Veterinary Cancer Medicine

Edited by

Gordon H. Theilen, D.V.M.

Professor of Surgery
Head, Clinical Oncology Section

and

Bruce R. Madewell, V.M.D., M.S.

Professor, Clinical Oncology Section
Diplomate, American College of Veterinary Internal Medicine

Department of Veterinary Surgery
School of Veterinary Medicine
University of California, Davis
Davis, California

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Part II

PAPILLOMATOSIS AND FIBROMATOSIS

GORDON H. THEILEN AND BRUCE R. MADEWELL

PAPILLOMAS

Papillomas (warts, verruca vulgaris, fibropapillomas) are usually classified as benign cutaneous tumors caused by either noninfectious irritants or infectious DNA viruses belonging to the papovaviridae family.[2,144] The naturally occurring noninfectious papilloma is usually solitary and reported most frequently on the body surface of laboratory animals, dogs, and occasionally other species.[124] The infectious types are usually multiple and seen in a variety of species at several different anatomic sites. They were first recognized in horses in 1901 by Cadeac, in humans in 1894 by Variot, in 1907 by Ciuffo and 1919 by Wile and Kingrey, and in cattle as a filtrable agent in 1929 by Creech.[21,35,138A,145] Of considerable medical and biologic interest is the growing evidence that several types of viral-induced papillomas transform to carcinomas when the host is exposed to various promoting factors.[38,108] Several excellent reviews have given virologic and biologic details of papillomavirus infections in man and animals.[76,108,146,147] One of the main handicaps in studying papillomatosis is the general lack of cell cultures that support papillomavirus replication. Most virologic and molecular studies must be done on primary tumor tissue obtained from affected hosts.

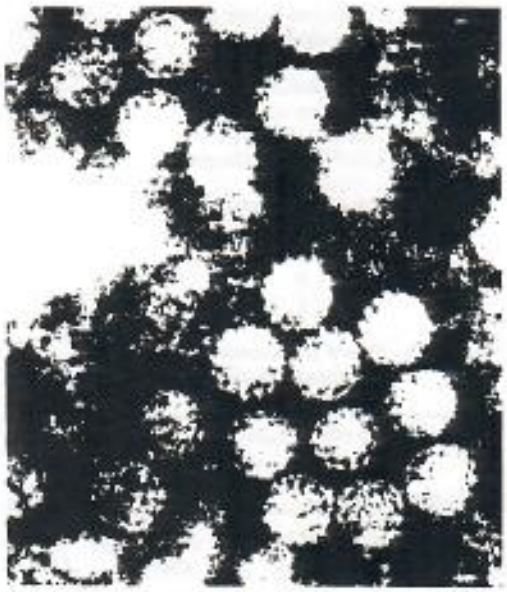

Fig. 14–32. Electron micrograph of bovine papillomavirus concentrated by cesium chloride gradient ultracentrifugation and prepared for electron microscopy by phosphotungstic acid staining. Virions have a naked icasahedral capsid measuring about 50 nm in diameter (×179,000).

Viral Properties

Papovaviruses are small, ether resistant, double-stranded DNA-containing viruses which have been classified into the family papovaviridae, consisting of two genera: papillomavirus and polyomavirus (Fig. 14–32).[47] Papillomaviruses are hardy and can be preserved for several months at 40°C in equal parts of physiologic saline and glycerine. To preserve material for molecular hybridization studies, it is recommended to quick freeze tumors to −70°C or lower temperatures. Papillomaviruses are naturally oncogenic and induce papillomas usually only on the species of origin. Virions have a naked icosahedral capsid about 55 nm in diameter.[134] Those of the cottontail rabbit, human, and chaffinch have 72 capsomeres. Empty and full particles have been isolated from wart tissue with full particles having sedimentation coefficients of 296 to 300 s and empty particles 168 to 172 s.[34] Their mass is about 5×10^6 daltons adequate to code for about 300,000 daltons of protein and the guanine + cytosine content of papillomavirus DNA ranges from 41 to 49% being similar to host species DNA.[49] Virion protein represents 88% of the mass of the particle and it has been shown that 10 major polypeptides can be resolved by sodium dodecylsulfate (SDS) polyacrilamide gel electrophoresis.[33]

The main capsid protein has a molecular weight of 50,000 to 63,000.[75,104,128] Four low-molecular-weight polypeptides associated with viral DNA appear to be similar to cell histones.[43] No serologic cross-reactivity has been detected among papillomaviruses of different species as assayed by immunodiffusion, and it was also shown that no DNA sequence homology has been detected between selected members of this virus group.[33,50,75,81]

Several different papillomaviruses infecting mammals and one avian genus have been characterized (Table 14–8). There are no other known DNA tumor viruses that cause such a variety of natural and experimentally induced neoplastic tissue responses. These animals include cattle, sheep, deer, horses, dogs, cottontail rabbits (*Sylvilagus spp*), domestic rabbits (oral papillomavirus), European elk (*Alces alces*) (referred to as moose in North America), chaffinch and human beings.[76,103,126,127] Most infectious papillo-

Table 14–8. Papillomaviruses

Virus	Host	Site	Histology
Human papillomaviruses 30 subtypes	human	various	papilloma
Equine papillomaviruses	horse	cutaneous (lips)	papilloma
Canine papilloma–viruses	dog	oral mucosa and cutaneous	papilloma
Cottontail rabbit (Shope) papillomavirus	rabbit	cutaneous	papilloma
Rabbit oral papillomavirus	rabbit	oral mucosa	papilloma
Mastomus natalensis papillomavirus	rodent	cutaneous	papilloma
Chaffinch papilloma–virus	bird	cutaneous	papilloma
Bovine papillomavirus			
Type 1	cattle	cutaneous	fibropapilloma
2	cattle	cutaneous	fibropapilloma
3	cattle	cutaneous	papilloma
4	cattle	alimentary tract	papilloma
5	cattle	teat	papilloma
Sheep papillomavirus	sheep	cutaneous	fibropapilloma
European elk papilloma	moose*	cutaneous	fibropapilloma
Deer fibromavirus	deer	cutaneous	fibroma

*Referred to as elk in Europe.

mas will spontaneously regress although some are associated with malignant transformation to squamous cell carcinomas, i.e., Shope papillomas of wild and domestic rabbits, papillomas in a specific strain of *Mastomys natalensis* (an African rodent), several types of human warts (epidermodysplasia verruciformis, cervical flat wart, condylomata acuminatum and laryngeal papilloma), bovine alimentary warts associated with ingestion of bracken fern *Pteridium aquilinum*, and papilloma of the mammary gland in goats with non-pigmented skin.[60,73,90,127,147] Rarely, papillomas in other species will progress to carcinomas, however, it was reported in a dog with oral papilloma.[142] Genetic factors, immune suppression, possible hormonal factors, excessive exposure to sunlight especially in animals with nonpigmented skin, UV light, or unidentified chemicals in unnatural foodstuffs like cattle foraging on bracken fern may act as co-carcinogens to promote transformation of warts to malignancy.[60,76,108,136]

The viral cause and infectious nature of warts were reported and studied in detail in rabbits for both cutaneous and oral types,[103,127] and also in cattle,[76,108] European elk (*Alces alces*),[15,88] horses,[72] dogs,[37] mastomys,[90] and sheep.[48] Fibromas in deer are caused by a papovavirus having viral properties similar to bovine papilloma viruses.[52,78,125] Extracts of wart tissue from coyotes (*Canis latrans*) was transmissible to beagle puppies.[51,121] Typical papovaviruses have been demonstrated by use of electron microscopy in wart tissue from nutrias (*Myocastor coypus molina*),[65] beaver (*Castor canadensis*),[24] impala (*Aepyceros melampus*),[67] giraffe (*Giraffa camelopardalis*),[67] opossum (*Didelphis virginiana*),[68] serow (*Capricornis spp.*),[91] and chaffinch (*Fringilla coelebs*).[83,100] In addition to the above species with proven or highly probable viral causes, multiple papillomas studied in lesser detail occur in a broad spectrum of animal species from different families and orders; in these cases, however, cause has not been completely established. These species include free-living animals and non-human primates,[11,84] goats,[36,89] wolves (*Canus lupus*),[121] barren-ground caribou (*Rangifer tarandus groenlandicus*),[17] newt (*Cynops pyrrhogaster*),[18] white croakers (*Genyonemus lineatus*),[111] eels (*Anguilla anguilla L.*),[106] several types of fish[57,143] and reptiles.[115]

TISSUE AFFINITY. Comparative studies have demonstrated that certain strains of PV have affinity to infect only specific epithelial tissues of cutaneous stratified epithelium (i.e., human, equine, chaffinch, and cottontail rabbit). The canine and rabbit oral papillomaviruses constitute a second group that is confined to induction of hyperplasia of mucosal epithelium, and a third group consists of bovine (BPV-1 and 2), ovine, and European elk papillomaviruses which induce both dermal and epidermal hyperplasia (fibropapillomas). The deer fibromavirus induces dermal hyperplasia (fibromas) with minimal epidermal stimulation.[76,125] The anatomic location of lesions according to species and subtype of papillomavirus are given in Table 14–9. Several distinct subtypes may affect the same location. Experiments conducted with rabbit Shope papillomavirus (RSPV) show that this virus precisely recognizes tissue boundaries that are

of cottontail rabbits *(Sylvilagus floridanus)* caused by cell-free preparations persisted as benign papillomas, regressed completely, or progressed to metastasizing carcinomas.[127] The rabbit papilloma virus is now referred to as Shope (rabbit) papilloma virus (SRPV). It is indigenous to central United States in cottontail rabbits, rarely found in other rabbit species and orders, although readily transmissible to domestic strains.[73] The virus is rarely recovered from papillomas of domestic rabbit species inoculated with SPV and never from carcinomas.[73] The SRPV-induced papilloma of rabbits is a useful comparative model for neoplasia because of the predictable and sequential stages of tumor progression for the following reasons: (1) progression occurs at independent rates for identical tumors even in a common environment. Thus, multiple papillomas on the same rabbit will progress independently; (2) individual characteristics of a given tumor progress independently and these features depend on clonal tumor-cell proliferation. Antigens, enzymes, and structural proteins are lost independently from different papillomas, which results in varying alterations in immunogenicity or immunosensitivity, metabolic changes, anaplasia, and increased aggressiveness evidenced by invasion and metastasis; and (3) tumor progression is potentially unlimited and without clearly defined end points in autochthonous systems.[73]

Several investigators have studied the pathobiology of SRPV and it was suggested as early as 1934 that regression may be affected by means of an immune mechanism.[108] Regression was not correlated with antibody titer against the virus but rather with cellular factors;[41] however, both antiviral and antitumor immunologic mechanisms are present.[42] The pathogenesis of Shope papilloma-carcinoma complex is illustrated as a model in domestic rabbits in 4 stages. Stage 0 is the time period when SRPV is applied to scarified skin. Papillomas appear at all inoculation sites in 2 to 3 weeks (stage I), and tumor growth occurs for the next 1 to 2 months (stage II). Spontaneous regression occurs in 10 to 40% of the rabbits about 1 to 3 months after inoculation. Animals with persistent papillomas enter stage III and no additional regression occurs. In approximately 20 to 30% of all hosts, tumors remain as benign papillomas, however, primary epidermoid carcinomas (stage IV) develop in one or more papillomas in 40 to 60% of progressor rabbits which occurs about 1 to 2 years after inoculation. Pulmonary metastases will occur in long-term survivors.[73]

Host factors are important in tumor progression. Domestic rabbits experience up to 75%, while cottontails rarely exceed 25% stage IV tumors. Younger domestic rabbits develop stage IV tumors somewhat sooner. Fetuses inoculated in utero with SRPV have developed carcinomas 7 months after birth.[133] Host resistance in other rabbits may retard carcinomatous transformation. The SRPV genome probably plays a key role in transformation because it persists in carcinoma cells; the rate of neoplastic progression is directly proportional to presence of co-carcinogens, i.e., SRPV superinfected tarred rabbit skin produces a high incidence of carcinomas, whereas tar treatment alone produces none.[43,119,120] Immunologic responses occurring in tumor regression (Stage II rabbits) have been studied in detail. Key factors are systemic, specific, anamnestic and transferrable to isologous hosts with immunocytes or sera, and also, leukocytes of various types are associated with target cells at the reaction site.[72]

The cottontail rabbit papilloma-sarcoma complex has been a useful model in studying papillomatosis of other mammalian species. In domestic rabbits it has been used mainly as an experimentally induced disease that has given insight into the role of papillomaviruses from which a tissue replicating virus cannot be isolated.

Oral Rabbit Papillomatosis

An oral strain of rabbit papillomavirus studied by Parsons and Kidd would not induce papillomas on non-oral epithelial tissue.[105] The oral rabbit papilloma virus is spread naturally in domestic rabbits and induces typical squamous papillomas.[105] The virus will also induce oral papillomas in experimentally inoculated cottontail rabbits *(Sylvilagus spp.)*, jack rabbits *(Lepus californicus* and snowshoe hares *(Lepus americanus)*.[105] The incubation period was approximately 6 to 38 days and warts persisted for generally 30 to 40 days and in some hosts up to a year.

Bovine Papillomatosis

Bovine Papillomavirus (BPV)

Five BPV subgroups are now described. BPV 1, 2, and 5 make up one group. Subtypes 1 and 2 show extensive cross-hybridization of about 45% when tested by DNA-DNA reassociation kinetics.[75] BPV-5 seems to be distantly related, revealing at most 5% DNA homology with BPV-2.[22] In all 3 subtypes common sequences are equally distributed over the whole genome, as deduced from Southern blot hybridization labeled probes and subgenomic restriction enzyme fragments.[22,80,107,110] Common sequences seem to be highly conserved as shown by thermal denaturation experiments with BPV-2 and BPV-5 DNAs.[22] BVP-1 and BVP-2 are antigenically cross reactive, but titers of monospecific animal antisera differed both on hem-

Table 14–9. Location of Papilloma Lesion According to Virus Type

Species	Cutaneous	Nose	Oral	Laryngeal	Feet	Hands	Alimentary	Genital	Mammary Gland
Rabbit	+		+						
Cattle*	BPV-1, 2, 3						BPV-4	BPV-1	BPV-1, 5
Dog	+		+						
Sheep	+								
European Elk	+								
Mastomys	+								
Horse	+	+							
Human*	HPV-3			+	HPV-2	HPV-1a		HPV-6	
	HPV-5				HPV-3	1b, 1c			

*Subtypes of BPV and only a few HPV are given

difficult for a microscopist to recognize microscopically. RSPV of cutaneous origin inoculated into a line of sites beginning at the buccal mucosa and extending across the mucocutaneous junction and ending on the outer lip has produced papillomas only at the latter site.[73] Such experiments have not been done so precisely in other animal species, however, rabbit and canine oral papillomas exist only on oral mucosa, and naturally occurring mammary gland warts of goats exist only in non-pigmented skin. Cutaneous papillomas of calves affect a broader cutaneous area although lesions are often restricted to the head and neck regions.

VIRAL MATURATION. Epidermal cells are nonpermissive for papillomaviruses in the begining of their differentiation process and become more permissive with increasing differentiation. Of the four epidermal layers, cytopathogenic effects are most prominent in the stratum granulosum. Mature virus particles are first seen in association with the nucleoli of cells in the stratum spinosum, while in the stratum granulosum virions are spread throughout the nuclei and appear in paracrystalline arrays. After dissolution of cell structures, aggregates of virus are embedded in keratin in the stratum corneum where infectious virions are shed into the environment.[108] In situations in which fibroblastic responses occur in the dermis, i.e., fibropapilloma of cattle and sheep and fibroma of deer, no virus particles can be detected in transformed proliferating fibroblasts.

HOST RANGE OF VARIOUS PAPILLOMAVIRUSES. Most PV are species specific although bovine, European elk and sheep papillomaviruses will, under experimental conditions, induce tumors in heterologous hosts, i.e., the Syrian hamster and mouse.[28,130] Tumors induced in hamsters are fibromas and fibrosarcomas; these tumors have a latent period of 9 to 18 months, which is a much longer period of time than fibropapillomas of cattle induced after exposure to BPV-1 and BPV-2.[96] Bovine PV will induce neoplasms of various histologic types when injected into the brains of calves,[91] fibromatous changes in urinary bladder of calves,[95] and sarcoid-like lesions, a fibromatous change, in the dermis of the horse.[97] Lancaster and co-workers demonstrated that naturally occurring equine sarcomas had a high degree of DNA-DNA homology with BPV suggesting, on a biochemical basis, that equine sarcoids are induced by BPV.[77,79]

MEDICAL AND ECONOMIC CONSEQUENCES OF PAPILLOMATOSIS. The economic impact in agriculture is not well documented, however, sums must be considerable from chronic effects of papillomas. In animals, discomfort from warts may lead to reduced milk production, weight loss, or death from wart progression to carcinoma. Animals with immune deficiencies may have warts that persist.[39] Deer with fibromatosis and other wild *Cervidae* with papillomatosis may be severely debilitated as a result of being partially or totally blinded by tumor growth around eyelids leading to inability to find feed.

Warts in humans may cause incapacitation if growths are severe on the plantar surface of the feet (plantar warts), and debility may result from generalized cutaneous warts or warts progressing to carcinoma. "Butcher's warts" may be an incapacitating disease described as extensive warts on the hands of some persons who work in the butchers trade. These warts are associated with human papillomaviruses substrains 2 and 3.[111] Presently there is no proof that animal papillomaviruses cause "human warts."

CRITERIA FOR ESTABLISHMENT OF SPECIFIC PAPILLOMAVIRUS SUBTYPES. Molecular cloning, either in bacterial plasmids or in bacteriophage lambda, are used to show homology between different strains. Isolates that show more than 50% cross-hybridization are regarded as subtypes even if their restriction endonuclease cleavage patterns differ. On this basis 30 HPV and 5 bovine papillomavirus subtypes have been differentiated.[16,108]

Lapine Papillomatosis

Rabbit papillomas with cutaneous horn resulting from aberrant squamous cells of the epidermis were first reported by Seton in 1909.[124] Shope and Hurst reported in 1933 that naturally occurring papillomas

agglutination inhibition and complement fixation assays by a considerable difference.[73]

BPV-3 and BPV-4 form the second group of cattle papillomaviruses.[21,109] Their DNAs do not crosshybridize with probes from the other group and there is no serologic cross reactivity. The DNAs of both viruses have a molecular weight of about 4.4×10^6 which is smaller than BPV-1, 2, and 5 DNA's (4.5×10^6). A comparison of physical nucleotide maps reveals a close relationship between BPV-1 and BPV-2.[74] Heteroduplex analysis confirmed that both DNAs are in register and are broadly homologous throughout most of their length when aligned at their single Hind III sites.[23] The transforming region of the BPV-1 genome proved to be almost completely homologous to BPV-2, whereas only partial homology was observed with the segment coding for structural proteins.[23]

The two subtypes (BPV 1 and 2) induce fibropapilloma, a fibroblastic as well as epithelial response in development of cutaneous warts.[8] Three types (BPV 3, 4, 5) induce only epithelial changes similar to the cellular effects found in human, rabbit, dog, and horse papillomas. Warts of cattle and humans are usually described anatomically by location. For cattle, warts are divided into (1) typical cutaneous fibropapilloma, (2) genital fibropapillomas, (3) teat and mammary gland warts, (4) atypical cutaneous squamous papillomas, and (5) alimentary warts. The various pathologic changes resulting from infection of known BPV subtypes are given in Table 14–10.

Clinicopathologic Aspects of Bovine Papillomatosis

BOVINE CUTANEOUS FIBROPAPILLOMAS. The traditionally described bovine papilloma is induced by BPV-2 (Figs. 14–33 and 14–34). BPV-2 shares about 50% of its DNA nucleotide sequences with BPV-1 and they cross-react immunologically. BPV-1 and 2 have a DNA molecular weight of 5×10^6, and they have oncogenic potential in such heterospecies as Syrian hamsters and mice.[76] In vitro, BPV-1 and 2 are able to transform mouse cell lines NIH 3T3 and C 127 with focus formation that shows growth in soft agar and tumorigenicity in the nude mouse.[40]

Once premises are infected with BPV-2, papillomatosis may recur yearly, developing in calves from 4 to 12 months of age, or it may occur in alternate years. The disease may have economic importance depending on the severity of infection. BPV-2 induced cutaneous warts generally occur in susceptible cattle from 4 to 18 months of age, and are found on the head, chin, periorbital region, neck, dewlap, and shoulders.

Natural transmission occurs by contact with contaminated objects that cause injury to the skin, such as fence posts, stanchions, brushes, halters, and tattoo instruments.[96,107] For instance, when ear tattooing at time of vaccination for brucellosis, veterinarians using a contaminated instrument can induce papillomas in the ears of tattooed calves. Insect vectors transmit rabbit Shope papilloma viruses, and inapparent viral carriers may be responsible for endemic outbreaks in cattle.

Field exposure usually results in an incubation period of 1 to 12 months before warts appear, although the disease can be produced experimentally in 1 month (Figs. 14–33 and 14–34).[96] In most cases, spontaneous regression occurs within 4 to 8 weeks after the first appearance of warts, but occasionally the disease may persist for long periods of time.[4] Cattle can be infected with other subtypes especially 3, 4 and 5, even if there is an immunity to subtype BPV-2, as was described several years ago.[5]

Experimentally, BPV will induce tumors in the bovine brain and urinary bladder,[50,95] and BPV (probably BPV-2) will also produce sarcoid-like tumors in

Table 14–10. Clinicopathologic Description of Lesions Induced by Various Subtypes of Bovine Papilloma Viruses

Type	Reference	Age	Location	Histology	Description
BPV-1	64	Young adult to adult	Teat, penis, vulva	Fibropapilloma	Typical fibropapilloma of penis, vulva and teat. 50% sequence homology with BPV-2 under stringent hybridization conditions
BPV-2	64	4–18 months	Head, neck, dewlap	Fibropapilloma	Typical cutaneous fibropapilloma
BPV-3*	55	Adult	Cutaneous	Squamous papilloma	Atypical cutaneous wart
BPV-4	64	Young adult	Tongue, oropharynx, esophagus, rumen	Squamous papilloma	Alimentary warts may progress to carcinoma. Some DNA sequence homology with BPV-3 under stringent conditions
BPV-5	64	Adult	Teat, udder	Squamous papilloma	About 35% sequence homology to BPV-2 and 5% to BPV-1

*Other atypical warts have been described although not yet associated with a particular strain of BPV (Ref. 7).

Fig. 14–33. Papillomas arising from multiple injection sites on neck of yearling Hereford steer experimentally inoculated with BPV. Virus was inoculated 50 days previously and papillomas were clinically apparent for 4 weeks. (From Olson, C: J Am Vet Med Assoc 135:339, 1959.)

horses.[97] Horses with BPV induced tumors have demonstrable neutralizing antibodies in their serum against BPV.[123] Lancaster and co-workers described the cross DNA homology between BPV and equine sarcoids.[77,79] It is now generally accepted on the basis of these molecular hybridization studies that BPV-2 and perhaps other BPV subtypes are associated with the development of equine sarcoids (see Equine Sarcoid, Chapter 14, Part III).

The Syrian hamster *(Mesocricetus auratus)* is susceptible to experimental injection of BPV, developing fibromas and fibrosarcomas of the skin, chondromas of the ear, and meningiomas in the brain according to inoculation site of virus.[116] Tumors first appear in hamsters from 5 to 13 (average 9) months after injection of BVP. No infectious BPV can be recovered from BPV-induced tumors in hamsters. Metastasis to lung occurs in approximately 10% of the BPV-induced hamster tumors. One strain of mice (C_3H/eB) developed fibromas when inoculated with BPV.[12]

Multiple large warts covering 10% or more of the body can lead to unthrifty young animals. This may lead to lowered resistance to infection and secondary disease, such as severe lice infestation and during summer months to myiasis. Lesions are circumscribed, varying in size from several millimeters in diameter to large dry, horny, whitish, cauliflower-like growths 10 cm or more in diameter. Fronds are deep and the papilloma is hairless. Histologically the lesion is a cutaneous fibropapilloma.

Fig. 14–34. One-year-old purebred Angus bull with naturally occurring infectious papillomas and severe fly strike; bull was raised on premises with seasonal papillomatosis.

BOVINE GENITAL FIBROPAPILLOMA. Induced by BPV-1, this fibropapilloma is typically characterized by warts of the penis and vulva (Fig. 14–35). Because the virus is transmitted at coitus, genital fibropapillomas are found in adult cattle. Lesions on the penis and vulva are glittering and smooth-surfaced and do not develop deep fronds as found in mature cutaneous fibropapillomas induced by BPV subtype-2.

BOVINE ATYPICAL CUTANEOUS PAPILLOMA. Barthold and co-workers first described atypical warts in cattle and reported several herds in Washington state and one herd in Minnesota.[7] An outbreak of atypical warts occurred in adult polled Hereford cattle in a herd in Washington state and approximately 15 years later began to appear in younger beef cattle in the same herd and in some Holstein-Friesian cattle on the same premises. Tumors usually persisted but in some cases regressed and reappeared on the same animal. Many young cattle 6 to 18 months of age developed typical fibropapillomas similar to those induced with BPV-2 which regressed after several months. Preventive vaccination with commercial or autogenous wart vaccine controlled the typical BPV-2 wart problem but failed to alter the incidence of atypical papillomas.

Grossly, atypical warts are low, flat circular lesions with a broad base that often coalesce to cover large areas. The papular fronds are delicate, often with hair growing between them. Microscopically, atypical papillomas appear as areas of acanthosis with considerable hyperkeratosis. Epidermal rete pegs extend only superficially into the dermis. Most of the dermal adnexae are preserved and functional. All experimental attempts to transmit warts with virus isolates from atypical warts have failed. The skin and brain of hamsters and brain of calves were resistant to atypical wart virus yet highly susceptible to strain BVP-2, the typical wart virus. Atypical warts frequently recurred or appeared at new sites on certain animals but 12 tumor bearing animals experimentally inoculated with atypical wart material resisted tumor induction, indicating difficulty in transmission.

PAPILLOMAS OF THE MAMMARY GLAND AND TEAT. A detailed survey of teat papillomatosis in British cattle was documented by Meischke.[86] There are two distinct histologic types of teat and mammary

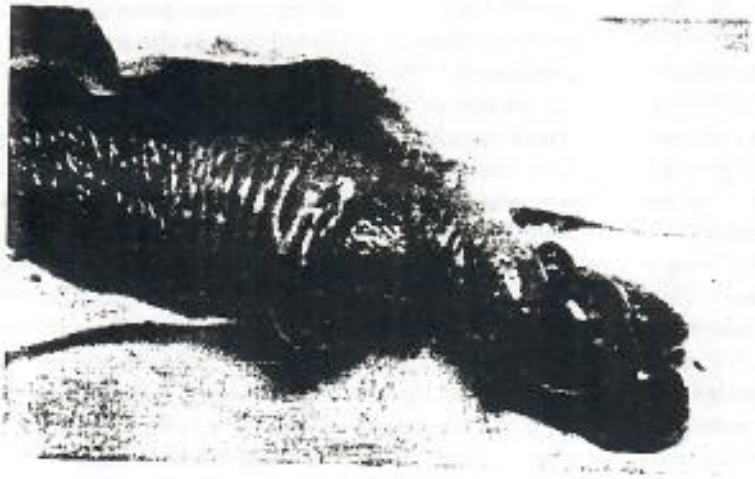

Fig. 14–35. Fibropapilloma of glans of bull's penis. Virus isolated from this lesion produced papillomas in experimentally inoculated calf. (From Olson, C: Arch Environ Hlth 19:827, 1969.)*

gland papillomas: (1) fibropapilloma or papillary type associated with BPV-1 infection and (2) squamous papilloma associated with BPV-5 infection (Table 14–10).[64] Warts of this type are found on the teats and mammary gland, are often multiple, and occur in adult cattle (Fig. 14–36). They are pedunculated with deep fronds, and smaller in size than typical cutaneous warts. They may be multiple, and do not involve the teat canal but may be located around the teat orifice. This type occurs primarily in adult dairy cattle, and to a lesser degree in males. Some lesions, particularly on the mammary gland, are broad, diffuse, and flat. The other type induced by BPV-5 produces a squamous papilloma that appears grossly as a rice grain-type lesion on the teat and occasionally involves the mammary gland (Fig. 14–37). BPV-5 has approximately 35% sequence homology to BPV-2 and 5% to BPV-1.[64] The rice grain papilloma is seen in adult cattle and may persist for months or years.

BOVINE ALIMENTARY PAPILLOMATOSIS. Thorsen and co-workers indicated that multiple alimentary tract warts were described as early as 1875 in Germany and subsequently in France and the United States.[137] Papillomas associated with carcinomas of the pharynx and upper alimentary tract have been reported in regions of Brazil, Kenya, Britain, and other areas of the world where bracken fern (*Pteridium acquilinum*) grows in abundance.[38,61,112,113] Döbereiner and Plowright suggested that alimentary papillomas progressed to carcinomas.[38,113] A recent detailed study of multiple alimentary papillomas and carcinomas was described by Jarrett and co-workers in Britain in cattle that had foraged on bracken fern for extended periods of time.[60,63] Whereas Döbereiner and Plowright were unsuccessful in isolating a papilloma virus, Jarrett's group in Scotland succeeded in 1980.[63] The new isolate has only slight DNA homology with BPV-1, BPV-2, and BPV-5 but does cross hybridize with BPV-3.[61] Electron microscopically the virus measures 55 nm, and has a buoyant density of 1.35 g/ml in

Fig. 14–36. Infectious papillomas on udder of 3-year-old Holstein-Friesian cow, caused by BPV-1.

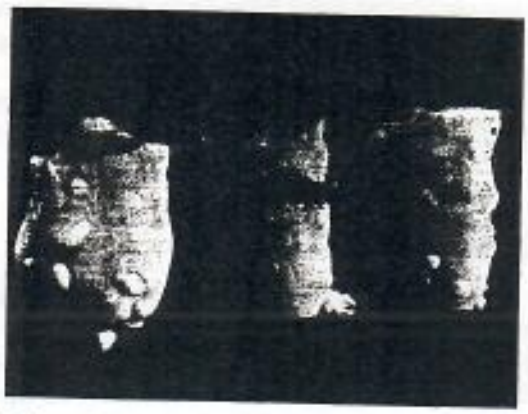

Fig. 14–37. "Rice-grain" papillomas on teats of cow induced by BPV-5. (Courtesy of Dr. W.F.H. Jarrett.)

$CsCl_2$. Virus can be isolated from tongue, mouth, gingiva, esophagus and rumen (forestomach).[64] Warts will frequently progress to squamous cell carcinoma when cattle also forage on bracken fern. Papillomas are found in young cattle but carcinomas occur only in adult cattle generally 5 years and older. Epidemiologic evidence suggests a co-carcinogenic role for bracken fern in association with BPV-4 infection.

Twenty percent of cattle of all ages going to slaughter in Britain had alimentary tract papillomas.[62] They had less than three papillomas per site, however, whereas in the endemic area where cattle foraged on bracken fern, 80% of the animals had papillomas and 90% had more than three lesions. In cases with carcinoma, 96% had papillomas and 90% had more than three lesions. Further, 65% had tumors at more than one site. Carcinomas occurred on the tongue, soft palate, oropharynx, esophageal groove and cardiac area of the rumen in cattle 5 years and older. Cattle in the cancer area had a high concurrent frequency of intestinal adenoma and adenocarcinoma, and bladder carcinoma.

Alimentary papillomas induced by BPV-4 are of the squamous type histologically similar to atypical cutaneous papillomas reported in both Australia and the USA. BPV-1 and BPV-2 will induce fibropapillomas of the alimentary tract.

Ovine Papillomatosis

Papillomas have infrequently been described in sheep.[48,53,59] Little is known about the epidemiology and natural spread of ovine papilloma virus (OPV) infection. Gibbs and co-workers in Britain isolated an OPV that is molecularly distinct from BPV but has similar biologic properties.[48]

Papillomas primarily affect the skin of the head and ears. The sheep papillomavirus induces a fibropapilloma response similar to BPV-2.[48] Squamous cell carcinomas may develop in the same location, in which case cutaneous horn frequently projects from the surface of such carcinomatous lesions giving the appearance of a small aberrant ram's horn growing from the ear.[2] Inoculated hamsters developed fibromas in 9 months but transmission was not successful to cattle.[48]

Caprine Papillomatosis

Goat papillomas are described infrequently although there are several reports of spread within a herd.[36,89,136] Affected animals are primarily of breeds that lack pigmented skin, i.e., saanen, angora, alpine, or goats with cross-breeding resulting in nonpigmented skin.[136] Three types have been described. The most commonly reported type is the mammary gland papilloma.[9,45,136] The second documented type is a squamous papilloma occurring on the head, neck and trunk regions. Grossly it appears as a circumscribed, flat, round lesion. It is usually persistent, multicentric, and looks somewhat similar to fungal dermatitis. Both types occur more frequently in goats with nonpigmented skin 2 years or older, and appear to spread within the herd.[36,55,89] A third type occurs on the genitalia, histologically being a typical fibropapilloma.[136]

With extrapolation of data from Shope rabbit papillomatosis, one can hypothesize that there appear to be different stages of caprine papillomas of the mammary gland.[73] The first stage includes does that show total regression of warts after a course of 2 to 4 months. A second stage involves warts that partially or totally regress during winter months, usually during pregnancy, and recur with return of intense sunlight in the summer months. The third stage includes those goats that after recurrence, warts become persistent, or develop persistent warts from the onset. A fourth stage includes those goats with warts that progress to carcinomas (Fig. 14–38). It is unrecorded what percentage of affected animals with warts have total regression and those that are grouped into stages II-IV. Some of the etiologic facts related to squamous cell carcinoma of other species appear similar to those of goat mammary gland papillomas, namely: (1) lack of pigmented skin; (2) excessive exposure to sunlight; and (3) adult age. Goat mammary gland papillomas seem to arise after contact with a yet undefined transmissible agent as well as the above mentioned factors.

European Elk Papillomatosis

The virus causing elk papillomas was isolated by Swedish workers in 1981.[15] It causes fibropapillomas similar to those induced in cattle by BPV-2, and it will

Fig. 14–38. Five-year-old Saanen doe. Persistent papillomas progressed after two years during the summer months to squamous cell carcinoma on the caudal aspect of the right mammary gland.

induce tumors in hamsters similar in type and incubation time to those induced with BPV-2. The elk papillomatosis virus has physical and biologic characteristics similar to other ruminant papillomaviruses and may produce a severe debilitating disease (Fig. 14–39).[86]

Deer Fibromatosis

Deer fibromavirus (DFV) induces a fibroblastic response in white-tailed deer *(Odocoileus virginianus)*, mule deer *(Odocoileus hemionus)* and black-tailed deer *(Odocoileus hemionus columbianus)*.[54,114,139] Fibromas have been reported from several areas of the USA and also in other deer, moose, and caribou.[25,54,132,139] The physical and biologic characteristics of the virus are similar to other papillomaviruses; two subtypes have been described and there is a high degree of relatedness with BPV-1 and BPV-2 suggesting that evolutionary diversity occurred only recently.[76]

In most animals, the lesions regress spontaneously, but some progress to fibrosarcomas with metastases.[69] Transmission experiments of DFV in cattle and horses have been unsuccessful; however, the virus is oncogenic in the hamster.[70] The mode of natural transmission is unknown but probably occurs similarly to bovine papillomaviruses by contact with contaminated objects such as bushes and tree trunks along the trail. There is a higher frequency in males suggesting rubbing antlers on contaminated objects and fighting during the rutting season are reasons for sex differences.[132]

Fibromas occur most frequently on the head, neck, and shoulder regions of the body in deer 2 years or younger. They are firm, round, and nodular, measuring up to 1 cm in diameter. They are often dark brown to black with a smooth or verrucous surface. Unpigmented, tan, to white fibromas are occasionally observed on areas of the body where the hair is white or buff color. Massive fibromas can be induced when DFV is experimentally transmitted.[125]

Fig. 14–39. Naturally occurring fibropapilloma involving large portions of the lateral head and neck regions of a young European elk calf. (Courtesy of Dr. J Moreno-Lopez.)

Equine Papillomatosis

Papillomas are frequently recognized tumors of horses and generally are located on the lips, nose and legs of one to 3-year-old horses (Fig. 14–40).[6,131,135] Papillomas have been reported in other locations including mouth, ocular regions, male and female genitalia.[66] They usually exist in clusters and measure 2 to 10 cm in diameter although solitary warts are found in ocular and genital locations. Squamous cell carcinomas and papillomas often affect similar anatomic locations and may arise de novo or by progression from papillomas.[66]

In a retrospective study of 135 horses with papillomas and squamous cell carcinomas, papillomas occurred in neonatal to 20-year-old horses with a mean age of 8.3 years.[66] Congenital cutaneous papillomas have been reported in foals ranging in age at time of biopsy from 1 hour to 5 days.[3,47,92,93,122] It appears from these reports that papillomavirus infection can occur in utero.

Equine papillomavirus (EPV) induces papillomas by experimental inoculation and natural transmission occurs by contact with contaminated fomites.[20,32] Following exposure to EPV under experimental conditions, the incubation period is about 60 days, with growth and regression of papillomas taking another 45 to 60 days. When lesions mature, they quickly become necrotic and usually disappear within 1 to 2 weeks. Persistent warts are often multicentric on the ear or legs. These locations are atypical and may result

Fig. 14–40. Multiple papillomas on muzzle of yearling quarter horse colt. The papillomas had been present for 8 weeks, and the ranch had an endemic history.

from infection with a different subtype of virus, or perhaps by infection with typical EPV but in an immunosuppressed host. Grossly, lesions are elevated, circumscribed, horny masses having fronds similar in appearance to the surface of a cauliflower. Histologically, reactive tissue is squamous epithelium. The verrucous-type sarcoid, when small, may be mistaken for warts.

EPV has similar properties to those of other species.[46] Structural antigens were detected in 12 of 45 papillomas but none of 90 squamous cell carcinomas using a peroxidase-antiperoxidase technique.[66] Diverse anatomic sites of equine papillomas suggest there might be several subtypes of EPV similar to the situation in human beings and cattle. Papilloma viruses of other species have distinct predilections for certain types of epithelial tissue.

Canine Papillomatosis

Solitary canine papillomas are located in the cutaneous regions of the body and most probably are of non-infectious origin. Multiple canine papillomas are of three types: (1) oral, (2) cutaneous—usually occurring on medial aspects of legs, posterior ventral abdomen and occasionally elsewhere on the body, and (3) ocular lesions occurring on the cornea and conjunctiva. Solitary types are usually found in older dogs and multiple types in young (6 month to 3-year-old) animals. The multiple types are associated with virus infection and occasionally will be solitary. Typical oral papillomas have been reported in domestic dogs, coyotes *(Canis latrans)*, and wolves *(Canis lupus)*.[37,44,51,85,91,105,121] Multiple papillomatous growths on internal genital organs have been reported in bitches administered the synthetic estrogen dimethyl diethylstilbane.[102] Papillomas occurred on the serosal surface of the uterus and ovaries and regressed completely within 200 days after treatment was discontinued.

Canine Cutaneous Papillomas

Little is known about multiple canine cutaneous papillomas (CCP). They occur infrequently, the reasons for which are not known. Early lesions may have a smooth glistening surface and flat donut-shaped appearance (Fig. 14–41). As CCP mature they develop a typical wart-like appearance with deep rough-surfaced fronds. Watrach demonstrated presence of papilloma virus in CCP growths.[140] There is no record of experimental transmission, however, the presence of papilloma virus gives good evidence of their infectious nature.

Fig. 14–41. Multiple cutaneous papillomas on the skin of the abdomen of a 12-year-old mixed breed dog. The lesions have a flat "donut-shaped" appearance.

Canine Oral Papillomatosis

Canine oral papillomas (COP) have received considerable attention.[1,26,27,44,71,85,105,141] The infectious nature of COP was described by Penberthy in 1898 from a kennel of foxhound puppies.[105] McFadyean and Hobday experimentally transmitted COP from two of Penberthy's puppies to unrelated dogs using a cellular homogenate as inoculum.[85] The virus etiology was experimentally demonstrated some 30 years later when cell free filtrates were used as inoculae to transmit COP to puppies.[44] DeMonbreun and Goodpasture could easily transmit COP with cell free preparations in scarified regions of the oral mucosa including lips, gingiva, tongue, and epiglottis.[37] They were unable to transmit COP to gastrointestinal tract, cutaneous portions of abdomen, penis or vulva of the dog or to guinea pigs, rabbits, rats or mice. The structural characteristics and viral properties of the COP were later found to be similar to other papillomaviruses.[71,138,141] The virus has not been studied with molecular probes to the same degree as have human, bovine, and European elk papillomaviruses.

Grossly, COPs appear on the mucosa as pale, smooth elevations that soon develop a rough surface (Figs. 14–42 and 14–43). Within 3 weeks after appearance, oral papillomas become pedunculated and develop characteristic deep fronds. The older warts of 3 to 4 weeks' duration usually have deep and closely packed fronds. Regressing warts become dark gray and begin to shrivel. Scars are not evident upon complete regression, which takes only 1 to 2 weeks.

A detailed study of the cytology of canine oral papilloma was published.[29] Microscopically, COP involves squamous epithelial cells similar to the tissue responses found in warts of human beings, rabbits, and horses.[29,37] The first tissue response to COP virus

Fig. 14–42. Multiple papillomas on lips, gingiva, and dorsal tongue of 6-month-old puppy. Smooth lesions are early growths and roughened cauliflower-like ones are advanced tumors; all regressed within 6 weeks.

infection is an increase in mitotic activity resulting in acanthosis and hyperkeratosis. As lesions enlarge, some cells seem to be diverted from keratization to a role of virus production. A few cells in the acanthotic stratum spinosum become swollen and have a stronger affinity for pyronin than do surrounding cells. It is these cells that show formation of inclusion material and failure of cytoplasmic differentiation into tonofibrils and keratohyaline which occurs in the stratum granulosum. Cytoplasmic degeneration ensues and eventually leads to cell death and persistence of virus embedded in strands of keratin. The majority of basal layer cells, however, differentiate into keratogenic normal cells.

Fig. 14–43. Extensive oral papillomatosis of the oral cavity in an 8-month-old black and tan coon hound. The lesions regressed spontaneously.

Transition of COP to carcinoma has been reported in progressive and persistent oral papillomas of a 1 ½-year-old male beagle.[142] The carcinoma was at the level of the posterior portion of the jaw. This is the only known report of COP progressing to carcinoma which probably indicates the rarity of this event in canine oral papilloma. Oral carcinoma of unknown causes are common although to date there is no evidence they arise from COP.

Canine Ocular Papillomas

Naturally occurring multiple ocular papillomas have been reported in several dogs.[13] They are found in relatively young dogs 6 months to 4 years of age. Virus particles have been demonstrated in these lesions.[108] It is not established whether ocular papillomas are caused by a third subtype or whether COPV is the agent responsible since experimentally injected COPV will induce ocular papillomas.[14]

Papilloma Diagnostic Procedures

Gross appearance is sufficient for an accurate diagnosis of papillomas in most instances, although histopathologic examination is necessary to determine the diagnosis in some atypical types such as fibropapillomas of the bovine penis and vulva and those occurring on the udder of goats.

Infectious papillomas are usually multiple and are also divided into fibrous and squamous types. Squamous viral papillomas occur in multiple sites and have a thickened epidermis with deep penetrating rete pegs. This type is found in dogs, horses, and other animals except cattle, sheep, and European elk.

The fibrous papilloma type is found in the dermis of cattle, sheep, and European elk. The fibrous papilloma type is best studied in cattle. After induction in the skin, the first reaction is a granulomatous response with hemorrhage, edema, leukocyte infiltration, and fibroblastic reaction. After 1 week, the granulomatous response subsides and continuous fibroplasia occurs. The fibroblasts infiltrate the papillary layer of the dermis and replace the normal adnexa of the corium. Four to 6 weeks after induction, acanthosis and proliferation of the epithelium occur over the area of fibroplasia. Fibropapillomas typically develop in 60 to 90 days following inoculation of scarified skin, which results from a hyperplastic reaction of epithelium overlying the dermal fibroma.[96]

The BPV appears to first transform the fibroblasts of the dermis into cells with a capacity for unrestricted growth, followed by changes in the overlying epithe-

lium. Some of the epithelial cells proliferate while others degenerate, which leads to replication of virus.[96] A histochemical study to elucidate these changes indicated prominent enzyme activity of DPNH-diaphorase, TPNH-diaphorase, cytochrome oxidase, succinic dehydrogenase, and esterase in the hyperplastic epithelial cells of the acanthotic stage.[92] Less epithelial cell response is found in the bovine penis and vulva.

Principles of Treatment and Prognosis

Because most warts disappear spontaneously, no treatment is usually needed. Treatment is advocated only in cases of severe secondary disease such as occlusion of air passageways from extensive oral pharyngeal warts in dogs or in an attempt to accelerate remission in calves or horses used for show purposes. Surgical excision and electrosurgical treatment are successful in removing pharyngeal tumors in anesthetized dogs. Removal of 6 to 10 warts may induce a rapid remission of other warts (referred to as autovaccination). An autogenous wart vaccine was first reported in 1931.[10] Its efficacy in cattle has been investigated, but its benefit has been questioned.[19,56,98,99] An autogenous vaccine is made by mincing and freeze-thawing tissue 2 to 3 times. One part of tumor tissue is mixed with nine parts of 0.85% saline solution. The homogenate is filtered through gauze and then stored at 4°C until used. Autogenous vaccine has been used in cattle, dogs, and horses. In dogs, vaccine is injected intradermally (0.5 to 1 ml) once weekly for three treatments. In horses and cattle, intradermal injection of 1 to 5 ml once weekly for three treatments is used.

Prognosis is favorable for infectious cutaneous warts of cattle, horses, and dogs. The duration of papillomas usually will be 1 to 2 months. A longer period is seen in animals when reinfection takes place because of loss of immunity or infection with an immunologically different strain of papilloma virus. Prognosis should be guarded if 20% or more of the bovine body is involved.

In goats, sheep, and rabbits the prognosis should be guarded because lesions may develop into carcinomas. This may also occur on the eyelid of cattle and oral cavity of dogs.[142] In persistent papillomatosis the pathologist should look carefully for squamous metaplasia or early signs of squamous cell carcinoma at the margins of each lesion, which may help explain persistence.

Prevention and Control

Vaccination in all endemic situations will help control subsequent outbreaks. Vaccine made from wart tissue on the premises is of greatest value; however, commercially prepared bovine wart vaccine of bovine cell origin usually is effective in prevention of bovine warts.[19,98] Wart vaccine is species-specific and is not useful in heterospecies other than from the point of view of a nonspecific foreign protein reaction; therefore, it is not advisable to use bovine wart vaccine to treat canine or other species with papillomatosis.

Vaccine is made in a manner similar to the manufacture of autogenous vaccine. Tissue extracts are not frozen and thawed but rather added to the final concentration of 0.5% phenol or formalin to inactivate the virus. Animals are vaccinated at 2 to 3 months of age. Foals and calves are given 5 to 10 ml of vaccine by subcutaneous or intramuscular injection 2 to 3 times at 10-day intervals. Puppies are given 2 to 3 doses of 2 ml of vaccine by subcutaneous or intramuscular injection at 10-day intervals.

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