4. Studies of Cancer in Animals

Due to the species specificity of papillomaviruses, infection of experimental animals with HPVs is not possible. However, understanding the natural history and carcinogenic potential of HPVs is assisted by the study of several animal papillomaviruses.

In the analysis of the association of animal papillomavirus with naturally occurring or experimentally induced neoplasia in various species, benign tumours (warts) rather than cancer are often taken as the end-point, often on the grounds that: (i) the incidence of warts is higher than that of cancer and it is therefore easier to monitor; (ii) it is difficult to follow the course of disease in wild animals; (iii) domestic animals, such as cattle, are usually killed before the onset of malignancy; and (iv) papillomavirus-associated cancer may ultimately derive from warts exposed to the action of cofactors, and thus the presence of warts can be considered as an indication of possible incipient neoplastic progression. All of the reported genomic sequences of animal papillomaviruses are available in the EMBL and GeneBank data.

For each of the animal papillomaviruses included in this section, naturally occurring warts and their progression to cancer are described first, followed by experimental production of tumours in natural and in heterologous hosts.

4.1 Non-human primate papillomaviruses

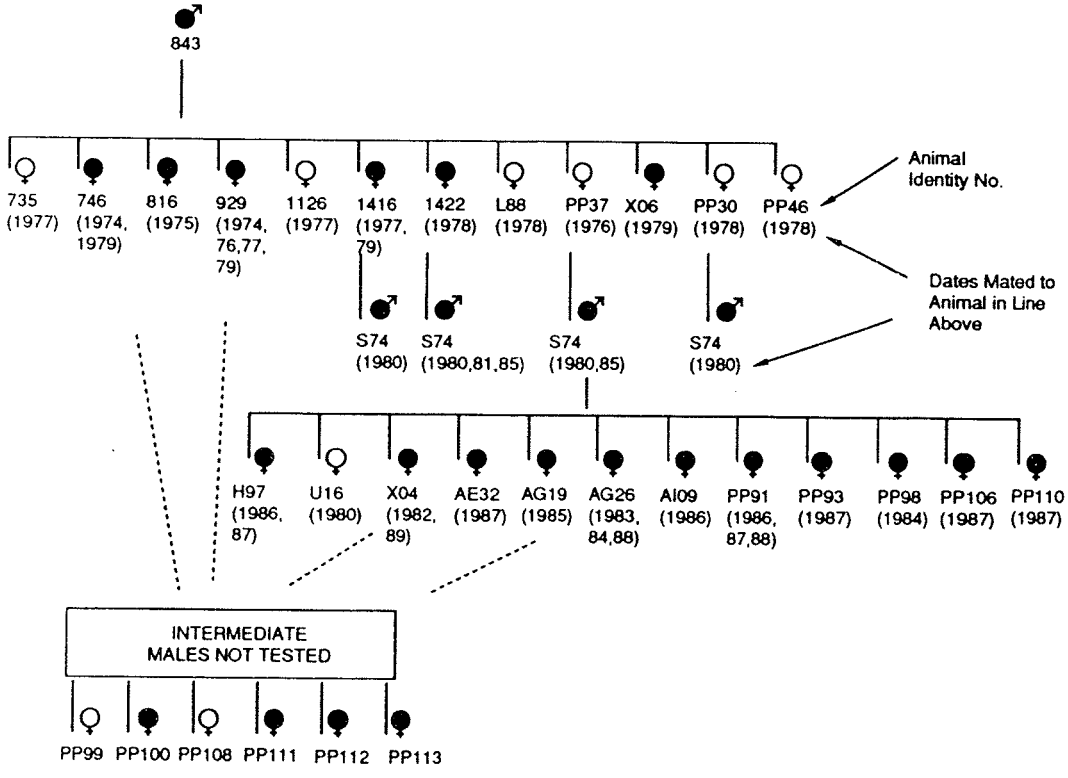
Two different types of papillomavirus were isolated from papillomas of the colobus monkey (*Colobus guereza*): CgPV-1 from a penile papilloma and CgPV-2 from a cutaneous papilloma (O'Banion *et al.*, 1987; Kloster *et al.*, 1988). A different papillomavirus was isolated from 5/8 cases of focal epithelial hyperplasia in the pygmy chimpanzee (*Pan paniscus*) and called PCPV (Van Ranst *et al.*, 1991). This virus is related to HPV-13 which induces focal epithelial hyperplasia in humans.

Rhesus monkey genital papillomavirus

Kloster *et al.* (1988) isolated and cloned an integrated papillomavirus genome from a lymph node metastasis of a penile squamous-cell carcinoma in a rhesus monkey (*Maccaca mulatta*). The viral DNA was designated RhPV-1 and used as a probe in a retrospective study of a rhesus colony (Ostrow *et al.*, 1990). The authors analysed individuals that had either mated directly with the 'index male' or with intermediate sexual partners. They analysed by PCR biopsies or scrapes from 30 females, one male and the index male, all belonging to the same group, from four mature females from a different group and from seven virgin females. The direct (6/12) and indirect (15/18) mates of the index male were found to be positive for viral DNA, clinical lesions or histopathology (Figure 20). One intermediate male analysed by PCR was positive for RhPV-1 DNA and four intermediate males were all clinically positive (Figure 20). The lesions

displayed various degrees of atypia, ranging from koilocytosis, CIN I, koilocytosis plus CIN I, to invasive squamous-cell carcinomas of the penis and the cervix. The virgin females and those from the outside group showed no RhPV-1 infection. These results strongly linked, in a causal way, infection by RhPV-1 with genital neoplasia.

Figure 20. RhPV-1 transmission and mating relationships in rhesus monkeys



The most direct sexual relationships are shown with the dates of interactions. Filled symbols represent animals for which at least one clinical, histopathological or molecular result indicate papillomavirus infection. Open symbols represent negative individuals. Virgin females and females mated outside the group are not shown. Reproduced from Ostrow *et al.* (1990) with the permission of the authors.

In a recent study, Ostrow *et al.* (1995) analysed a number of fresh or archival genital tissues of rhesus monkeys from three geographically distinct regions for evidence of papillomavirus infection. By PCR, sequences related to RhPV-1 DNA were found in 12/59 (20%) animals from the three areas. The serological status of the animals was also investigated and 34/59 (57.6%) animals were positive for at least one RhPV-1 antigen. Most of the RhPV-1 DNA-positive animals were also serologically positive (8/10 tested cases), whereas most serologically positive animals were RhPV-1 DNA negative (23/31 tested cases). Histopathological analysis showed that the vast majority of cervical smears and biopsies were clinically normal, with the occasional presence of mild-to-moderate chronic inflammation and focal squamous metaplasia. Four cases showed features of papillomavirus infection; of these one was classified as CIN I, and another was the only case concordant with seropositivity; all cases were RhPV-1 DNA negative. The

situation parallels HPV infection in humans, where the detection of seropositivity is higher than the detection of viral DNA.

4.2 Bovine papillomavirus

4.2.1 Heterogeneity of bovine papillomavirus

Bovine papillomaviruses (BPVs) make up a heterogeneous group of viruses with world-wide distribution. They induce papillomatosis of the skin, the genital and paragenital area, the eye, the alimentary canal and the bladder. Six types (BPV-1 to -6) have been described in detail (Jarrett *et al.*, 1984a), but many more remain to be identified and isolated. The six well-characterized BPVs fall into two subgroups, A and B, depending on the size of the viral genome and the degree of their molecular and immunological relationship. The molecular and immunological differences between the members of the two subgroups underlie biological differences. Thus, subgroup A members (BPV-1, BPV-2 and BPV-5) induce fibropapillomas, warts with fibroblastic and epithelial components, whereas subgroup B members (BPV-4 and BPV-6) induce purely epithelial papillomas (Campo *et al.*, 1980; Jarrett *et al.*, 1984a); BPV-3 has been found in epithelial papillomas (Pfister *et al.*, 1979a) (Table 61).

Table 61. Bovine papillomaviruses and their tumours

Subgroup	Virus type	Associated tumours
A	BPV-1	Fibropapillomas of the paragenital areas
	BPV-2	Fibropapillomas of the skin
		Fibropapillomas of the alimentary canal[a]
	BPV-5	Fibropapillomas of teats and udders
B	BPV-3	Papillomas of skin
	BPV-4	Papillomas of the alimentary canal
	BPV-6	Papillomas of teats and udders

[a]Alimentary canal fibropapillomas do not produce virus
From Pfister *et al.* (1979a), Campo *et al.* (1980, 1981) and Jarrett *et al.* (1984a)

4.2.2 BPV-1

BPV-1 induces primarily fibropapilloma of the penis of bulls and of the teats and udders of cows and can also infect adjacent skin and the muzzle, leading to the same histological lesions (Campo *et al.*, 1981). BPV-1 has been used extensively in transmission experiments, where the 'take' in cattle can be up to 100% (Jarrett, 1985). Olson *et al.* (1969) were the first to perform transmission experiments with BPV and, although at that time the multiplicity of BPV was not known, it is reasonable to assume that the virus was either BPV-1 or BPV-2, as it was extracted from fibropapillomas of the skin. In addition to transmitting BPV to skin, Gordon and Olson (1968) induced meningiomas in 17/19 calves (89.5%) by injection of the virus into the brain. The latency period was nine months and the take in the brain was the same as that in the skin.

A review of early transmission experiments both in homologous and heterologous hosts is given in Olson (1987).

(a) BPV-1 in hamsters

Inoculation of BPV-1 into Syrian hamsters (*Mesocricetus auratus*) induced subcutaneous fibromas and fibrosarcomas, chondromas of the ear and meningiomas of the brain, depending on the site of injection; metastases to internal organs were relatively frequent particularly in the lungs (10% of the animals) (Olson *et al.*, 1969). In a typical experiment, Pfister *et al.* (1981b) extracted BPV-1 from an udder fibropapilloma of a cow and inoculated approximately 10^9 viral particles/ml subcutaneously in the back of six two-month-old hamsters. Two of the animals developed fibrosarcomas at the site of injection, with a latency period of about 14 months. Both fibrosarcomas contained viral DNA, which was present in multiple episomal copies, but not structural viral antigens or virus particles. Both tumours were transplantable to other hamsters.

(b) BPV-1 in transgenic mice

BPV-1 transgenic mice have been generated by Lacey *et al.* (1986). A partial tandem duplication of the BPV-1 genome was used, containing two copies of the early transforming region and one of the late structural genes. Two transgenic mice were obtained, one of which died soon after birth. The other mouse had approximately five copies of integrated viral DNA in head-to-tail tandem structures. The heterozygous progeny of this mouse were used to generate homozygous animals. The homozygotes were normal during development and early adulthood. However, when about eight months old, all animals developed skin tumours, initially benign fibromas, in multiple body locations. Fibromas also developed on the tails of heterozygotes where they had been clipped for DNA analysis. The fibromas became malignant and invasive with age. No virion or viral structural antigen was detected in the fibromas or fibrosarcomas. Whereas in young normal mice and in normal skin the viral DNA was integrated into the cellular DNA, in the tumours the viral DNA was episomal and amplified. Viral transcription activity increased during tumour progression from fibroma to fibrosarcoma (Bossy-Wetzel *et al.*, 1992; Christofori & Hanahan, 1994).

4.2.3 BPV-2

BPV-2 induces typical skin warts of the neck and shoulder in cattle (Campo *et al.*, 1981). The histology of these skin warts is similar to that of BPV-1 warts (Jarrett, 1985). BPV-2 is also found in fibropapillomas of the oesophagus and rumen (Jarrett *et al.*, 1984b), but these tumours, contrary to the fibropapillomas of the skin, do not produce virus and appear to be the result of abortive infection. Transmission experiments of BPV-2 to the skin have a take of 100% (Jarrett, 1985).

(a) BPV-2 in bladder cancers

In a study in Scotland, 30% of the cattle with squamous-cell carcinoma (80 animals) of the upper alimentary tract had concurrent bladder tumours. Animals can have more than one type of bladder tumour. Haemangioendotheliomas were found in 23%, transitional-cell carcinomas in 8%, fibromas in 4% and adenocarcinomas in 1% (Jarrett *et al.*, 1978a). The same histological

types of bladder tumours, in addition to papillomas and squamous-cell carcinomas, have been found in other parts of the world in association with a diet of bracken fern (Pamukcu, 1963; Rosenberger, 1971). Bracken fern contains mutagenic (Evans *et al.*, 1982a) and immunosuppressive compounds (Evans *et al.*, 1982b).

Injection of a 10% suspension of bovine wart tissue in the urinary bladder of two- to three-month-old calves induced fibromas and polyps in 13/15 animals (Olson *et al.*, 1959). The calves were killed from 40 to 80 days after inoculation and no malignant progression was observed. In another experiment (Olson *et al.*, 1965), suspensions of six naturally occurring bladder tumours (2 haemangiomas, 1 haemangioma plus papilloma, 2 papillomas, 1 papilloma plus adenocarcinoma plus squamous carcinoma — this latter case was accompanied by metastasis to the iliac node) were inoculated in the skin, the vagina and the urinary bladder of young calves. Of 17 innoculated calves, 10 developed skin fibropapillomas, seven developed fibropapillomas of the vagina and five developed polyps and fibromas of the urinary bladder. These experiments demonstrated both the presence of BPV in tumours of the urinary bladder and the ability of the virus to induce bladder tumours. At the time, the heterogeneity of BPV was not known and the identity of the virus used in the above experiments is uncertain.

Campo *et al.* (1992) reported that the virus involved in bladder cancer in cattle was BPV-2. Multiple copies of episomal BPV-2 DNA were found in 7/15 biopsies (46%) of naturally occurring bladder tumours from animals from bracken fern-infested areas. Eight of 10 normal bladder biopsies were negative and, of the remaining two biopsies, one was positive for BPV-2 DNA and the other for an unidentified papillomavirus. In an experiment designed to reproduce the papilloma–carcinoma syndrome of the upper alimentary canal (see below), further evidence was obtained of the involvement of BPV-2 and of its synergism with bracken fern in the induction of urinary bladder malignancies. Calves approximately three to five months old were immunosuppressed either by treatment with azathioprine (see IARC, 1987e) (10 animals) or by a diet of bracken fern (12 animals) (Table 62). Some of the animals were infected with BPV-4 (see below), but not with BPV-2. All the immunosuppressed calves developed urinary bladder tumours starting approximately two years after the beginning of the experiment. However, in the animals immunosuppressed with azathioprine, the tumours were benign haemangiomas, whereas in the animals fed with bracken fern, the tumours were malignant and representative of the whole range of naturally occurring bladder cancers. Bladder biopsies were analysed for the presence of BPV DNA in three animals from the azathioprine group and in 10 animals from the bracken fern group. BPV-2 DNA was found in tumour biopsies of 9/13 animals, including the haemangiomas of the azathioprine-treated animals (Table 62). The negative biopsies were from four animals of the bracken fern group. In the cases with multiple tumour types, the tumours were either all positive or all negative. As in the natural bladder cancers, no virus or structural viral antigens was detected in the experimental tumours. It was concluded that immunosuppression favoured the establishment of premalignant viral lesions, but mutagens present in the fern promoted their malignant progression.

(b) BPV-2 latency

The above experiment also suggested the presence of latent BPV-2, which could be reactivated by immunosuppressive treatment, as in the bladder and/or by skin damage (Campo *et al.*,

Table 62. Bladder cancers, alimentary canal tumours and viral latency in experimental calves

Group	Animal	Treatment	Bladder cancer	Alimentary papilloma	Alimentary cancer	Site of skin warts
1	1	BPV-4		Yes		
	2			Yes		
	3			Yes		
	4			Yes		
	5			Yes		
	6			Yes		Bleeding site (BPV-1)
2	7	BPV-4 +	Yes (BPV-2)	Yes (++)		Scarification site (BPV-1)
	8	azathioprine	Yes (BPV-2)	Yes (++)		Scarification site (BPV-1)
	9	(2 mg/kg	Yes (ND)	Yes (++)		
	10	bw/day)	Yes (ND)	Yes (++)		
	11		Yes (ND)	Yes (++)		
	12		Yes (BPV-2)	Yes (++)		
3	13	Azathioprine	Yes (ND)			Scratch on neck (BPV-2)
	14	(2 mg/kg	Yes (ND)			
	15	bw/day)	Yes (ND)			
	16		Yes (ND)			Lips (BPV-2)
4	17	None				
	18					
	19					
	20					
5	21	Bracken fern	Yes (BPV-2)			
	22		Yes (BPV-2)			
	23		Yes (no BPV)			
	24		Yes (ND)			
	25		Yes (BPV-2)			Scratch on neck (BPV-2)
	26		Yes (no BPV)			
6	27	BPV-4 +	Yes (BPV-2)	Yes (+)		
	28	bracken fern	Yes (BPV-2)	Yes (+)		
	29		Yes (ND)	Yes (+)		
	30		Yes (no BPV)	Yes (+)	Yes (no BPV)	
	31		Yes (no BPV)	Yes (+)	Yes (no BPV)	
	32		Yes (BPV-2)	Yes (+)		
7	33	Quercetin	No			Scarification site (BPV-1)
	34		No			Scarification site (BPV-1)
8	35	BPV-4 +	No	Yes		Scarification site (BPV-1)
	36	quercetin	No	Yes		Scarification site (BPV-1)

From Campo *et al.* (1992, 1994a,b)
Degree of alimentary papillomatosis is indicated by +
ND, not determined

1994a). Four of 10 of the azathioprine-treated animals developed skin warts; two of the warts contained BPV-1 and two BPV-2. One of the 12 bracken fern-fed animals developed a BPV-2 wart. All the warts developed at sites of damaged skin (Table 62). All of four animals that had been administered the flavonoid quercetin (see IARC, 1987f) but were fully immunocompetent developed BPV-1 warts at the site of damaged skin (Table 62), indicating that wounding, with the attendant cell proliferation, is sufficient for reactivation of latent virus.

(c) BPV-2 in hamsters

A 10% suspension of a BPV-2 fibropapilloma taken from the neck of a cow was injected into the back of one hamster. After two years, a fibropapilloma developed at the injection site, which contained multiple episomal copies of BPV-2 DNA but no virus or structural viral antigens (Moar *et al.*, 1981).

4.2.4 BPV-3

BPV-3 was isolated from an epithelial skin papilloma of a calf in Australia (Pfister *et al.*, 1979b). Nothing is known about its natural history and no transmission experiment has been performed.

4.2.5 BPV-4

BPV-4 is the causative agent of alimentary tract papillomas (Campo *et al.*, 1980). In a survey of 7746 cattle from local abattoirs, Jarrett *et al.* (1978b) found alimentary canal tumours in 19% of the animals. Of these, 78% had epithelial squamous papillomas and 22% had fibropapillomas; 79% of the affected animals had papillomas at one site and the remaining 21% at more than one site. The epithelial papillomas were productive for and induced by BPV-4 (Campo *et al.*, 1980), whereas BPV-2 DNA, but not the virus, was present in the fibropapillomas (Jarrett *et al.*, 1984b).

(a) BPV-4 and alimentary tract cancer

In the above abattoir survey, 80 cases of squamous-cell carcinomas of the upper alimentary canal (7% tongue, 4% palate, 8% pharynx, 41% oesophagus and groove, 30% (sic) rumen) were observed in animals from the so-called cancer farms, the grazing ground of which was infested with bracken fern: 96% of the animals with cancers had papillomas and 13% of the animals with papillomas had squamous-cell carcinomas; all the histological stages from papillomas to squamous carcinomas were observed (Jarrett, 1978): 36% of the animals with upper alimentary cancers had metastases (liver and/or spleen), 56% had large intestine tumours (polyps, adenomas and adenocarcinomas) and 30% had urinary bladder cancers. In a survey of 366 cattle from the cancer farms, 39% had squamous oral papillomas; of these, 24% had papillomas at one site and 65% had widespread papillomas at multiple sites (Jarrett, 1978; Jarrett *et al.*, 1978a,b).

The papilloma–carcinoma syndrome was reproduced experimentally by Campo *et al.* (1994b) in an experiment that lasted 13 years. Of 32 calves three to five months old, six were infected in the palate with BPV-4, six were infected with BPV-4 and immunosuppressed with azathioprine, four were immunosuppressed with azathioprine, six were kept on a diet of bracken fern, six were infected with BPV-4 and fed bracken fern and four were kept as controls

(Table 62). All calves infected with BPV-4 developed squamous papillomas at the sites of intramucosal injection into the palate. However, the animals immunosuppressed either by azathioprine or by bracken-fern developed florid and persistent papillomatosis with papillomas that spread from the inoculation site, particularly in the azathioprine-treated animals. The last surviving animal from the BPV-4-and-bracken-fern group still had papillomas 13 years after infection and these had spread from the mouth to the lower oesophagus and the rumen. The virus-infected azathioprine-treated animals had to be killed on humanitarian grounds and no progression from papilloma to carcinoma was observed. Two of six animals from the virus-and-bracken-fern group developed cancers of the upper alimentary canal and the lower intestine, six and 10 years, respectively, after the start of the experiment. The two animals had typical papillomas, foci of carcinoma in the oesophagus infiltrating the subjacent tissue and polyps, adenomas and adenocarcinomas of the duodenum, jejunum and colon. No malignancy of the alimentary tract was detected in animals of the other groups. As already observed for the naturally occurring alimentary canal cancers (Campo *et al.*, 1985), no BPV-4 DNA could be detected in the experimental cancers. It was concluded from this experiment that immunosuppression prevented rejection of papillomas and allowed their expansion, while other compounds present in the bracken fern promoted their neoplastic progression.

(b) BPV-4 in mouse xenografts

The tumorigenic potential of BPV-4 has been studied in nude mice xenografts. Chips of bovine fetal palate tissue infected with BPV-4 were implanted in nude mice either under the kidney capsule or subcutaneously and induced virus-producing papillomas in 19/21 mice within 22 weeks. One of the xenograft papillomas underwent spontaneous transformation to a squamous-cell carcinoma which infiltrated the kidney with metastasis to the spleen (Gaukroger *et al.*, 1989, 1991). The malignant cells were confirmed to be of bovine origin by MHC typing and by the nucleotide sequence of the bovine *ras* gene. No BPV-4 DNA was detected either in the primary or in the metastatic cancer. Spontaneous conversion of papillomas in the xenograft system is a very rare event as it was observed only once out of approximately 100 papilloma-bearing mice generated in different experiments. In a further experiment, neoplastic progression was greatly accelerated by the implantation in the recipient mice of slow releasing pellets of either 7,12-dimethylbenz[*a*]anthracene (DMBA) or 12-*O*-tetradecanoylphorbol 13-acetate (TPA). When the mice were exposed to DMBA, the progression of BPV-4 papillomas to carcinomas was observed in 13/20 (65%) implants and when mice were exposed to TPA, in 4/33 (12%) implants. No tumours (papillomas or carcinomas) were found in mice receiving implants of the chemicals alone (DMBA, 0/10; TPA, 0/25). It was concluded that BPV-4 could synergize with both a classical tumour initiator and a classical tumour promoter (Gaukroger *et al.*, 1993).

(c) BPV-4 in hamsters

Six young hamsters were injected with a 10% suspension of a BPV-4 papilloma in the right buccal pouch and intradermally on the skin of the back. One hamster developed a liposarcoma at the site of injection on the back 20 months later. The tumour showed no evidence of fibrocytic transformation, which was in agreement with the inability of the virus to transform fibroblasts *in vivo*; it was positive for viral DNA, which was present in multiple episomal copies, but not for virions or structural antigens (Moar *et al.*, 1986).

4.2.6 BPV-5 and BPV-6

BPV-5 induces 'rice grain' fibropapillomas on the teats and udders of cattle, so called because of their appearance (Campo *et al*, 1981). BPV-6 also induces epithelial papillomas (Jarrett *et al.*, 1984a). These two viruses have not been found in any other body location so far and the tumours produced have not been reported to undergo malignant conversion, although the BPV-6 papillomas are very persistent and natural regression has not been observed (Jarrett, 1985).

In a survey of 1657 cattle from local abattoirs (Lindholm *et al.*, 1984), 37.3% of the animals were found to have at least one teat or udder papilloma. Of the affected animals, 28.4% had BPV-1 warts, 88.5% had BPV-5 warts and 92.3% had BPV-6 warts; 58.6% had double infections with BPV-5 and BPV-6 and 22.9% had triple infections with BPV-1, BPV-5 and BPV-6; 13.9% were infected by only one virus — most often BPV-6 (8.7%) followed by BPV-5 (4.4%) and then by BPV-1 (0.8%). BPV-6 is the most frequent infection of the udder and teats of cattle (Table 63).

Table 63. Incidence (%) of BPV types in teat and udder papillomas

Multiplicity of papillomas	BPV-1	BPV-5	BPV-6
Single	0.8	4.4	8.7
Double — BPV-1 with...	–	2.6	2.1
Double — BPV-5 with...	2.6	–	58.6
Triple	22.9	22.9	22.9
Overall	28.4	88.5	92.3

From Lindholm et al. (1984)

4.2.7 Bovine ocular squamous-cell carcinoma (OSCC)

In Australia, some herds of cattle are commonly affected by OSCC (Spradbrow & Hoffman, 1980). The carcinomas derive from papillomas, and malignant transformation of papillomas is particularly noticeable in lightly pigmented animals, implicating ultraviolet light as a co-carcinogen. Viral particles, strongly resembling papillomavirus, were detected in 8/25 early lesions, including one conjunctival plaque, five conjunctival papillomas, one eyelid papilloma and one eyelid keratinized horn (Ford *et al.*, 1982). The viral DNA has not been cloned, so it is not known which BPV type is responsible for the precursor lesions of OSCC.

4.2.8 Bovine skin carcinoma

Australian herds are commonly affected also by skin cancer (Spradbrow *et al.*, 1987). As in the case of OSCC, the cancers derive from precursor lesions. Thirteen cattle, four to 15 years old, were studied. All animals had lesions of different degrees of severity, from early lesions, such as cutaneous horns with acanthosis and hyperkeratosis, to advanced lesions, such as squamous-cell carcinomas and basal-cell carcinomas. Four animals were observed for three

years and in two of these progression of early lesions to squamous-cell cancer was observed. Viral DNA, hybridizing to BPV-1 in low stringency conditions, was found in 10/11 keratotic lesions and in 5/8 neoplasias, two of which were squamous cancers and three basal-cell carcinomas. The viral DNA has not been cloned, so the virus type cannot be defined; the virus has not been isolated and no transmission experiments have been done. However, as for OSCC, progression of early lesions to carcinomas has been observed and it was concluded that a type of papillomavirus, in conjunction with ultraviolet light, was responsible for the skin cancers.

4.2.9 BPV in equine sarcoids

Sarcoids are commonly found in horses (*Equus equus*) and donkeys (*Equus asinus*). Equine sarcoids are locally invasive non-metastatic fibropapillomas that are rarely rejected by the host. The histological similarity between equine sarcoids and bovine fibromas suggested a link between BPV and the equine disease. Infection of horses with bovine papilloma material induced sarcoids similar to those occurring naturally (Olson & Cook, 1951) but the experimental sarcoids regressed contrary to the natural ones.

Lancaster *et al.* (1977) were the first to detect BPV DNA in natural equine sarcoids. Neither the natural nor the experimental sarcoids contained virus or structural viral antigens. More recent analyses of these equine neoplasms confirmed those original findings (Table 64).

Table 64. DNA positivity for BPV types in equine sarcoids

Reference	BPV-1 (%)	BPV-2 (%)	Overall (%)
Angelos *et al.* (1991)	66.7	21.2	87.9
Otten *et al.* (1993)[a]	92.7	7.3	100
Bloch *et al.* (1994)	60.4	13.3	73.7
Reid *et al.* (1994)[b]	83.3	16.7	100

[a] Survey includes 32 horses and two donkeys.

[b] Survey includes 18 donkeys.

Angelos *et al.* (1991) found BPV DNA in 12/13 sarcoids from horses from New York State and in 17/20 sarcoids from horses from Switzerland. The viral DNA was BPV-1-like in 22 biopsies and BPV-2-like in seven biopsies. BPV DNA was also found in one biopsy each of fibrosarcoma, fibropapilloma and pyogranulomatous dermatitis. No biopsy showed a restriction enzyme pattern of viral DNA identical to reference BPV-1 or BPV-2 DNA, indicating the presence of BPV subtypes or variants.

Bloch *et al.* (1994) conducted a retrospective analysis of equine sarcoids by polymerase chain reaction (PCR) on DNA from formalin-fixed paraffin embedded sections of archival samples. They detected BPV DNA in 56/76 (74%) samples; of these 82% were BPV-1-like and 18% were BPV-2-like.

Reid and Smith (1992) and Reid *et al.* (1994) analysed 24 sarcoid samples from six horses and 18 donkeys by PCR. These authors found that all the biopsies contained BPV DNA and that again BPV-1-like sequences were more prevalent than BPV-2-like sequences. There was no correlation between viral type, clinical type and anatomic location of the lesions or sex of the animals.

The virtual absence in these surveys of BPV-1 or BPV-2 DNA sequences with restriction patterns identical to those of the reference genomes might suggest that particular variants or subtypes of BPV infect horses specifically. However this was not confirmed in another study. Otten *et al.* (1993) analysed by PCR 58 sarcoids from 32 horses and two donkeys. They found BPV-1 DNA in 55 biopsies and BPV-2 DNA in three biopsies. One horse had two sarcoids, one with BPV-1 DNA and the other with BPV-2 DNA. The BPV sequences in the sarcoids had the same restriction enzyme patterns found in BPV-1 and BPV-2 isolates from cutaneous bovine papillomas in cattle from the same geographical area, and the relative incidence of BPV-1 and BPV-2 infection was the same in cattle and horses, suggesting that the BPV variants found in equine sarcoids are not specific for horses. The involvement of BPV in equine sarcoids is the only known instance of naturally occurring infection in which papillomaviruses cross species barriers.

4.3 Equine papillomavirus (EqPV)

In addition to sarcoids, horses can develop cutaneous, genital, oral and ocular papillomas (Olson, 1987). Paraffin sections from 135 equine neoplasms were analysed for the presence of viral structural antigen (Junge *et al.*, 1984). The tumours were 45 papillomas from penis, vulva, skin, eye and oral cavity and 90 carcinomas from eyelid, cornea, genital area, oral cavity, maxillary sinus and skin. Antigen was detected in seven cutaneous and five genital papillomas but not in the carcinomas.

A papillomavirus, EqPV, has been isolated from cases of cutaneous papillomas of one poney and one horse (O'Banion *et al.*, 1986). The same viral type was found in papillomas of the muzzle and the leg but not in penile papillomas from four different horses; the authors concluded that the latter were possibly caused by a different equine papillomavirus.

4.4 Papillomaviruses in cervidae

Papillomavirus was isolated from fibropapillomas of European elk (*Alces a. alces*) (EEPV or EPV; Moreno-Lopez *et al.*, 1981), reindeer (*Rangifer tarandus*) (RPV; Moreno-Lopez *et al.*, 1987), red deer (*Cervus elaphus*) (RDPV; Moar & Jarrett, 1985), mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) (deer fibromavirus subtypes a and b respectively; Groff *et al.*, 1983). EEPV and RPV induced fibrosarcomas after experimental infection of young Syrian hamsters by subcutaneous injection (Stenlund *et al.*, 1983; Moreno-Lopez *et al.*, 1987). The cervidae papillomaviruses have recently been shown to contain a novel open-reading frame encoding transforming activity (Eriksson *et al.*, 1994).

4.5 Ovine papillomatosis

Norval *et al.* (1985) found that 25/200 sheep from local Scottish abattoirs had rumenal fibropapillomas. One animal had a squamous-cell carcinoma. Six of 10 biopsies of the rumenal tumours had few cells (1–3) positive for common antigen (Table 65).

Table 65. Papillomavirus in tumours of sheep

Reference	Lesion	Viral DNA[a]	Viral antigen[a,b]	Virus[a]
Norval *et al.* (1985)	Rumenal fibropapilloma	0/30[c]	6/10	0/20
Vanselow *et al.* (1982)	Cutaneous papillomas	ND	ND	2/3
Vanselow & Spradbrow (1983)	Hyperkeratotic scales	ND	ND	0/1
Trenfield *et al.* (1990)	Cutaneous and vulvar lesions[d]	11/83	ND	ND
Tilbrook *et al.* (1992)	Perineal squamous-cell carcinomas and papillomas	20/26	0/17	ND
Hayward *et al.* (1993)	Cutaneous filiform papillomas	1/1	9/9	Yes (NR)

[a]Positive lesions/analysed lesions
[b]Common structural antigen
[c]Only tested with HPV-1 DNA
[d]Keratinized horns, papillomas and fibropapillomas
ND, not determined; NR, not reported

Other surveys of sheep were performed in Australia (Table 65). Vanselow *et al.* (1982) reported the isolation of a papillomavirus from the cutaneous papillomas of two Merino sheep. Two more sheep with papillomas were followed for several months and during the observation period the papillomas progressed to squamous-cell carcinomas. The same authors (Vanselow & Spradbrow, 1983) reported on another Merino sheep with squamous-cell carcinomas on a lower eyelid and on the vulva. Hyperkeratotic lesions in which virions were detected were present near both cancers but virus was not isolated.

In a later survey, Trenfield *et al.* (1990) analysed 67 ear lesions (cutaneous horns, papillomas, fibropapillomas) from 51 sheep and 16 lesions from other skin sites from 15 sheep. Ten ear lesions and one vulvar lesion were analysed for viral DNA using BPV-1 DNA as a probe. The vulvar lesion and 8/10 of the ear lesions were positive. The viral DNA gave a BPV-2 restriction pattern with eight enzymes and a BPV-1 restriction pattern with two enzymes, reminiscent of the situation found in horses (see section 4.2.9).

A similar survey was performed by Tilbrook *et al.* (1992). Five of 10 premalignant biopsies and 15/16 squamous-cell carcinomas, all from the perineal region of sheep, were found to contain papillomavirus-like DNA, by using both BPV probes and HPV probes.

The occurrence of filiform squamous papillomas on sheep was reported by Hayward *et al.* (1993), also in Australia. These papillomas were not of the fibropapilloma type but histologically resembled verruca vulgaris; they were present in less than 1% ($N = 2660$) of young sheep, always on the lower fore legs. Papillomavirus was visualized by electron microscopy and

viral DNA was detected by hybridization with an HPV-16 DNA probe. All papillomas analysed were positive for the common viral antigen.

4.6 Cottontail rabbit papillomavirus (CRPV)

CRPV was the first papillomavirus to be identified and isolated, and the CRPV system was the first in which progression from benign papillomas to malignant carcinomas was observed and the synergism between virus and cofactors was documented (Rous & Beard, 1934; Rous & Kidd, 1938; Rous & Friedwald, 1944). In nature, CRPV infects primarily cottontail rabbits (*Sylvilagus floridanus*) and occasionally jackrabbits (*Lepus californicus*). The first phase of infection lasts from one to six weeks, during which papillomas (cutaneous horns) grow. Ninety-five to 100% of the infected animals develop papillomas. In 71% of the animals, the papillomas are permanently benign, in 6% of the animals the papillomas regress and in 23% of the animals the papillomas progress to squamous cancer within 12–18 months. Experimental infection of the domestic rabbit (*Oryctalagus cunniculus*) follows a different course. After one to six weeks of growth, 95–100% of rabbits have papillomas. Papilloma regression takes place in 10–40% of the rabbits after one to three months; in 20–30% of the rabbits, the papillomas remain permanently benign, but in 40–60% of the animals they progress to squamous-cell carcinomas in six to 12 months (Kreider, 1980; Wettstein, 1987). In domestic rabbits, therefore, more papillomas progress more rapidly to cancer than in cottontail rabbits, implicating the genetic background of the host in malignant conversion. Infectious virus is found in cottontail rabbit papillomas but not generally in domestic rabbit papillomas or in cancers in either species; however, viral DNA is present in the nonproductive lesions.

4.6.1 CRPV and co-carcinogens

The progression rate of papillomas induced by CRPV is influenced by other carcinogens (Rous & Kidd, 1938; Rous & Friedwald, 1944). When tar was applied to the skin of the ears of rabbits, hyperplastic skin and papillomas developed. The skin reverted to normal and the papillomas regressed or remained indolent when tar treatment was suspended. Only 1/90 tar-treated rabbits developed a carcinoma after two years of treatment. On the contrary, infection with CRPV of tar-treated rabbits either by scarification of hyperplastic skin or tar papillomas or by intravenous inoculation of virus resulted in the rapid appearance of highly malignant cancers in more than half of 70 rabbits (Rous & Kidd, 1938). In an experiment typical of a large series, eight rabbits had tar applied to the ears for 89 days, were infected with CRPV by intravenous injection and by scarification and then tarred again twice weekly for 25 days. Large numbers of papillomas developed rapidly on tarred skin in all rabbits; the papillomas continued growing even when tar treatment was discontinued. In 2/5 rabbits, the papillomas progressed to squamous and anaplastic carcinomas; in one rabbit, there was metastasis to local lymph nodes. None of five control rabbits that had been tarred but not infected with CRPV developed malignancies, although they did develop tar papillomas (Rous & Kidd, 1938).

Rapid malignant progression was observed also when CRPV papillomas were treated with carcinogens. Eight rabbits were infected with CRPV by scarification on six patches of skin, three patches on either side of each animal. Seven days later, healing was complete and each

animal was swabbed on one of the three patches on one side with methylcholanthrene, tar plus methylcholanthrene or tar alone; the patches on the other side were swabbed with solvents only or left untreated; areas of skin not infected with CRPV were swabbed with carcinogens. All sites inoculated with the virus developed papillomas after 14 days, but the papillomas were larger and faster growing in the carcinogen-treated areas. Cancer started to appear as early as 63 days in two animals on sites treated with methylcholanthrene and with tar plus methylcholanthrene in one of the animals. After 85 days, all eight rabbits had developed cancers in the carcinogen-treated areas; these ranged from malignant papillomas to squamous-cell carcinomas, cystic squamous carcinomas and highly anaplastic carcinomas. Two rabbits had metastases to lymph nodes. There were more cancers in areas treated with methylcholanthrene and tar plus methylcholanthrene than in areas treated with tar. During the observation period, no cancer developed from untreated papillomas or from noninfected skin treated with carcinogens. From their extensive series of experiments, the authors concluded that CRPV and carcinogens synergize powerfully in inducing malignant conversion of papillomas (Rous & Friedwald, 1944).

4.6.2 CRPV latency

In an experiment designed to study latency and reactivation of CRPV, Amella *et al.* (1994) infected rabbits with serial dilutions of CRPV. With undiluted virus, 6/7 injection sites in seven rabbits developed papillomas. With virus diluted from 1:2 to 1:8, 8/8 sites in eight animals developed papillomas. With virus diluted from 1:10 to 1:100, 9/34 sites in 18 animals developed papillomas, while with virus diluted from 1:1000 to 1:100 000, none of 14 sites in 14 animals developed papillomas. However, 6/6 biopsies of the negative sites contained viral DNA detected by PCR. Virus was used at a dilution of 1:20 in order to generate papillomas only in a subset of sites. After virus injection, 24/43 sites (55.8%) were mildly irritated by subtherapeutic photodynamic therapy; 2/19 of the nonirritated sites (10.5%) developed papillomas, which contrasts with 11/24 of the irritated sites (45.8%) which did. PCR of injection sites that did not develop papillomas showed the presence of viral DNA. It was concluded that infection with low doses of virus results in the establishment of viral latency, and that virus can be reactivated by skin injury (see also section 4.2.8).

4.6.3 CRPV in transgenic rabbits

CRPV in conjunction with activated *ras* has been used to generate transgenic rabbits (Peng *et al.*, 1993). Three transgenic rabbits were obtained. Two rabbits had only CRPV DNA and one had both CRPV DNA and activated *ras*. The two CRPV transgenic rabbits were phenotypically normal up to two weeks after birth; then they started developing epidermal hyperkeratosis. Small papillomas appeared when the animals were 20–30 days old that developed and spread all over the body. The rabbits died of pneumonia and septicaemia at 40 and 75 days, respectively. No malignant changes were detected in the papillomas. The third rabbit, transgenic for both CRPV DNA and *ras*, had thickened skin at birth and died at day 3. It was covered by epidermal papillomas that had already undergone highly malignant progression. The entire skin was described by the authors as 'an extended squamous carcinoma'. No neoplasia was detected in other organs. Integrated CRPV DNA was detected in all tissues but it was episomal and greatly amplified in tumours in all three rabbits. In contrast, there was no difference in *ras* transgene

copy number between normal and tumour tissues. CRPV DNA was transcribed in papillomas and carcinomas but not in normal tissue, while *ras* was transcribed only in the cancers. It was concluded by the authors that CRPV-induced papillomas and that the rapid progression of papillomas to carcinomas was due to synergism between CRPV oncogenes and activated *ras*.

4.7 Domestic rabbit oral papillomavirus

Oral papillomas were found in 31% of 51 New Zealand white rabbits from two commercial sources. The virus was isolated and inoculated into the tongue and vulva of three noninfected rabbits. All rabbits developed papillomas of the tongue but not of the vulva, demonstrating site specificity. No cross-immunity was observed between skin (CRPV) and oral virus and it was concluded that the viruses have separate identities. When the oral papillomavirus was inoculated into baby hamsters, 9/10 hamsters developed fibromas (Sunberg *et al.*, 1985).

4.8 *Mastomys natalensis* papillomavirus (MnPV)

Mastomys natalensis is a common rodent in southern Africa. Colonies have been established in several laboratories. The animals of the Giessen colony and the Heidelberg colony harbour a latent papillomavirus, MnPV (Amtmann *et al.*, 1984; Amtmann & Wayss, 1987; Tan *et al.*, 1994b). Certain strains of the animals develop keratoacanthomas and papillomas of the skin, in an age-dependent manner. The tumours never appear in animals younger than 50 weeks old, but by 16 months of age 80% of the animals have tumours. Occasionally, the benign lesions progress to malignant cancers in older animals. The rate of malignant progression depends on the genetic susceptibility of the host — in the Heidelberg colony progression is only sporadic, while in the Giessen colony 11% of the animals undergo progression. The viral genome increases dramatically in copy number during tumour formation, from approximately 0.1 copy per cell in 16-week-old animals to more than 3000 copies per cell in 80-week-old animals. Amtmann *et al.* (1984) showed that treatment of the skin with TPA increased DNA copy number and lowered the age at tumour appearance to as early as 14 weeks. The same result was obtained when the skin was irritated with sandpaper (Siegsmund *et al.*, 1991).

4.9 Mouse papillomavirus (MmPV)

The only known mouse papillomavirus was isolated from a zoo colony of European harvest mice (*Micromys minutus*) (Sundberg *et al.*, 1988). Adult mice of each sex developed acanthomas, papillomas, inverted papillomas, sebaceous carcinomas and pulmonary keratinaceous cysts. The virus (MmPV) was detected in two papillomas, viral DNA in 28/28 biopsies, both benign and malignant, and structural antigen in 20/31 biopsies. The MmPV could be transmitted to one of two harvest mice but not to laboratory mice (CAF or C3H strains) or to wild deer mice (*Peromyscus maniculatus gambeli*).

4.10 Canine oral papillomavirus (COPV)

Dogs can be affected by oral papillomatosis, particularly if kept in kennels in large numbers. The incubation period of the oral papillomas varies from four to 10 weeks and regression usually follows in three to 14 weeks (Olson, 1987). Progression to squamous cancer is rare (Watrach *et al.*, 1970). Recently, three identical isolates of a papillomavirus, COPV, were isolated from oral papillomas from three dogs (*Canis familiaris*) and one coyote (*Canis latrans*). The COPV isolated from the coyote was transmitted to 2/3 dogs. One of the dog isolates was transmitted to 2/3 dogs and a second dog isolate to 1/3 dogs. The third dog isolate was not tested (Sundberg *et al.*, 1991).

Several thousands of kennel beagles were prophylactically vaccinated with a suspension of live COPV at 10–14 weeks of age. The vaccine was injected into the gluteal muscles. The great majority of dogs were protected from natural infection, but 5/500 dogs kept in the authors' laboratory and 7/4000 dogs kept in the breeder's kennels developed cancers at the site of vaccine inoculation. The cancers comprised 10 highly invasive squamous-cell carcinomas, one basal-cell epithelioma and one epidermal pseudocarcinomatous hyperplasia. Five of 12 cancers were positive for COPV structural antigen, but all were negative for virus. The biopsies were not analysed for viral DNA. As COPV does not naturally infect the skin, it was concluded that the cancers had been induced by the live virus in the vaccine (Bregman *et al.*, 1987).

4.11 Feline papillomas

Two Persian cats, 10 and 13 years old, respectively, both under steroid immunosuppressive therapy, developed sessile hyperkeratotic skin lesions, that were positive for papillomavirus, common viral structural antigen and viral DNA (Carney *et al.*, 1990). Both cats were negative for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV). Transmission of the papillomavirus was attempted but was unsuccessful. The viral DNA was not cloned. In another study, one six-year-old cat positive for FIV developed wart-like lesions on the skin. The lesions were positive for papillomavirus and for viral structural antigen (Egberink *et al.*, 1992). It was concluded that cats display clinical papillomavirus lesions when immunosuppressed either by FIV infection or by therapy.

4.12 Avian papillomavirus

A papillomavirus (FPV) was isolated from a leg papilloma of a chaffinch (*Fringilla coelebs*) (Osterhaus *et al.*, 1977) but could not be transmitted to other finches or hamsters (Moreno-Lopez *et al.*, 1984).