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From Cell Biology to Tissue Engineering

Cytokeratin expression profiles of canine epithelial tissues

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Summary. Cytokeratins (CKs) are intermediate filaments of epithelial cells. In humans, different types of epithelia as well as their neoplasms show distinct CK expression profiles.

The aim of this study was to establish a panel of CKs for the identification of specialized canine epithelia that can be integrated in a routine diagnostic setting. Immunohistochemistry was performed on 42 formalin-fixed paraffin-embedded (FFPE) canine unaltered tissues including all epithelial tissues by using an antibody panel detecting CKs 7, 8, 13, 14, 17, 19 and 20 and the pancytokeratin marker AE1/AE3.

Using this antibody panel, a differentiation scheme for the identification of canine tissues was developed. This allowed the identification of 23 out of the 42 examined canine tissues and the distinction of 9 groups of specialized epithelia. The statistical validation revealed high variations in the immunoreactivity for CKs 7, 8, 14, 17 and 20 between the donor dogs. The antibody detecting CK 7 (OV-TL 12/13) showed a decrease in immunostaining after a fixation time of 3 and 4 days.

To the best of the authors' knowledge this is the first study that characterizes all canine epithelial tissues for their expression of CKs 7, 8, 13, 14, 17, 19 and 20 and the pancytokeratin marker AE1/AE3. Results of this study are an important prerequisite for comparative histology and for the investigation into similarities/differences of the cytokeratin expression between normal and neoplastic epithelia. Since this study was performed on FFPE tissue, it can be included in the workflow of a routine diagnostic laboratory.

Key words: Cytokeratin expression profiles, Epithelial tissues, Immunohistochemistry

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Introduction

Keratins are structural proteins belonging to the heterogeneous family of intermediate filaments (Geisler and Weber, 1986; Moll et al., 2008). They are classified into hard and soft keratins; soft keratins are also named as cytokeratins (Franke et al., 1978; Lynch et al., 1986). Cytokeratins (CKs) are mainly expressed in epithelial cells (Rungger-Brändle and Gabbiani, 1983; Jockusch et al., 1986; Oriolo et al., 2007) and are grouped into type I and type II keratins. Type I keratins, i.e. CKs 9-20, are acidic with a molecular weight of 40 to 64 kDa. In comparison, type II keratins, i.e. CKs 1-8, are neutral to basic and have a molecular weight ranging from 52 to 68 kDa (Fuchs et al., 1981; Moll et al., 1982; Schiller et al., 1982).

Intracellular CK filaments are obligatory heterodimers of keratin I and II (Franke et al., 1983; Sun et al., 1984; Hatzfeld and Franke, 1985; Hatzfeld and Weber, 1990). Most commonly, these heterodimers are composed of constant partners, for example CK 8/CK 18, CK 5/ CK 14 and CK 4/CK 13. An exception is CK 19 that usually associates with CK 7. In the absence of CK 7, however, CK 19 and CK 8 form heterodimers (Moll et al., 2008).

Each epithelial cell contains also a variable portion of soluble keratins (Flitney et al., 2009). Their aggregation to filaments is triggered by mechanical

stress (Flitney et al., 2009); this enables epithelial cells to adapt to different environmental conditions (Windoffer et al., 2011). Further, cytokeratins participate in intracellular signal transduction pathways (Caulin et al., 2000; Hesse et al., 2000; Jaquemar et al., 2003; Ku et al., 2003; Tong and Coulombe, 2006; Moll et al., 2008).

In human beings, it is known that epithelia of different organ systems express different CKs. Moll (1993) established a cytokeratin panel for the identification of the main types of specialized epithelia. Furthermore, the author showed that the tissue specific cytokeratin expression profile of epithelia is commonly well preserved in carcinomas of these organs (Moll, 1993). This can be helpful for the identification of carcinomas of unknown tissue origin.

Similarly, in dogs specialized epithelial cells of multiple organs also show differences in their cytokeratin expression profile (Vos et al., 1992a-c; Espinosa de los Monteros et al., 1999). There are several cytokine panels which are available in canine tissues already, but the studies are not complete (Vos et al., 1989; Cardona et al., 1989; Suter et al., 1990; Desnoyers et al., 1990; Vos et al., 1992a-c; Espinosa de los Monteros et al., 1999).

Therefore, the aim of this study was to establish a cytokeratin panel that allows the identification of the main types of specialized epithelia in dogs.

The availability of such a panel would be of value for comparative histology and can serve as a diagnostic tool for the identification of specialized epithelial cells under in vivo and in vitro conditions. In cases of disseminated cancer, it may assist to find out the location of the primary tumor (Moll, 1993; Moll et al., 2008).

Materials and methods

Animals and tissue samples

This study included 1280 canine tissue samples. These were obtained from 50 dogs that were between 2 months and 15 years of age (average age: 6.7 years). Of the dogs, 22 were male, 28 female. Samples (42 different tissues/organs) of 30 dogs were collected during the post mortem examination and were immediately fixed in 10% buffered formalin. Those of the remaining dogs were received as surgical specimens that had been fixed in 10% buffered formalin. The fixation period of samples with a known fixation time (n=1260) varied between 24 hours and 4 days. Examined were 36 tissues/organs with epithelial cell populations and 6 nonepithelial tissues/organs. The former included all epithelial tissues that can serve as origin for epithelial tumors (with the exception of claw, ocular globe and teeth) listed in the international histological classification of tumors of domestic animals of the World Health Organization (Slayter et al., 1994; Goldschmidt et al., 1998; Hendrick et al., 1998; Kennedy et al., 1998; Dungworth et al., 1999; Koestner et al., 1999; Misdorp et al., 1999; Valli et al., 2002; Wilcock et al., 2002; Head et al., 2003; Meuten et al., 2004). The latter represented those nonepithelial tissues/organs that express cytokeratins physiologically and/or after neoplastic transformation in either dogs or human beings, i.e. mesothelium (Cagle et al., 1989), synovial membrane (Miettinen, 1991; Miettinen et al., 2000), meningothelial cells of the arachnoid mater (Miettinen und Peatau, 2002), ependyma (Baumgärtner and Peixoto, 1987) as well as the choroid plexus (Ribas et al., 1989).

Histological procedure

Formalin fixed tissue samples were processed routinely, embedded in paraffin wax, sectioned with a microtome (3 μm) and stained with haematoxylin-eosin.

Light microscopy

All 1280 canine tissue samples were examined by the use of a light microscope (Fa. Olympus, BH-2) to detect those with autolytic changes or histopathological alterations, i.e. inflammation, degenerative lesions or tumor growth. These (778) were excluded from the immunohistochemical investigation.

Immunohistochemical procedure

Only tissue samples without pathomorphological alterations or autolytic changes were immunostained.

Table 1. Monoclonal mouse anti-human anti-cytokeratin antibodies used for the immunohistochemical examination of canine epithelial tissues.

Detection	Clone	Company	Dilution	Antigen retrieval	Method
CKs 1-8,10,14,15,16,19	AE1/AE3	Dako	1:50	95°C, citrate buffer	PAP
CK 7	OV-TL 12/30	Dianova	1:100	protease	PAP
CK 8	TS1	Novocastra	1:50	95°C, citrate buffer	PAP
CK 13	AE8	Santa Cruz	1:50	95°C, citrate buffer	PAP
CK 14	LL002	Novocastra	1:20	95°C, citrate buffer	Histofine
CK 17	E3	Santa Cruz	1:20	95°C, protease	Histofine
CK 19	NCL-CK19	Novocastra	1:100	protease	PAP
CK 20	KS 20.8	Dianova	1:10	95°C, citrat buffer	PAP

CK: Cytokeratin; Dako: Hamburg, Germany; Dianova: Hamburg, Germany; Santa Cruz: Heidelberg, Germany; Novocastra: Newcastle Upon Tyne, UK
PAP: peroxidase-antiperoxidase method; Histofine: Nichirei Biosciences Inc., Tokyo, Japan.

This method was performed on 10 unaltered tissue samples (each of these was obtained from a different donor dog) of every organ/tissue included in this study (36 tissues/organs with epithelial cell populations and 6 nonepithelial tissues/organs). The donor dogs for unaltered tissue samples were selected randomly from the pool of all animals.

The primary antibodies and the methods are listed in Table 1. Sections of formalin-fixed paraffin-embedded (FFPE) tissues were dewaxed and rehydrated. The endogenous peroxidase was blocked with 3% H_2O_2 for 30 minutes (min) at room temperature (RT). After antigen retrieval and a subsequent washing step, sections were incubated with the primary antibody overnight at 4°C and rinsed again. The peroxidase-antiperoxidase (PAP) method included their treatment with rat anti-mouse immunoglobulin G (1:100, Dianova, Hamburg, Germany) for 30 min at RT, washing and the application of mouse-PAP-complex (1:100, Dianova, Hamburg, Germany) for 30 min at RT. The detection system Histofine was applied for 30 min at RT. As chromogen, 3,3'-diaminobenzidine tetrahydrochloride (DAB) was used. To verify the binding specificity of primary antibodies, in the negative controls the primary antibody was replaced by a nonrelated monoclonal antibody (Hirschberger, 1987). All washing steps were performed with Tris-buffered saline (TBS). Antibodies and the PAP-complex were diluted in TBS containing 1% bovine serum albumen. Slides were counterstained with Papanicoloau's solution (Merck, Darmstadt, Germany).

Evaluation of the immunostained tissue sections

This step was performed separately for each applied antibody on the 10 tissue sections of all organs included in this study. Per tissue section all epithelial cells were analyzed by the use of the 40x objective of a microscope (Fa. Olympus, BH-2). Immunopositive cells were further evaluated in regard to their staining intensity (mild, moderate or marked). This procedure was repeated 3x by two veterinary pathologists, and the obtained consensus values were used for the determination of an immunoreactive score (IRS, Özgen et al., 1997). This IRS (range 0-10) is calculated in consideration of the numbers of immunopositive cells as well as their staining intensity. The IRS values were graduated as follows: <0.5: minimal immunoreactivity; 0.5-3.99: mild immunoreactivity; 4.0-6.99: moderate immunoreactivity; 7.0-10.0: marked immunoreactivity.

Relative staining intensity

The IRS values for a particular cytokeratin varied between the sections of identical organs that originated from different dogs or between different organs from the same dog. For a comparison of the observed variations of the IRS values, the relative staining intensity (RSI; formula provided below) was calculated. Thus, the received RSI is a tissue independent relative value relating to the highest obtained IRS in a particular tissue.

$$RSI = \left(\frac{IRSi}{IRS\max} \right) \times 100$$

IRSi=IRS in an organ of one particular donor dog; IRSmax=highest IRS value.

Evaluation of the antibodies for their immunostaining characteristics

Descriptive statistical analysis was used to compare the immunoreactivity of the applied antibodies independent of the respective tissue or donor dog. This was performed by comparing their RSI values that had been calculated from all examined tissues. The number of RSI values per antibody varied between 98 and 2135. For the analysis, the following RSI variables were used: mean value (MV) and standard deviation (SD), median, maximum and minimum values as well as the deviation of the mean and median values. An antibody was considered to show a highly variable immunoreactivity if at least one of the following criteria was detected: difference between mean and median values >10; RSI mean <40%; high number of negative tissue samples (RSI_{min}=0) >10%; RSI max <40%.

Determination of the influence of the duration of the formalin fixation

Epithelial tissues of different donor dogs were fixed for 24 hours (15 donor dogs), 3 days (5 dogs) and 4 days (5 dogs) and their RSI values were compared separately for each examined antibody. In total 373 tissues with a fixation time of 24 hours, 240 tissues fixed for 3 days and 77 tissues fixed for 4 days were evaluated.

Evaluation of differences in the immunoreactivity between the donor dogs

For each applied anti-cytokeratin antibody, the mean RSI values of the different dogs were compared. Per antibody, between 98 and 2135 tissue samples obtained from 16 to 25 donor dogs were analyzed. A descriptive statistical analysis was done as previously described. The used antibodies were considered to show high individual variations in their immunoreactivity between the donor dogs, if at least one of the following criteria was detected: RSI SD >20; minimum RSI <15%; difference between mean and median >5; RSI mean <40%.

Results

Cytokeratin expression profiles of examined tissues

By the applied anti-cytokeratin antibody panel, a specific identification of 23 out of the 42 examined organs/tissues was achieved (Fig. 1). According to their CK expression profiles, the following 9 epithelial

subtypes were distinguished: urothelium, respiratory epithelium, gastrointestinal epithelium, stratified squamous epithelium, epithelium of transitional zones, secretory epithelial cells of sweat glands, epithelial cells of sebaceous gland, myoepithelial cells of glandular acini and basal epithelial cells of excretory ducts (Table 2). Epithelial cells of the remaining tissues showed unique immunoreaction profiles that did not correlate with one of the 9 epithelial types listed above.

In addition, some nonepithelial cells showed an

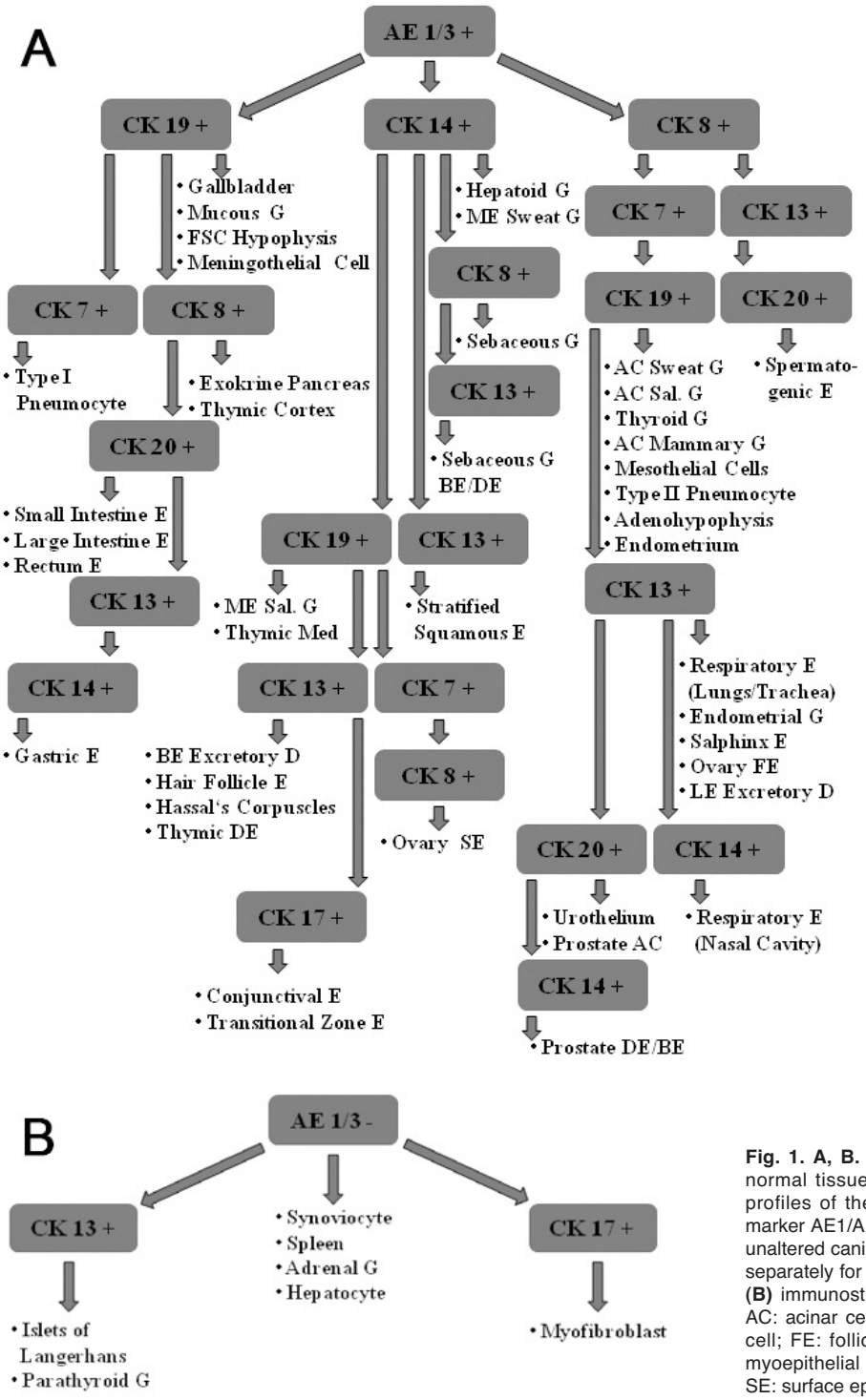

Fig. 1. A, B. Cytokeratin expression (CK) profiles of canine normal tissues. Depicted is a flow chart of the expression profiles of the examined cytokeratin panel (pancytokeratin marker AE1/AE3 as well as CKs 7, 8, 13, 14, 17, 19 and 20) in unaltered canine tissues. The expression profiles are illustrated separately for canine tissues with a positive **(A)** and a negative **(B)** immunostaining for the pancytokeratin marker AE1/AE3. AC: acinar cell; BE: basal epithelial cell; DE: ductal epithelial cell; FE: follicular epithelium; E: epithelium; G: gland; ME: myoepithelial cell; Med: medulla; RE: respiratory epithelium, SE: surface epithelium, FSCs: follicular stellate cell.

immunoreaction with the pancytokeratin marker AE1/AE3 as well as the anti-cytokeratin 19 antibody. The remaining investigated epithelial cells and nonepithelial cell populations were immunonegative for the applied antibody panel.

Urothelium

Investigated was the urothelium of the renal pelvis, urinary bladder and urethra. The urothelium of all investigated tissues stained immunopositive for the pancytokeratin marker AE1/AE3 as well as CKs 7, 8, 13, 19 and 20. The expression of pancytokeratin marker AE1/AE3 as well as CKs 7, 8 and 19 was identical within the basal and the suprabasal layers. In comparison, the immunoreactivity for CK 13 was minimal within suprabasal layers and mild within the basal layer, whereas CK 20 showed the opposite immunoreactivity within these two layers. The concurrent expression of CK 13 and CK 20 together with the absence of CK 14 is a characteristic feature of urothelium.

Respiratory epithelium

Examined was the respiratory epithelium of the nasal cavity, trachea and bronchi. An expression of the pancytokeratin marker AE1/AE3 as well as CKs 7, 8, 13 and 19 was characteristic for respiratory epithelium of all examined tissues. Notably, the pancytokeratin marker AE1/AE3 as well as CKs 7, 8 and 19 was expressed in all cell populations of the respiratory epithelium including basal cells, ciliated epithelium and goblet cells.

Gastrointestinal epithelium

The epithelium of the stomach, small and large intestines, rectum and the zona columnaris of the anal canal was immunopositive for the pancytokeratin marker AE1/AE3 and showed a concurrent expression of CKs 8, 19 and CK 20 (Fig. 2). The different cell populations of the gastric and intestinal epithelium varied in regard to the degree of their immunoreactivity for the investigated markers. A unique feature of gastrointestinal epithelium was the CK 20 immunstaining together with the absence of CK 7 expression. Within the intestines, the CK immunoreactivity decreased from oral to aboral; in the colon and rectum only scattered epithelial cells of the luminal surface were immunopositive. CKs 13 and 14 were only detected within the gastric crypt epithelium.

Stratified squamous epithelium

Examined were sections of the skin and the cutaneous mucosa of the nose and lip, the gingiva, the tongue, the oesophagus, the cardia of the stomach, the palatine tonsil and the penis. The stratified squamous epithelium was consistently immunopositive for the pancytokeratin marker AE1/AE3. In the skin as well as in the examined mucous membranes, cells of the basal

Table 2. Cytokeratin expression patterns (immunoreactive score) in different types of canine specialized epithelia.

Type of epithelium	Cytokeratin (CK) expression profile	Epithelial components	Immunoreactive scores (IRS)							
			AE1/AE3	CK7	CK8	CK13	CK14	CK17	CK19	CK20
Urothelium	AE1/AE3, CKs 7, 8, 13, 19, 20	Suprabasal L.	++	+	+	-(+)	-	-	++	+
		Basal L.	++	+	+	+	-	-	++	-(+)
Respiratory	AE1/AE3, CKs 7,8,13,19	Ciliated	+++	+	++	-(+)	-	-	+++	-
		Goblet cells	++	+	+	-	-	-	++	-
		Basal cells	++	+	+	+	-	-	+++	-
Gastric	AE1/AE3, CKs 8,13,14,19,20	Foveolar	+++	-	++	-	-	-	+++	+
		Crypt	++	-	+	+	+	-	++	-(+)
		Parietal cells	-	-	-	-	-	-	-	-
Intestinal	AE1/AE3, CKs 8, 19, 20	Surface/villous	++	-	+	-(E)	-	-	++	+
		Basal/cryptal	+	-	-(+)	-(E)	-	-	+	-(+)
Stratified, squamous	AE1/AE3, CKs 13, 14	Suprabasal L. (skin)	++	-	-	-	-	-	-	-
		Suprabasal (mucosa)	++	-	-	+	-	-	-	-
		Basal	+	-	-	+	++	-	-	-
Transitional zones	AE1/AE3, CKs 13,14,17,19	Superficial L.	++	-	-	++	-	+	++	-
		Intermediate L.	++	-	-	++	-(+)	++	++	-
		Basal L.	+	-	-	+	++	-	++	-
Myoepithelium	AE1/AE3, CKs 14,19	Myoepithelial cells	++	-	-	-	++	-	+	-
Sweat gland	AE1/AE3, CKs 7,8,19	Acinar cells	+++	++	++	-	-	-	+++	-
Sebaceous gland	AE1/AE3, CKs 8,14	All cells	+	-	-(+)	-	+++	-	-	-
Excretory ducts	AE1/AE3, CKs 13,14,19	Basal myoepithelium	+	-	-	+	++	-	+	-

- (+): IRS <0.5; +: IRS 0.5-3.99; ++: IRS 4.0-6.99; +++: IRS 7.0-10; AE1/AE3: Pancytokeratin marker; E: exceptions; L.: Layers.

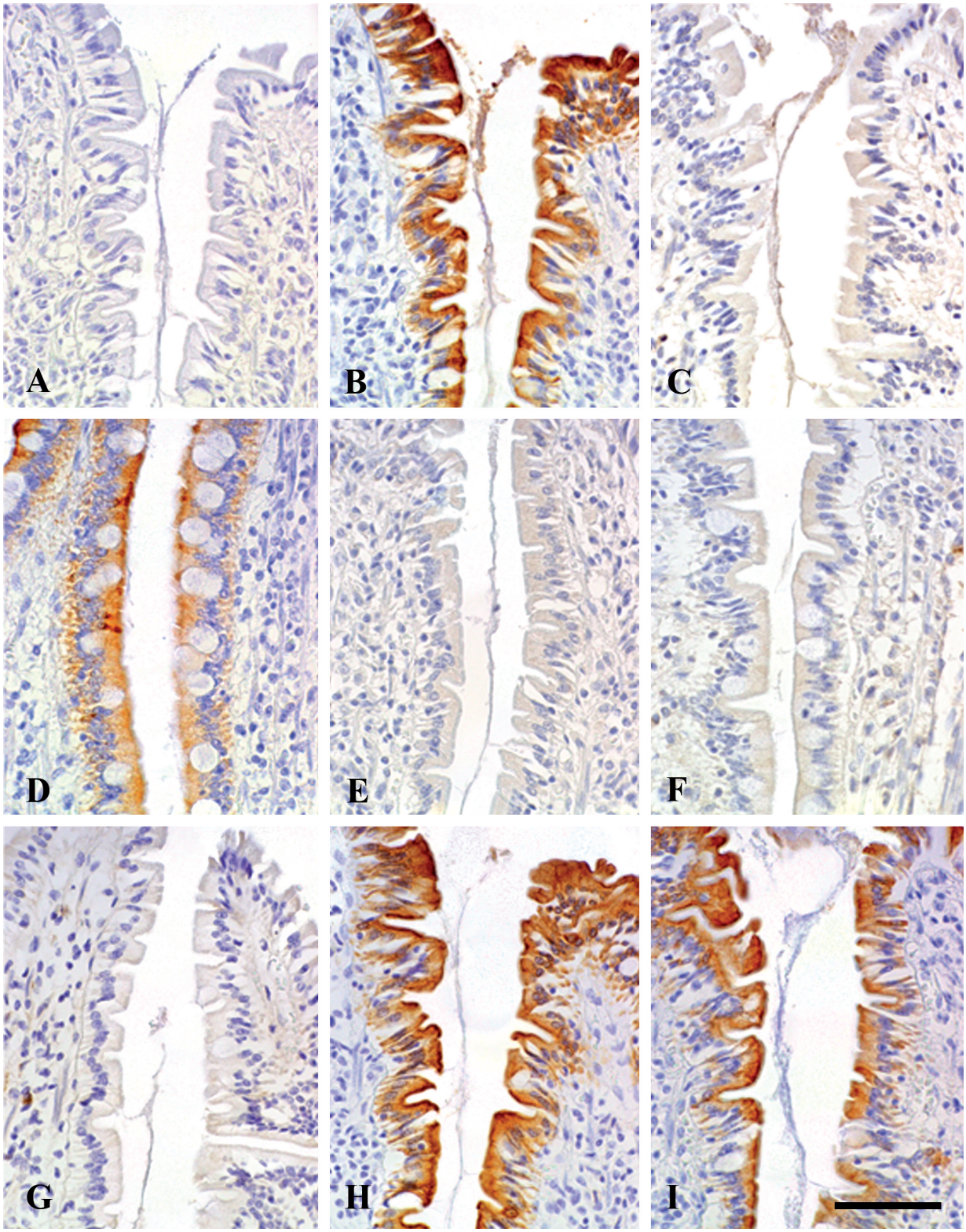

Fig. 2. Canine intestinal epithelium: Results of the immunostaining for the pancytokeratin marker AE1/AE3 as well as cytokeratins 7, 8, 13, 14, 17, 19 and 20. **A.** Depicted is the negative control. The primary antibody was replaced by a non-binding isotype matched antibody. **B.** Intestinal epithelial cells are strongly immunopositive for the pancytokeratin marker AE1/AE3. **C.** There is no immunostaining for cytokeratin 7 in the epithelium mucosae. **D.** Epithelial cells display a diffuse mild to moderate cytoplasmatic immunostaining for cytokeratin 8. **E.** Intestinal epithelial cells are negative for cytokeratin 13. **F.** Intestinal epithelial cells lack an expression of cytokeratin 14. **G.** The immunolabelling for cytokeratin 17 reveals a negative result. **H.** Immunostaining for cytokeratin 19 shows a diffuse moderate cytoplasmatic reaction with an apical accentuation. **I.** A diffuse moderate cytoplasmatic labelling with an apical accentuation is also observed for cytokeratin 20. Scale bar: 40 μm.

layer were CK 13 and CK 14 immunopositive. Immunolabelling for CK 13 was also observed in the suprabasal layers of mucosal locations, but it was absent in the skin. In comparison epithelium of hair follicles (inner and outer root sheath) showed an additional mild positive reaction with the antibody against CK 19.

Epithelium of transitional zones

Investigated were the transitional epithelium of the nasal cavity, the anorectal junction, the transition zone between the penile urethra and the cutaneous mucosa of the glans penis, portio vaginalis uteri, cervix uteri and conjunctiva. Unique for the epithelium at transitional zones was the immunoreactivity for CK 17. In addition, this type of epithelium stained immunopositive for CKs 13, 14 and 19 as well as the pancytokeratin marker AE1/AE3. Most of these markers were expressed in each of the three epithelial layers. The superficial layer, however, lacked immunoreactivity for CK 14, and the basal layer was CK 17 immunonegative (Fig. 3).

Secretory acinar cells of sweat glands

Investigated were apocrine sweat glands of the skin, anal sac glands and the mammary gland. The secretory

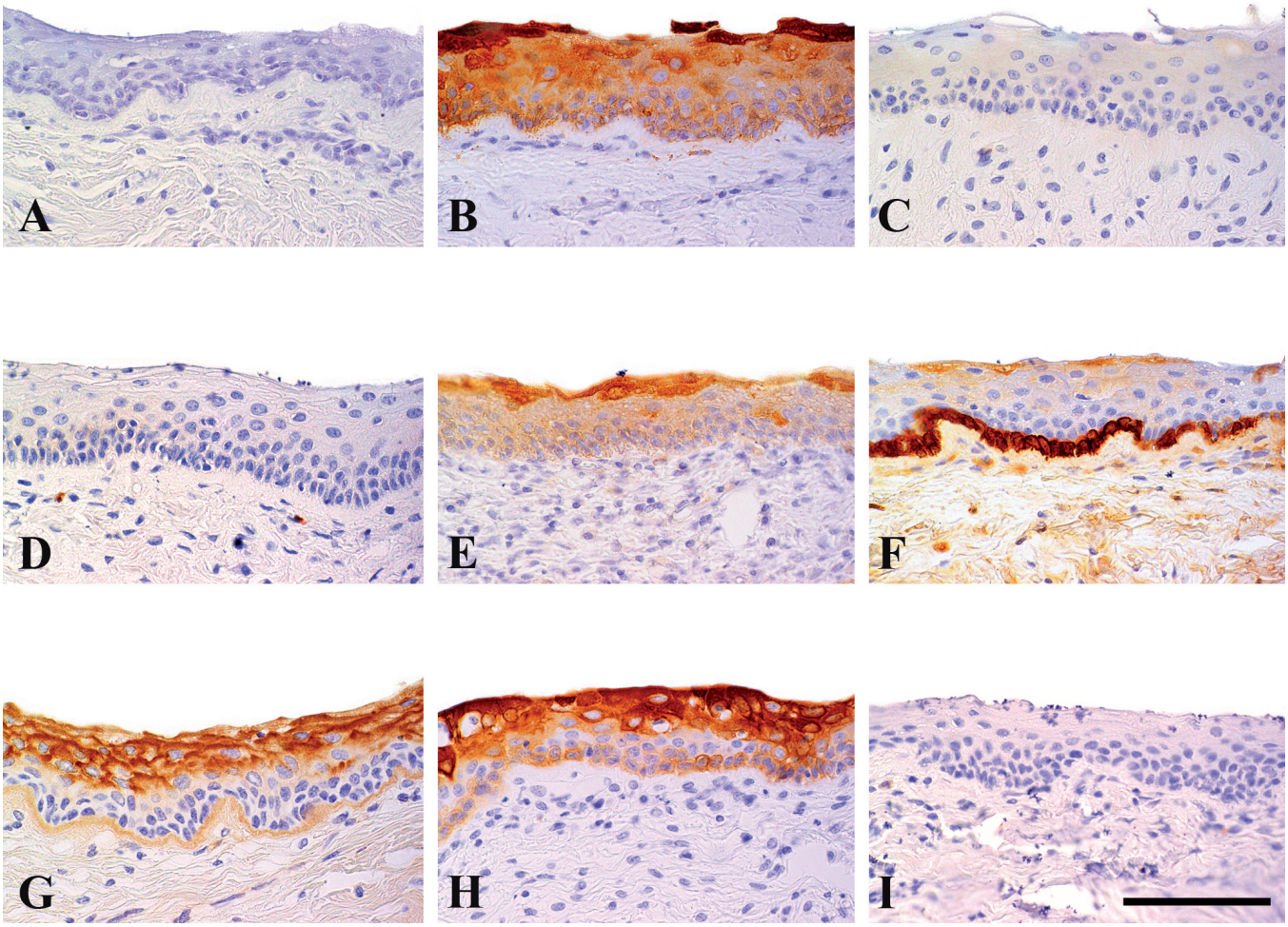

Fig. 3. Canine transitional zone epithelium (portio vaginalis): results of the immunostaining for the pancytokeratin marker AE1/AE3 as well as cytokeratins 7, 8, 13, 14, 17, 19 and 20. **A.** Depicted is the negative control. The primary antibody was replaced by a non-binding isotype matched antibody. **B.** Application of the pancytokeratin marker AE1/AE3 results in a diffuse moderate cytoplasmatic immunostaining with a stronger labelling of the superficial cell layers. **C.** There is no immunostaining for cytokeratin 7. **D.** The immunostaining for cytokeratin 8 is negative. **E.** The transitional zone epithelium shows a mild diffuse cytoplasmatic immunstaining for cytokeratin 13 with a superficial moderate accentuation. **F.** Cytokeratin 14 is expressed within the basal epithelial layer. The cytoplasmic immunostaining is moderate with a stronger labelling at the periphery of the cell. **G.** The suprabasal epithelial layers display a moderate cytoplasmic immunostaining for cytokeratin 17 with a peripheral accentuation. **H.** The cytokeratin 19 immunolabelling is located within the cytoplasm of epithelial cells and shows a membranous accentuation. It is mild within the basal layer and moderate within suprabasal layers. **I.** Transitional zone epithelial cells lack an immunostaining for CK 20. Scale bar: 40 μm.

acinar cells were immunopositive for AE1/AE3 and CKs 7, 8 and 19. An identical immunoreaction pattern was also observed in the biliary duct epithelium.

Sebaceous glands

Sebaceous glands of the skin, perianal (hepatoid) glands and Meibomian glands (tarsal glands) were immunostained. These glands were immunopositive for CKs 8 and 14 as well as the pancytokeratin marker AE1/AE3. This immunoreactivity was observed in basally located reserve cells as well as mature sebocytes. Within the latter, the immunostaining was accentuated peripherally below the cell membrane due to the intracellular presence of lipid vacuoles.

Acinar myoepithelial cells

Myoepithelial cells of submucosal glands of nasal conchae, trachea and bronchi, pharynx and oesophagus as well as the parotideal gland, ceruminal glands and the mammary gland were examined. These cells can be identified by their immunoreactivity for CKs 14 and 19 and the pancytokeratin marker AE1/AE2.

Basal epithelial cells of excretory ducts

Investigated were mammary gland, anal sac glands, anal glands, parotideal gland, pancreas as well as serous and/or mucinous glands of the pharynx, nasal cavity, trachea and oesophagus. Although these were also immunopositive for CKs 14 and 19 and the pancytokeratin marker AE1/AE3, they differed from alveolar myoepithelial cells by their additional CK 13 immunostaining. The remnants of the branchial ducts/thymic duct within the involuting thymus showed an identical cytokeratin expression profile.

Additional immunopositive cell populations

A positive reaction for the pancytokeratin marker AE1/AE3 as well as CK 19 was detected in additional epithelial cells (Fig. 1) and nonepithelial cells, i.e. meningothelial cells, folliculo-stellate cells of the anterior hypophysis and mesothelial cells. As CK 20 is known to be specific for Merkel cells (Moll et al., 1993), all dermal sections were routinely screened for basally located CK 20 positive cells. In the examined sections only a few CK20 positive cells were detected that may

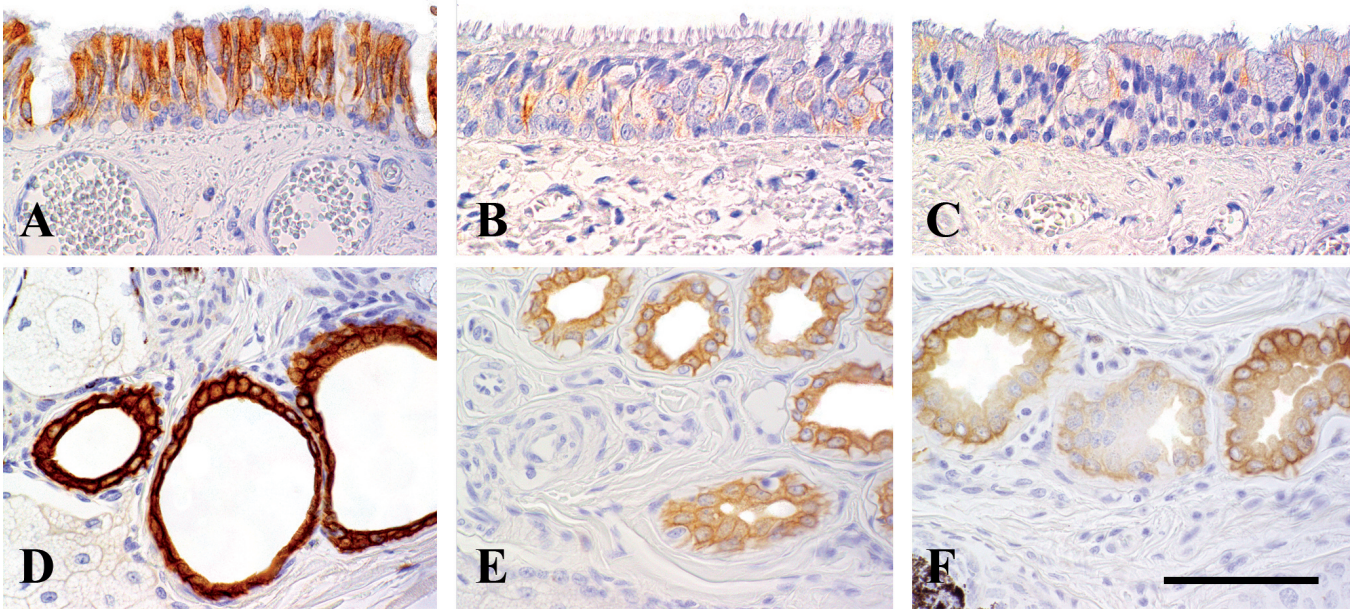

Fig. 4. A-C. Differences in the cytokeratin immunoreactivity between the donor dogs. As an example, the CK 8 immunostaining of the tracheal epithelium of three different dogs is depicted. Respiratory epithelial cells of one dog show a diffuse moderate to marked cytoplasmatic immunostaining **(A)**. In comparison, the respiratory epithelium of another dog displays a diffuse mild cytoplasmic and partially apical accentuated immunolabelling, whereas in the remaining dog a mild immunostaining is restricted to a few individualized or clustered epithelial cells **(C)**. **D-F.** Influence of the fixation time on the epithelial immunoreactivity for CK 7. Exemplarily depicted is the cytokeratin 7 immunostaining of sweat gland acinar cells in skin samples. The fixation was performed for 24 hrs **(D)**, 3 days **(E)** and 4 days **(F)**. In general, glandular epithelial cells show a diffuse cytoplasmatic to membranous accentuated immunostaining for cytokeratin 7. A marked immunoreaction is detected with a 24 hrs fixation time **(A)**, whereas it is mild to moderate after a 3 day fixation **(E)** and minimal to mild fixed for 4 days **(F)**. Scale bar: 40 μm.

represent Merkel cells. The small number of positive cell may be due to the screened skin lokations. Biliary duct epithelium showed a positive reaction for AE1/AE3, CKs 7, 8 and 19.

Immunonegative cell populations

The following epithelial cells showed a lack of immunostaining for all applied antibodies: hepatocytes as well as epithelial cells of the adrenal cortex and medulla, the parathyroid gland and the choroid plexus. A negative immunoreaction with the investigated antibodies was also observed in the spleen (reticular cells), the ventricular ependyma and synoviocytes of the joint capsule.

Statistical validation of the applied method

Antibody specific immunostaining characteristics

RSI values were used to evaluate the reliability of the applied antibodies and the elected methodical approach. Notably, all examined antibodies showed a moderate to high reactivity with canine tissue (RSI mean values 40-75%), except CK 20 (mean RSI <30%). The intensity of the immunoreaction, however, varied widely as indicated by the high standard deviation (SD) of the RSI mean values (SD 30-36). The antibodies detecting CKs 14, 17 and 20 displayed a remarkably high variation, i.e. the difference between the mean and median values was >10. This indicates that these data may not be normally distributed. In addition, the antibodies against CKs 7, 8, 13, 14, 17 and 20 had a sensitivity <90%, this equals the presence of negative results in 14- 48% of the cases (Table 3).

Influence of preanalytic factors on the cytokeratin immunostaining (fixation time)

The immunoreactivity of almost all investigated antibodies was not influenced by the fixation time (24 hours, 3 days, 4 days). The only exception was the antibody detecting CK 7 (OV-TL 12/30): compared to the staining intensity after 24 hours (hrs) fixation, a mild

Table 3. Variations in the immunoreactivity of the applied antibodies.

Immunostaining	RSI Mean*	RSI SD	RSI Max*	RSI Min*(#*)	RSI Median*	Mean-Median	Nos. samples
AE1/AE3	59.46	30.2	100 (14.75)	0 (0.8)	60	0.54	2135
CK 7	49.85	36.38	100 (14.95)	0 (**15.85**)	52.63	2.78	776
CK 8	40.66	35.19	100 (12.53)	0 (**17.27**)	33.33	7.33	886
CK 13	45.21	33.55	100 (14.12)	0 (**14**)	40	5.21	857
CK 14	73.70	31.14	100 (18.69)	0 (3.03)	88.89	**15.19**	792
CK 17	41.76	36.09	100 (17.35)	0 (**14.28**)	25	**16.76**	98
CK 19	66.54	31.2	100 (19.95)	0 (4.4)	76	9.46	1499
CK 20	**26.07**	36.26	100 (12.12)	0 (**47.5**)	1.25	**24.82**	99

The presented statistical data describe the variations in the immunoreactivity that existed for the used antibodies. These data were calculated for each antibody over all examined tissues by using all RSI obtained for the respective antibody and are therefore not influenced by the donor dog or the tissue/organ. AE1/AE3: pancytokeratin marker, i.e. detection of cyfortokeratins 1-8, 10, 14, 15, 16, 19. CK: cytokeratin; Nos.: numbers; Max: tissue sample with the maximal RSI value; Min: tissue sample with the minimal RSI value; SD: standard division; *: Values shown in percentage (%); #: percentage of the entire number of samples per antibody. ____: difference between mean and median >10; RSI mean <40%; high number of negative tissue samples (RSI_{min}=0)>10%; RSI Max <40%.

Table 4. Individual differences in the expression of cytokeratins.

Immunostaining	RSI Mean*	RSI SD	RSI Max*	RSI Min*	RSI Median*	Mean-Median	Nos. dogs
AE1/AE3	64.8	12.7	96.48	41.75	62.01	2.79	25
CK 7	52.02	18.48	82.04	**5.5**	50.74	1.28	25
CK 8	45.32	19.79	89.74	**10.43**	42.66	2.67	22
CK 13	46.2	18.18	98.33	21.04	44.17	2.92	22
CK 14	58.38	**32.5**	98.15	**2.8**	74.04	**15.66**	21
CK 17	**36.94**	**24.44**	83.34	**0**	39.2	2.26	18
CK 19	67.22	12.45	100	39.87	64.76	2.46	23
CK 20	**26.22**	**27.1**	85.24	**0**	18.27	**8.29**	16

Shown are the statistical data of the tissue independent individual differences in the immunohistochemical expression of the examined cytokeratins (CKs) between the donor dogs. For each analysed CK, the mean value of the relative staining intensity (RSI) was calculated from the RSI values of all examined tissues per donor dog. AE1/AE3: pancytokeratin marker, i.e. detection of cytokeratins 1-8, 10, 14, 15, 16, 19. CK: cytokeratin; Max: dog with the maximal RSI mean value; Min: dog with the minimal RSI mean value; SD: standard division; Nos.: numbers.; *: Values shown in percentage (%). _____: RSI mean <40%; RSI SD >20; RSI Min <15%; difference between mean and median >5.

to moderate reduction in immunostaining was noted in tissues fixed for 3 and 4 days, respectively. The mean RSI values of CK 7 were 24 hrs: 56.66 with a standard deviation (SD) of 19.70, 3 days: 50.57 (SD 15.65) and 4 days: 39.15 (SD 11.79), respectively. All other analyzed factors (age, sex, breed, time of death) had no significant influence on the immunoreactivity of the examined antibodies.

Differences in the immunoreactivity between the donor dogs

RSI values of all examined antibodies varied between the donor dogs. The comparison of the mean RSI values showed high individual differences for CKs 7, 8, 14, 17 and 20 and mild variations for AE1/AE3 and CKs 13 and 19 (Table 4). This indicates individual differences in the CK expression of the examined dogs.

Discussion

This investigation identified the cytokeratin expression profiles of normal canine epithelial and nonepithelial cell populations. The results of this study are of great value not only for comparative investigations of basic science, but also for pathomorphological investigations, in particular the possible identification of the tissue origin of tumor cells. The applied antibodies were elected under consideration of the antibody panel recommended by Moll (1993) for the characterization of human specialized epithelia, i.e. monoclonal antibodies detecting CKs 5, 7, 8, 18, 19 and CK 20. In the present study, the following modifications were applied: The antibody against CK 5 was replaced by an antibody detecting its partner cytokeratin 14. CK 18 was not examined, because an antibody detecting its partner keratin, i.e. CK 8, was included in panel. This selection of antibodies was supplemented by the pancytokeratin marker AE1/AE3 and antibodies against CKs 13, 17 and 20. The pancytokeratin marker AE1/AE3 detects all CKs except some hair keratins and CKs 13, 17 and 20. All elected antibodies represented cross-reactive anti-human antibodies that had been previously established for use on canine tissues (Espinosa de los Monteros et al., 1999; Walter, 2001). In comparison to the study of Moll (1993), the present investigation was performed on FFPE tissue samples, since this method is routinely used for histological and histopathological studies in human medicine as well as veterinary medicine. Thus, the successful use of the selected antibody panel on FFPE tissues will be an important prerequisite for its future application on surgical specimens submitted for routine histopathological examinations.

Results of the immunostaining by using the elected antibodies

This is the first investigation on a wide range of normal FFPE canine epithelial and nonepithelial tissues by using a comprehensive antibody panel. In contrast, previous studies examined only a small number of tissues for the presence of different cytokeratins (Cardona et al., 1989; Vos et al., 1989, 1992a-c; Desnoyer et al., 1990; Suter et al., 1990; Espinosa de los Monteros et al., 1999). Several of these studies used native unfixed tissue samples (Suter et al., 1990; Vos et al., 1992a-c) or different clones of the applied antibodies (Vos et al., 1989, 1992a-c; Suter et al., 1990). Although the cytokeratin expression profile of human normal tissues has been reported by Moll (1993), a comparable catalog for canine tissues does not exist.

Pancytokeratin marker AE1/AE3

Since this marker binds the majority of type I and II CKs (CKs 1-10, 14, 15, 16 and 19) (Lai et al., 2008), it will identify numerous epithelial tissues. This antibody, however, will not immunolabel epithelial tissues solely expressing CKs 13, 17 and 20. In previous studies on canine tissues endocrine active cells were immunonegative (Cardona et al., 1989). Similarly, in this study canine endocrine pancreas and parathyroid gland were AE1/AE3 negative and CK 13 positive. The negative staining of hepatocytes for AE1/AE3 in formalin-fixed canine liver confirms the results of Desnoyer et al. (1990). Since a positive immunoreaction with this antibody was observed in unfixed frozen human and canine liver (Moll, 1993; Ijzer et al., 2010; Schotanus et al., 2009), the negative immunostaining was likely attributed to the formalin fixation; it was already observed in liver tissue fixed for only 24 hrs.

Cytokeratin 7

The monoclonal antibody of the present study was also used by Espinosa de los Monteros et al. (1999) in 35 different FFPE canine tissues, as well as in normal canine skin (Kozaki et al., 2001) and in canine liver (Schotanus et al., 2009). The results of these three studies were mostly comparable to the present results. Although Espinosa de los Monteros et al. (1999) detected an immunosignal for CK 7 in canine kidney (Bowman's capsule and proximal tubules), in the present study these cell populations were immunonegative.

Cytokeratin 8

In accordance with the results in human and canine tissues this keratin is characteristic for simple epithelia (Vos et al., 1992a-c, Moll, 1993). This study, however, failed to detect CK 8 in hepatocytes (see above). The applied antibody had not been used in canine tissues previously. It was elected, since the antibody clones that had been used by other authors to detect CK 8 also labelled other cytokeratins (Vos et al., 1992a-c, Moll,

1993). By using CAM 5.2 (CKs 8 and CK 18) and RCK 102 (CKs 5 and 8) Vos et al. (1992a-c) detected a positive reaction in the cervical epithelium and kidney, that could not be confirmed by this study.

Cytokeratin 13

The present study shows that the expression profile of this cytokeratin markedly differs between human and canine tissues. In comparison to findings on human tissues (Moll, 1993), in this study CK13 was expressed in all stratified and pseudostratified epithelia including the skin, basal cells of all excretory glands and their ducts, as well as endocrine active cells (i.e. parathyroid gland) and sebaceous glands. In another study on FFPE normal canine skin and adnexal structures, which used the same monoclonal antibody (AE8), a positive immunostaining was not detected (Walter 2001). Using different detection methods, in human mucous membranes CK 13 was exclusively observed in suprabasal layers of uncornified stratified epithelia (Moll, 1993; Moll et al., 2008). The present study indicates that in dogs CK 13 - similar to CK 14 - has to be considered as a reliable stem cell marker with a longer expression during the differentiation process.

Cytokeratin 14

The detected expression profile of CK 14 in dogs is comparable to the known data in man (Moll, 1993) and canine skin (Walter, 2001), mammary gland (Gama et al., 2010) and prostate (Lai et al., 2008). It labels basal layers of stratified and pseudostratified epithelia, myoepithelial cells and sebaceous glands. Pieper et al. (2015) compared the expression of CK 14 in nonneoplastic epidermis and cutaneous epithelial neoplasms and observed CK 14 expression also in suprabasal layers of nonneoplastic skin. The authors interpret this finding as likely related to the presence of hyperplastic epidermal changes (Pieper et al., 2015). Similarly, Moll et al. (1993) described an expression of CK 14 in hyperplastic human epidermis.

Cytokeratin 17

Notably, the observed expression pattern of this cytokeratin differs completely in location and activation during differentiation process from the data described in humans (Moll, 1993). In canine normal tissues it was exclusively expressed in intermediate layers of epithelia of transitional zones (see below). In contrast to humans (Troyanovsky et al., 1989, 1992; Smedts et al., 1992; Moll, 1993; Moll et al., 2008), an immunoreaction in basal cells of sebaceous glands, excretory ducts of the pancreas, urothelium, myoepithelial cells as well as sensory Merkel cell-associated "haarscheiben" organs was not detected in dogs. To the authors' knowledge this is the first description of CK 17 expression in canine tissues.

Cytokeratin 19

Comparable to the findings observed in human tissues (Moll, 1993), the expression of this keratin can be detected in a wide range of epithelial and nonepithelial canine tissues. Only stratified epithelia were immunonegative. In contrast to the study of Moll (1993) on human tissue, in dogs also the acinar cells of exocrine pancreas, meningothel cells and folliculo-stellar cells of the adenohypophysis were immunopositive. In contrast to the findings of neoplastic tissue (Miettinen, 1991; Miettinen et al., 2000), normal canine synoviocytes were immunonegative.

Cytokeratin 20

In human beings (Moll, 1993) and canines (Espinosa de los Monteros et al., 1999; this study) CK 20 is expressed in intestinal cells and urothelial cells. In contrast to data from human tissues (Moll, 1993), by using the same antibody a positive reaction of CK 20 in the germ cells of testis, scattered epithelial cells of endometrium and salphinx, respiratory epithelial cells and excretory glands was observed (this study; Espinosa de los Monteros et al., 1999).

Tissue specific cytokeratin expression profiles

The results of this study show that the selected antibody panel can be used to distinguish between different types of canine epithelia. This is in accordance with the established work of Moll (1993) in human tissues. About 80% of the cytokeratin expression profiles detected in dogs match the data reported in man. Some of the observed differences may be explained by methodical differences and/or the variations in the used antibodies. In regard to the latter, Moll (1993) used CKs 5 and 18, whereas in the present study their partner cytokeratins, i.e. CKs 14 and CK 8, were applied. Moreover, this investigation examined more tissues/organs for their cytokeratin expression profile than the study of Moll (1993) on human tissue.

Epithelium of transitional zones

A positive immunoreaction for CKs 13, 14, 17 and 19 as well as AE1/AE3 is restricted to epithelia that represented a transition between stratified epithelium and organ specific functional epithelium, the latter is often a simple columnar epithelium. Almost all investigated tissue locations show also ectodermal to endodermal transitions (Kiecker et al., 2016). Since the common feature of the investigated tissue areas is their location at transitional zones (two different types of epithelia, transition between ectoderm and entoderm), they are collectively designated as epithelium of transitional zones. In canines, CK 17 is exclusively detected in epithelia of transitional zones, whereas in human tissues CK 17 is found in basal and myoepithelial

cells of complex tissues (Troyanovsky et al., 1989).

Influence of the formalin-fixation time on the immunoreactivity

The fixation-time dependent reduction of immunostaining for CK 7 (applied clone: OV-TL 12/30) has been also observed in other studies on human and canine tissues (Vos et al., 1989; Ramos-Vara et al., 2003; Webster et al., 2009). Since a prolonged fixation may result in a false negative reaction, a known fixation time is an important prerequisite for the correct interpretation of the immunstaining with this antibody.

Individual differences in the immunoreactivity between the different donor dogs

The differences in the immunostaining intensity for certain cytokeratins (CKs 7, 8, 14, 17 and 20) between the donor dogs may be explained by genetic polymorphisms. These could result in modifications of cytokeratin protein and subsequently influence the antibody binding (Walter, 2001). Such differences, however, are not known in man and keratins are a highly conserved phylogenetically old group of genes/proteins (Moll et al., 2008). Therefore another possible explanation are individual differences in the quantitative amount of tissue specific cytokeratins (Walter, 2001). Further, the respective condition of the cytokeratin protein (soluble or condensed) could influence the antibody binding and thus the immunoreactivity as well (Flitney et al., 2009; Windoffer et al., 2011).

Future application of the data obtained from this study

The results of the present study provide the basis for a wide variety of future investigations. They may help to identify different epithelial cell populations within particular tissues, e.g. secretory epithelial cells and myoepithelial cells in mammary gland tissue. Although the suggested panel will have to be evaluated in canine neoplastic tissue, Moll (1993) proposed a high alienability of the results to neoplastic tissue. Further applications on canine tissues might be the differentiation between hyperplasia and neoplasia (Harnden et al., 1999), the detection of early lymph node metastases (Höinghaus et al., 2007) and the tissue origin of malignant cells (Moll et al., 2008). In accordance with Moll (1993), the results of this study suggest that the distribution of cytokeratins in canine tissues is related to their cellular function and does not necessarily refer to their embryonal origin, i.e. 1) all examined cytokeratins were detected in tissues of all three germ layers, but they showed a variable expression in the same epithelial organ and 2) a similar cytokeratin expression profile was detected in cells with the same biological function.

The observation that the cytokeratin expression profile is influenced by cellular functions could provide a useful tool to stage tumors according to their biological behavior (Destexhe et al., 1993; Takei et al., 1995; Schaller et al., 1996; van Sprundel, 2010). In human medicine cytokeratins are also used to monitor the amount of carcinoma cells in the circulation and thus the success of therapy in cancer patients (Moll et al., 2008).

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References

Baumgärtner W. and Peixoto P.V. (1987). Immunohistochemical demonstration of keratin in canine neuroepithelioma. Vet. Pathol. 24, 500-503.

Cagle P.T., Truong L.D., Roggli V.L. and Greenberg S.D. (1989). Immunohistochemical differentiation of sarcomatoid mesotheliomas from other spindle cell neoplasms. Am. J. Clin. Pathol. 92, 566-571.

Cardona A., Madewell B.R., Naydan D.K. and Lund J.K. (1989). A comparison of six monoclonal antibodies for detection of cytokeratins in normal and neoplastic canine tissues. J. Vet. Diagn. Invest. 1, 316-323.

Caulin C., Ware C.F., Magin T.M. and Oshima R.G. (2000). Keratin-dependent, epithelial resistance to tumor necrosis factor-induced apoptosis. J. Cell Biol. 149, 17-22.

Desnoyers M.M., Haines D.M. and Searcy G.P. (1990). Immunohistochemical detection of intermediate filament proteins in formalin fixed normal and neoplastic canine tissues. Can. J. Vet. Res. 54, 360-365.

Destexhe E., Lespagnard L., Degeyter M., Heymann R. and Coignoul F. (1993). Immunohistochemical identification of myoepithelial, epithelial, and connective tissue cells in canine mammary tumors. Vet. Pathol. 30, 146-154.

Dungworth D.L., Hauser B., Hahn F.F., Wilson D.W., Haenichen T. and Harkema (1999). Histological Classification of Tumors of the Respiratory System of Domestic Animals. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume VI.

Espinosa de los Monteros A., Fernández A., Millán M.Y., Rodríguez F., Herráez P. and Martín de las Mulas J. (1999). Coordinate expression of cytokeratins 7 and 20 in feline and canine carcinomas. Vet. Pathol. 36, 179-190.

Franke W., Weber K., Osborn M., Schmid E. and Freudenstein C. (1978). Antibody to prekeratin: Decoration of tonofilament-like arrays in various cells of epithelial character. Exp. Cell Res. 116, 429-445.

Franke W.W., Schiller D.L., Hatzfeld M. and Winter S. (1983). Protein complexes of intermediate-sized filaments: melting of cytokeratin complexes in urea reveals different polypeptide separation characteristics. Proc. Natl. Acad. Sci. USA 80, 7113-7.

Flitney E.W., Kuczmarski E.R., Adam S.A. and Goldman R.D. (2009). Insights into the mechanical properties of epithelial cells: the effects of shear stress on the assembly and remodeling of keratin intermediate filaments. FASEB J. 23, 2110-2119.

Fuchs E.V., Coppock S.M., Green H. and Cleveland D.W. (1981). Two distinct classes of keratin genes and their evolutionary significance. Cell 27, 75-84.

Gama A., Alves A. and Schmitt F. (2010). Expression and prognostic significance of CK19 in canine malignant mammary tumours. Vet. J. 184, 45-51.

Geisler N. and Weber K. (1986). Structural aspects of intermediate

filaments. In: Cell and molecular biology of the cytoskeleton. Shaw J.W. (ed). Plenum Press, New York-London. pp 41-68.

Goldschmidt M.H., Dunstan R.W., Stannard A.A., von Tscharner C., Walder E.J. and Yager J.A. (1998). Histological Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals. Washington D.C.: Armed Forces Institute of Pathology. Second Series, Volume III.

Harnden P., Mahmood N., and Southgate J. (1999). Expression of cytokeratin 20 redefines urothelial papillomas of the bladder. Lancet 353, 974-977.

Hatzfeld M. and Franke W.W. (1985). Pair formation and promiscuity of cytokeratins: formation in vitro of heterotypic complexes and intermediate-sized filaments by homologous and heterologous recombinations of purified polypeptides. J. Cell Biol. 101, 1826-1841.

Hatzfeld M. and Weber K. (1990). Tailless keratins assemble into regular intermediate filaments in vitro. J. Cell Sci. 97, 317-324.

Head K.W., Cullen J.M., Dubielzig R.R., Else R.W., Misdorp W., Patnaik A.K., Tateyama S. and van der Gagg I. (2003). Histological Classification of Tumors of the Alimentary Sytsem of Domestic Animals. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume X.

Hendrick M.J., Mahaffey E.A., Moore F.M., Vos J.H. and Walder E.J. (1998). Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals. Washington D.C.: Armed Forces Institute of Pathology. Second Series, Volume II.

Hesse M., Franz T., Tamai Y., Taketo M.M. and Magin T.M. (2000). Targeted deletion of keratins 18 and 19 leads to trophoblast fragility and early embryonic lethality. EMBO J. 19, 5060-5070.

Hirschberger J. (1987). Herstellung und Charakterisierung monoklonaler Antikörper gegen T-Lymphozyten des Huhnes [Dissertation med. vet.]. Gießen: Justus-Liebig-Universität Gießen.

Höinghaus R., von Wasielewski R., Hewicker-Trautwein M., Freund M. and Mischke R. (2007). Immunocytological detection of lymph node metastases in dogs with malignant epithelial tumours. J. Comp. Pathol. 137, 1-8.

Ijzer J., Schotanus B.A., Vander Borght S., Roskams T.A., Kisjes R., Penning L.C., Rothuizen J. and van den Ingh T.S. (2010). Characterisation of the hepatic progenitor cell compartment in normal liver and in hepatitis: an immunohistochemical comparison between dog and man. Vet. J. 184, 308-314.

Jaquemar D., Kupriyanov S., Wankell M., Avis J., Benirschke K., Baribault H. and Oshima R.G. (2003). Keratin 8 protection of placental barrier function. J. Cell Biol. 161, 749-756.

Jockusch B.M., Füchtbauer A., Wiegand C. and Höhner B. (1986). Probing the cytoskeleton by mirkoinjection. In: Cell and molecular biology of the cytoskeleton. Sefaw J.W. (ed). 1 Edt. New York: Plenum Press. pp 1-40.

Kennedy P.C., Cullen J.M., Edwards J.F., Goldschmidt M.H., Larsen S., Munson L. and Nielson S. (1998). Histological Classification of Tumors of the Genital System of Domestic Animals. Washington D.C.: Armed Forces Institute of Pathology. Second Series, Volume IV.

Kiecker C., Bates T. and Bell E. (2016). Molecular specification of germ layers in vertebrate embryos. Cell. Mol. Life Sci. 73, 923-947.

Koestner A., Bilzer T., Fatzer R., Schulman F.Y., Summers B.A. and Van Winkle T.J. (1999). Histological Classificatio if Tumors of the Nervous System of Domestic Animals. Washington D.C.: Armed Forces Institute of Pathology. Second Series, Volume V.

Kozaki M., Nakamura Y., Iguchi M., Kano R., Watanabe S., Fujiwara K. and Hasegawa A. (2001). Immunohistochemical analysis of cytokeratin expression in dog skin. J. Vet. Med. Sci. 63, 1-4.

Ku N.O., Darling J.M., Krams S.M., Esquivel C.O., KeeVe E.B., Sibley R.K., Lee Y.M., Wright T.L. and Omary M.B. (2003). Keratin 8 and 18 mutations are risk factors for developing liver disease of multiple etiologies. Proc. Natl. Acad. Sci USA 100, 6063-6068.

Lai C.L., van den Ham R., van Leenders G., van der Lugt J., Mol J.A. and Teske E. (2008). Histopathological and immunohistochemical characterization of canine prostate cancer. Prostate 68, 477-488.

Lynch M.H., O'Guin W.M., Hardy C., Mak L. and Sun T.T. (1986). Acidic and basic hair/nail ("hard") keratins: their colocalization in upper cortical and cuticle cells of the human hair follicle and their relationship to "soft" keratins. J. Cell Biol. 103, 2593-2606.

Meuten D.J., Everitt J., Inskeep W., Jacobs R.M., Peleteiro M. and Thompson K.G. (2004). Histological classification of tumors of the urinary system of domestic animals. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume XI.

Miettinen M. (1991). Subsets in spindle cell sarcomas. Keratins are widespread but synovial sarcoma contains a distinctive keratin polypeptide pattern and desmoplakins. Am. J. Pathol. 138, 505-513.

Miettinen M., Limon J., Niezabitowski A. and Lasota J. (2000). Patterns of keratin polypeptides in 110 biphasic, monophasic, and poorly differentiated synovial sarcomas. Virchows Arch. 437, 275-283.

Miettinen M. and Paetau A. (2002). Mapping of the keratin polypeptides in meningiomas of different types: an immunohistochemical analysis of 463 cases. Hum. Pathol. 33, 590-598.

Misdorp W., Else R.W., Hellmén E. and Lipscomb T.P. (1999). Histological Classification of Mammary Tumors of the Dog and the Cat. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume VII.

Moll R. (1993). Cytokeratine als Differenzierungsmarker : Expressionsprofile von Epithelien und epithelialen Tumoren. [Habil. med.]. Mainz: Univ. Mainz.

Moll R., Franke W.W., Schiller D.L., Geiger B. and Krepler R. (1982). The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31, 11-24.

Moll I., Troyanovsky S.M. and Moll R. (1993). Special program of differentiation expressed in keratinocytes of human haarscheiben: an analysis of individual cytokeratin polypeptides. J. Invest. Dermatol. 100, 69-76.

Moll R., Divo M. and Langbein L. (2008). The human keratins: biology and pathology. Histochem. Cell. Biol. 129, 705-733.

Özgen S., Rasch K., Kropp G., Schoon H.-A., Aupperle H. and Sieme H. (1997). Aetiopathogenesis and therapy of equine hydromucometra: preliminary data. Pferdeheilkunde. 13, 533-534.

Oriolo A.S., Wald F.A., Ramsauer V.P. and Salas P.J. (2007). Intermediate filaments: a role in epithelial polarity. Exp. Cell Res. 313, 2255-2264.

Pieper J.B., Stern A.W., LeClerc S.M. and Campbell K.L. (2015). Coordinate expression of cytokeratins 7 and 14, vimentin, and Bcl-2 in canine cutaneous epithelial tumors and cysts. J. Vet. Diagn. Invest. 27, 497-503.

Ramos-Vara J.A., Miller M.A., Boucher M., Roudabush A. and Johnson G.C. (2003). Immunohistochemical detection of uroplakin III, cytokeratin 7, and cytokeratin 20 in canine urothelial tumors. Vet. Pathol. 40, 55-62.

Ribas J.L., Mena H., Braund K.G., Sesterhenn I.A. and Toivio-Kinnucan M. (1989). A histologic and immunocytochemical study of choroid

plexus tumors of the dog. Vet. Pathol. 26, 55-64.

Rungger-Brändle E. and Gabbiani G. (1983). The role of cytoskeletal and cytocontractile elements in pathologic processes. Am. J. Pathol. 110, 361-392.

Schaller G., Fuchs I., Pritze W., Ebert A., Herbst H., Pantel K., Weitzel H. and Lengyel E. (1996). Elevated keratin 18 protein expression indicates a favorable prognosis in patients with breast cancer. Clin. Cancer Res. 11, 1879-1885.

Schiller D.L., Franke W.W. and Geiger B. (1982). A subfamily of relatively large and basic cytokeratin polypeptides as defined by peptide mapping is represented by one or several polypeptides in epithelial cells. EMBO J. 1, 761-769.

Schotanus B.A., van den Ingh T.S., Penning L.C., Rothuizen J., Roskams T.A. and Spee B. (2009). Cross-species immunohistochemical investigation of the activation of the liver progenitor cell niche in different types of liver disease. Liver Int. 29, 1241-1252.

Slayter M.V., Boosinger T.R., Pool R.R., Dämmrich K., Misdorp W. and Larsen S. (1994). Histological Classification of Bone and Joint Tumors of Domestic Animals. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume I.

Smedts F., Ramaekers F., Troyanovsky S., Pruszczynski M., Robben H., Lane B., Leigh I., Plantema F. and Vooijs P. (1992). Basal-cell keratins in cervical reserve cells and a comparison to their expression in cervical intraepithelial neoplasia. Am. J. Pathol. 140, 601-612.

Sun T.T., Eichner R. and Bonitz P. (1984). Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. J. Cell Biol. 98, 1388-1396.

Suter M.M., Greenberger L.J., Wilkinson J.E. and Lewis R.M. (1990). Differential expression of cell surface antigens of canine keratinocytes defined by monoclonal antibodies. J. Histochem. Cytochem. 38, 541-549.

Takei H., Iino Y., Horiguchi J., Kanoh T., Takao Y., Oyama T. and Morishita Y. (1995). Immunohistochemical analysis of cytokeratin #8 as a prognostic factor in invasive breast carcinoma. Anticancer Res. 15, 1101-1105.

Tong X. and Coulombe P.A. (2006). Keratin 17 modulates hair follicle cycling in a TNFalpha-dependent fashion. Genes. Dev. 20, 1353-1364.

Troyanovsky S.M., Guelstein V.I., Tchipysheva T.A., Krutovskikh V.A. and Bannikov G.A. (1989). Patterns of expression of keratin 17 in human epithelia: dependency on cell position. J. Cell Sci. 93, 419-426.

Troyanovsky S.M., Leube R.E. and Franke W.W. (1992). Characterization of the human gene encoding cytokeratin 17 and its expression pattern. Eur. J. Cell Biol. 59, 127-137.

Valli V.E., Jacobs R.M., Parodi A.L. and Vernau W. (2002). Histological Classification of Hematopoietic Tumors of Domestic Animals. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume VIII.

van Sprundel R.G., van den Ingh T.S., Desmet V.J., Katoonizadeh A., Penning L.C., Rothuizen J., Roskams T. and Spee B. (2010). Keratin 19 marks poor differentiation and a more aggressive behaviour in canine and human hepatocellular tumours. Comp. Hepatol. 9, 4-15.

Vos J.H., van den Ingh T.S., Misdorp W., Ramaekers F.C., van Mil F.N. and de Neijs M. (1989). Keratin staining of canine epithelial tissues by a polyclonal antiserum. Zentralbl. Veterinarmed A. 36, 374-385.

Vos J.H., van den Ingh T.S., de Neijs M., van Mil F.N., Ivanyi D. and Ramaekers F.C. (1992a). Immunohistochemistry with keratin and smooth muscle actin monoclonal antibodies in canine digestive tract and extramural glands. Zentralbl. Veterinarmed A. 39, 241-257.

Vos J.H., van den Ingh T.S., de Neijs M., van Mil F.N., Ivanyi D. and Ramaekers F.C. (1992b). Immunohistochemistry with keratin monoclonal antibodies in canine tissues: urogenital tract, respiratory tract, (neuro-)endocrine tissues, choroid plexus and spinal cord. Zentralbl. Veterinarmed A. 39, 721-740.

Vos J.H., van den Ingh T.S., Ramaekers F.C., de Neijs M., van Mil F.N. and Ivanyi D. (1992c). Keratin and vimentin distribution patterns in the epithelial structures of the canine anal region. Anat. Rec. 234, 391-398.

Walter J.H. (2001). Cytokeratins in the canine epidermis. Vet. Dermatol. 12, 81-87.

Webster J.D., Miller M.A., Dusold D. and Ramos-Vara J. (2009). Effects of prolonged formalin fixation on diagnostic immunohistochemistry in domestic animals. J. Histochem. Cytochem. 57, 753-761.

Wilcock B., Dubielzig R.R. and Render J.A. (2002). Histological Classification of Ocular and Otic Tumors of Domestic Animals. Washington D.C. Armed Forces Institute of Pathology. Second Series, Volume IX.

Windoffer R., Beil M., Magin T.M. and Leube R.E. (2011). Cytoskeleton in motion: the dynamics of keratin intermediate filaments in epithelia. J. Cell Biol. 194, 669-678.

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