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CASE REPORT

Horses and other equids

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

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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.

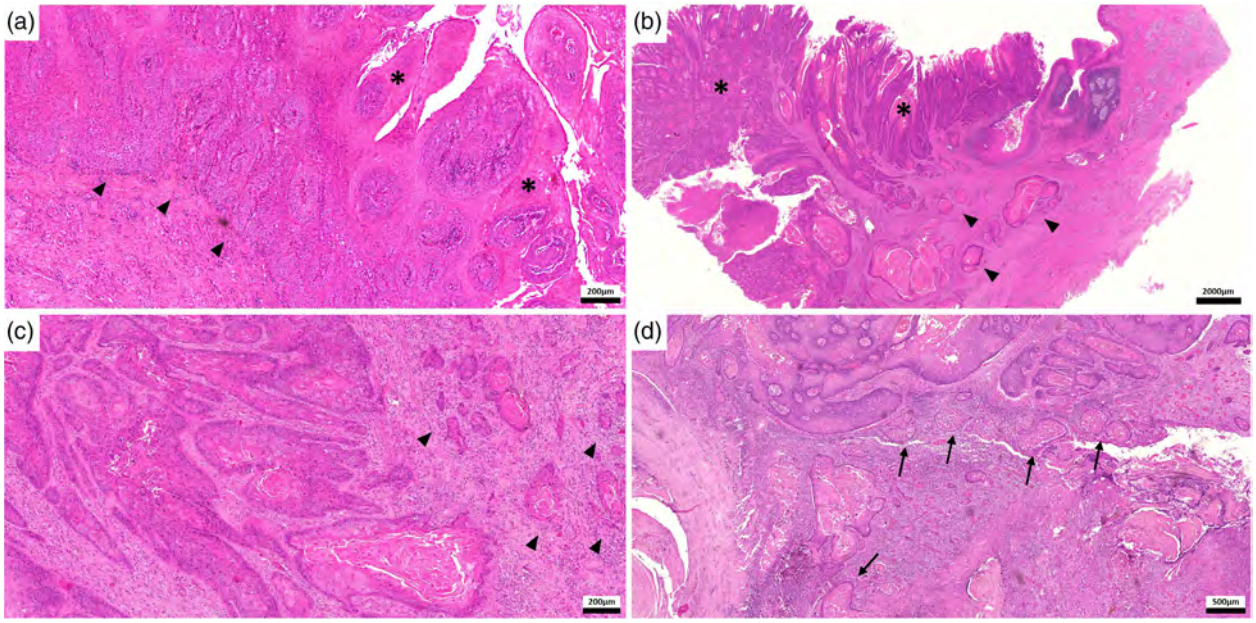

FIGURE 1 Histopathological images of the penile carcinoma in situ (CIS) and laryngopharyngeal squamous cell carcinoma (SCC). (a) CIS (penis): Exophytic growth (asterisk) but no evidence of invasion of neoplastic cells into the adjacent stroma (arrowheads). (b) SCC: Transition of the laryngopharyngeal 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.

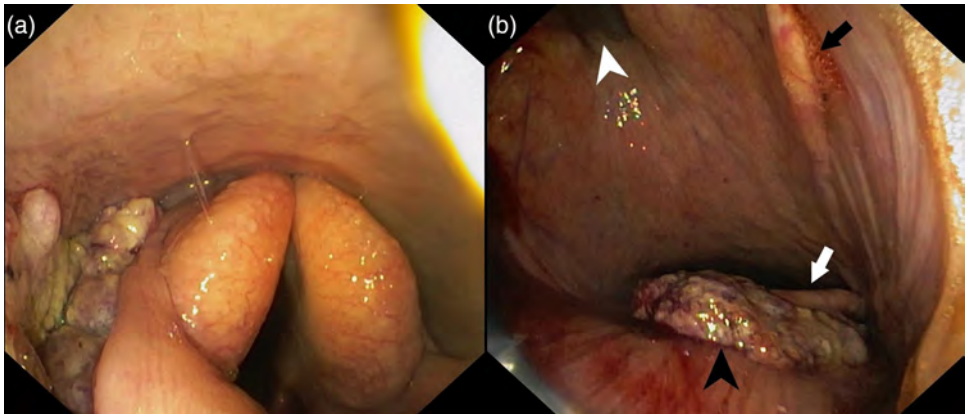

FIGURE 2 Endoscopic images of the laryngopharyngeal lesion. (a) The beginning of the lesion from the most caudal part of the laryngopharynx. (b) The extension of the lesion to the most rostral part of the laryngopharynx (black arrowhead). Notice pharyngeal recess (white arrowhead), epiglottis (white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

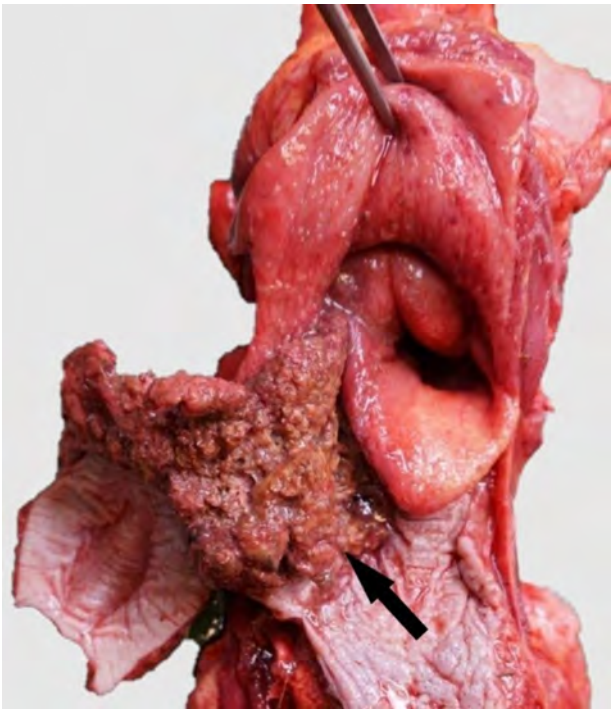

FIGURE 3 Gross image of the larynx and pharynx, including the exophytic lesion (black arrow) adherent to the lateral pharyngeal wall and soft palate.

(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

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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.

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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.