Urothelial tumors of cattle: a biological model of comparative oncology



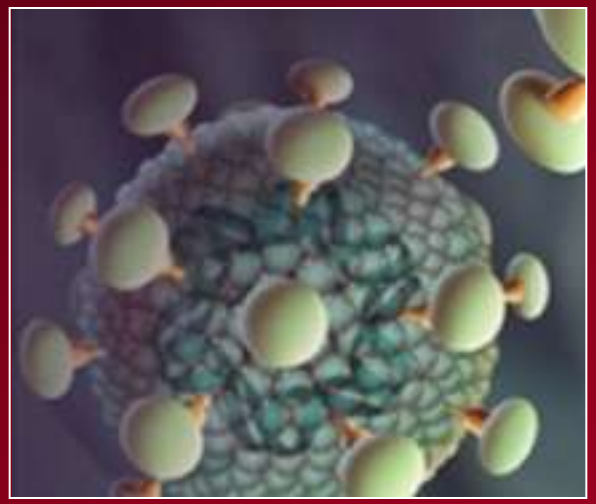
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Bracken fern

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Bovine Papillomavirus type 2

A high level of red meat consumption has been defined as a risk for colon carcinogenesis (zur Hausen, 2006)



Fig. 15. Red meat cooked "raw".

In meat prepared medium or raw (Fig. 15), temperatures in the central portions do not exceed 55 to 65 °C. At least members of the polyoma- and papillomavirus families readily survive these temperatures without significant loss of their infectivity (Lelie et al., 1987, Sauerbrei and Wutzler, 2009).



Fig. 11. Some human viruses are carcinogenic for several animal species. Do animal viruses exist potentially carcinogenic in humans?

Toxicity of bracken fern





The toxicity of bracken fern, *Pteridium* spp, one of the most common plants in the world, is very complicated as the plant elaborates several toxic substances that are implicated in different and severe pathological manifestations of animal livestock and represent an hazard for human health too. It is generally believed that its consumption is responsible for a number of well-recognized acute and chronic toxic syndromes in domestic animals (Smith, 1990) and increases the oncogenic risk in man as well (Fenwick, 1988; Potter and Baird, 2000).

Clastogenic effect of bracken fern (*Pteridium aquilinum v. arachnoideum*) diet in peripheral lymphocytes of human consumers: preliminary data

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Abstract

Ingestion of bracken fern (*Pteridium aquilinum v. arachnoideum*) is associated with digestive tract cancer in different regions of Japan, Venezuela and Brazil. In view of reports that dietary bracken fern causes chromosomal instability in cattle, the clastogenic effect of bracken fern was investigated, in a preliminary study, in peripheral lymphocytes obtained from habitual consumers and a control group of non-consumers, which were carefully investigated about cancer history or family cancer history, negative in both cases, using protocols comparable to those previously described in studies in cattle raised on bracken pastures. Cytogenetic analysis showed significant increased levels of chromosomal abnormalities, such as chromatid breaks, in cultured peripheral lymphocytes of the consumer group. There was no correlation with subjects, gender, smoking habits or alcohol consumption, and the only correlation was with prolonged exposure to dietary bracken.

Toxicity of bracken fern

In cattle, the chronic poisoning of bracken fern causes neoplastic lesions of the alimentary canal as well as urothelial and mesenchymal tumours of the urinary bladder. An interaction of the bracken carcinogens with bovine papillomavirus type 4 (BPV-4) (Campo et al., 1980; Borzacchiello et al., 2003) and type 2 (BPV-2) (Campo et al., 1992; Borzacchiello et al., 2003) is known to occur in alimentary and bladder tumours, respectively.

Toxicity of bracken fern

The most important clinical manifestation of the latter ones is the presence of blood in the urine thus characterizing a syndrome known as bovine enzootic hematuria (BEH).



Toxicity of bracken fern

Despite the fact that the toxicity and carcinogenicity of bracken fern was clearly documented by Evans and Mason as from the middle sixties, the carcinogenic fraction of bracken was uncertain until a norsesquiterpenoid glycoside, named ptaquiloside (PT) (Aquilide A), responsible for mutagenic, clastogenic and carcinogenic activity, was simultaneously isolated in Japan and the Netherlands (Niwa et al., 1983; Van der Hoeven et al., 1983).



Toxicity of bracken fern

It has been suggested that the different distribution and concentrations of PT in the genotype of bracken may be the reason for the geographically localized occurrence of BEH (Smith et al., 1988).

PT is believed to be carcinogenic via DNA alkylation only at specific organs and in a sequence selective fashion when it is activated to its unstable reactive dienone form – activated PT (APT) – under alkaline conditions (Prakash et al., 1996; Alonso-Amelot and Avendaño, 2002).



Toxicity of bracken fern

Ileum and urinary bladder of cattle are known to be alkaline and provide ideal conditions for the formation of APT. It has been shown that in vivo in bracken-fed cattle APT alkylates DNA forming a number of DNA adducts in the ileum and urinary bladder which result in H-ras 1 mutation via initial adenine alkylation in codon 61 leading adenine to pyrimidine (T and C) transversion. The alkylating group present in APT compound is a cyclopropyl ring (Prakash et al., 1996).

Toxicity of bracken fern

Activation of this oncogene in codon 61 has been also observed in tumours of mammary glands of rats induced by 7,12-dimethyl-benz(a)anthracene (DMBA). It is worthwhile to note that H-ras 1 gene is also activated in papillomavirus-associated carcinomas of the upper alimentary canal in cattle (Campo et al., 1990).



Toxicity of bracken fern

More recently, immunohistochemical expression of H-ras protein was detected in some pathological urinary bladders in cattle naturally exposed to bracken fern (Sardon et al., 2005).

In addition, mutations in codon 59 resulting in G to A transition or G to T transversion and consequent change from Ala to Thr or Ala to Ser are known to occur.



Toxicity of bracken fern

They were shown in preneoplastic tissues of mammary glands of rats after dosing ATP by intravenous (iv) route suggesting that H-ras activation is an early event in the PT carcinogenesis (Shahin et al., 1998). The ras product p21 has GTPase activity and plays a role in the control of cell cycle.



Toxicity of bracken fern

In vitro mutagenesis studies have shown that H-ras mutations occurring in codons 12-13 and codons 59-63 are responsible for the alteration of the protein conformation thus reducing its activity (Benjamin and Vogt, 1991).
Interestingly, several DNA adducts were described in tissues of mice treated with *Pteridium aquilinum* subsp. *aquilinum*.



Toxicity of bracken fern

These are different from the adducts produced by the reactive APT suggesting that bracken may contain genotoxins other than ptaquiloside (Freitas et al., 2001). *Pteridium aquilinum* contains tannins and phenolic acid in up to 10% of its dry weight (Alonso-Amelot and Avendaño, 2002). The flavanoid quercetin is one of the most potent mutagens present in the fern.

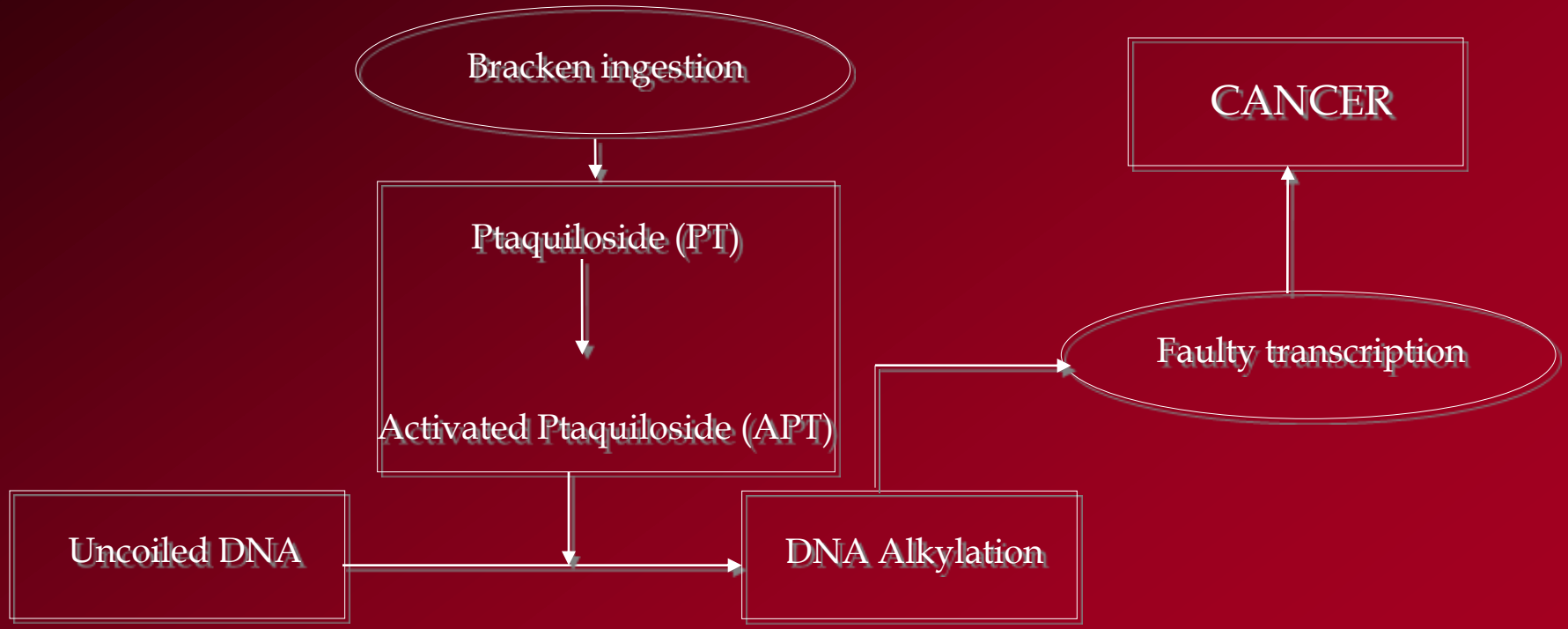


Sixth IARC Monographs Advisory Group on priorities for future evaluations - Priority List of agents and exposures to consider in future IARC Monographs

Food additives, contaminants and components		
Urethane (2B)	high	- new mechanistic data - NTP report will soon be available Within the framework of a re-evaluation of 'alcoholic beverages'
Naturally occurring substances		
Growth hormones (GH)	low	- children with growth deficiencies are given GH - possible human exposure because GH is given to cattle - animal data show increase in mammographic densities and prostatic hyperplasia in macaques
Methyl eugenol	low	- widespread but low human exposure - NTP animal carcinogenicity study
Nitrate, nitrite and endogenous nitrosation	high	- epidemiological studies on fertilizer workers and on nitrate in drinking water An *ad hoc* planning meeting is needed to define the scope of this Monograph
Ptaquiloside and bracken fern	high	- new animal carcinogenicity data and some epidemiology in relation to bracken fern
Insulin-like growth factors (IGF)	low	- increase in endogenous IGF concentration in serum would lead to increase in breast, prostate and colon cancer. This is supported by on-going *in vitro* studies - IGF-1 present in cows' milk, possibility of human exposure - data on transgenic animals are available

Toxicity of bracken fern

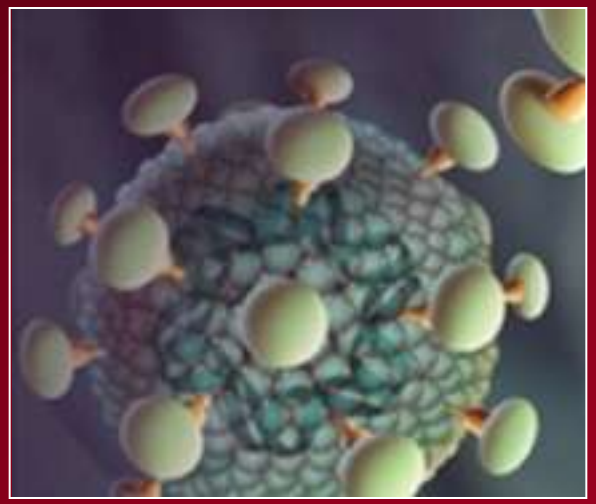
Schematically, the following mechanism can elucidate the oncogenic potential of bracken.





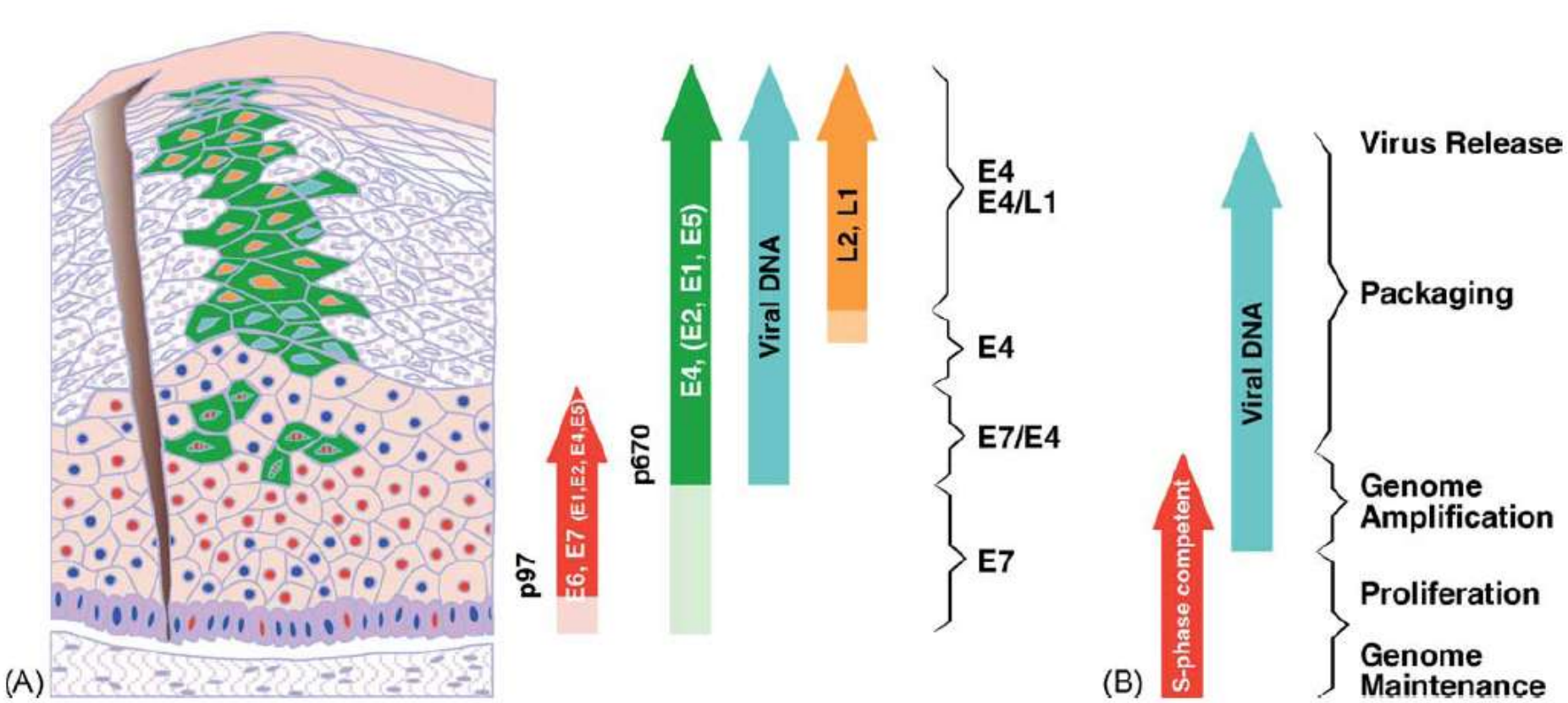
Bracken fern

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Bovine Papillomavirus type 2

Life cycle organization during productive infection by HPV types



The new classification of Bovine Papillomavirus (BPVs)

BPV-1, -2 and -13 :
genus Deltapapillomavirus

BPV-3, -4, -6, -9, -10, -11 and -12 :
genus Xipapillomavirus

BPV-5 and -8 :
genus Epsilonpapillomavirus



BPV-7 : not yet assigned

Bovine papillomavirus type 2

At the **genus Deltapapillomavirus**, composed of four species, also belong:

- European elk papillomavirus (EEPV) and Reindeer papillomavirus (RPV), species 1;
- Deer papillomavirus (DPV), species 2;
- Ovine papillomavirus type 1 and type 2 (OvPV-1 and OvPV-2), species 3;

Bovine papillomavirus type 2

The main biological properties of BPV-2 are characterized by inducing cutaneous fibropapillomas; and, unique among PVs, BPV-2 can be responsible for a trans-species transmission causing equine sarcoids, and neoplastic proliferations in buffaloes, cats, African lions, zebras, giraffes and sable antelopes.

Bovine papillomavirus type 2

Shortly, three oncogenes of PVs E5, E6, and E7 modulate cell transformation, E1 and E2, that are genes coding regulatory proteins, modulate transcription and replication, and two genes, L1 and L2 code some structural proteins responsible for the formation of viral capsid.



Bovine papillomavirus type 2

BPV E5 is the main gene responsible for the cell transformation; it is also capable of transforming human fibroblasts and keratinocytes.
E5 is expressed during the early stages of infection and its expression is often extinguished as the lesion progresses toward malignancy (Venuti and Campo, 2002).

Bovine papillomavirus type 2

The E5 protein is localized in the Golgi Apparatus (GA), endoplasmic reticulum and occasionally the plasma membrane of the host cells.

BPV E5 expression is responsible for the retention of major histocompatibility complexes class I in the Golgi and the inhibition of their transport to cell surface.



Bovine papillomavirus type 2

BPV2 is also responsible for neoplastic transformation of urothelial cells thus causing urothelial tumours in cattle grazing on bracken fern-infested lands. Papillomavirus-associated bladder tumours have been reported in many European areas (Campo, 1992; Carvalho et al., 2006; Roperto et al., 2003; 2005; 2008; 2011; 2012).

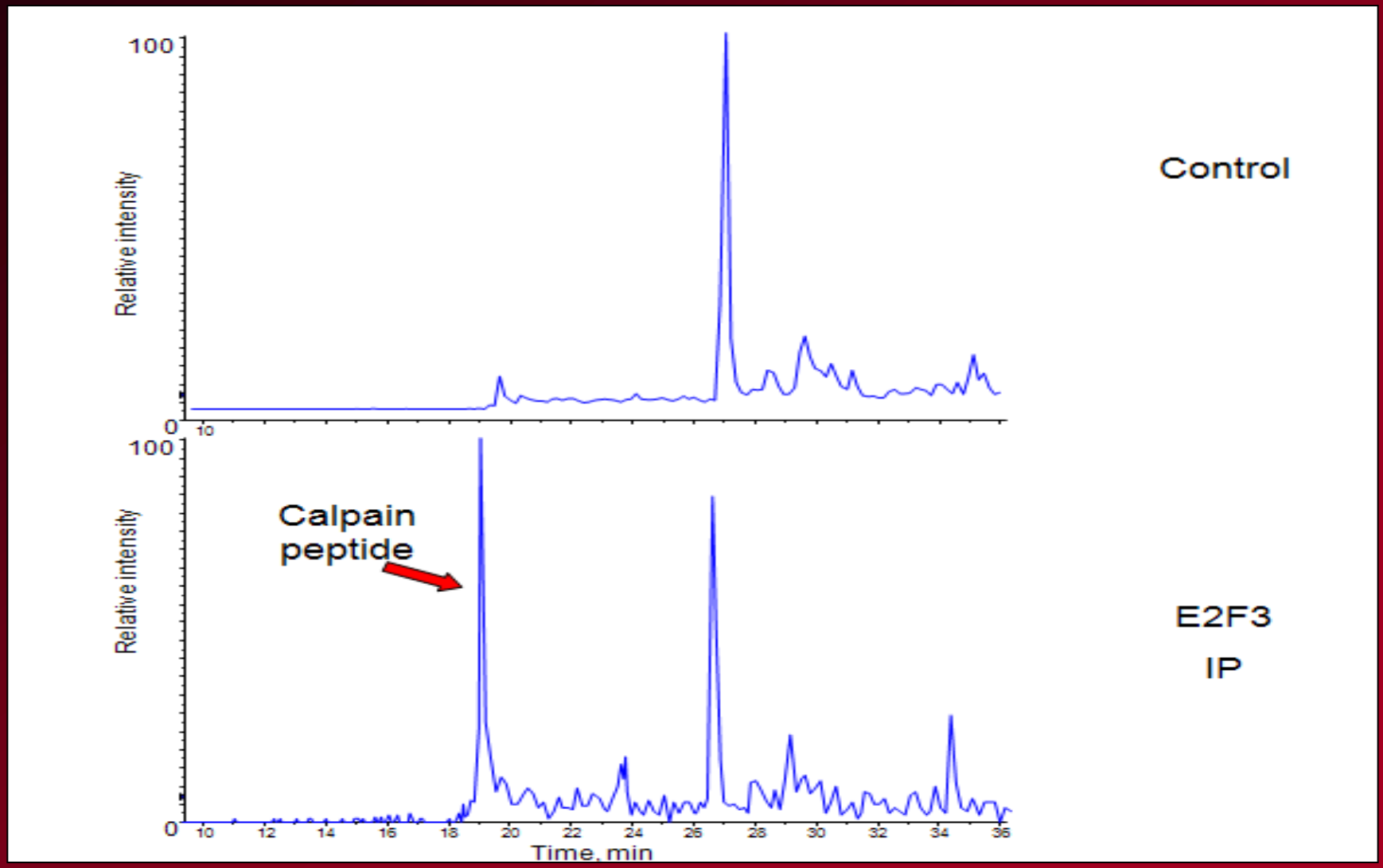
Bovine papillomavirus type 2

Recently, for the first time, urothelial tumours expressing BPV2 E5 oncoprotein have been described in the urinary bladder of water buffaloes in Turkey (Roperto. - J Gen Virol, 2013).

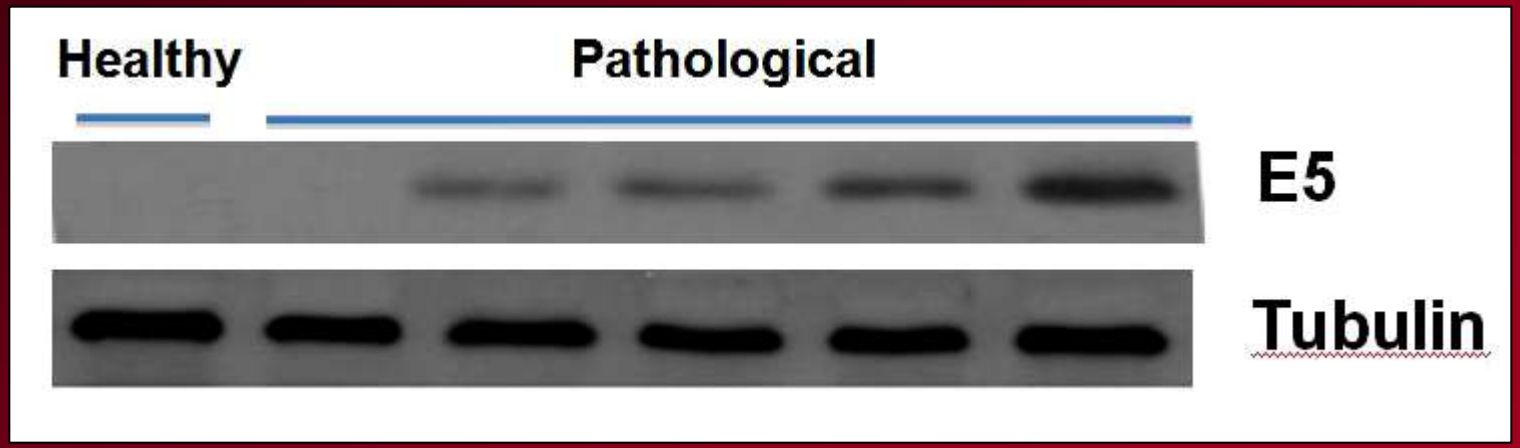
Bovine papillomavirus type 2

In spite of many molecular studies are under way to better understand pathogenetic mechanism(s) of disease of PVs, it is not well-known, step by step, how the BPV-2 works in inducing urinary bladder tumours.

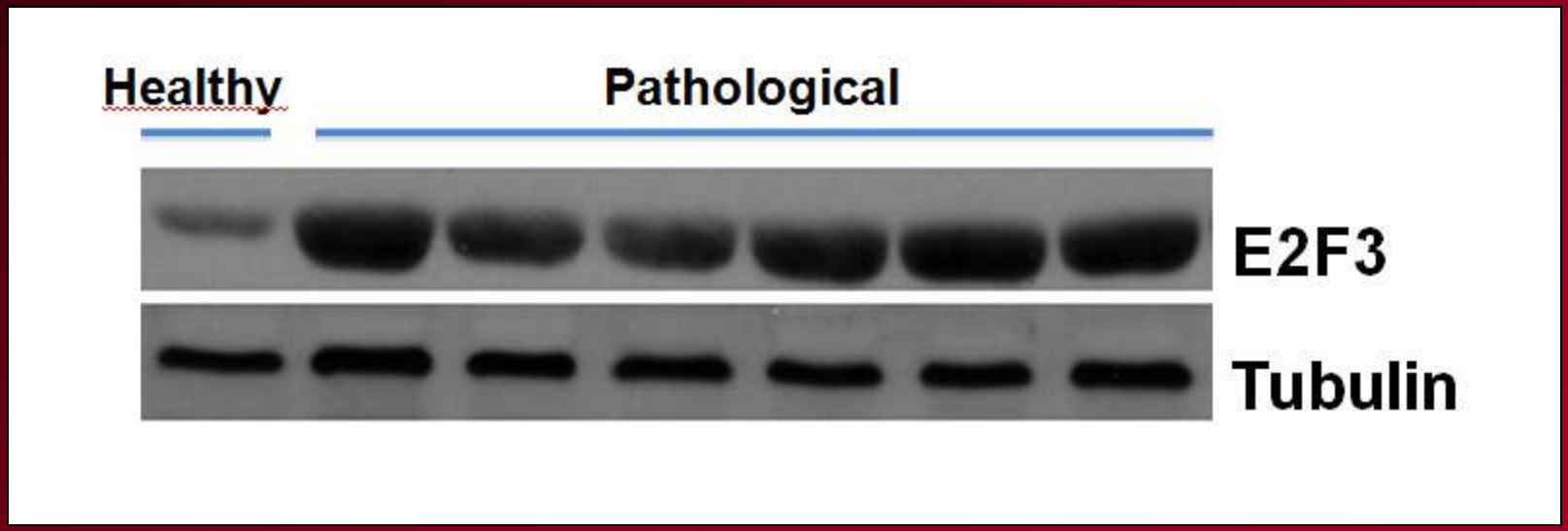
Bovine papillomavirus type 2



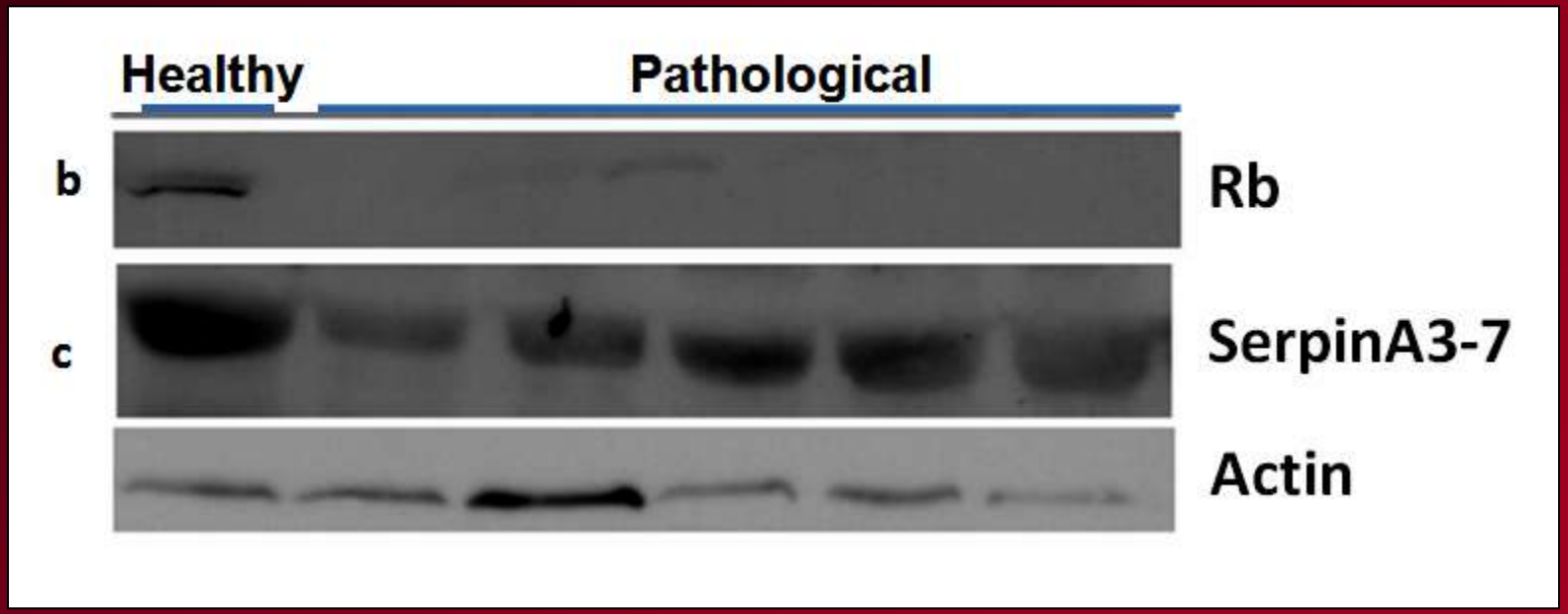
Bovine papillomavirus type 2



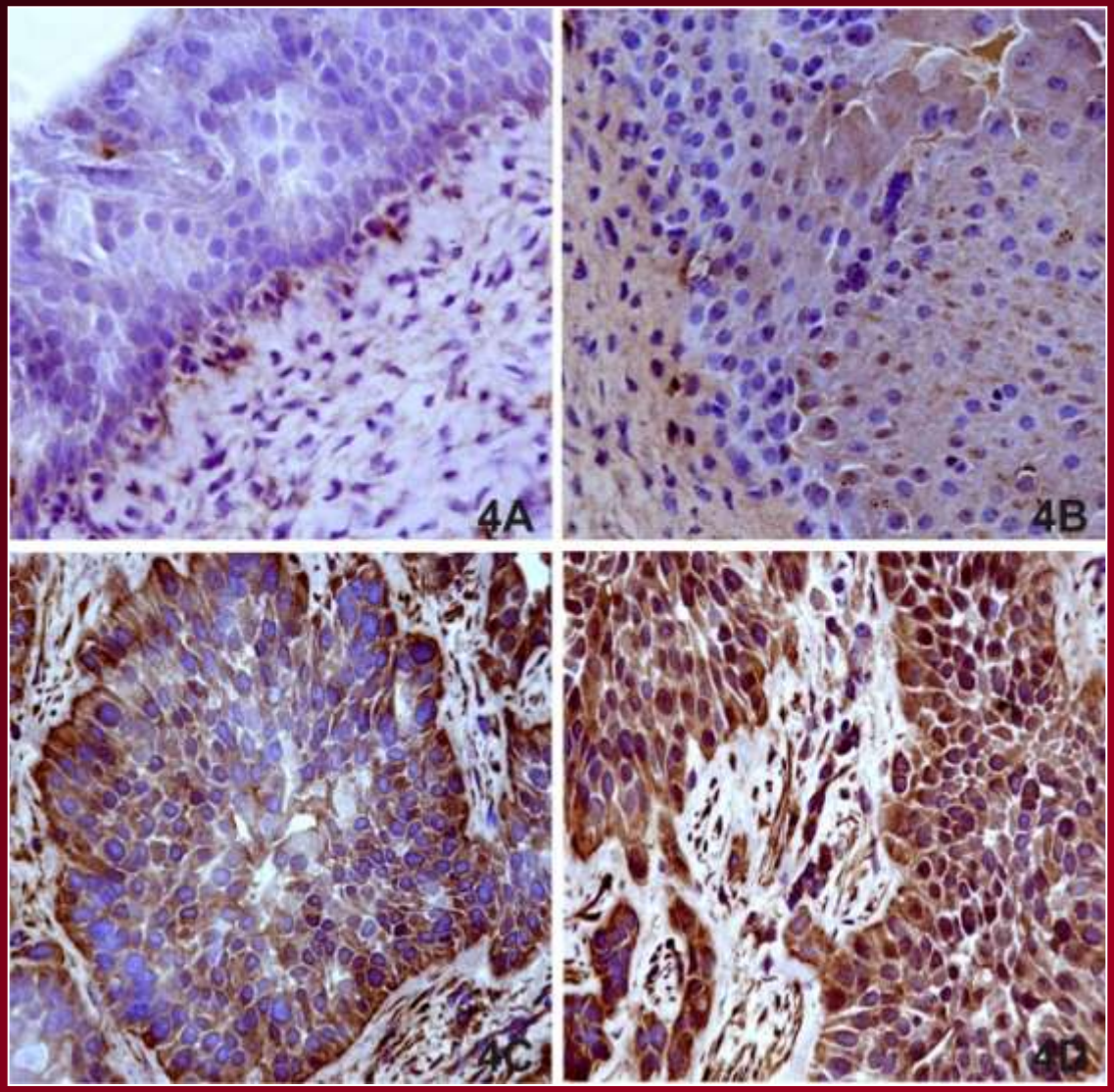
Bovine papillomavirus type 2



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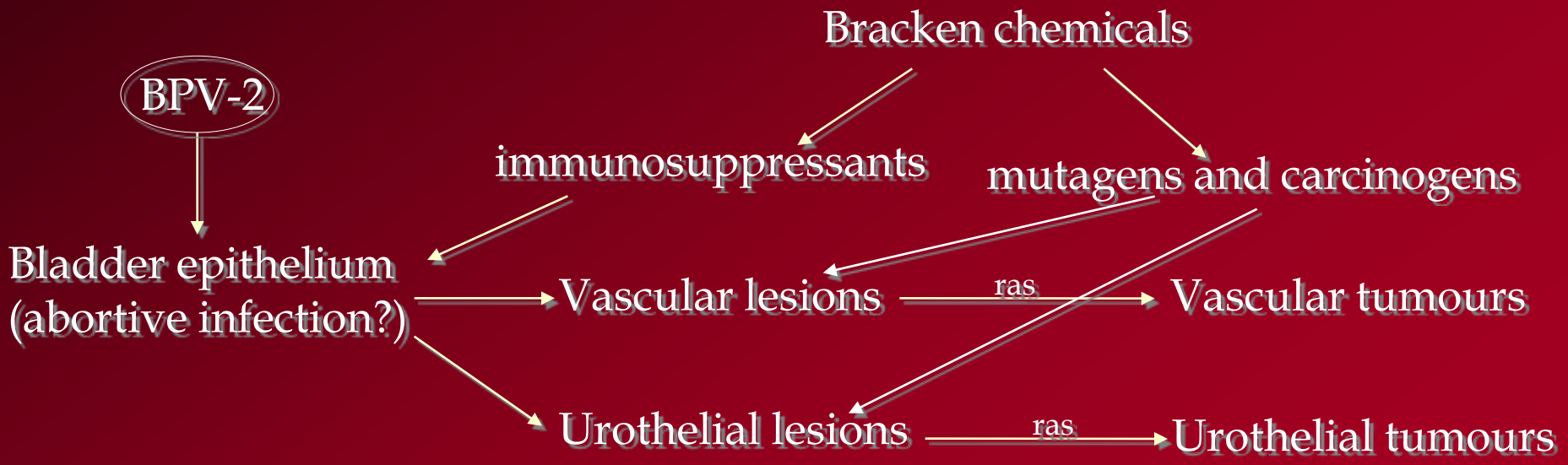


Bovine papillomavirus type 2

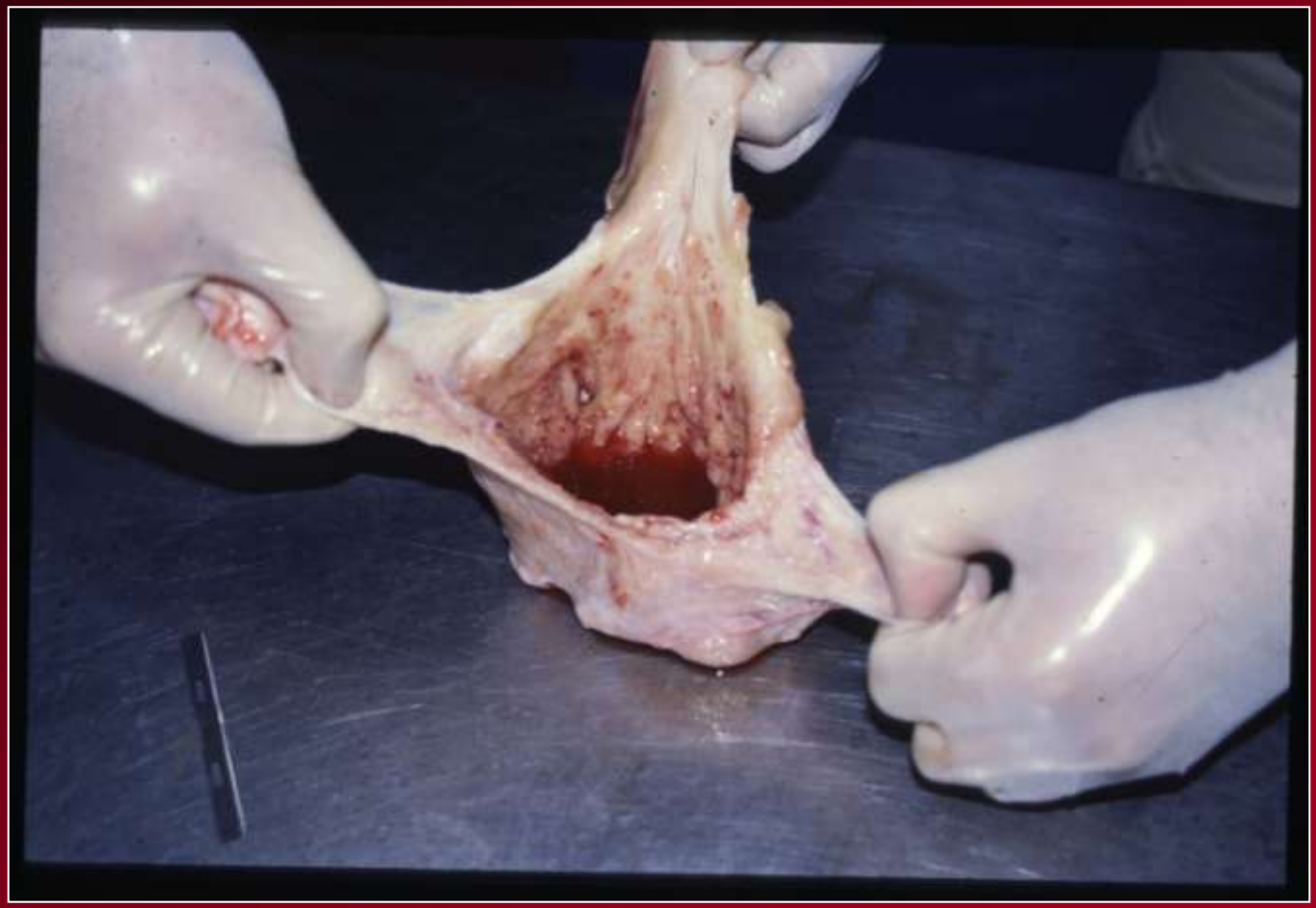


Bovine papillomavirus type 2

It has been suggested the following scheme to justify the involvement of BPV-2 in urinary bladder carcinogenesis.



Some macroscopic aspects of urothelial tumours









Thank you for your attention