3. Studies of Animal Papillomaviruses

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

Whereas cancer is the end-point to assess carcinogenicity in the study of HPV, benign tumours (warts and papillomas) are often used as the end-point in the analysis of the association of papillomavirus with naturally occurring or experimentally induced neoplasia in animals. This is based on the grounds that: (*a*) the incidence of warts is higher than that of cancer and is therefore easier to monitor; (*b*) it is difficult to follow the course of disease in wild animals; (*c*) domestic animals, such as cattle, are usually killed before the onset of malignancy; and (*d*) papillomavirus-associated cancer ultimately derives from warts, and thus the presence of warts can be considered as an indication of possible incipient neoplastic progression.

For each of the animal papillomaviruses discussed below, naturally occurring warts and their progression to cancer are considered primarily, followed by experimental reproduction in natural and heterologous hosts and tumour production in transgenic animals.

3.1 Non-human primate papillomaviruses (Table 75)

Two different types of papillomavirus were isolated from papillomas of the colobus monkey (*Colobus guereza*): CgPV 1 from a penile papilloma (O'Banion *et al.*, 1987) and CgPV 2 from a cutaneous papilloma (Kloster *et al*., 1988). CgPV 1 is a typical genital alpha-papillomavirus, whereas CgPV 2 belongs to the cutaneous beta-papillomaviruses (Chan, S.Y. *et al*., 1997a).

Another papillomavirus was isolated from five of eight cases of focal epithelial hyperplasia in pygmy chimpanzees (*Pan paniscus*) and was called PCPV (van Ranst *et al*., 1991). This virus is evolutionarily related to HPV 13 (85% sequence homology), which is associated with oral focal epithelial hyperplasia in humans (van Ranst *et al*., 1992a). Recently, oral focal epithelial hyperplasia was also diagnosed in a neotropical primate, the howler monkey (*Alouatta fusca*). Whereas the group-specific papillomavirus antigen (denatured L1) was identified by immunohistochemistry, in-situ hybridization with various HPV probes did not reveal any DNA, which suggests the possible presence of a papillomavirus that is specific for the howler monkey (Sá *et al.*, 2000).

Cervical and vaginal epithelial neoplasms, including cervical cancer, were identified in 20/385 (5.2%) female cynomolgus macaques (*Macaca fascicularis*). All lesions were positive when stained with antibodies against bovine papillomavirus 1 (BPV 1), HPV 1, 6, 11,

Table 75. Papillomaviruses in non-human primates

Non-human primate	Papillomavirus	Genus and species[a]	Greatest homology to	Reference
Colobus guereza	CgPV 1	Alpha 9/7	HPV-16/18	O'Banion *et al*. (1987)
	CgPV 2	Beta 1	HPV-5/8	Kloster *et al*. (1988)
Pan paniscus	PCPV	Alpha 10	HPV-13	van Ranst *et al*. (1991)
Alouatta fusca	HMPV	Alpha[b]	–	Sá *et al.* (2000)
Macaca mulatta	RhPV 1	Alpha 12	HPV-16	Kloster *et al.* (1988)
	RhPV a to m	Alpha	–	Chan, S.Y. *et al*. (1997b)

CgPV, *Colobus guereza* papillomavirus; HMPV, howler monkey papillomavirus; PCPV, pygmy chimpanzee papillomavirus; RhPV, rhesus monkey papillomavirus
[a] For a definition of genus and species, see de Villiers *et al*. (2004a).
[b] Uncertain

16, 18 or 31 and HPV 16 E6. Although a DNA fragment was amplified by polymerase chain reaction (PCR) using degenerate primers from nine of 16 cases, sequencing was not successful and the nature of the amplified fragment remains doubtful (Wood *et al.*, 2004).

Rhesus monkey genital papillomavirus

Kloster *et al*. (1988) isolated and cloned an integrated papillomavirus genome (designated RhPV 1) from a lymph node metastasis of a penile squamous-cell carcinoma in a rhesus monkey (*Macaca mulatta*). Viral DNA was fully sequenced and it was determined that the integration site in the viral genome was within the L1 open reading frame (ORF; Ostrow *et al*., 1991). A series of different RhPV genomes (RhPV a to RhPV m) were subsequently isolated and, similarly to RhPV 1, these were found to belong to the alpha-papillomaviruses (Chan, S.Y. *et al*., 1997b).

Ostrow *et al.* (1990) performed a retrospective study of a colony of rhesus monkeys to assess the extent of RhPV 1 infection in individuals that had either mated with the index male or with intermediate sexual partners. Biopsies or scrapes were analysed from 30 females, the index male and four intermediate males that all belonged 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. One of the four intermediate males analysed by PCR was positive for RhPV 1 DNA; four intermediate males were all clinically positive. The lesions displayed various degrees of neoplasia, ranging from koilocytosis, grade 1 cervical intraepithelial neoplasia (CIN1) and koilocytosis plus CIN1 to invasive squamous-cell carcinomas of the penis and the cervix. Virgin females and those from the outside group showed no RhPV 1 infection. These results strongly indicated that infection by RhPV 1 is a cause of genital neoplasia. Subsequently, 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, RhPV 1 DNA sequences were found in 12/59 (20.3%) 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 antigen. There was concordance between viral DNA positivity and seropositivity in 10 cases. Histopathological analysis showed that the majority of the samples was 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 CIN1 and another was the only case that concorded with seropositivity. All cases were RhPV DNA-negative. This situation parallels HPV infection in humans, in which most cases of infection are undetected clinically and the concordance between seropositivity and viral DNA positivity is not complete.

3.2 Bovine papillomavirus (BPV)

3.2.1 *Heterogeneity of BPV* (Table 76)

BPVs are a heterogeneous group of viruses that are distributed worldwide. They induce papillomatosis of the skin, the genital and paragenital area, the eye, the upper gastrointestinal tract and the urinary bladder. Six members (BPV 1–6) have been described in detail (Jarrett *et al*., 1984a,b; Jarrett, 1985), and a further 13 types were identified recently (Antonsson & Hansson, 2002; Ogawa *et al*., 2004), which more than trebles the heterogeneity of BPVs (Table 76).

The six well-characterized BPVs were originally classified into two subgroups (A and B), based on their genomic structure and recognized pathology. Subgroup A comprised BPV 1, 2 and 5, which were commonly defined as fibropapillomaviruses — that is, viruses that infect both the epithelium and the underlying derma and give rise to fibropapillomas. Subgroup B comprised BPV 3, 4 and 6, defined as purely epitheliotropic BPVs that infect only the epithelium and induce true papillomas. Papillomaviruses have recently been reclassified (de Villiers *et al*., 2004a) following the Greek letter nomenclature used for other virus families. According to the new nomenclature, the epitheliotropic BPVs 3, 4 and 6 are defined as xi-papillomaviruses and BPVs 1 and 2 as delta-papillomaviruses. The genome of BPV 5 appears to share homology with both xi- and delta-papillomaviruses (Bloch & Breen, 1997) but BPV 5 appears to have a dual pathology and causes both fibropapillomas and epithelial papillomas (see below; Bloch *et al*., 1994a). These two observations have led to the re-classification of BPV 5 as the only member of the epsilon-papillomavirus genus (de Villiers *et al*., 2004a).

The new BPV types were found in teat papillomas and in healthy teat skin but their pathology and whether they are delta-, xi- or epsilon-papillomaviruses are not yet known. BPVs 1, 3 and 6 were also found in healthy teat skin, which strongly suggests latent or subclinical infection (see Section 3.2.3(*b*)).

Table 76. Heterogeneity of bovine papillomaviruses (BVPs) and their tumours

BPV type	Old classification	New classification[a]	Benign tumours/healthy skin	Malignant tumours in natural host	Malignant tumours in experimental hosts
BPV 1	Subgroup A	Delta 4	Skin fibropapillomas, including penis and teats	Penile carcinoma	Hamsters; transgenic mice
BPV 2	Subgroup A	Delta 4	Skin and oesophageal fibropapillomas	Urinary bladder cancer	Hamsters; SCID mice
BPV 3	Subgroup B	Xi	Skin epithelial papillomas	NK	NT
BPV 4	Subgroup B	Xi	Upper gastrointestinal tract epithelial papillomas	Upper gastrointestinal tract carcinoma	Hamsters; nude mice
BPV 5	Subgroup A	Epsilon	Skin epithelial and fibropapillomas (teats, udder and face)	NO	NT
BPV 6	Subgroup B	Xi	Skin epithelial papillomas (teats and udder)	NO	NT
BAA 1 to 5 BAPV 1 to 10 BAPV 11MY	NA	Delta/Xi[c]	Skin papillomas, healthy skin	NK	NT
BPV[b]	NA	NA	Skin fibropapillomas	Meningiomas[d]	
BPV[b]	NA	NA	Epithelial papillomas	Eye carcinoma	
BPV[b]	NA	NA	Skin hyperkeratosis	Skin carcinoma and basaliomas	

NA, not applicable; NK, not known; NO, never observed; NT, not tested
[a] For new classification and a definition of genus and species, see de Villiers *et al*. (2004a)
[b] Unidentified type
[c] To be established
[d] Meningiomas were experimentally produced in calves by injecting the virus into the brain.

3.2.2 *BPV 1*

BPV 1 induces primarily fibropapillomas of the penis of bulls and of the teats and udders of cows but can also spread to adjacent skin and to the muzzle (Campo *et al*., 1981). It has been used extensively in transmission experiments, in which the rate of infection in cattle can be up to 100% (Jarrett, 1985). Olson *et al*. (1969) were among the first to perform transmission experiments with BPV. In addition to transmitting BPV to skin, Gordon and Olson (1968) induced meningiomas in 17/19 calves (89.5%) by injecting the virus into the brain. These tumours were found as early as 33 days after inoculation and the incidence of neoplasms in the brain was similar to that of warts in the skin.

(*a*) *BPV 1 in hamsters*

Inoculation of BPV 1 into Syrian golden hamsters (*Mesocricatus auratus*) induced fibromas and fibrosarcomas of the skin, 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). Pfister *et al*. (1981) extracted BPV 1 from a cow udder fibropapilloma and inoculated 10^9 viral particles subcutaneously into the back of each of six 2-month-old hamsters. Fourteen months later, one of the animals developed a fibrous histiocytoma with some areas of fibrosarcoma at the site of injection, and another developed a fibroma with partially atypic fibroblasts. Both tumours were positive for BPV 1 DNA, which was present in multiple episomal copies, but not for structural viral antigens or virus particles.

(*b*) *BPV 1 in transgenic mice*

BPV 1 transgenic mice (BPV 1:69 mice) were first generated by Lacey *et al*. (1986). A partial tandem duplication of the BPV 1 genome that contained two copies of the early transforming region and one of the late structural genes was used. One of these transgenic mice 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. At 8 months of age, all animals developed tumours (initially benign fibromas) in multiple body locations. Tumours were most frequently found in the face and head area and on the end of the tails of heterozygotes where they had been clipped for DNA analysis. The tumours became malignant and locally invasive with age. No virion or viral structural antigens were detected in the fibromas or fibrosarcomas. Whereas in young normal mice and in normal skin the viral DNA was integrated into the cell DNA and transcriptionally inactive, the viral DNA in the tumours was extrachromosomal and transcriptionally active (Lacey *et al.*, 1986; Sippola-Thiele *et al.*, 1989). The same transgenic mice were further analysed for specific chromosomal abnormalities that emerged during the carcinogenic process. In contrast to fibromas, fibrosarcomas consistently showed trisomy or duplication of chromosome 8 and/or monosomy or translocation of chromosome 14, which suggests that these chromosomal losses and/or duplications accompany and contribute to neoplastic transformation (Lindgren *et al*., 1989).

3.2.3 *BPV 2*

BPV 2 induces classical skin warts (Campo *et al*., 1981) that are histologically similar to those induced by BPV 1 (Jarrett, 1985). It also induces fibropapillomas of the oesophagus and rumen, which, contrary to fibropapillomas of the skin, do not produce viruses and appear to be the result of abortive infection (Jarrett *et al.*, 1984a). In experiments in which BPV 2 is transmitted to the skin, the virus produces warts in 100% of the animals (Jarrett, 1985).

(*a*) *BPV 2 and cancers of the urinary bladder* (Table 77)

In Scotland, 30% of cattle that had squamous-cell carcinoma of the upper gastrointestinal tract (see below) had concurrent bladder tumours (Jarrett *et al*., 1978a): haemangioendotheliomas (23%), transitional-cell carcinomas (8%), fibromas (4%) and adenocarcinomas (1%). The same histological types of tumour, including the Pagetoid variant of urothelial carcinoma, have been found in cattle in other parts of the world and were associated with bracken fern in the diet, which contains highly immunosuppressive and mutagenic chemicals (Pamukcu, 1963; Rosenberger, 1971; Hirono, 1986; Borzacchiello *et al*., 2001).

Injections of a 10% suspension of homogenized bovine wart tissue, either alone or in combination with 3-hydroxy-kynurenine and/or 3-hydroxyanthranilic acid, into the wall of the urinary bladder of 2–3-month-old calves induced fibromas and polyps in 13/15 animals examined cystoscopically at intervals starting 14 days after inoculation. Simultaneous intradermal injections of the same suspensions or application on scarified skin in the same animals induced fibropapillomas in the skin of 12 calves in 33–83 days. No malignant tumours were observed in six calves examined histopathologically from 40 to 81 days after inoculation (Olson *et al*., 1959). In another experiment (Olson *et al*., 1965), suspensions of six naturally occurring bladder tumours (two haemangiomas, one haemangioma plus

Table 77. Bovine papillomavirus (BPV) in urinary bladder cancers

BPV type	Naturally occurring cancers (%)	Experimentally induced cancers (%)	Control cases	Reference
NK		13/15 (87)	NA	Olson *et al*. (1959)[a]
BPV 2	7/15 (46%)	9/13 (69%)	2/10 (20%)	Campo *et al*. (1992)
BPV 2	1[b]		NA	Borzacchiello *et al*. (2001)
BPV 2	46/60 (75%)*		17/34 (50%)*	Borzacchiello *et al*. (2003a)
BPV 2	11/27 (40%)		NK	Lioi *et al*. (2004)

NA, not applicable; NK, not known

[a] The cancers were induced with a suspension of bovine wart tissue.

[b] Single case report

* $p < 0.01$

papilloma, two papillomas, one papilloma plus adenocarcinoma plus squamous carcinoma — this latter case was accompanied by metastasis to the iliac node) were inoculated into the skin, vagina and urinary bladder of young calves. Of 17 inoculated 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 that 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) and, more recently, Borzacchiello *et al*. (2003a) and Lioi *et al*. (2004) showed that the virus that is involved in bladder cancer in cattle in Italy and the United Kingdom is BPV 2. Campo *et al*. (1992) found multiple copies of episomal BPV 2 DNA in seven of 15 biopsies (46%) of naturally occurring bladder tumours from animals in bracken-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 contained an unidentified papillomavirus. Borzacchiello *et al*. (2003a) found BPV 2 DNA in 46/60 (75%) biopsies of bladder cancer and 17/34 (50%) biopsies of normal bladder epithelium. Despite the high incidence of BPV 2 DNA in normal urothelium, the difference between pathological and normal samples was statistically significant ($p < 0.01$). Lioi *et al*. (2004) found BPV 2 DNA in 11/27 (40%) bladder tumour biopsies; there was no information on BPV DNA positivity in normal samples of the urinary bladder.

In an experiment designed to reproduce the progression of papillomas to carcinomas of the upper gastrointestinal tract (see below), further evidence of the involvement of BPV 2 and its synergism with bracken fern in the induction of urinary bladder malignancies was obtained. Calves approximately 3–4 months of age were immunosuppressed by either treatment with azathioprine (10 animals) or a diet with bracken fern (12 animals). Some of the animals were infected with BPV 4 (see below), but not with BPV 2. All of the immunosuppressed calves developed urinary bladder tumours approximately two years after the beginning of the experiment. However, the animals immunosuppressed with azathioprine developed benign haemangiomas, whereas the animals fed bracken fern developed malignant tumours that were representative of the whole range of naturally occurring bladder cancers. Bladder biopsies from three animals in the azathioprine-treated group and 10 animals in the group fed bracken fern were analysed for the presence of BPV DNA. BPV 2 DNA was found in biopsies of tumours from nine of 13 animals (69%), including haemangiomas of the azathioprine-treated animals. Biopsies from four animals of the group fed bracken fern were negative. Biopsies from cases with multiple tumour types were either all positive or all negative. As in the naturally occurring bladder cancers, no virus or structural viral antigens, no evidence of abortive infection and no production of virus were detected in the experimental tumours, as in cases of fibropapillomas of the upper gastrointestinal tract (see above). It was concluded that immunosuppression favoured the establishment of premalignant viral lesions, and that mutagens present in the bracken fern promoted their malignant progression (Campo *et al*., 1992).

The viral DNA in bladder lesions is infectious and can initiate a replicative cycle in the permissive environment of the skin; extracts from urinary bladder cancers induced skin warts (Olson *et al*., 1965). The viral oncoprotein E5 is expressed in the tumour tissue (Borzacchiello *et al*., 2003a), the *Ras* gene is activated at early stages of ptaquiloside carcinogenesis (Shahin *et al*., 1998; Campo, 2002) and expression of the tumour suppressor fragile histidine tetrads (*FHIT*) locus is down-regulated (Borzacchiello *et al*., 2001). Fragile sites are often disrupted by integration of HPV DNA in cervical cancers (Butler *et al*., 2000) and alterations of *FHIT* expression have been observed in many cervical carcinomas (Takizawa *et al*., 2003). The immunosuppression induced by bracken fern prevents tumour rejection and the mutagens in fern contribute to destabilization of the genome, particularly when BPV is involved. Ingestion of bracken fern has been deemed to be the cause of chromosomal abnormalities, which include acentric fragments, rearrangements and chromatid and chromosome breaks and gaps (Moura *et al*., 1988; Stocco dos Santos *et al*., 1998; Lioi *et al*., 2004). The incidence of chromosomal abnormalities increases when the bladder is infected with BPV 2 (Lioi *et al*., 2004).

(*b*) *Latency of BPV 2*

Experimental reproduction of tumorigenesis of the urinary bladder also showed the presence of latent BPV 2 (Campo *et al.*, 1992), which could be reactivated by immunosuppressive treatment, as in the bladder, and/or by damage to the skin (Campo *et al*., 1994a). Four of 10 azathioprine-treated cattle developed skin warts, two contained BPV 1 and two showed the presence of BPV 2. One of 12 animals fed bracken fern developed a BPV 2 wart. All the warts developed at sites of damaged skin. Four fully immunocompetent animals developed BPV 1 warts at the site of damaged skin, which indicated that wounding, with the attendant cell proliferation, is sufficient for reactivation of the latent virus (Campo *et al.*, 1994a).

Epithelia may not be the only site where latent papillomavirus is located. BPV DNA has been found in the episomal form in circulating lymphocytes of three of five experimental cattle with warts and in lymphocytes of the general cattle population in the presence or absence of warts (seven of 18) (Campo *et al*., 1994a). Latent BPV 2 infection of lymphocytes has also been established in cattle (10/11) by transfection of blood from three donors that had BPV 2 in their lymphocytes (3/3) (Stocco dos Santos *et al*., 1998).

(*c*) *BPV 2 in mouse xenografts*

The ability of BPV 2 DNA to induce tumours was confirmed in xenografts. BPV 2 DNA extracted from bovine fibropapillomas was injected into bovine scrotal skin before it was grafted onto the back of severe combined immunodeficient (NOD- SCID) mice. All of the 14 grafts developed fibropapillomas that produced mature infectious virus. When the experiment was repeated with molecularly cloned BPV 2 DNA, only 50% of 140 grafts developed epithelial papillomas that had no fibroblastic component and did not produce virus. Similar results were observed with molecularly cloned BPV 1 DNA. The differences between the tumours induced by ‘natural’ BPV DNA and recombinant BPV DNA was

ascribed to a different methylation pattern of the viral genome and it was concluded that production of the virus requires a fibroblastic component (Pawellek *et al.*, 2002).

(*d*) *BPV 2 in hamsters*

A 10% suspension of a spontaneous fibropapilloma that contained BPV 2 and was removed from the neck of a cow was injected subcutaneously into the back of a hamster. After 2 years, a subcutaneous fibrous tissue nodule was excised from the injection site. The induced tumour was diagnosed as a fibrosarcoma that contained multiple extrachromosomal copies of complete BPV 2 DNA, but no virus or structural viral antigens (Moar *et al*., 1981).

3.2.4 *BPV 3*

BPV 3 was isolated from epithelial skin papillomas in Australian cattle (Pfister *et al*., 1979). It produced warts on the skin but not in the conjunctiva or at other sites. Nothing is known about its natural history and no transmission experiments have been performed.

3.2.5 *BPV 4*

BPV 4 is the causative agent of papillomas of the upper gastrointestinal tract in cattle (Campo *et al*., 1980). In a survey of 7746 cattle from local abattoirs, Jarrett *et al*. (1978b) found upper gastrointestinal tract tumours in 19% of the animals. One hundred unselected serial papillomas were taken for histological examination; 78% were found to be true epithelial squamous papillomas and 22% were fibromas or fibropapillomas; 79% of the affected animals had papillomas at one site and the remaining 21% had papillomas at more than one site. BPV 2 DNA but no virus was present in the fibropapillomas (Jarrett *et al*., 1984a), while the BPV 4-induced epithelial papillomas produced virus (Campo *et al*., 1980). BPV 4 was also found in 47/75 (62.2%) papillomas of the upper gastrointestinal tract in cattle (Borzacchiello *et al*., 2003b).

(*a*) *BPV 4 and cancer of the upper gastrointestinal tract*

In the above-mentioned survey, 80 cases of squamous-cell carcinomas of the upper gastrointestinal tract [7% tongue, 5% palate, 8% pharynx, 50% oesophagus, 30% rumen] were selected in so-called ‘cancer farms’ [total incidence not given], the grazing grounds of which were infested with bracken fern. Of 366 cattle from ‘cancer farms’, 39% had squamous papillomas. Ninety six per cent of the animals that had squamous-cell carcinomas also had squamous papillomas; 36% of the animals that had squamous-cell carcinomas had metastases (liver and/or spleen), 56% had tumours of the large intestine (polyps, adenomas and adenocarcinomas) and 30% had urinary bladder cancers. All stages of progression from papilloma to squamous-cell carcinoma were observed (Jarrett, 1978; Jarrett *et al.*, 1978a,b). It was concluded that, in animals that grazed on bracken fern, BPV 4 papillomas were widespread, persistent and prone to progress to cancer, probably

due to the synergistic interaction between the virus and the chemicals present in the bracken fern (Jarrett *et al*., 1978a).

Widespread and persistent papillomatosis of the upper gastrointestinal tract can also occur in the absence of bracken fern when cattle are immunosuppressed by other factors, such as infection with bovine diarrhoea virus (Tsirimonaki *et al*., 2003).

The progression from papilloma to carcinoma was reproduced by Campo *et al*. (1994b) in an experiment that lasted 13 years. Of 32 calves, 3–5 months of age, 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 untreated controls. The virus-infected azathioprine-treated animals had to be killed after 2 years. All calves infected with BPV 4 developed squamous papillomas at the site of injection. However, the animals that were immunosuppressed either by azathioprine or by bracken fern developed florid and persistent papillomatosis with papillomas that spread away from the inoculation site, particularly in the azathioprine-treated animals. The last surviving animal from the group treated with BPV 4 and bracken fern still had papillomas 13 years after infection which had spread from the mouth to the lower oesophagus and the rumen. No progression from papilloma to carcinoma was observed. Two of six animals from the virus-treated group fed bracken fern developed cancers of the upper gastrointestinal tract and lower intestine 6 and 10 years, respectively, after the start of the experiment. Both animals had typical papillomas, foci of carcinoma in the oesophagus that infiltrated the underlying tissue and polyps and adenomas and adenocarcinomas of the duodenum, jejunum and colon. No malignancies of the gastrointestinal tract were detected in animals of the other groups. As observed previously for naturally occurring upper gastrointestinal tract cancers (Campo *et al*., 1985), in which only one of approximately 100 cases of cancer examined was found to be positive for BPV 4 DNA, no viral 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 mutagens present in the bracken fern were responsible for neoplastic conversion of papilloma cells and promoted their neoplastic progression. The almost total absence of BPV 4 DNA in the cancers suggests that the continuous presence and expression of the viral genome is not necessary for maintenance of the neoplastic state.

(*b*) *BPV 4 in mouse xenografts*

The tumorigenic potential of BPV 4 was studied in nude mouse xenografts. Chips of fetal bovine palate tissue infected with BPV 4 were implanted into nude mice either under the kidney capsule or subcutaneously and induced virus-producing papillomas (Gaukroger *et al*., 1989). One of the xenograft papillomas underwent spontaneous transformation to squamous-cell carcinoma with metastasis to the spleen (Gaukroger *et al*., 1991). The malignant cells were confirmed to be of bovine origin by major histocompatibility complex typing and by the nucleotide sequence of the bovine *Ras* gene. No BPV 4 DNA was detected in either the primary or metastatic cancer. Spontaneous conversion of papillomas in the

xenograft system is very rare and was observed only once in approximately 100 mice bearing papillomas generated in different experiments. Neoplastic progression was, however, greatly accelerated by the implantation in the recipient mice of slow-releasing pellets of either the initiator 7,12-dimethylbenz[*a*]anthracene (DMBA) or the promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The progression of BPV 4 papillomas to carcinomas was observed in 13/20 (65%) implants in mice exposed to DMBA and in four of 33 (12%) implants in mice exposed to TPA (Gaukroger *et al*., 1993).

(*c*) *BPV 4 in hamsters*

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

3.2.6 *BPV 5 and BPV 6*

BPV 5 induces 'rice grain' fibropapillomas (so called because of their appearance) on the teats and udders of cattle (Campo *et al*., 1981) and BPV 6 induces epithelial papillomas (Jarrett *et al*., 1984b). In the United Kingdom, these two viruses have not been found in any other location in the body and the tumours they produce have not been reported to undergo malignant conversion, although 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% of the animals were found to have papillomas on the teats and udders. Of the affected animals, 28% had BPV 1 warts, 88% had BPV 5 warts and 92% had BPV 6 warts; 58% had double infections with BPV 5 and 6 and 23% had triple infections with BPV 1, 5 and 6; only 14% were infected by only one virus, and this was most frequently BPV 6 (8.7%) followed by BPV 5 (4.4%) and then by BPV 1 (0.8%). BPV 4 was never found and it was therefore concluded that there is no association between alimentary and teat papillomas.

Although BPV 5 had been detected only in fibropapillomas in the United Kingdom (Jarrett, 1985), a later survey conducted in Australia revealed that it can cause both fibropapillomas and epithelial papillomas (Bloch *et al*., 1994a).

3.2.7 *Unknown BPV types that cause cancer in cattle*

(*a*) *Bovine ocular squamous-cell carcinoma*

In Australia, approximately 10–20% of some herds are affected by ocular squamous-cell carcinoma (Spradbrow & Hoffman, 1980). The carcinomas derive from papillomas, the malignant transformation of which is particularly noticeable in lightly pigmented ani-

mals and implicates the role of ultraviolet (UV) light as a co-carcinogen. Viral particles that strongly resemble papillomavirus were detected in eight of 25 early lesions, including one conjunctival plaque, five conjunctival papillomas, one eyelid papilloma and one eyelid keratinized horn (Ford *et al*., 1982).

(*b*) *Bovine skin carcinoma*

Australian herds are affected commonly by skin cancer (Spradbrow *et al*., 1987). Similarly to ocular squamous-cell carcinomas, the cancers derive from precursor lesions. Of a herd of 4–15-year-old cattle, all 13 had lesions with differing degrees of severity, from early lesions, such as cutaneous horns with acanthosis and hyperkeratosis, to advanced lesions, such as squamous-cell carcinomas and basaliomas. In two of four animals that were observed for 3 years, progression of early lesions to squamous-cell cancer was observed. Viral DNA that hybridized to BPV 1 under low-stringency conditions was found in 10/11 keratotic lesions and five of eight neoplasms (two squamous cancers and three basaliomas). Similarly to bovine ocular squamous-cell carcinomas, it was concluded that an unknown papillomavirus in conjunction with UV light was responsible for the skin cancers.

3.2.8 *BPV in equine sarcoids* (Table 78)

Equids can be infected by BPV and the infection leads to a fibroblastic tumour called a sarcoid. Sarcoids are a common disease of horses (*Equus equus*) and donkeys (*Equus asinus*); they are locally invasive, non-metastatic tumours 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 BPV induced sarcoids

Table 78. Bovine papillomavirus (BPV) DNA in equine sarcoids

No. of positive cases	Predominant (like)-type	Reported equine 'variants'	Geographical location	Reference
12/14 (86%)	BPV 1	Yes	Australia	Trenfield *et al*. (1985)
12/13 (92%)	BPV 1	Yes	USA	Angelos *et al*. (1991)
17/20 (85%)	BPV 1	Yes	Switzerland	Angelos *et al*. (1991)
24/24 (100%)	BPV 1	Yes	United Kingdom	Reid & Smith (1992); Reid *et al*. (1994)
58/58 (100%)	BPV 1	No	Switzerland	Otten *et al*. (1993)
56/76 (73%)	BPV 1	Yes	Australia	Bloch *et al*. (1994b)
41/41 (100%)	BPV 1	No	Belgium	Martens *et al*. (2001a)
94/96 (98%)	BPV 2	No	USA	Carr *et al.* (2001a)
39/41 (95%)	BPV 1	Yes	United Kingdom, Switzerland	Chambers *et al*. (2003a,b)

similar to those that occur naturally; however, in contrast to natural sarcoids, experimental sarcoids regressed (Olson & Cook, 1951).

Lancaster *et al*. (1977) first detected BPV DNA in natural equine sarcoids in the USA. Neither natural nor experimental sarcoids contained virus or structural viral antigens. More recent analyses of these equine tumours throughout the world have confirmed the original findings (Table 78).

Trenfield *et al*. (1985) reported the presence of BPV DNA in 12/14 (86%) equine sarcoids from Australia. The restriction enzyme pattern of the BPV sequences was not identical to that of BPV 1 or BPV 2, which suggested the presence of variants or subtypes.

Angelos *et al*. (1991) found BPV DNA in 12/13 (92%) sarcoids from horses from New York State and in 17/20 (85%) sarcoids from horses from Switzerland. The viral DNA was similar to BPV 1 in 22 biopsies and similar to BPV 2 in seven biopsies. BPV DNA was also found in one biopsy each of a fibrosarcoma, a fibropapilloma and a pyogranulomatous dermatitis. No biopsy showed a restriction enzyme pattern of viral DNA identical to reference BPV 1 or BPV 2 DNA, which indicated the presence of BPV subtypes or variants.

Reid and Smith (1992) and Reid *et al*. (1994) analysed 24 sarcoid samples from six horses and 18 donkeys from the United Kingdom. All the biopsies contained BPV DNA and BPV 1-like sequences were more prevalent than BPV 2-like sequences; however, the sequences were not identical to either BPV 1 or BPV 2. There was no correlation between viral type, clinical type or anatomical location of the lesions or sex of the animals.

Otten *et al*. (1993) analysed 58 sarcoids from 32 horses and two donkeys. BPV 1 DNA was found in 55 biopsies and BPV 2 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 as those found in BPV 1 and BPV 2 isolates from cattle from the same geographical area, and the relative incidence of BPV 1 and BPV 2 infection was the same in cattle and horses, which suggested that the BPV variants found in equine sarcoids are not specific for horses.

Bloch *et al*. (1994b) conducted a retrospective analysis of equine sarcoids from Australian horses. BPV DNA was detected in 56/76 (73%) samples; of these, 82% were similar to BPV 1 and 18% were similar to BPV 2.

Martens *et al*. (2001) found BPV DNA in all of 41 samples of sarcoids from 19 Belgian horses. Thirty-four sarcoids harboured BPV 1 DNA and seven contained BPV 2 DNA.

Carr *et al*. (2001a) found BPV DNA in 94/96 (98%) sarcoid samples from American horses; BPV 2 DNA was present in 62% of the positive samples and the viral nucleotide sequences had 100% homology with reference BPV 1 or 2.

More recently, Chambers *et al*. (2003a,b) found BPV DNA in 39/41 (95%) samples of sarcoids from horses in the United Kingdom and Switzerland. BPV 1 DNA was present in 34/37 positive samples, although variations from the established nucleotide sequences of BPV 1 or BPV 2 were consistently observed. BPV DNA was also found in four of five samples of granulomatous dermatitis, as reported previously by Angelos *et al*. (1991). It is unclear what role this condition might play in sarcoid pathogenesis.

In all surveys but one (Carr *et al*., 2001a), BPV 1-like DNA has been found more often than BPV 2 DNA (Table 78). Furthermore, the absence in most surveys of BPV 1 or BPV 2 DNA sequences identical to those of the reference genomes suggests the existence of 'equine-adapted' variants of BPV that specifically infect horses.

The causal involvement of BPV in equine sarcoids has been confirmed by the expression of viral oncogenes. Nasir and Reid (1999) analysed sarcoids for the expression of BPV *E2*, *E5*, *E6*, *E7* and *L1* genes and found that 18/20 tumour samples examined were positive for E2 expression and 10 were positive for L1 expression. Viral oncogene *E5*, *E6* and *E7* transcripts were detected in 16, nine and 12 tumours, respectively. Carr *et al*. (2001b) found that 23/23 sarcoids were positive for expression of the E5 oncoprotein. Chamber *et al*. (2003b) detected genomic BPV E5 DNA in 39/41 (95%) sarcoids and BPV E5 mRNA transcripts in 34/41 (85%) samples which confirmed active viral transcription.

3.3 Equine papillomavirus (EqPV)

Horses can also develop genital, cutaneous, ocular, and oral papillomas and squamous-cell carcinomas. Papillomavirus antigen was detected in papillomas but not in carcinomas (Junge *et al*., 1984; Olson, 1987). An EqPV was isolated from cutaneous papillomas of one pony and one horse. The same viral type was found in papillomas of the muzzle and the leg but not in penile papillomas, which suggests the existence of two different types of EqPV (O'Banion *et al.*, 1986).

3.4 Papillomavirus in cervidae

Papillomavirus DNA was isolated from fibropapillomas or fibromas of different members of the cervidae family, such as 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*) (DPV; deer fibromavirus subtypes a and b, respectively; Groff *et al*., 1983). While DPV and EEPV have been reported to induce tumours with a prominent fibroblastic component *in vivo*, RPV mainly induces fibromas in its natural host.

The genomes of EEPV or RPV induced fibrosarcomas after experimental infection of young Syrian hamsters by subcutaneous injection. The tumours contained multiple, non-integrated copies of the EEPV or RPV genome (Stenlund *et al*., 1983; Moreno-Lopez *et al*., 1987).

3.5 Cottontail rabbit papillomavirus (CRPV)

The discovery of the viral etiology of naturally occurring warts in cottontail rabbits (Shope & Hurst, 1933) and the subsequent demonstration that benign papillomas induced by the virus progress into carcinomas (Rous & Beard, 1935) constitute hallmarks in the

history of viral oncology. A number of important properties of papillomaviruses such as the role of E6 and E7 viral genes in the development of papillomas and carcinomas (Georges *et al*., 1984; Nasseri & Wettstein, 1984; Danos *et al*., 1984) and the synergism between virus and chemical co-carcinogens (Rous & Beard, 1934; Rous & Kidd, 1938; Rous & Friedwald, 1944) were established for the first time using CRPV.

3.5.1 *Species specificity*

In nature, CRPV infects primarily cottontail rabbits (*Sylvilagus floridanus*) and occasionally jackrabbits (*Lepus californicus*), and rabbit papillomatosis is endemic in certain parts of the USA (Syverton, 1952; Stevens & Wettstein, 1979; Kreider & Bartlett, 1981). Infectious virus has been obtained from papillomas induced in jackrabbits and snowshoe hares (*Lepus americanus*) (Beard & Rous, 1935), and the host range has even been extended to rats under experimental conditions (Kreider & Bartlett, 1981). Warts of naturally infected cottontail rabbits usually contain large amounts of virion, in spite of a great variation in viral content (Beard, 1956).

3.5.2 *Viral multiplication and tumour induction*

The first phase of infection in cottontail rabbits lasts from 1 to 6 weeks, during which time papillomas grow; 95–100% of the infected animals develop papillomas that are permanently benign in 71%, regress in 6% and progress to squamous cancer within 12–18 months in 23% of the animals. Experimental infection of domestic rabbits (*Oryctolagus cunniculus*) follows a different course: 95–100% of domestic rabbits developed papillomas after 1–6 weeks and regression of the papillomas occurred in 9% of the rabbits after 1–3 months. In 25% of the rabbits, papillomas remained permanently benign but, in 66% of the animals, they progressed to squamous-cell carcinomas within 6–12 months and were mainly accompanied by metastatic spread to the lung (Wettstein, 1987). A larger number of papillomas progress more rapidly to cancer in domestic rabbits than in cottontail rabbits, which implies that the genetic background of the host is involved in malignant conversion. Infectious virus is found in papillomas of cottontail rabbits but not in those of domestic rabbits or in cancers in either species; however, viral DNA is present in the non-productive lesions at low copy numbers (Shope & Hurst, 1933; Beard, 1956; Noyes & Mellors, 1957; Orth *et al*., 1971; Nasseri & Wettstein, 1984; Wettstein, 1987; Zeltner *et al*., 1994).

3.5.3 *Co-factors for tumour induction and progression*

Malignant transformation of CRPV-induced papillomas is accelerated and its frequency is increased when scarified skin is exposed to chemical carcinogens.

The application of tar to the ears of rabbits produces generalized epidermal hyperplasia and papillomatosis, but the papillomas never become malignant and often regress after cessation of application. However, when administered intravenously to rabbits,

CRPV induces carcinomas on tarred skin. Rapid malignant progression was also observed when CRPV-induced papillomas were treated with methylcholanthrene (Rous & Kidd, 1938; Rous & Friedewald, 1944; Rogers & Rous, 1951). In addition, the much higher frequency of tumour progression observed in domestic rabbits compared with cottontail rabbits implies that the genetic background of the host is an intrinsic co-factor in the malignant progression of persistent warts, which has been linked to genes in the class II region of the major histocompatibility index (Han *et al.*, 1992, 1994).

3.5.4 *Latency of CRPV*

In an experiment designed to study the latency and reactivation of CRPV, Amella *et al*. (1994) scarified and inoculated domestic rabbits with serial dilutions of CRPV. With undiluted virus, six of seven injection sites in seven rabbits developed papillomas. With virus diluted from 1:1000 to 1:100 000, none of 14 sites in 14 animals developed papillomas. PCR on tissue from 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 the virus can be reactivated by skin injury.

Zhang *et al*. (1999) reported that highly diluted preparations of CRPV lead to the establishment of a subset of latent infections in New Zealand white rabbits that can be activated by UV-radiation shortly after infection. Sites that did not form papillomas within 3 months after irradiation were CRPV DNA-positive and showed transcripts from the E1 region, but were E6/E7 RNA-negative. Attempts to activate infections that remained latent by repeating UV irradiation at the end of the 3-month observation period were, however, unsuccessful.

3.5.5 *CRPV in transgenic rabbits*

CRPV in conjunction with activated *Ras* was used to generate three transgenic New Zealand white rabbits. Two rabbits had CRPV DNA only and one had both CRPV DNA and activated *Ras*. The two CRPV transgenic rabbits were phenotypically normal up to 2 weeks after birth, but then started to develop epidermal hyperkeratosis. When the animals were 20–30 days of age, small papillomas appeared 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 that was transgenic for both CRPV DNA and *Ras*, had thickened skin at birth and died at day 3. It was covered with 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 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 tissues, while *Ras* was transcribed only in the cancers. It was concluded

by the authors that the rapid progression of papillomas to carcinomas was due to synergism between CRPV oncogenes and activated *Ras* (Peng *et al.*, 1993).

3.6 Domestic rabbit oral papillomavirus (ROPV)

Oral papillomas were initially found in 31% of 51 New Zealand white rabbits from two commercial sources. The virus was isolated and inoculated into the tongue, vulva and bulbar conjunctiva of three non-infected rabbits. All rabbits developed papillomas of the tongue but not of the conjunctiva or vulva. No cross-immunity was observed between the cutaneous (CRPV) and oral viruses and it was concluded that they have separate identities. Nine of 10 neonatal hamsters inoculated in the thoracombular region with oral papillomavirus developed fibromas (Sundberg *et al*., 1985). Tissue fragments from New Zealand white rabbit tongue, larynx, cervix, vulva/vagina and penis that were infected with extracts prepared from oral papillomas induced by ROPV and subsequently placed subrenally into athymic mice were tested by southern blot analysis and found to be positive for ROPV. Viral production was observed in subrenal xenografts from penile and vulvar tissue. After direct penile inoculation of adult rabbits with ROPV, 10/17 rabbits produced small raised lesions (papillomas) of approximately 1 mm^3 that were ROPV-positive by both in-situ hybridization and southern blot analyses and were also positive for viral capsid antigen by immunohistological staining. These lesions quickly regressed within 50–60 days (Christensen *et al.*, 1996c; Harvey *et al*., 1998). In another experiment, ROPV-induced benign papillomas at oral and genital sites regressed in 100% of infected domestic rabbits approximately 60 days after infection (Christensen *et al*., 2000).

3.7 Ovine papillomatosis (OVP) (Table 79)

In Australia, Hawkins *et al.* (1981) first described squamous-cell carcinomas in sheep. They occurred more commonly on areas that were poorly covered by wool and lacking pigmentation — the vulva, tail and perineum — which implied that UV light played as an important role as a co-factor in their etiology.

Vanselow *et al*. (1982) reported the apparent transformation of ovine facial papillomas into carcinomas and the presence of virions that resembled papillomaviruses in one of them. Further support for UV as a co-factor came from a study that correlated the increased use of the total and partial removal of tails with an increased prevalence of neoplasias in sheep in Australia. These procedures expose the entire perineal area, including the vulval labia, to direct sunlight. These observations suggested that the progression from virally induced papilloma to carcinoma first demonstrated with CRPV also occurs in sheep (Vanselow & Spradbrow, 1983).

Trenfield *et al*. (1990) analysed 67 benign precancerous cutaneous ear lesions (cutaneous horns, papillomas, fibropapillomas) from 51 sheep and 16 lesions from other cutaneous 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 eight of the 10 lesions were posi-

Table 79. Papillomavirus in tumours of sheep and goats

Lesion	Viral DNA[a]	Viral antigen[a,b]	Virus[a]	Reference
Cutaneous papillomas	ND	ND	2/3	Vanselow *et al*. (1982)
Hyperkeratotic scales	ND	ND	0/1	Vanselow & Spradbrow (1983)
Rumenal fibropapilloma	0/30[c]	6/10	0/20	Norval *et al*. (1985)
Cutaneous and vulvar lesions[d]	11/83	ND	ND	Trenfield *et al*. (1990)
Perineal squamous-cell carcinomas and papillomas	20/26	0/17	ND	Tilbrook *et al*. (1992)
Cutaneous filiform papillomas	1/1	9/9	Yes (NR)	Hayward *et al*. (1993)
Mammary papillomas of the goat udder	3/20	0/20	ND	Manni *et al*. (1998)
Papillomatosis	ND	5/5	5/5	Uzal *et al*. (2000)

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

tive. The viral DNA had a BPV-like restriction enzyme pattern similar to that of equine sarcoids (see Section 3.2.8). A similar survey was performed by Tilbrook *et al*. (1992) who found that five of 10 premalignant biopsies and 15/16 squamous-cell carcinomas, all from the perineal region of sheep, contained papillomavirus-like DNA using both BPV probes and HPV probes. The filiform squamous papillomas on sheep reported by Hayward *et al*. (1993) were not of the fibropapilloma type but histologically resembled verruca vulgaris; they were present in less than 1% of 2660 young sheep and were always found on the lower forelegs. Papillomavirus was visualized by electron microscopy and viral DNA was detected by low-stringency hybridization with an HPV 16 DNA probe. All papillomas analysed were positive for the common viral antigen. Similar observations have been made in Patagonia and support the possible existence of a second OPV (Uzal *et al*., 2000). The occurrence of rumenal fibropapillomas in 25/200 sheep from local abattoirs in Scotland was reported by Norval *et al*. (1985), who also described one animal with a squamous-cell carcinoma of the rumen.

Manni *et al*. (1998) reported evidence for the existence of papillomavirus-like sequences in mammary papillomas of goats. No viral particles were found in papillomatous lesions from the mammary skin of goats, but reverse blot hybridization revealed cross-hybridization between DNA extracted from goat mammary papillomas and HPV 8, 10 and 16. Southern blot, using OPV and BPV 4 DNA probes under conditions of reduced stringency, detected homologous sequences in 40% of the biopsies.

3.8 *Mastomys natalensis* papillomavirus (MnPV)

Mastomys natalensis is a common rodent in southern Africa. Interest in these animals was stimulated by the early observation of Oettlé (1957) that 28–53% of *Mastomys* that were older than 1 year suffered from stomach cancer. As the incidence of stomach cancer is extremely low in other laboratory rodents and was highly variable in the different *Mastomys* laboratory colonies, exogenous causal factors were postulated. Extrachromosomal MnPV DNA was found in various tissues from animals of such colonies, whereas colonies that did not display a high rate of spontaneous stomach tumour formation were free from MnPV (Amtmann *et al*., 1984; Amtmann & Wayss, 1987).

In addition, a high rate of spontaneous epithelial skin tumours in colonies of *Mastomys* was first described by Burtscher *et al*. (1973). Malignant conversion rates of 11% were detected in some colonies (Giessen colony), whereas inbred strains derived from other colonies (Heidelberg colony) were free from malignancies. The animals of the Giessen and the Heidelberg colonies were found to harbour the latent papillomavirus, MnPV, the genomic sequence (7687 base pairs) of which was determined by Tan *et al*. (1994). Almost half of the tumours failed to give a uniform picture by histological examination. They were composed of keratoacanthomas, papillomas and epithelial proliferation. MnPV-infected animals developed keratoacanthomas and papillomas of the skin, in an age-dependent manner. The tumours never appeared in animals under 50 weeks of age but, by 16 months of age, 80% of the animals had tumours. The viral genome copy number increased markedly (30 000-fold) during tumour formation (Amtmann *et al.*, 1984; Amtmann & Wayss, 1987; Tan *et al.*, 1994). Amtmann *et al*. (1984) showed that treatment of the skin with TPA increased the DNA copy number (100-fold) and lowered the age at tumour appearance to as early as 14 weeks. Similar results were obtained when the skin was irritated with sandpaper, which indicated that the wound healing processes are prequisite for activation of latent papillomavirus genomes (Siegsmund *et al*., 1991). When the purified virions isolated from benign as well as from malignant tumours were used to infect the scarified skin of young *Mastomys*, 11/30 infected animals developed tumours (Müller & Gissmann, 1978).

Extrachromosomal MnPV DNA was found in all cutaneous DNA samples. In addition, viral DNA persisted in tissues other than the skin. After studying the effects of different chemical carcinogens, Amtmann *et al*. (1984) concluded that the activation of MnPV *in vivo* is mediated by a cellular mechanism that is correlated to second-stage tumour promotion, since transition from benign keratoacanthomas to malignant tumours was not induced by tumour promoters or DMBA.

Recently, transgenic mice that carry the oncogene E6 of MnPV were generated (Helfrich *et al*., 2004) and used in two-stage skin carcinogenesis experiments with DMBA and TPA. In this system, squamous-cell carcinomas developed in nearly 100% of MnPV E6 transgenic mice compared with 10% of their non-transgenic littermates, from which it can be concluded that the MnPV *E6* transgene favours malignant progression of chemically induced tumours.

3.9 Mouse papillomavirus (MmPV)

The only known MmPV was isolated from a zoological 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. MmPV was detected in 28/28 benign and malignant biopsies and structural antigen in 20/31 biopsies. 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*).

3.10 Canine oral papillomavirus (COPV)

Dogs can be affected by oral papillomatosis, particularly if kept in kennels in large numbers. The incubation period for oral papillomas varies from 4 to 10 weeks and regression usually follows in 3–14 weeks (Olson, 1987). Progression to squamous cancer is rare (Watrach *et al*., 1970), but has been observed. Analysis of the lesions by light and electron microscopy (Watrach *et al*., 1969) showed features typical of papillomavirus infection. The genomic sequence of COPV was determined by Delius *et al.* (1994). In addition to benign lesions, squamous-cell carcinomas of both cutaneous (Bregman *et al*., 1987) and oral mucosal tissue (Teifke *et al*., 1998) have been associated with COPV. Vaccination with 'live' COPV extract occasionally resulted in the development of various epithelial neoplasms at the injection site. Although a large majority of dogs were protected from natural infection, 12/5400 dogs 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 viral particles by electron microscopy (Bregman *et al*., 1987). More recent investigations included 19 cutaneous and mucocutaneous papillomas, as well as 29 oral and 25 non-oral squamous-cell carcinomas in dogs. Immunohistological analysis provided evidence for the presence of papillomavirus antigens in more than 50% of the oral and cutaneous papillomas, while no papillomavirus antigen were demonstrated in venereal papillomas. In addition, one squamous-cell carcinoma was papillomavirus antigen-positive and overexpression of p53 was detectable in approximately 35% of all squamous-cell carcinomas (Teifke *et al*., 1998).

3.11 Feline papillomas

Two Persian cats, 10 and 13 years of age, respectively, both of which received steroid immunosuppressive therapy, developed sessile hyperkeratotic skin lesions that were positive for papillomavirus, group-specific viral structural antigen (denatured L1) and viral BPV 1 DNA (Carney *et al*., 1990). Both cats were negative for feline leukaemia virus and feline immunodeficiency virus (FIV). Twelve years later, the *Felis domesticus* papillomavirus was cloned from hyperkeratotic cutaneous lesions of a Persian

domestic cat and sequenced (Tachezy *et al*., 2002a). In another study, a 6-year-old cat that was positive for FIV developed skin lesions consisting of slightly raised pigmented plaques, 2-7 mm in diameter, with a rough, slightly verrucous surface. The lesions were positive for papillomavirus and for viral structural antigen (Egberink *et al*., 1992). From both studies, it could be concluded that cats display clinical papillomaviral lesions when immunosuppressed either by FIV infection or by steroid therapy.

3.12 Avian papillomavirus

In a large survey of 25 000 captured chaffinches (*Fringilla coelebs*) in the Netherlands, papillomas were found on the bare part of the leg of 1.3% of the birds (Lina *et al*., 1973). The DNA of a *Fringilla* papillomavirus (FPV) was isolated from such cutaneous papillomas (Osterhaus *et al.*, 1977) but could not be transmitted to other chaffinches, canaries or hamsters (Moreno-Lopez *et al*., 1984). From that DNA, two partial sequences of FPV were determined (Osterhaus *et al*., 1977; Moreno-Lopez *et al*., 1984).

Recently, an avian papillomavirus genome has been cloned from a cutaneous exophytic papilloma from an African grey parrot, *Psittacus erithacus* (PePV; Tachezy *et al*., 2002b). The PePV genome (7304 base pairs) represents the first complete avian papillomavirus genome.