration of a foreign body. In this case, given the history of previous neoplasia, the macroscopic appearance (friable, exophytic-ulcerative lesion), the location and the initial results of the cytological and histopathological examinations, SCC was suspected.

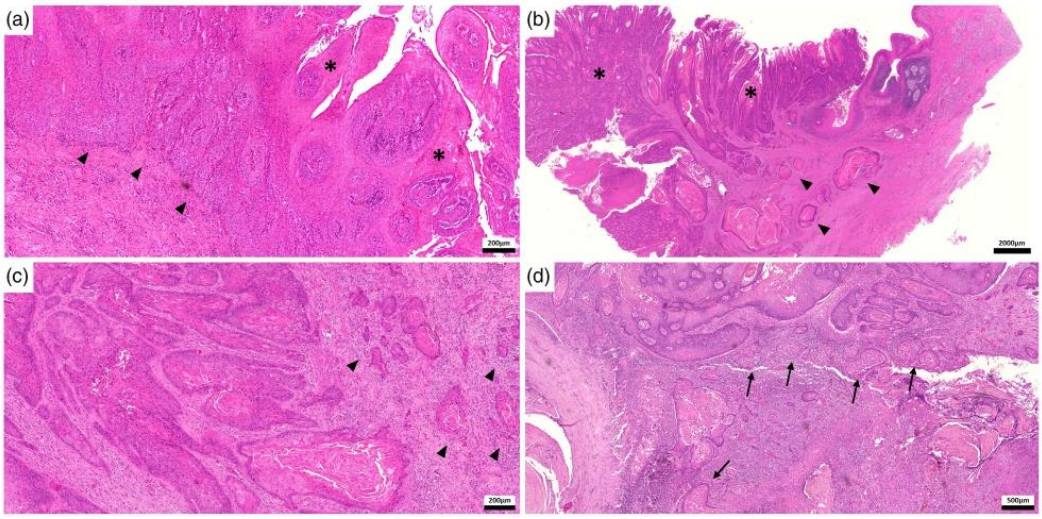

FIGURE 1 Histopathological images of the penile carcinoma in situ (CIS) and laryngopharyngeal squamous cell carcinoma (SCC). (a) CIS (penis): Exophytic growth (asterisk) but no evidence of invasion of neoplastic cells into the adjacent stroma (arrowheads). (b) SCC: Transition of the laryngopharyngeal mucosa into an SCC, showing both exophytic (asterisk) and invasive (arrowheads) growth patterns. (c) SCC: Detailed image of the neoplastic infiltration by invasive tumour islands (arrowheads). (d) SCC: Acantholytic pattern of some tumour islands embedded in the submucosal tissue (arrows), accompanied by severe lymphocytic infiltration within the stroma.

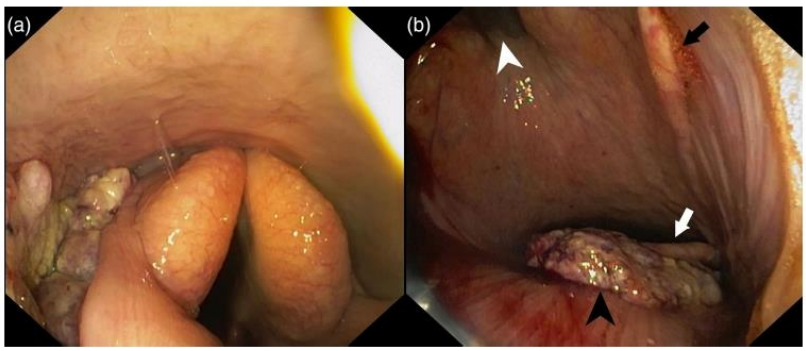

FIGURE 2 Endoscopic images of the laryngopharyngeal lesion. (a) The beginning of the lesion from the most caudal part of the laryngopharynx. (b) The extension of the lesion to the most rostral part of the laryngopharynx (black arrowhead). Notice pharyngeal recess (white arrowhead), epiglottis (white arrow) and opening of the guttural pouch (black arrow).

TREATMENT

Considering the location and extent of the laryngopharyngeal lesion, complete surgical excision was not considered possible. No therapeutic attempt was initiated.

OUTCOME AND FOLLOW-UP

The horse had been treated at the University Equine Hospital for a penile CIS already in 2017. Two years later, the horse was presented with a laryngopharyngeal SCC. On both presentations, complaint of progressive deterioration of body condition was noted. Based on the poor prognosis, the owners opted for euthanasia.

The body was submitted to the Institute of Pathology for postmortem examination. Inspection of the laryngopharyngeal cavity revealed a well-circumscribed, superficially friable, exophytic lesion (approximately 8 × 5 × 2.2 cm), with multifocal erosions and ulcerations located on the right side and rostro-laterally adjacent to the epiglottis (Figure 3). Based on inspection of the abdominal and thoracic cavities, there was no macroscopic evidence of a metastatic process.

The histopathology of the penile CIS diagnosed 2 years earlier revealed no evidence of invasion of neoplastic cells into the adjacent stroma (Figure 1a). In contrast, the laryngopharyngeal SCC revealed an abrupt transition of the squamous epithelium into a malignant, exophytic, but also infiltrative neoplastic lesion with broad trabeculae, as well as solitary, variably sized islands spreading into the submucosa (Figure 1b,c). The mitotic count varied between zero and eight mitoses per high power field (HPF), some of which were bizarre. Anisocytosis and anisokaryosis showed moderate to marked presence. Based on the histopathology, the tumour was classified as a grade I–II SCC (Broder's grading system),[26] with a multifocal, pronounced, acantholytic pattern

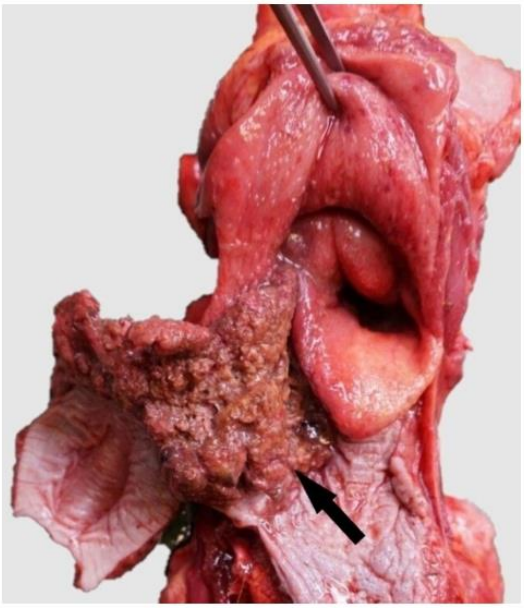

FIGURE 3 Gross image of the larynx and pharynx, including the exophytic lesion (black arrow) adherent to the lateral pharyngeal wall and soft palate.

(Figure 1d). There was no evidence of metastases in the mildly enlarged regional lymph nodes. Secondary microscopic findings were multifocal, extensive neutrophilic inflammation, multifocal foci of bacterial colonisation, as well as desmoplasia and marked to severe multifocal extensive lymphoplasmacytic inflammation within the submucosa.

EcPV2 E6 DNA was detected in both tumours by means of PCR. Fresh frozen tissue of the laryngopharyngeal SCC and formalin-fixed, paraffin-embedded tissue of the penile CIS were analysed as described previously.[10] Bidirectional sequencing of both amplimers (395 nt in size) revealed four 329–353 nt-sized sequences. Sequence alignment revealed 100% identity between both amplicon sequences on a 394 nt-sized E6 fragment. Using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi), 100% nucleotide sequence identity to the corresponding region (nt 43–436) of the EcPV2 DNA isolate Zurich_2009 (GenBank ID HM461973.1)[27] was detected. This nucleotide sequence was also identical to the corresponding region of the EcPV2 putative E6 and E7 genes variant (GenBank ID JN664040.1),[9] the E6 protein variant IV Icelandic (GenBank ID KX349721.1) and variant V (GenBank ID KX349722.1).[11,20] Parts of this sequence were previously described as Icelandic E6 variants (GenBank ID HM153759.1).[12]

DISCUSSION

Elderly horses (average age range: 12.4–21 years), as in this case, have the highest incidence for SCCs.[7] Forty-five percent of equine SCCs involve the penile region, while only 7% are diagnosed in the oronasal, pharyngeal and laryngeal regions.[4,5,28,29] Similar to humans, PV infection and chronic inflammation are risk factors for development of genital precursor lesions and SCCs.[4,11,30] Reported cases of equine HSCCs are limited.[15-17,28,31,32] Up to 100% of genital SCCs and precursor lesions and approximately 20% of HSCCs are associated with EcPV2.[9,11,12,15,20] Similarly, in humans, up to 50% of penile SCCs and 25% of HSCCs are related to HPV infections.[33–35]

The horse in this report was diagnosed with penile CIS in 2017, which was subsequently surgically removed, and in 2019 with laryngopharyngeal SCC. EcPV2 DNA was found in both lesions, with a high likelihood of the same EcPV2 variant being present in both tumours. This case raises questions about infection and transmission of EcPV2, the development of associated neoplasms, as well as the implications for management of such cases.

Similar to other reports of co-occurring EcPV2-associated lesions in the same horses, the route of transmission and pathogenesis remain speculative. One case report described both a gastric SCC and vaginal precursor lesions (vulval papilloma and CIS) in an elderly mare.[36] Another horse developed epithelial hyperplasia of the mucosa of the lips after licking and biting an EcPV2-associated SCC on its penis.[9] A horse that tested PCR-positive for EcPV2 DNA in its smegma but did not show any genital lesions, later developed HSCC.[20] Genital EcPV2 infection, with or without cancerous lesions, and SCC or precursor lesions of the upper digestive tract were present in all these cases. Thus, it is warranted to further investigate potential underlying mechanisms of transmission in the future.

The laryngopharyngeal SCC in this case could have been the result of metastasis. This, however, seems unlikely because of the distant localisation of the initial penile lesion that was resected en bloc. Lymphatic spread to regional inguinal lymph nodes would therefore be more likely. In addition, CIS does not show infiltrative growth and is limited in its extent to the epithelium.[37] Hence, the metastatic spread of neoplastic cells appears unlikely. The histological images of the penile CIS showed no infiltrative growth of neoplastic cells into the adjacent tissues. The detection of EcPV2 nucleic acid within both lesions rather supports an infectious aetiology.

EcPV2 transmission is generally not well understood. Horses with productive EcPV2 infection might shed infectious virus and could be a source of EcPV2 for in-contact horses. It is known that high EcPV2 DNA loads can be harboured in smegma, representing a potential virus reservoir.[11,12] In our case, no in-contact horses were reported to show mucocutaneous lesions. EcPV2 surveillance was not carried out, and it remains unknown whether any in-contact horses were indeed infected.

Both lesions in this gelding could have developed independently following contact with the virus. Licking and biting of EcPV2-containing smegma and/or infected genital lesions or contact with any virus-contaminated source (bedding, hay and/or insects) can result in infection of naive horses.[11] Therefore, a separate virus source, for example an infected co-stabled horse, would be as likely as the transmission of infectious viral particles from the penile lesion to the pharynx before the surgical resection.

An alternative possibility is that the two tumours developed in response to the same initial virus infection. This could have occurred months to years before tumorigenesis. Equine PV infection may spread within the host, as reported for HPV

infections.[19,38] Mild elevation of the soft palate rostral to the epiglottis was noted during the first endoscopic evaluation of the nasopharynx in 2017. It cannot be excluded that this finding represented the initial lesion, which progressed into the laryngopharyngeal SCC. The time of progression of EcPV2-associated precursor lesions to SCC has been reported to be several months.[9,15-17]

The laryngopharyngeal SCC was detected 2 years after the penile CIS. This raises the question of required time for EcPV2 infections to have a role in carcinogenesis and further related co-factors. The duration of the delay between initial EcPV2 infection and tumour development is not known.[9,11] This period is known to depend on initial control of the infection by the immune system in humans. Failure to control the infection may lead to tumour progression.[39] While some PVs can be ubiquitous in healthy humans and various animals,[21,22,40,41] the prevalence of asymptomatic EcPV2 infection on various areas of equine skin ranges between 6.6% and 29% in different studies.[9,15,23,24] Clearance of the infection as a result of immune response might be present in horses, as seropositivity for EcPV2 in horses was reported to be 36%.[23,24] Apart from this, EcPV2 can escape the immune system by several mechanisms, remain latent and become active at a later point in time.[11] Additionally, there is some evidence of host-related factors in humans and animals (such as genetic immunodeficiency or genetic predisposition), which might have a role in PV infections and subsequent development of SCCs.[42–44] This warrants further investigations in EcPV2-associated SCCs in horses.

Although the initial penile CIS was resected en bloc by partial phallectomy, a second tumour occurred that may have been unrecognised but present at the time of initial diagnosis. This indicates that horses with EcPV2-associated lesions need to be carefully checked for possible metastatic spread, and closely monitored after radical excisional surgery. Early detection of further precursor lesions, SCCs and/or metastasis in other regions could improve welfare and treatment success. Specifically, given the reported association of EcPV2 with HSCCs, regular monitoring of this region should be considered. Should further lesions occur, histological classification to identify early-stage malignant transformation is recommended.[10] Laser ablation could be useful to completely excise a precursor lesion or small HSCC following early diagnosis.[5] This could prolong the lifespan and quality of life of an affected horse. Thus, early recognition and intervention are essential in affected horses.

AUTHOR CONTRIBUTIONS

Dilara Lale drafted the manuscript and performed the clinical and laboratory investigations. Antonia Geyer performed the postmortem examination, histopathology and revised the manuscript. Christoph Jindra performed the virological investigations and revised the manuscript. Jessika-Maximiliane V. Cavalleri supervised the clinical and laboratory investigations and revised the manuscript. Anna Sophie Ramsauer conceptualised the case report, performed the virological investigations and revised the manuscript.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

FUNDING INFORMATION

The authors have not declared funding obtained for this research from any funding agency in the public, commercial, or not-for-profit sectors.

ETHICS STATEMENT

This study did not involve animal experiments. Horse sample material was derived from lesional tissue that was resected for diagnostic purposes or postmortem. The resected material was used for research purposes with the owner's consent. All procedures were performed by experienced horse surgeons in accordance with the standard operating procedures of the Vetmeduni Vienna. No ethical review and approval was required.

ORCID

Dilara Lale https://orcid.org/0000-0003-2360-8898

REFERENCES

1. Valentine BA. Survey of equine cutaneous neoplasia in the Pacific Northwest. J Vet Diagn Invest. 2006;18(1):123–6.
2. Scott DW, Miller WH. Equine dermatology. 2nd ed. Maryland Heights, MO: Elsevier/Saunders; 2011. http://site.ebrary.com/lib/alltitles/docDetail.action?docID=10494758
3. van den Top JGB, Heer N de, Klein WR, Ensink JM. Penile and preputial squamous cell carcinoma in the horse: a retrospective study of treatment of 77 affected horses. Equine Vet J. 2008;40(6):533–7.
4. Taylor S, Haldorson G. A review of equine mucocutaneous squamous cell carcinoma. Equine Vet Educ. 2013;25(7):374–8.
5. Jones DL. Squamous cell carcinoma of the larynx and pharynx in horses. Cornell Vet. 1994:84:15–24.
6. Brinsko SP. Neoplasia of the male reproductive tract. Vet Clin North Am Equine Pract. 1998;14(3):517–33.
7. van den Top JGB, Ensink JM, Gröne A, Klein WR, Barneveld A, van Weeren PR. Penile and preputial tumours in the horse: literature review and proposal of a standardised approach. Equine Vet J. 2010;42(8):746–57.
8. Mair TS, Walmsley JP, Phillips TJ. Surgical treatment of 45 horses affected by squamous cell carcinoma of the penis and prepuce. Equine Vet J. 2000;32(5):406–10.
9. Bogaert L, Willemsen A, Vanderstraeten E, Bracho MA, de Baere C, Bravo IG et al. EcPV2 DNA in equine genital squamous cell carcinomas and normal genital mucosa. Vet Microbiol. 2012;158(1–2):33–41.
10. Ramsauer AS, Wachoski-Dark GL, Fraefel C, Tobler K, Brandt S, Knight CG et al. Paving the way for more precise diagnosis of EcPV2-associated equine penile lesions. BMC Vet Res. 2019;15(1):356.
11. Sykora S, Brandt S. Papillomavirus infection and squamous cell carcinoma in horses. Vet J. 2017;223:48–54.
12. Scase T, Brandt S, Kainzbauer C, Sykora S, Bijmholt S, Hughes K et al. *Equus caballus* papillomavirus-2 (EcPV-2): an infectious cause for equine genital cancer? Equine Vet J. 2010;42(8):738–45.
13. Knight CG, Munday JS, Peters J, Dunowska M. Equine penile squamous cell carcinomas are associated with the presence of equine papillomavirus type 2 DNA sequences. Vet Pathol. 2011;48(6):1190–4.
14. Zhu KW, Affolter VK, Gaynor AM, Dela Cruz FN, Pesavento PA. Equine genital squamous cell carcinoma: in situ hybridization identifies a distinct subset containing *Equus caballus* papillomavirus 2. Vet Pathol. 2015;52(6):1067–72.
15. Knight CG, Dunowska M, Munday JS, Peters-Kennedy J, Rosa BV. Comparison of the levels of *Equus caballus* papillomavirus type 2 (EcPV-2) DNA in equine squamous cell carcinomas and non-cancerous tissues using quantitative PCR. Vet Microbiol. 2013;166(1–2):257–62.
16. Kainzbauer C, Rushton J, Tober R, Scase T, Nell B, Sykora S et al. Bovine papillomavirus type 1 and *Equus caballus* papillomavirus 2 in equine squamous cell carcinoma of the head and neck in a Connemara mare. Equine Vet J. 2012;44(1):112–5.
17. Hibi H, Hatama S, Obata A, Shibahara T, Kadota K. Laryngeal squamous cell carcinoma and papilloma associated with *Equus caballus* papillomavirus 2 in a horse. J Vet Med Sci. 2019;81(7):1029–33.

18. Alloway E, Linder K, May S, Rose T, DeLay J, Bender S et al. A subset of equine gastric squamous cell carcinomas is associated with *Equus caballus* papillomavirus-2 Infection. Vet Pathol. 2020;57(3):427–31.
19. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55–70.
20. Sykora S, Jindra C, Hofer M, Steinborn R, Brandt S. Equine papillomavirus type 2: an equine equivalent to human papillomavirus 16? Vet J. 2017;225:3–8.
21. Antonsson A, Hansson BG. Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. J Virol. 2002;76(24):12537–42.
22. Antonsson A, McMillan NAJ. Papillomavirus in healthy skin of Australian animals. J Gen Virol. 2006;87(Pt 11):3195–200.
23. Greenwood S, Chow-Lockerbie B, Ramsauer S, Wachoski-Dark G, Knight C, Wobeser B. Prevalence of *Equus caballus* papillomavirus type-2 infection and seropositivity in asymptomatic Western Canadian horses. Vet Pathol. 2020;57(5):632–41.
24. Fischer NM, Favrot C, Birkmann K, Jackson M, Schwarzwald CC, Müller M et al. Serum antibodies and DNA indicate a high prevalence of equine papillomavirus 2 (EcPV2) among horses in Switzerland. Vet Dermatol. 2014;25(3):210–54.
25. Doles J, Williams JW, Yarbrough TB. Penile amputation and sheath ablation in the horse. Vet Surg. 2001;30(4):327–31.
26. Broders AC. Squamous cell epithelioma of the skin. Ann Surg. 1921:20:141–60.
27. Lange CE, Tobler K, Ackermann M, Favrot C. Identification of two novel equine papillomavirus sequences suggests three genera in one cluster. Vet Microbiol. 2011;149(1–2):85–90.
28. Schuh JC. Squamous cell carcinoma of the oral, pharyngeal and nasal mucosa in the horse. Vet Pathol. 1986;23(2):205–7.
29. Howarth S, Lucke VM, Pearson H. Squamous cell carcinoma of the equine external genitalia: a review and assessment of penile amputation and urethrostomy as a surgical treatment. Equine Vet J. 1991;23(1):53–8.
30. Hakenberg OW, Dräger DL, Erbersdobler A, Naumann CM, Jünemann K-P, Protzel C. The diagnosis and treatment of penile cancer. Dtsch Arztebl Int. 2018;115(39):646–52.
31. Monteiro S, Lemberger K, Gangl M. Mandibular squamous cell carcinoma in a young horse. Equine Vet Educ. 2009;21(8):406–10.
32. Morrison ML, Groover E, Schumacher J, Newton J, Pereira MM. Lingual squamous cell carcinoma in two horses. J Equine Vet Sci. 2019;79:35–8.
33. Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, Miyazaki T. A review of HPV-related head and neck cancer. J Clin Med. 2018;7(9):241.
34. Zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology. 2009;384(2):260–5.
35. Canete-Portillo S, Sanchez DF, Cubilla AL. Pathology of invasive and intraepithelial penile neoplasia. Eur Urol Focus. 2019;5(5):713–7.
36. Porcellato I, Modesto P, Cappelli K, Varello K, Peletto S, Brachelente C et al. *Equus caballus* papillomavirus type 2 (EcPV2) in co-occurring vulvar and gastric lesions of a pony. Res Vet Sci. 2020;132:167–71.
37. van den Top JGB, Ensink JM, Barneveld A, van Weeren PR. Penile and preputial squamous cell carcinoma in the horse and proposal of a classification system. Equine Vet Educ. 2011;23(12):636–48.
38. Ryndock EJ, Meyers C. A risk for non-sexual transmission of human papillomavirus? Expert Rev Anti Infect Ther. 2014;12(10):1165–70.
39. Doorbar J. Host control of human papillomavirus infection and disease. Best Pract Res Clin Obstet Gynaecol. 2018;47:27–41.
40. Antonsson A, Erfurt C, Hazard K, Holmgren V, Simon M, Kataoka A et al. Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents. J Gen Virol. 2003;84(Pt 7):1881–6.
41. Antonsson A, Forslund O, Ekberg H, Sterner G, Hansson BG. The ubiquity and impressive genomic diversity of human skin papillomaviruses suggest a commensalic nature of these viruses. J Virol. 2000;74(24):11636–41.
42. Magnusson PK, Lichtenstein P, Gyllensten UB. Heritability of cervical tumours. Int J Cancer. 2000;88(5):698–701.
43. Wei T, Buehler D, Ward-Shaw E, Lambert PF. An infection-based murine model for papillomavirus-associated head and neck cancer. mBio. 2020;11(3):e00908–20.
44. Staiger EA, Tseng CT, Miller D, Cassano JM, Nasir L, Garrick D et al. Host genetic influence on papillomavirus-induced tumors in the horse. Int J Cancer. 2016;139(4):784–92.

How to cite this article: Lale D, Geyer A, Jindra C, Cavalleri J-MV, Ramsauer AS. Equine papillomavirus type 2-associated, carcinomatous lesions of the penis and laryngopharynx of an elderly Icelandic horse gelding. Vet Rec Case Rep. 2022;e508. https://doi.org/10.1002/vrc2.508

MULTIPLE-CHOICE QUESTION

Which of the following statements regarding equine papillomavirus type 2 (EcPV2) is correct?

POSSIBLE ANSWERS TO MULTIPLE-CHOICE QUESTION

A) EcPV2 infection immediately results in the development of squamous cell carcinomas in the genital and pharyngeal regions of horses.
B) EcPV2 is associated with genital squamous cell carcinomas and a subset of pharyngeal squamous cell carcinomas in horses.
C) After complete surgical removal of EcPV2-associated squamous cell carcinomas, there is no risk of recurrence.
D) EcPV2 is associated with genital squamous cell carcinomas, but it is not detected in squamous cell carcinomas at other locations of the equine body.
E) EcPV2 usually results in subclinical infection, and there is no disease association described to date.

CORRECT ANSWER

A) Wrong: EcPV2 infection can also be detected in healthy horses, and it is not known how long it takes from initial infection to tumour occurrence.
B) Correct.
C) Wrong: Due to the viral aetiology, the risk of recurrence cannot be excluded, even after complete surgical removal, routine monitoring would be recommended.
D) Wrong: There are reports of EcPV2-associated tumours also in the pharyngeal region.
E) Wrong: EcPV2 is also detected in healthy horses (subclinical infection), but there is an association with the development of squamous cell carcinomas in the genital region and a subset of tumours in the pharyngeal region.

3. ABSTRACT IN GERMAN

Papillomaviren können Menschen sowie bestimmte Tiere infizieren und zur Entstehung von tumorösen Läsionen beitragen. Bei Menschen ist die Assoziation mit dem Humanen Papillomavirus (HPV) mit der Entstehung des zervikalen Platenepithelkarzinoms gegeben. Auch ist es bekannt das Platenepithelkarzinome in ein Drittel der Fälle im penilen Bereich und ein Viertel der Fälle im Kopfbereich mit dem HPV in Verbindung gesetzt werden. Das Equine Papilloma Virus 2 (EqPV2) wird mit der Ätiopathogenese von genitalen Platenepithelkarzinomen und Präkanzerosen bei Pferden in Verbindung gebracht. Etwa 20 % der berichteten Fälle von Plattenepithelkarzinomen bei Pferden im oronasalen, laryngealen und pharyngealen Bereich deuten auf eine aktive Rolle von EcPV2 bei der Pathogenese hin. Derzeit gibt es nur wenige Daten über EcPV2-Infektionen in Verbindung mit Plattenepithelkarzinom des Kopfes bei Pferden. Derzeit gibt es nur wenige Daten über EcPV2-Infektionen im Zusammenhang mit Plattenepithelkarzinomen des Kopfes bei Pferden. Die Früherkennung und Behandlung von Plattenepithelkarzinomen am Kopf können bei Pferden eine große Herausforderung darstellen. Die möglichen Wege der EcPV2-Infektion und -Übertragung innerhalb eines Pferdes, die zur Tumorentstehung führen, sind nicht vollständig enthüllt. Pferde mit Plattenepithelkarzinom oder Vorläuferläsionen können eine EcPV2-Infektion beherbergen, die zu einer neoplastischen Progression führen könnte.
In diesem Fallbericht wird ein Patient mit einer EcPV2-assoziierten Tumorentwicklung zunächst im Genitalbereich und später im Laryngopharyngealbereich beschrieben.
Der 28-jährige Islandpferdewallach wurde mit einem laryngopharyngealen Plattenepithelkarzinom vorgestellt. Zwei Jahre zuvor wurde aufgrund eines *Carcinoma in situ* am Penis eine partielle Phallektomie durchgeführt. Die PCR Untersuchung von Tumor-DNA und die anschließenden Amplikon-Sequenzierungen ergaben, dass die E6-Onkogensequenzen der EcPV2 beider Läsionen identisch waren. Dieses Pferd entwickelte nacheinander ein peniles *Carcinoma in situ* und Plattenepithelkarzinom des Laryngopharynx, das offenbar durch dieselbe EcPV2-Variante induziert wurde. In diesem Zusammenhang wurde die Bedeutung einer frühzeitigen Erkennung von Läsionen und möglichen EcPV2-Infektionswegen (wie z.B hämo-lymphatische und/oder durch Kontakt infizierte Läsionen) diskutiert.

4. ABSTRACT

Papillomaviruses can infect humans as well as certain animals and contribute to the development of tumorous lesions. In humans, the association of the human papillomavirus (HPV) with development of cervical squamous cell carcinoma (SCC) is well known. It is also known that one-third of penile and one-fourth of cervical squamous cell carcinomas are associated with HPV. Equine Papilloma Virus 2 (EqPV2) is implicated in the aetiopathogenesis of genital squamous cell carcinoma and precancerous lesions in horses. About 20% of reported cases of equine squamous cell carcinoma in the oronasal, laryngeal and pharyngeal areas indicate an active role of EcPV2 in pathogenesis. Currently, there are few data on EcPV2 infection associated with squamous cell carcinoma of the head in horses. Early detection and treatment of squamous cell carcinoma of the head can be challenging in horses. The possible pathways of EcPV2 infection and transmission within a horse leading to tumorigenesis are not fully revealed. Horses with squamous cell carcinomas or precursor lesions may harbour EcPV2 infection that could lead to neoplastic progression. This case report describes a patient with EcPV2-associated tumour development first in the genital region and later in the laryngopharyngeal region.

The 28-year-old Icelandic horse gelding presented with laryngopharyngeal squamous cell carcinoma. Two years previously, a partial phallectomy had been performed due to a carcinoma in situ on the penis. PCR examination of tumour DNA and subsequent amplicon sequencing revealed that the E6 oncogene sequences of EcPV2 of both lesions were identical. This horse successively developed penile carcinoma in situ and squamous cell carcinoma of the laryngopharynx, apparently induced by the same EcPV2 variant. In this context, the importance of early detection of lesions and possible EcPV2 infection pathways (such as haemo-lymphatic and/or contact-infected lesions) are discussed

5. REFERENCES

Alloway E, Linder K, May S, Rose T, DeLay J, Bender S, Tucker A, Luff J, 2020. A Subset of Equine Gastric Squamous Cell Carcinomas Is Associated With Equus Caballus Papillomavirus-2 Infection. Veterinary pathology, 57 (3), 427–431.

Antonsson A, Hansson BG, 2002. Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. Journal of virology, 76 (24), 12537–12542.

Antonsson A, McMillan NAJ, 2006. Papillomavirus in healthy skin of Australian animals. The Journal of general virology, 87 (Pt 11), 3195–3200.

Armando F, Godizzi F, Razzuoli E, Leonardi F, Angelone M, Corradi A, Meloni D, Ferrari L, Passeri B, 2020. Epithelial to Mesenchymal Transition (EMT) in a Laryngeal Squamous Cell Carcinoma of a Horse: Future Perspectives. Animals : an open access journal from MDPI, 10 (12).

Astori G, Merluzzi S, Arzese A, Brosolo P, Pretis G de, Maieron R, Pipan C, Botta GA, 2001. Detection of human papillomavirus DNA and p53 gene mutations in esophageal cancer samples and adjacent normal mucosa. Digestion, 64 (1), 9–14.

Auer JA (ed.), 2012. Equine surgery (4th ed.). Elsevier Saunders, St. Louis, Mo., 1536 p.

Bogaert L, Willemsen A, Vanderstraeten E, Bracho MA, Baere C de, Bravo IG, Martens A, 2012. EcPV2 DNA in equine genital squamous cell carcinomas and normal genital mucosa. Veterinary microbiology, 158 (1-2), 33–41.

Brandwein M, Zeitlin J, Nuovo GJ, MacConnell P, Bodian C, Urken M, Biller H, 1994. HPV detection using "hot start" polymerase chain reaction in patients with oral cancer: a clinicopathological study of 64 patients. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc, 7 (7), 720–727.

Brinsko SP, 1998. Neoplasia of the Male Reproductive Tract. Veterinary Clinics of North America: Equine Practice, 14 (3), 517–533.

Canete-Portillo S, Sanchez DF, Cubilla AL, 2019. Pathology of Invasive and Intraepithelial Penile Neoplasia. European urology focus, 5 (5), 713–717.

Chambers G, Ellsmore VA, O'Brien PM, Reid SWJ, Love S, Campo MS, Nasir L, 2003. Association of bovine papillomavirus with the equine sarcoid. The Journal of general virology, 84 (Pt 5), 1055–1062.

Chaux A, Velazquez EF, Amin A, Soskin A, Pfannl R, Rodríguez IM, Barreto JE, Lezcano C, Ayala G, Netto GJ, Cubilla AL, 2012. Distribution and characterization of subtypes of penile intraepithelial neoplasia and their association with invasive carcinomas: a pathological study of 139 lesions in 121 patients. Human pathology, 43 (7), 1020–1027.

Chaux, Torres, Pfannl, Barreto, Rodriguez, Velazquez, Cubilla, 2009. Histologic Grade in Penile Squamous Cell Carcinoma. Am J Surg Pathol, 33, 1042–1048.

Cramer, 2011. Pathology in practise. American College of Veterinary Pathologists.

Dalianis T, 2014. Human papillomavirus and oropharyngeal cancer, the epidemics, and significance of additional clinical biomarkers for prediction of response to therapy (Review). International journal of oncology, 44 (6), 1799–1805.

Dayyani F, Etzel CJ, Liu M, Ho C-H, Lippman SM, Tsao AS, 2010. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). Head & neck oncology, 2, 15.

Doorbar J, 2005. The papillomavirus life cycle. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology, 32 Suppl 1, S7-15.

Doorbar J, 2006. Molecular biology of human papillomavirus infection and cervical cancer. Clinical science (London, England : 1979), 110 (5), 525–541.

Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA, 2012. The biology and life-cycle of human papillomaviruses. Vaccine, 30 Suppl 5, F55-70.

Downes MR, 2015. Review of in situ and invasive penile squamous cell carcinoma and associated non-neoplastic dermatological conditions. Journal of clinical pathology, 68 (5), 333–340.

Durham, 1997. Squamous cell carcinoma of the epidermal laminae. Equine Veterinary Education, 30–33.

Fischer NM, Favrot C, Birkmann K, Jackson M, Schwarzwald CC, Müller M, Tobler K, Geisseler M, Lange CE, 2014. Serum antibodies and DNA indicate a high prevalence of equine papillomavirus 2 (EcPV2) among horses in Switzerland. Veterinary dermatology, 25 (3), 210-e54.

Gillison et al., 2012. Evidence for a Causal Association Between Human Papillomavirus and a Subset of Head and Neck Cancers.

Gray DT, 1997. Trends in the population-based incidence of squamous cell carcinoma of the skin first diagnosed between 1984 and 1992. Archives of Dermatology, 133 (6), 735–740.

Greenwood S, Chow-Lockerbie B, Ramsauer S, Wachoski-Dark G, Knight C, Wobeser B, 2020. Prevalence of Equus caballus Papillomavirus Type-2 Infection and Seropositivity in Asymptomatic Western Canadian Horses. Veterinary pathology, 57 (5), 632–641.

Hakenberg OW, Dräger DL, Erbersdobler A, Naumann CM, Jünemann K-P, Protzel C, 2018. The Diagnosis and Treatment of Penile Cancer. Deutsches Arzteblatt international, 115 (39), 646–652.

Hibi H, Hatama S, Obata A, Shibahara T, Kadota K, 2019. Laryngeal squamous cell carcinoma and papilloma associated with Equus caballus papillomavirus 2 in a horse. The Journal of veterinary medical science, 81 (7), 1029–1033.

Jones, 1994. Jones 1994 Oropharyngeal SCC in horses. Cornell Veterinary, 1994.

Kainzbauer C, Rushton J, Tober R, Scase T, Nell B, Sykora S, Brandt S, 2012. Bovine papillomavirus type 1 and Equus caballus papillomavirus 2 in equine squamous cell carcinoma of the head and neck in a Connemara mare. Equine veterinary journal, 44 (1), 112–115.

Kim J, Park MK, Li W-Q, Qureshi AA, Cho E, 2019. Association of Vitamin A Intake With Cutaneous Squamous Cell Carcinoma Risk in the United States. JAMA dermatology, 155 (11), 1260–1268.

King TC, Priehs DR, Gum GG, Miller TR, 1991. Therapeutic management of ocular squamous cell carcinoma in the horse: 43 cases (1979-1989). Equine veterinary journal, 23 (6), 449–452.

Knickelbein KE, Lassaline ME, Singer-Berk M, Reilly CM, Clode AB, Famula TR, Michau TM, Bellone RR, 2020. A missense mutation in damage-specific DNA binding protein 2 is a genetic risk factor for ocular squamous cell carcinoma in Belgian horses. Equine veterinary journal, 52 (1), 34–40.

Knight CG, Dunowska M, Munday JS, Peters-Kennedy J, Rosa BV, 2013. Comparison of the levels of Equus caballus papillomavirus type 2 (EcPV-2) DNA in equine squamous cell

carcinomas and non-cancerous tissues using quantitative PCR. Veterinary microbiology, 166 (1-2), 257–262.

Knight CG, Munday JS, Peters J, Dunowska M, 2011. Equine penile squamous cell carcinomas are associated with the presence of equine papillomavirus type 2 DNA sequences. Veterinary pathology, 48 (6), 1190–1194.

Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, Miyazaki T, 2018. A Review of HPV-Related Head and Neck Cancer. Journal of clinical medicine, 7 (9).

Kreimer, 2004. Human Papillomavirus Types in Head and Neck Squamous Cell Carcinomas Worldwide: A Systematic Review.

Lafaurie GI, Perdomo SJ, Buenahora MR, Amaya S, Díaz-Báez D, 2018. Human papilloma virus: An etiological and prognostic factor for oral cancer? Journal of investigative and clinical dentistry, 9 (2), e12313.

Lai K, Killingsworth M, Matthews S, Caixeiro N, Evangelista C, Wu X, Wykes J, Samakeh A, Forstner D, Niles N, Hong A, Lee CS, 2017. Differences in survival outcome between oropharyngeal and oral cavity squamous cell carcinoma in relation to HPV status. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology, 46 (8), 574–582.

Lange CE, Tobler K, Lehner A, Grest P, Welle MM, Schwarzwald CC, Favrot C, 2013. EcPV2 DNA in equine papillomas and in situ and invasive squamous cell carcinomas supports papillomavirus etiology. Veterinary pathology, 50 (4), 686–692.

Mair et al., 2000. Surgical treatment of 45 horses affected by squamous cell carcinoma of the penis and prepuce.

Marks, 1996. Squamous cell carcinoma.

McBride AA, 2022. Human papillomaviruses: diversity, infection and host interactions. Nature Reviews Microbiology, 95–108.

Mellin H, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E, 2000. Human papillomavirus (HPV) DNA in tonsillar cancer: Clinical correlates, risk of relapse, and survival. International Journal of Cancer, 89 (3), 300–304.

Motaparthi K, Kapil JP, Velazquez EF, 2017. Cutaneous Squamous Cell Carcinoma: Review of the Eighth Edition of the American Joint Committee on Cancer Staging Guidelines,

Prognostic Factors, and Histopathologic Variants. Advances in anatomic pathology, 24 (4), 171–194.

Munday JS, French A, Harvey CJ, 2015. Molecular and immunohistochemical studies do not support a role for papillomaviruses in canine oral squamous cell carcinoma development. Veterinary journal (London, England : 1997), 204 (2), 223–225.

Muñoz N, Castellsagué X, Berrington de González A, Gissmann L, 2006. Chapter 1: HPV in the etiology of human cancer. Vaccine, 24 Suppl 3, S3/1-10.

Parkin et al., 2006. Chapter 2: The burden of HPV-related cancers. Vaccine, 24 Suppl 3, S3/11-25.

Patterson LJ, May SA, Baker JR, 1990. Skeletal metastasis of a penile squamous cell carcinoma. The Veterinary record, 126 (23), 579–580.

Petersen I, Klein F, 2008. HPV bei nichtgynäkologischen Tumoren. Der Pathologe, 29 Suppl 2, 118–122.

Ramsauer AS, Kubacki J, Favrot C, Ackermann M, Fraefel C, Tobler K, 2019a. RNA-seq analysis in equine papillomavirus type 2-positive carcinomas identifies affected pathways and potential cancer markers as well as viral gene expression and splicing events. The Journal of general virology, 100 (6), 985–998.

Ramsauer AS, Wachoski-Dark GL, Fraefel C, Tobler K, Brandt S, Knight CG, Favrot C, Grest P, 2019b. Paving the way for more precise diagnosis of EcPV2-associated equine penile lesions. BMC veterinary research, 15 (1), 356.

Reed SM, Bayly WM, Sellon DC (eds.), 2018. Equine internal medicine (Fourth edition). Elsevier, St. Louis, Missouri, 1566 p.

Riddel C, Rashid R, Thomas V, 2011. Ungual and periungual human papillomavirus-associated squamous cell carcinoma: a review. Journal of the American Academy of Dermatology, 64 (6), 1147–1153.

Rowe DE, Carroll RJ, Day CL, 1992. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Journal of the American Academy of Dermatology, 26 (6), 976–990.

Ryan D. Rosen, Amit Sapra ., 2020. TNM Classification. StatPearls, Bookshelf ID: NBK553187 PMID: 31985980

Scase T, Brandt S, Kainzbauer C, Sykora S, Bijmholt S, Hughes K, Sharpe S, Foote A, 2010. Equus caballus papillomavirus-2 (EcPV-2): an infectious cause for equine genital cancer? Equine veterinary journal, 42 (8), 738–745.

Schuh, 1986. Squamous Cell Carcinoma of the Oral, Pharyngeal and Nasal Mucosa in the Horse.

Scott DW, 2011. Equine dermatology (2nd ed.). Elsevier/Saunders, Maryland Heights, Mo, 536 p.

Scott and Hughes, 2017. Diagnosis of equine penile and preputial masses: A clinical and pathological perspective. Equine Veterinary Education, 29 (1), 10–14.

Sobin and Fleming, 1997. TNM classification of malignant tumors, fifth edition (1997).

Sykora and Brandt, 2017. Equine papillomavirus type 2: An equine equivalent to human papillomavirus 16? Veterinary journal (London, England : 1997), 225, 3–8.

Sykora et al., 2017. Papillomavirus infection and squamous cell carcinoma in horses. Veterinary journal (London, England : 1997), 223, 48–54.

Taylor et al, 2013. A review of equine mucocutaneous squamous cell carcinoma. Equine Veterinary Education, 25 (7), 374–378.

Valentine BA, 2006. Survey of equine cutaneous neoplasia in the Pacific Northwest. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 18 (1), 123–126.

van den Top JGB, Ensink JM, Barneveld A, van Weeren PR, 2011. Penile and preputial squamous cell carcinoma in the horse and proposal of a classification system. Equine Veterinary Education, 23 (12), 636–648.

van den Top JGB, Ensink JM, Gröne A, Klein WR, Barneveld A, van Weeren PR, 2010. Penile and preputial tumours in the horse: literature review and proposal of a standardised approach. Equine veterinary journal, 42 (8), 746–757.

van den Top JGB, Heer N de, Klein WR, Ensink JM, 2008. Penile and preputial squamous cell carcinoma in the horse: a retrospective study of treatment of 77 affected horses. Equine veterinary journal, 40 (6), 533–537.

Van den Top et al., 2014. Van den Top Standardised approach.

Varnai AD, Bollmann M, Griefingholt H, Speich N, Schmitt C, Bollmann R, Decker D, 2006. HPV in anal squamous cell carcinoma and anal intraepithelial neoplasia (AIN). Impact of

HPV analysis of anal lesions on diagnosis and prognosis. International journal of colorectal disease, 21 (2), 135–142.

Villiers E-M de, 2013. Cross-roads in the classification of papillomaviruses. Virology, 445 (1-2), 2–10.

Weinstock, 1989. The Epidemic of squamous cell carcinoma. JAMA.

Zhu KW, Affolter VK, Gaynor AM, Dela Cruz FN, Pesavento PA, 2015. Equine Genital Squamous Cell Carcinoma: In Situ Hybridization Identifies a Distinct Subset Containing Equus caballus Papillomavirus 2. Veterinary pathology, 52 (6), 1067–1072.

zur Hausen H, 2009. Papillomaviruses in the causation of human cancers - a brief historical account. Virology, 384 (2), 260–265.

6. ACKNOWLEDGEMENTS

I would like to express my special thanks of gratitude to my supervisor Prof. Dr. Cavalleri who gave me this opportunity to shape my future. You supported me with great patience during my whole work.

Dear Sophie, a million thanks to you! You have handled me in bad and good times. Also supported me in any case of this work.

Also, I would like to thank my Grosi, Dede, Mami, Baladam, and Gucca without you I would not succeed.