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Clinical Commentary

Papillomaviruses in equids: A decade of discovery and more to come?

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Introduction

The case report that this clinical commentary accompanies (Vichi *et al*. 2022) describes the diagnosis and treatment of a 2-year-old horse with cutaneous papillomatosis lesions, attributed to *Equus caballus* papillomavirus 2 (EcPV2) infection, and concurrent cutaneous habronemiasis. The additional pathology caused by the presence of Habronematidae larvae necessitated surgical removal of the lesions. Without this coinfection, the papillomavirus infection and lesions could have progressed in several ways.

The number of known papillomaviruses is increasing for many species, including equids, as is the range of pathologies in which they are implicated. This article summarises known presentations of papillomavirus-associated infections in equids and outlines the underlying host-viral biology.

Progress in papillomavirus discovery

Papillomavirus (PV) infections may be latent, subclinical or result in lesion development. Active infections may cause self-resolving benign proliferative lesions, more severe or persistent benign disease, or malignant cancers (**Fig 1**). To date, nine *Equus caballus* papillomaviruses (EcPV1 to 9) and two asinine papillomaviruses (EaPV1 and 2) have been identified (Papillomavirus Episteme). Additionally, bovine papillomavirus (BPV) types 1, 2 (Chambers *et al*. 2003) and 13 (Lunardi *et al*. 2013) infect horses and donkeys and cause equine sarcoids, in an unusual example of a cross-species infection by this host-specific family of viruses.

In humans, 225 papillomaviruses (HPVs) have been identified (Papillomavirus Episteme), of which at least 14 can cause cancer with metastatic potential (high-risk HPVs (hrHPVs)) (World Health Organization 2020). An infectious cause of human cervical cancer was first suspected from the 1950s onwards, and hrHPVs 16 and 18 were characterised as causes of cervical cancer in the 1980s (Dürst *et al*. 1983; Boshart *et al*. 1984), a discovery for which Harald zur Hausen was awarded a Nobel Prize (Nobel Prize 2008). It is now known that hrHPVs are the cause of almost all cases of cervical cancer, as well as a significant proportion of other anogenital cancers and approximately 25% of head and neck cancers (de Martel *et al*. 2017). Moreover, hrHPVs are implicated in over 5% of human cancers worldwide (Berman and Schiller 2017). Given the much lower numbers of PVs so far discovered in veterinary species, for example, 23 in dogs and 27 in cattle (Papillomavirus Episteme), it seems there are many more veterinary PVs to discover, both of low and high clinical significance. Additionally, already known veterinary PVs could become newly implicated in pathological conditions.

There has been a considerable increase in the number of known equine-infecting PVs in the last decade, prior to which only EcPV1 (Ghim *et al*. 2004) and BPV1 and BPV2 (Chambers *et al*. 2003) were fully characterised. PVs were previously suspected to be involved in genital papillomas and carcinomas and aural plaques, but EcPV2 and EcPV3, respectively, were not fully characterised from samples of these lesions until 2011 (Lange *et al*. 2011). EcPVs 4 to 7 were first reported in 2013 (Lange *et al*. 2013), EcPV8 in 2018 (Linder *et al*. 2018) and EcPV9 in 2019 (Li *et al*. 2019). In 2013, BPV13 was linked with sarcoids after being detected in samples in Brazil (Lunardi *et al*. 2013). *Equus asinus* papillomavirus 1 (EaPV1) was discovered in 2014 in Asinara white donkeys with no proliferative lesions and appears to have low pathogenicity (Lecis *et al*. 2014), whereas EcPV8 was discovered in horses with severe, generalised papillomatosis (Linder *et al*. 2018). Furthermore, EcPV2 has recently been associated with an increasing range of squamous cell carcinomas (SCCs), its DNA having been detected in a subset of SCCs of the stomach (Alloway *et al*. 2020) and head (Sykora *et al*. 2017) as well as of the genitalia.

Papillomaviruses in benign proliferative disease

Most PVs are strictly epitheliotrophic, infecting dermal keratinocytes or squamous epithelium. A subset of PVs also infects mesenchymal cells; for example, BPV1, BPV2 and BPV13 infect and transform dermal fibroblasts as well as keratinocytes (Bocaneti *et al*. 2016). The life cycle of PVs is linked to epithelial differentiation, a strategy that enables virions to be shed during desquamation and helps the virus escape the immune response (**Fig 2**). PVs have a double stranded circular DNA genome, encoding early (E) genes involved in viral genome replication and oncogenesis, and late (L) genes which encode the capsid proteins (**Fig 3**). The early genes are expressed in deeper tissue layers and the late genes only more superficially (Nasir and Campo 2008).

Papillomavirus infections are productive, with virions shed by infected hosts, in classic papillomatosis. An example is EcPV1-associated "warts" which mostly occur on the face and distal limbs of young horses. These typically spontaneously regress within a few weeks, when the host clears the infection and becomes immune to reinfection (Dong *et al*. 2017). Benign dermal fibropapillomas in cattle induced by BPV1 and BPV2 follow the same course, but the growths include proliferating fibroblasts as well as keratinocytes. In a minority of cases, for example if the host is

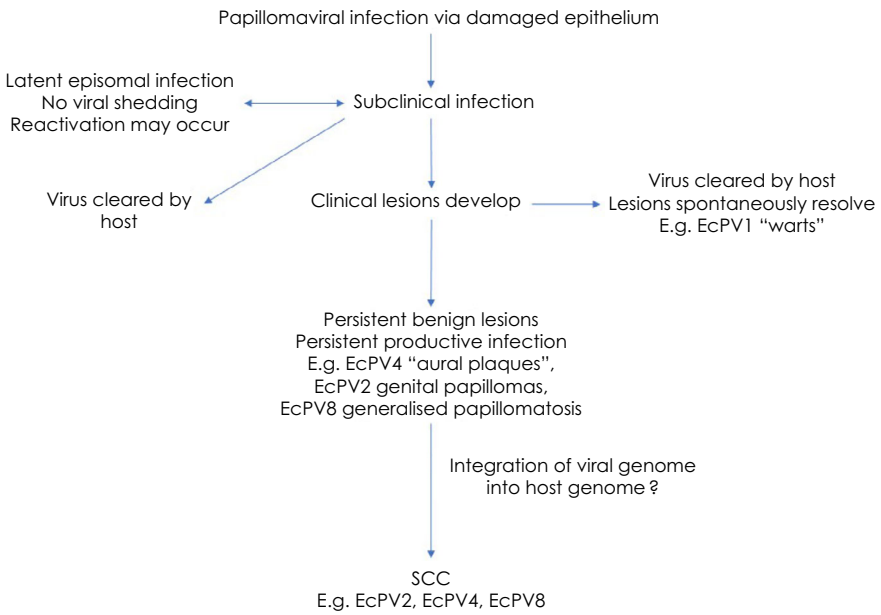

Fig 1: Possible outcomes for* Equus caballus *papillomaviral (EcPV) infections. NB more than one state may exist within a host, for example, there may be simultaneous productive and nonproductive infection.

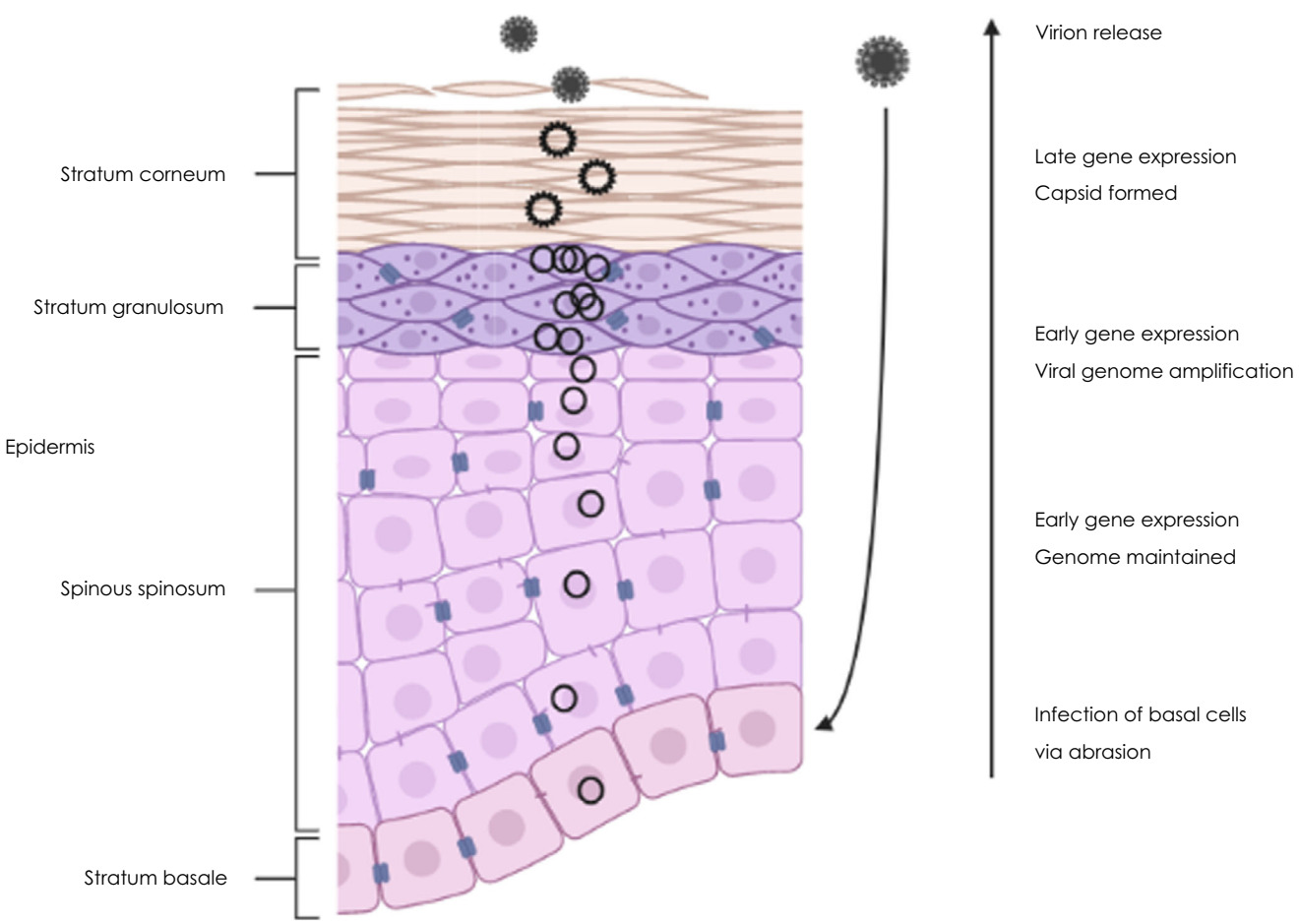

Fig 2: Schematic diagram of the productive life cycle of papillomaviruses (Created with BioRender.com).

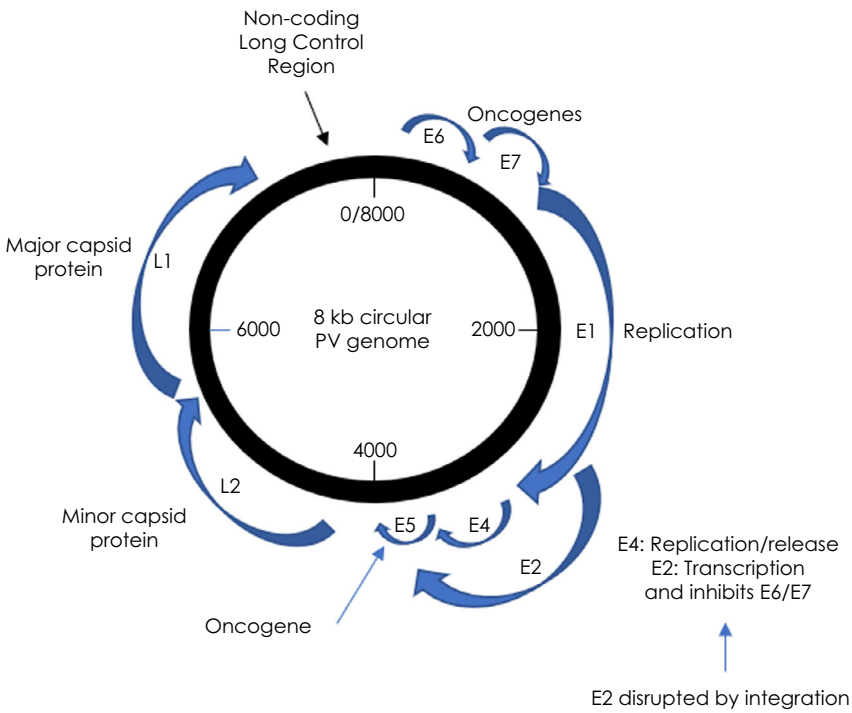

Fig 3: Schematic diagram of a papillomaviral genome, showing the open reading frame and function of each gene and the area disrupted if the viral genome integrates into the host genome.

immunocompromised, these infections are not cleared and the lesions become more severe and widespread (Turk *et al*. 2005). As discussed in the case report, co-infection with other pathogens may influence viral survival and pathogenesis.

Some equine PV infections involve persistent productive infections and lesions, for example, chronic genital papillomas associated with EcPV2 infection (Knight *et al*. 2011). Equine aural plaques are thought to be caused by EcPV3, EcPV4 and EcPV6, and co-infections with these viral types and with EcPV1 and EcPV5 appear common (Mira *et al*. 2018). As mentioned in the case report, the presence of one skin pathogen may aid invasion by another. Although not usually clinically significant, aural plaques typically do not regress and the finding of PV-like structures in the lesions by electron microscopy (Fairley and Haines 1992) suggests virion production. EcPV8 was reported to cause thousands of widespread coalescing hyperkeratotic papules and plaques, particularly on the trunk, in three horses. The lesions persisted for over a year in all three, after which one was euthanased and the others were lost to follow-up. Viral capsid protein was detected in the lesions, suggesting a productive infection in these cases too (Linder *et al*. 2018).

Papillomaviruses in invasive/metastatic disease

EcPV2 is being increasingly associated with some SCCs of the horse. Additionally, an aural SCC containing EcPV4 DNA was reported in an aged horse with multiple aural plaques (Peters-Kennedy *et al*. 2020) and a horse with EcPV8-containing lesions was reported to have multiple SCCs as well as extensive papillomas in the inguinal region (Peters-Kennedy *et al*. 2019).

Integration of the PV genome into the host genome is a dead end for the virus because the infection becomes nonproductive. However, it is an important step in hrHPV-induced oncogenesis and is seen in most, although not all, human cervical SCCs (Groves and Coleman 2015). It is also possible to have nonproductive infections in which the viral genome is maintained in its circular episomal form, as occurs in latency (De Leo *et al*. 2020). In equine sarcoids, the BPV genome appears to remain episomal and not to integrate, meaning expression of the viral genome alone is able to transform cells (Amtmann *et al*. 1980). Equids have traditionally been considered dead end hosts of BPV because infective virions have not been isolated from equine sarcoids as they can be from bovine papillomas (Hainisch *et al*. 2017). However, there are reports and evidence of apparent equid-equid transmission and sarcoid outbreaks (Ragland *et al*. 1966; Reid *et al*. 1994; Nasir and Campo 2008) and of equid-adapted BPV1 variants (Nasir *et al*. 2007). It is unclear how virions could be produced from fibroblasts, given virion production is linked to epithelial differentiation, and sarcoids are primarily tumours of fibroblasts with variable epidermal involvement (Martens *et al*. 2000). However, BPV1 infection has been found in the epidermis as well as the dermis of sarcoids, viral L1 capsid protein has been detected in sarcoid epidermis (Brandt *et al*. 2011), and approximately half of tested sarcoids are positive on an assay which detects BPV DNA complexed to L1 capsid protein (Brandt *et al*. 2008), suggesting at least some equids may be able to shed BPV1 and BPV2.

The case report unusually identified EcPV2 in cutaneous lesions on the face (Vichi *et al*. 2022). EcPV2 is usually associated with lesions in the genital area and more recently of the nasal and oral cavities (Sykora *et al*. 2017). However, it is possible that some cases of facial warts presumed to be caused by EcPV1 could contain EcPV2. EcPV2 DNA has been detected in normal samples and papillomas as well as in

precancerous lesions and SCCs (Bogaert *et al*. 2012). However, it is found in a much higher proportion of samples from SCCs than from healthy animals, suggesting it is an "oncogenic rather than a commensal virus" (summarised in Sykora and Brandt 2017). Additionally, an in situ hybridisation study using probes for EcPV2 detected viral material in 80–100% of neoplastic cells in a subset of genital SCCs, and in vessels of regional lymph nodes and metastases, providing stronger evidence that this virus is causative for SCCs than PCR positivity alone (Zhu *et al*. 2015). There is also some evidence that EcPV2 may integrate into the host genome, as hrHPVs do. In a qPCR (quantitative PCR) study, a subset of EcPV2-positive equine genital SCCs contained more copies of the viral E6 oncogene than the regulatory E2 gene, which is disrupted by integration (**Fig 3**), per host cell (Sykora *et al*. 2017).

Papillomavirus transmission

The anatomical distribution of many PV-induced diseases is suggestive of direct/fomite transmission. For example, EcPV1 lesions mostly occur on the face, and cattle (fibro)papillomas are often on the head and udder. PVs are nonenveloped viruses, resistant to freezing and desiccation (Roden *et al*. 1997), and may therefore persist in the environment for some time. Virions infect new hosts via abrasions as they cannot invade intact epithelium.

It is thought, although not yet proven, that EcPVs implicated in aural plaques are transmitted by flying insects, such as black flies (*Simulium* spp.), which are often seen in horses' ears (Fairley *et al*. 2014). The principle of flying insects acting as mechanical vectors for PVs has been experimentally proven in rabbits (Dalmat 1958). The anatomical distribution of sarcoids is also suggestive of an insect vector, and BPV1 and BPV2 DNA has been found on UK biting and nonbiting flies (Finlay *et al*. 2009). However, infective BPV virions have not been detected on flies to date.

Transmission mechanisms of other EcPVs to other anatomical sites are unclear. HPVs causing tumours of the head, neck and genitalia in humans are considered to be primarily sexually transmitted, but numerous other routes, both horizontal and vertical, have been proposed (Tumban 2019; Mchome *et al*. 2021). BPV1 and BPV2 DNA has been detected in the blood and semen of healthy equines (Silva *et al*. 2014), EcPV2 in smegma (Sykora *et al*. 2017) and EcPV9 in semen (Li *et al*. 2019). Additionally, given EcPV2 has now been found in gastric SCCs (Alloway *et al*. 2020) and SCCs of the equine head including of the larynx and guttural pouch (Hibi *et al*. 2019), there are multiple potential transmission mechanisms.

Vaccination

Effective prophylactic PV vaccinations have been developed for human patients, but post-exposure vaccination does not appear effective. HPV vaccines contain virus-like particles (VLP) which are empty viral capsid protein shells. Over 90% efficacy against persistent infections of HPV 16 and 18 has been demonstrated, and the vaccines also appear to offer cross-protection against similar HPV types (Cutts *et al*. 2007). BPV1 VLP vaccines have been shown to be safe and immunogenic in horses (Hainisch *et al*. 2012), and to protect horses from an experimental BPV1 or BPV2 challenge (Hainisch *et al*. 2017). Given that it may be possible to protect horses from PV-associated diseases, further research into the number of PVs affecting the horse, and the range of diseases they cause, is warranted. The potential market for such vaccinations should also be explored.

Author's declarations of interest

No conflicts of interest have been declared.

Ethical animal research

Not applicable to this clinical commentary.

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