

**Title: “Expression of the plasma membrane markers aquaporin 1 (AQP1), glucose transporter 1 (GLUT1) and Na, K-ATPase in canine mammary glands and mammary tumours”**

Paper submitted in part fulfillment of the requirements for the Diploma in Small Animal Surgery (Soft Tissue) By Alistair Freeman.

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## Short Communication

## Expression of the plasma membrane markers aquaporin 1 (AQP1), glucose transporter 1 (GLUT1) and Na, K-ATPase in canine mammary glands and mammary tumours

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## ABSTRACT

This study investigated the expression of the plasma membrane markers aquaporin 1 (AQP1), glucose transporter 1 (GLUT1) and the  $\alpha 1$  subunit of Na, K-ATPase in normal canine mammary glands and in benign and malignant mammary tumours, using immunohistochemistry and semi-quantitative histomorphometry. AQP1 immunoreactivity was absent from the majority of specimens studied. GLUT1 immunoreactivity was observed in normal mammary tissue and particularly in the epithelial and mesenchymal cells of benign, and in the epithelial cells of malignant tumours, respectively. Na, K-ATPase immunoreactivity was present in normal and neoplastic mammary epithelium and was significantly increased in the epithelium of both benign and malignant tumours. These results suggest that GLUT1 is more highly expressed in neoplastic epithelium and mesenchyme and that Na, K-ATPase is more highly expressed in neoplastic mammary epithelium. In consequence, these membrane proteins may have potential as diagnostic and prognostic biomarkers of canine mammary neoplasia.

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Recent research in tumour biology in humans and rodents has indicated altered expression of key membrane proteins involved in the transport of water, glucose and electrolytes in tumour cells compared to their normal counterparts (Brahimi-Horn and Pouyssegur, 2007). The altered growth of neoplastic cells necessitates alterations in cellular metabolism which, in turn, influences the cell surface expression of membrane proteins involved in water, nutrient and electrolyte homeostasis. Three such proteins are aquaporin 1 (AQP1), a water channel that controls the permeability of endothelial and epithelial barriers by facilitating water movement across cell membranes (Agre et al., 2002), the hypoxia-responsive glucose transporter GLUT1 (Airley and Mobasheri, 2007) and Na, K-ATPase, an electrogenic transmembrane P-type ATPase responsible for establishing and maintaining the high intracellular potassium ( $150 \text{ mEq l}^{-1} \text{ K}^+$ ) to low intracellular sodium ( $15 \text{ mEq l}^{-1} \text{ Na}^+$ ) ratio characteristic of all living cells (Mobasheri et al., 2000). Based on available evidence, we hypothesised that expression of these proteins might be altered in canine mammary tumours and that, in consequence, their expression might serve as a biomarker of canine mammary neoplasia. In this study we used

immunohistochemistry to examine the expression of these membrane proteins in normal and neoplastic canine mammary tissue and thus assess if their expression differed between normal and neoplastic epithelial and mesenchymal cells.

Twenty-seven samples of mammary tissue from 23 dogs of various breeds, aged between 5 and 14 years of age (average 10 years), were submitted for histopathological examination to The Department of Veterinary Pathology at The University of Liverpool. Standard  $3.5 \mu\text{m}$  thick sections were stained using haematoxylin and eosin and using immunohistochemical techniques. Samples were selected which contained benign or malignant mammary tumours with normal mammary tissue within the same section (Table 1).

For immunohistochemical examination, slides were dewaxed in xylene, rehydrated through ethanol solutions of decreasing concentration and then washed in distilled water. Tissue sections were encircled with paraffin and incubated with dual endogenous enzyme block for 15–30 min, rinsed briefly with distilled water, and then incubated with 0.5 mL tris buffered saline (TBS)–Tween wash containing 0.1 g/mL of protease-free bovine serum albumin. Slides were incubated overnight with 200 mL of  $1 \times$  TBS–Tween wash containing the primary antibodies. Affinity purified polyclonal antibody raised against the c-terminus of rat AQP1 (diluted 1:200) was a gift from Dr. David Marples (University of Leeds). Monoclonal antibody raised against the  $\alpha$  subunit of chicken Na,

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**Table 1**

Details of the histopathological diagnoses and grading for immunoreactivity (from '0' [no expression] to '3' [intense expression]) for the plasma membrane markers aquaporin 1 (AQP1), glucose transporter 1 (GLUT1) and Na, K-ATPase in canine mammary glands and mammary tumours. NEp, normal epithelium; NMes, normal mesenchyme; NeoEp, neoplastic epithelium; NeoMes, neoplastic mesenchyme; x, data not available for this marker in this sample.

Histopathological diagnosis	Classification	AQP1 immunoreactivity				Na, K-ATPase immunoreactivity				GLUT1 immunoreactivity			
		NEp	NeoEp	NMes	NeoMes	NEp	NeoEp	NMes	NeoMes	NEp	NeoEp	NMes	NeoMes
Benign mixed mammary tumour	Benign	0	0	1	1	0	0	0	0	1	2.5	0.5	0.5
Simple adenoma	Benign	0	0	0	0	0	0	0	0	0.5	0	0	1.5
Complex adenoma	Benign	x	x	x	x	0	0.5	0	0	1.5	2	0.5	2
Fibroadenoma	Benign	x	x	x	x	0	0	0	0	0.5	2	0	0
Benign mixed mammary tumour	Benign	0	0	0	0	0	0	0	0	0	1	0	0
Benign mixed mammary tumour	Benign	0	0	0	0	0	1.5	0	0	0.5	2	0	0
Complex adenoma	Benign	0	0	0	0	0	0	0	0	0	2.5	0	1
Benign mixed mammary tumour	Benign	0	0	0	0	0.5	1.5	0	0	1	3	0	0
Benign mixed mammary tumour	Benign	0	0	0	0	0	1.5	0	0	0.5	2.5	0	2
Benign mixed mammary tumour	Benign	0	0	0	0	0.5	2	0	0	0.5	2.5	0	1
Benign mixed mammary tumour	Benign	0	0	0	0	0.1	0.5	0	0	1	2	0	0
Simple adenoma	Benign	0	0	0	0	0	0.5	0	0	x	x	x	x
Complex adenoma	Benign	0	0	0	0	0	0.1	0	0	0.5	1.5	0	0
Benign mixed mammary tumour	Benign	0	0	0	0	0	0	0	0	0.5	2	0	0
Complex carcinoma	Malignant	0	0	0	0	0	0	0	0	1	0.5	0	0
Simple carcinoma	Malignant	0	0	0	0	0	0	0	0	1	2.5	0	0
Complex carcinoma	Malignant	0	0	0	0	0	1.5	0	0	0.5	2.5	0.5	0
Simple carcinoma	Malignant	0	0	0	0	0	0	0	0	0.5	1.5	0	0
Complex carcinoma	Malignant	0	0	0	0	0	1.5	0	0	0.5	2	0	0
Complex carcinoma	Malignant	0	0	0	0	0	1.5	0	0	0	2	0	1
Simple carcinoma	Malignant	0	0	0	0	0.5	2	0	0	1	2.5	0	1.5
Complex carcinoma	Malignant	0	0	0	0	0	1	0	0	0	2	0	1.5
Complex carcinoma	Malignant	0	0.5	0	0	0	2	0	0	1	3	0	0.5
Anaplastic carcinoma	Malignant	0	0	0	0	0	0	0	0	0	0	0	0
Simple carcinoma	Malignant	0	0	0	0	0	0	0	0	0.5	2.5	0	0
Complex carcinoma	Malignant	0	0	0	0	0	2	0	0	0.5	2.5	0	0

K-ATPase, the a5 monoclonal (used as neat hybridoma supernatant) originally developed by Douglas M. Fambrough (Johns Hopkins University) was obtained from the Developmental Studies Hybridoma Bank (DSHB) developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242.<sup>1</sup> Polyclonal antibody raised against the c-terminus of rat GLUT1 (diluted 1:200) was a gift from Dr. Steve Baldwin (University of Leeds). All three antibodies exhibit species cross-reactivity including canine tissue (Mobasher et al., 2003, 2005; Airley and Mobasher, 2007).

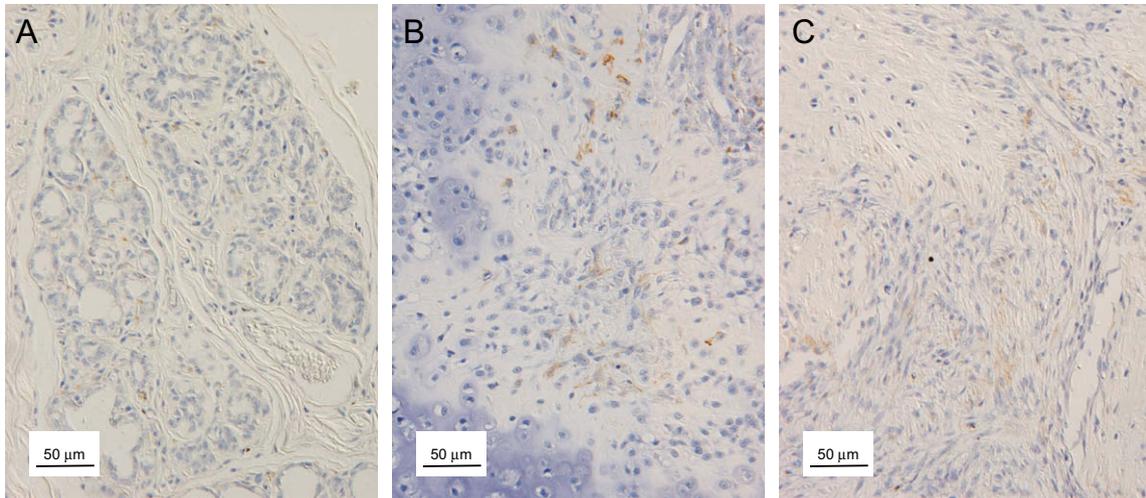
Control experiments were performed omitting the addition of the primary antibodies and using positive control tissue (canine kidney and liver). Immunohistochemical labelling was carried out using a DakoCytomation EnVision + Dual Link System, Peroxidase (DAB+) kit (Mobasher et al., 2003, 2005). Following overnight incubation, slides were agitated for three, 5 min periods in TBS-Tween and then incubated with diluted labelled polymer (DAKO) for 30 min at room temperature. Slides were again agitated for three, 5 min periods in TBS-Tween, incubated for 5–10 min with DAB + solution, rinsed with distilled water, counter-stained with haematoxylin, mounted and examined by light microscopy.

Mammary tumours were diagnosed according to the WHO classification system (Misdorp et al., 1999) and graded semi-quantitatively for intensity of antigen labelling from '0' (no expression) to '3' (intense expression). All sections were graded by the same pathologist (UH). Differences in staining intensity between normal and neoplastic epithelium and mesenchyme (expressed as grade for neoplastic tissue minus grade for normal tissue within the same section), were examined using Wilcoxon's one sample Signed Rank Test, matching for individual specimens. Statistical significance was where  $P < 0.05$  using a two-sided test and all analyses were performed in Minitab 15 (Minitab Inc.).

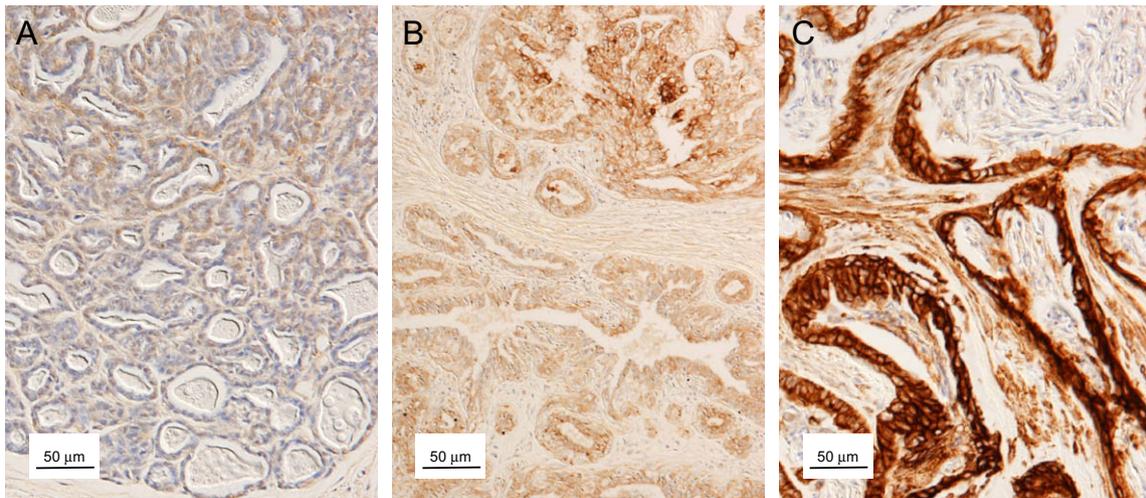
The histopathological diagnoses and results of the immunohistochemical grading are detailed in Table 1. AQP1 immunoreactivity was not evident in the majority of tissues examined, except for one sample of neoplastic epithelium and one sample that contained normal and neoplastic mesenchyme (Table 1, Fig. 1). There was no statistically significant difference in labelling for AQP1 between normal and neoplastic tissue. GLUT1 immunoreactivity was present in normal and neoplastic mammary epithelium and mesenchyme (Table 1, Fig. 2) and was significantly more intense in the epithelium of both benign ( $P = 0.001$ , median 1.5%, 95% confidence interval [CI] 1.25–1.75) and malignant ( $P < 0.01$ , median 1.63%, 95% CI 0.75–2.00) tumours. GLUT1 immunolabelling was also significantly greater in the mesenchyme of benign tumours ( $P = 0.03$ , median 0.50%, 95% CI 0.00–1.00) and there was a trend towards more intense immunoreactivity in the mesenchyme of malignant tumours ( $P = 0.069$ , median 0.25%, 95% CI 0.00–0.75). Na, K-ATPase immunolabelling was present in normal and neoplastic mammary epithelium but not in mammary mesenchymal tissue (Table 1, Fig. 3). Labelling for this antigen was significantly greater in the epithelium of both benign ( $P < 0.01$ , median 0.50%, 95% CI 0.05–0.80) and malignant ( $P = 0.011$ , median 0.75%, 95% CI 0.00–1.50) tumours.

The finding that GLUT1 is more highly expressed in neoplastic than in normal canine mammary epithelium and mesenchyme, is consistent with previous studies (Airley and Mobasher, 2007). Expression of GLUT1 in mammary tumours is regulated by the mammalian target of rapamycin (mTOR), a well-conserved serine/threonine kinase that regulates cell growth via alterations in mitochondrial oxygen consumption in response to nutrient status (Pankratz et al., 2009). Glucose transport and metabolism are essential for tumour cell survival and proliferation, where accelerated glycolysis and increased glucose uptake facilitate uncontrolled cell growth. There are reported associations between GLUT1 expression and proliferative indices and expression of this protein may be of prognostic significance. In humans,

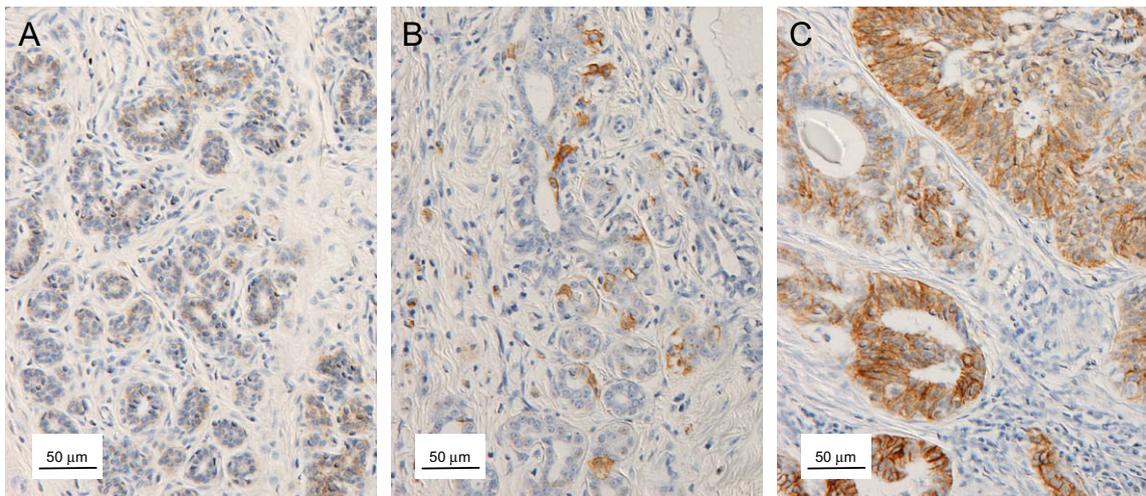
<sup>1</sup> See: [www.dshb.biology.uiowa.edu/a5](http://www.dshb.biology.uiowa.edu/a5).



**Fig. 1.** Immunohistochemical labelling of AQP1 in normal and neoplastic canine mammary tissue: (A) AQP1 in a normal mammary gland; (B) and (C) AQP1 in a benign mixed mammary gland tumour. Scale bar, 50 µm.



**Fig. 2.** Immunohistochemical labelling of GLUT1 in normal and neoplastic canine mammary tissue: (A) GLUT1 in a normal mammary gland; (B) GLUT1 in a benign mixed mammary gland tumour; (C) GLUT1 in a complex mammary carcinoma. Scale bar, 50 µm.



**Fig. 3.** Immunohistochemical labelling of Na, K-ATPase in normal and neoplastic canine mammary tissue: (A) Na, K-ATPase in a normal mammary gland; (B) Na, K-ATPase in a complex mammary adenoma; (C) Na, K-ATPase in a tubulo-papillary mammary carcinoma. Scale bar, 50 µm.

statistical analysis has revealed a close association between invasive mammary carcinomas and GLUT1 immunostaining (Alò et al., 2001).

Our findings indicate that Na, K-ATPase is more highly expressed in neoplastic compared to normal mammary epithelium in the dog. Recent work has shown that this protein is involved in cell adhesion and signal transduction and its aberrant expression and activity are implicated in the development and progression of several neoplasms (Mijatovic et al., 2008). Furthermore, ouabain and the related digitalis cardiac glycoside inhibitors of Na, K-ATPase possess potent anti-tumour activity (Prassas and Diamandis, 2008), particularly in breast cancer (Chen et al., 2006). The increased susceptibility of tumour cells to these compounds suggests their potential therapeutic use, which requires further study.

The finding that expression of AQP1 is limited in both normal and neoplastic canine mammary tissue is in contrast to studies of human mammary carcinomas that exhibited significantly elevated AQP1 expression, with immunoreactivity extending beyond microvascular structures to neoplastic cells in highly metastatic and poorly differentiated tumours (Mobasheri et al., 2005). AQP1 is strongly expressed in tumours of different cellular origin, particularly in aggressive and metastatic neoplasms (Verkman et al., 2008), and may account for vascular permeability and interstitial fluid pressure within these lesions (Endo et al., 1999). The findings of the current study suggest that aquaporin biology in mammary neoplasms may differ significantly between species.

Examination of a larger number of specimens of a smaller range of mammary tumour types would have reduced the effect of possible variation in the expression of these antigens between different tumours in the current study. Our findings in relation to GLUT1 expression support recent observations in the peri-necrotic regions of several human tumours (Airley et al., 2010). Furthermore, our observations highlight similarities and differences between the expression of these membrane biomarkers in humans, rodents and dogs and suggest that GLUT1 and Na, K-ATPase may be useful diagnostic and prognostic markers of canine mammary neoplasia. Future studies will investigate the expression of these proteins in canine neoplasms and correlate their expression with other markers of tumour metabolism and hypoxia.

#### Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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**Title: “The effect of silver impregnation of surgical scrub suits on surface bacterial contamination”**

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## The effect of silver impregnation of surgical scrub suits on surface bacterial contamination

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## ABSTRACT

Silver-impregnated fabrics are widely used for their antibacterial and antifungal effects, including for clinical clothing such as surgical scrub suits (scrubs). This study investigated whether silver impregnation reduces surface bacterial contamination of surgical scrubs during use in a veterinary hospital. Using agar contact plates, abdominal and lumbar areas of silver-impregnated nylon or polyester/cotton scrubs were sampled for surface bacterial contamination before (0 h) and after 4 and 8 h of use. The number of bacterial colonies on each contact plate was counted after 24 and 48 h incubation at 37 °C. Standard basic descriptive statistics and mixed-effects linear regression were used to investigate the association of possible predictors of the level of bacterial contamination of the scrubs with surface bacterial counts.

Silver-impregnated scrubs had significantly lowered bacterial colony counts (BCC) at 0 h compared with polyester/cotton scrubs. However, after 4 and 8 h of wear, silver impregnation had no effect on BCC. Scrub tops with higher BCC at 0 h had significantly higher BCC at 4 and 8 h, suggesting that contamination present at 0 h persisted during wear. Sampling from the lumbar area was associated with lower BCC at all three time points. Other factors (contamination of the scrub top with a medication/drug, restraint of patients, working in the anaesthesia recovery area) also affected BCC at some time points. Silver impregnation appeared to be ineffective in reducing bacterial contamination of scrubs during use in a veterinary hospital.

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## Introduction

Progressive contamination of clinical clothing with a mixture of bacteria from the wearer and the environment is a common occurrence (Babb et al., 1983; Boyce et al., 1997; Callaghan, 1998; Hambraeus, 1973; Loh et al., 2000; Perry et al., 2001; Speers et al., 1969; Wong et al., 1991) and is not necessarily prevented by wearing further protective clothing when contamination is perceived to be a risk (Kaplan et al., 2003). Furthermore, bacteria such as *Enterococcus* and *Staphylococcus* spp. can survive for more than 90 days on clothing worn by health care workers (Neely and Maley, 1999) and surgical scrub suits (scrubs) may be contaminated by bacteria even when freshly laundered (Moylan et al., 1975).

Although few studies have investigated the role of clinical clothing in the epidemiology of nosocomial bacterial infections, *Staphylococcus aureus* can be transferred from nurses' uniforms to patient bedding (Hambraeus, 1973) and bacteria cultured from the front of surgical scrubs preoperatively have subsequently been isolated from infected surgical wounds (Moylan et al., 1975). Taken together, these data suggest that surgical scrubs may be a vector for the spread of bacterial infection.

Silver ions have a direct antibacterial effect (Bragg and Rainnie, 1974; Feng et al., 2000; Matsumura et al., 2003; Schreurs and Rosenberg, 1982; Yamanaka et al., 2005) and silver impregnation of fabrics (e.g. cotton and polyester) has potent antibacterial and antifungal effects (Dastjerdi and Montazer, 2010; Ilic et al., 2009b; Lee and Jeong, 2004; Matyas-Zgondek et al., 2008; Rai et al., 2009) that persist through dying of the fabric (Ilic et al., 2009a). These properties have led to silver's wide clinical use as an antimicrobial (Rai et al., 2009). However, some authors have questioned the utility of silver coatings in a clinical setting, since some bacterial strains may be silver-resistant (Hendry and Stewart, 1979; McHugh et al., 1975; Neely and Maley, 2000) and the coating's effect may be altered by the presence or absence of water (Takai et al., 2002). To the authors' knowledge there are no previously-published veterinary studies assessing the use of silver-impregnated surgical scrubs. The present study investigated whether silver-impregnated surgical scrub tops carry less surface bacteria than traditional polyester/cotton tops while being worn by veterinary staff.

## Materials and methods

Surgical nursing and anaesthesia staff in the Small Animal Teaching Hospital (SATH), University of Liverpool, were randomly assigned to wear either silver-impregnated nylon scrubs (Buckley Lamb) or standard polyester/cotton scrubs

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(Urban Scrubs Ltd and Lundau Scrubs) on each day of the study. These staff groups were chosen because they were typically working inside the theatre suite for the whole day, allowing the mid-abdominal and mid-lumbar surface of each scrub top to be sampled for surface bacterial contamination prior to use (0 h), after 4 h use and after 8 h use.

Four centimetre diameter agar contact plates (Irradiated tryptone soya with Tween 80, lechithin, histidine and sodium thiosulfate contact plates, Southern Group Laboratory) were placed directly onto the fabric for 1–3 s. A different, adjacent section of fabric was sampled at each of the three time points to avoid sampling areas contaminated with agar from previous plates. Before and after each sampling, the researcher's hands were cleaned with chlorhexidine surgical scrub (Hibiscrub, AstraZeneca).

To allow statistical analysis of other possible influences on the level of bacterial contamination, participants each day completed a short questionnaire about factors that might affect how contaminated their scrubs had become (Table 1). If a participant had to change their scrub top during the course of the day (e.g. due to soiling), data for that participant for that day were excluded from the study.

Contact plates were incubated at 37 °C and the number of bacterial colonies on each plate was counted after 24 and 48 h incubation, allowing counting of both faster-growing and slower-growing colonies. Each day, two randomly-selected unused contact plates were incubated alongside the sample plates as negative controls. Both types of scrubs were laundered daily in a standard commercial washing machine (JLA 9277, JLA Limited) at 40 °C using a standard commercial biological washing powder (Persil biological washing powder, Lever Faberge), then tumble dried in a commercial drier (JLA 92830ELG). Both types of scrubs were folded and returned to the theatre changing area immediately after laundry and stored in open plastic baskets overnight.

To satisfy requirements for ethical approval, at the end of the study participants completed a short anonymous debriefing questionnaire about their participation. Each set of scrubs, participant, contact plate and questionnaire was assigned a unique identification number and all collected data were recorded against these numbers, ensuring that the researcher performing the bacterial colony counting was blinded to the type of scrub suit and participant's identity and job type.

#### Statistical analysis

Bacterial colony counts were recorded on a spreadsheet (Microsoft Excel) and transferred into STATA11 (StataCorp) for statistical analysis.

Data from contact plates where bacterial overgrowth had occurred and individual colonies could not be counted were coded as missing. If a contact plate contained 100 or more colonies, an accurate count could not be determined due to merging of adjacent colonies, so counts from these plates were recorded as '100+' and recoded to a value of 150 before statistical analysis. All counts were transformed to  $\log_{10}(\text{count} + 1)$ . After performing standard basic descriptive statistics, the association of the  $\log_{10}$  transformed cell count with possible predictors of the level of bacterial contamination of the scrubs (questionnaire data as shown in Table 1, participant's job role and the scrub type) was explored. Two approaches were considered: the first method offered all possible predictors to a backwards stepwise multiple regression model, retaining only those variables if their exclusion was significant at  $P \leq 0.2$ . These variables were then included in a mixed-effects linear regression model with staff identity and/or scrub suit identity as a random variable: the type of scrub suit (silver or not) was also forced into the model.

The second method included all possible predictors in a mixed-effects linear regression. In both cases, the only random effect needed by the model was staff identity. The Wald statistic was used to determine the significance of predictors in the final model. Graphical methods were used to examine the residuals for normality and equality of variance, revealing that the required assumptions were met. Since the two approaches gave very similar results, only the second is reported here. Predicted means were obtained using the STATA 'margins' command using

its default settings. For a given level of a factor (e.g. type of scrub suit), the predicted mean provided values estimated for the model that would have been obtained if all individuals had this level of the factor. Statistical significance was defined as  $P < 0.05$  on a two-sided null hypothesis.

All participants gave fully informed consent to take part in the study and were informed that they could withdraw from the study at any time for any reason. The study was approved by the University of Liverpool Research Ethics Committee.

## Results

Mixed-effects linear regression revealed that, at 0 h, samples taken from silver-impregnated scrubs had significantly lower BCC after both 24 and 48 h incubation (Tables 2–4). In samples taken at 4 and 8 h, silver impregnation had no effect on BCC (Tables 2 and 3). However, scrub tops with higher BCC at 0 h had significantly higher BCC at 4 and 8 h (Table 4). Samples taken from the lumbar area were associated with significantly lower BCC after 24 and 48 h incubation at all three sampling times (Tables 2–4). In samples taken at 4 h (after 48 h incubation) and 8 h (after 24 or 48 h incubation), contamination of the scrub top with a medication/drug was associated with significantly increased BCC (Table 4).

Restraint of patients during the day was associated with increased BCC in samples taken at 4 h and incubated for 24 or 48 h (Table 4). Two of the possible predictors were associated with lower BCC (Table 4): working in the anaesthesia recovery area during the day (in samples taken at 4 h incubated for 24 or 48 h) and contamination of the scrub top with urine (in samples taken at 8 h incubated for 48 h).

## Discussion

Although the antibacterial and antifungal properties of silver-impregnated fabrics are well known (Dastjerdi and Montazer, 2010; Ilic et al., 2009b; Lee and Jeong, 2004; Matyjas-Zgondek et al., 2008; Rai et al., 2009), our study suggests that silver impregnation is ineffective in reducing surface bacterial contamination of surgical scrub tops during use in a veterinary hospital. Data from human health care suggests that clean uniforms are progressively contaminated with a mixture of bacteria from the wearer, patients and the environment (Babb et al., 1983; Boyce et al., 1997; Callaghan, 1998; Hambraeus, 1973; Loh et al., 2000; Perry et al., 2001; Speers et al., 1969; Wong et al., 1991), with approximately one-third of bacterial contamination coming from the wearer (Speers et al., 1969). Observation of the contact plates in our study revealed a mix of colonies varying in shape (round to filamentous), colour (cream to bright yellow) and size (1–5 mm diameter), consistent with a mixed population of bacteria.

**Table 1**  
Data recorded in daily participant questionnaire.

Information
Participant identification
Scrub suit identification
Date
Length of time working in theatre suite (to nearest half hour)
If participant had restrained patients
If participant had prepared animals for surgery (clipping and scrubbing)
If participant left the theatre suite to go to the imaging suite while wearing scrubs and, if so, how many times and for how long (to the nearest half hour)
Whether or not the front of the participant's scrub top had been contaminated with blood, surgical scrub solution, urine, faeces, vomit, saliva, hair/fur, food, water or medications/drugs
If the participant was present in one of the operating theatres or procedure rooms during surgery and, if so, whether they were an observer, theatre nurse, anaesthetist or scrubbed surgical assistant.
Which areas of the theatre suite the participant had worked in (theatres 1–3, minor procedures theatre, endoscopy room, anaesthesia induction area, anaesthesia recovery area, intensive care unit and cleaning/sterilisation area)
Whether the participant had to change their scrub top during the day

**Table 2**

Uncorrected raw means, medians, standard errors of the mean (SEM) and interquartile ranges (IQR) of  $\log_{10}$  (bacterial colony count + 1) for normal and silver-impregnated scrubs and abdominal and lumbar sampling locations at sampling times of 0, 4 and 8 h. Number of observations ranged from  $n = 115$  to  $n = 127$ .

Sampling time (h)	Sample source	Incubation time (h)	Mean	SEM	Median	IQR
0	Normal scrubs	24	1.02	0.045	0.95	0.58
		48	1.10	0.044	1.04	0.60
	Silver scrubs	24	0.85	0.053	0.85	0.73
		48	0.95	0.050	0.87	0.68
	Abdominal area	24	1.15	0.047	1.11	0.75
		48	1.23	0.044	1.23	0.68
	Lumbar area	24	0.72	0.044	0.70	0.52
		48	0.81	0.042	0.78	0.56
4	Normal scrubs	24	1.20	0.047	1.18	0.67
		48	1.27	0.046	1.23	0.70
	Silver scrubs	24	1.27	0.045	1.28	0.70
		48	1.34	0.042	1.34	0.61
	Abdominal area	24	1.45	0.040	1.38	0.54
		48	1.52	0.038	1.52	0.53
	Lumbar area	24	1.02	0.043	1.02	0.62
		48	1.11	0.041	1.08	0.54
8	Normal scrubs	24	1.25	0.046	1.22	0.60
		48	1.31	0.043	1.26	0.57
	Silver scrubs	24	1.17	0.041	1.15	0.69
		48	1.23	0.042	1.18	0.72
	Abdominal area	24	1.40	0.042	1.40	0.63
		48	1.46	0.040	1.46	0.58
	Lumbar area	24	1.02	0.038	0.98	0.45
		48	1.09	0.038	1.04	0.43

**Table 3**

Predicted means and 95% confidence intervals for normal and silver-impregnated scrubs and abdominal and lumbar sampling locations at sampling times of 0, 4 and 8 h. estimated after mixed-effects linear regression analysis of  $\log_{10}$  (bacterial colony count + 1). Number of observations ranged from  $n = 115$  to  $n = 127$ .

Sampling time (h)	Sample source	Incubation time (h)	Predicted mean	95% Confidence interval
0	Normal scrubs	24	1.071	0.910–1.233
		48	1.142	0.988–1.296
	Silver scrubs	24	0.896	0.738–1.053
		48	0.983	0.833–1.133
	Abdominal area	24	1.197	1.039–1.355
		48	1.276	1.125–1.427
	Lumbar area	24	0.771	0.613–0.929
		48	0.85	0.700–1.001
4	Normal scrubs	24	1.181	1.041–1.320
		48	1.246	1.138–1.355
	Silver scrubs	24	1.243	1.107–1.379
		48	1.352	1.247–1.457
	Abdominal area	24	1.391	1.254–1.528
		48	1.479	1.375–1.583
	Lumbar area	24	1.036	0.901–1.171
		48	1.128	1.026–1.231
8	Normal scrubs	24	1.253	1.137–1.369
		48	1.321	1.212–1.430
	Silver scrubs	24	1.149	1.035–1.264
		48	1.209	1.102–1.316
	Abdominal area	24	1.343	1.229–1.457
		48	1.412	1.306–1.518
	Lumbar area	24	1.068	1.229–1.457
		48	1.125	1.022–1.228

In our study, sampling from the abdominal area of the scrubs was consistently associated with higher BCC than sampling from the lumbar area and patient restraint was associated with increased BCC. This suggested that patients and the wearer's hands were the main source of contamination of the scrub tops, since the abdominal area is most likely to come into contact with the patient during restraint or lifting and is more likely than the lumbar area to be touched by the wearer. Identification of the bacteria contaminating the scrubs might have allowed confirmation of the source of contamination but the large number of colonies grown on the contact plates precluded their identification in this study.

Silver appears to exert its antibacterial effect by several mechanisms, including denaturation and condensation of DNA (Feng et al., 2000), denaturation of ribosomes and reduced expression of respiratory enzymes (Yamanaka et al., 2005), direct interaction with sulfhydryl groups on proteins (Feng et al., 2000), generation of reactive  $O_2$  species and damage to the cell envelope (Feng et al., 2000). Together, these prevent DNA replication (Feng et al., 2000), inhibit bacterial respiration (Bragg and Rainnie, 1974) and cause altered metabolism of various substances, including phosphate, mannitol, succinate, glutamine and proline (Schreurs and Rosenberg, 1982).

**Table 4**  
Statistically significant results from mixed-effects linear regression analysis of effect of possible predictors on  $\log_{10}$  (bacterial colony count + 1) at sampling times of 0, 4 and 8 h. A negative coefficient indicates that the presence of the predictor reduces bacterial colony count, whereas a positive coefficient indicates that presence of the predictor increases colony count. Number of observations ranged from  $n = 115$  to  $n = 127$ .

Sampling time (h)	Possible predictor	Incubation time (h)	Coefficient	P value	95% Confidence interval
0	Silver impregnation of scrubs	24	-0.176	0.004	-0.296 to -0.055
		48	-0.159	0.006	-0.271 to -0.046
	Sample taken from lumbar area of scrub top	24	-0.426	0.000	-0.538 to -0.314
		48	-0.425	0.000	-0.530 to -0.320
4	Colony count at time 0	24	0.155	0.014	0.032–0.278
		48	0.132	0.037	0.008–0.257
	Sample taken from lumbar area of scrub top	24	-0.359	0.000	-0.475 to -0.244
		48	-0.352	0.000	-0.465 to -0.240
	Wearer restrained patients	24	0.360	0.007	0.099–0.620
		48	0.327	0.011	0.074–0.578
	Wearer entered anaesthesia recovery area	24	-0.235	0.029	-0.446 to -0.024
	48	-0.266	0.010	-0.469 to -0.063	
8	Scrub top contaminated with a medication	24	0.211	0.021	0.032–0.391
		48	0.207	0.001	0.087–0.327
	Colony count at time 0	24	0.207	0.001	0.087–0.327
		48	0.177	0.005	0.054–0.300
	Sample from lumbar area of scrub top	24	-0.281	0.000	-0.394 to -0.168
		48	-0.289	0.000	-0.400 to -0.177
	Scrub top contaminated with a medication	24	0.216	0.018	0.037–0.395
48		0.234	0.009	0.057–0.410	
Scrub top contaminated with urine	48	-0.187	0.032	-0.357 to -0.016	

Exposure to silver ions takes several hours to significantly reduce bacterial numbers (Yamanaka et al., 2005), suggesting that silver's lack of efficacy in our study may have been due to ongoing contamination of the scrubs (e.g. from patient contact during restraint or movement of patients around the theatre suite) resulting in contamination with bacteria which the silver in the fabric had insufficient time to kill before sampling took place. This hypothesis is supported by the observation that silver impregnation was associated with lower BCC at 0 h, suggesting that silver was effective in reducing surface bacterial contamination during storage of the scrubs.

An alternative hypothesis is that some of the bacteria contaminating the scrubs were silver-resistant. Silver resistance has been identified in several strains of bacteria (Hendry and Stewart, 1979; McHugh et al., 1975; Neely and Maley, 2000) and is apparently conferred by a plasmid (pMG101) carrying genes for a periplasmic silver-binding protein, two transmembrane silver efflux pumps and a combination of a membrane kinase and transcriptional regulatory responder that together act as a 'silver sensor' (Silver, 2003). However, previous reports suggest that the levels of silver resistance in bacteria isolated from human and veterinary patients are low (Ip et al., 2006; Woods et al., 2009) and, if a substantial number of our hospital population of bacteria were silver-resistant, one might also expect BCC at 0 h to be unaffected by silver impregnation. Further work to investigate the prevalence of silver resistance in our hospital population of bacteria could clarify this issue.

Interestingly, some bacteria were present on the surface of both types of scrubs at 0 h. Although bacteria may survive laundry of scrubs (Moyle et al., 1975), evidence from human healthcare suggests that the laundry process employed here should have been effective in killing most common pathogens (Jurkovich, 2004; Patel et al., 2006). We therefore consider it likely that the majority of the bacteria detected at 0 h were acquired during handling and storage of the scrubs post-laundry or from the wearer's hands during the process of dressing, with the relative importance of these two sources depending on the scrub type. Higher BCC at 0 h were associated with higher bacterial counts at later sampling times, suggesting that at least some of the bacteria identified in later samples were present on the scrubs when they were first put on. This indicates that surgical scrubs should be laundered, stored

and donned as hygienically as possible, since bacterial contamination acquired at these times may persist while the scrubs are in use.

Contamination of the scrub top with a medication or drug was associated with increased BCC in samples taken after 4 and 8 h wear. This is surprising, since medications should be sterile and therefore not a major source of bacterial contamination. Due to the nature of the drugs being handled (e.g. anaesthetic induction agents, intravenous antibacterials) medications in our theatre suite are usually only handled for a short period around the time that they are administered to the patient. Thus, contact with medications implies close patient contact and thus possibly increased scrub suit contamination. This hypothesis is supported by the association between handling of patients and increased BCC in the samples taken after 4 h wear.

Previous data have suggested that one of the other predictors associated with patient contact (visible contamination of the scrubs with organic material such as hair/fur, vomit or faeces) should be associated with increased BCC, since contamination with organic materials reduces the effectiveness of silver as an antibacterial agent (Takai et al., 2002). In our study, this association was not identified, however, possibly because transfer of bacteria to the surface of the scrubs from the patient or environment occurred without visible contamination with these organic materials. Also, SATH uniform and work-wear guidelines state that clothing visibly contaminated with organic matter should be changed immediately, which would remove the effect of these types of contamination from the study because data from scrubs changed during the day were excluded from the analysis. Alternatively, it is possible that workers in perceived 'high-risk' areas, or carrying out activities with a perceived increased risk of contamination, worked more carefully or took additional precautions, such as wearing disposable plastic aprons. This might also account for the association between working in the anaesthesia recovery area (an area where patients were frequently handled while kennelled and thus perhaps with a high perceived risk of contamination) and lower BCC in samples taken after 4 h wear. However, it is also possible that some associations were missed due to limitations of the study (see below). Further work visually monitoring staff movements and patient contacts might clarify why contamination with a medication resulted in increased BCC.

Contamination of the surgical scrubs with urine was associated with lower BCC in samples taken at 8 h and incubated for 48 h. We believe this association to be due to chance and not causal because it only occurred at this one time point and visible contamination of scrubs with urine should have prompted the scrub top to be changed, thus excluding data from it on that day from the study. Also, there may have been errors in reporting (see below).

There are several possible limitations to this study. First, the number of samples in each group was lower than that assumed in the power calculation, possibly resulting in type II errors; this may account for restraint of patients being associated with higher BCC in samples taken at 4 h, but not at 8 h, for example. Unfortunately, more samples could not be taken in the time available for the study. Second, although the questionnaires were completed anonymously (to encourage full disclosure) and as soon as was practical (at the end of the working day), there may have been errors or omissions in the responses, leading to over or under-reporting of some types of contamination. However, any such errors should not alter any effects based on scrub type, sampling location, participant identification or date, since the accuracy of these data was not dependent on reporting by the participants. Third, the difficulty in counting large numbers of bacterial colonies due to colony merging and overgrowth may have resulted in underestimation of the number of colonies on some sample plates, which may have affected the results.

## Conclusions

This study suggests that silver impregnation of fabric is ineffective in reducing surface bacterial contamination of surgical scrub tops during use in a veterinary surgical suite. Furthermore, bacterial contamination of scrub suits present when they are first put on may persist through the working day, although silver impregnation does appear to be effective in reducing bacterial contamination on the scrubs during storage. The apparent lack of efficacy of silver in the face of ongoing bacterial contamination may be clinically significant, since in a busy theatre suite bacteria could be transferred between patients via the scrubs. Precautions such as wearing disposable plastic aprons during patient contact, regularly changing surgical scrubs during the working day and strict hand hygiene may be more effective in reducing bacterial contamination than buying specialist 'antibacterial' scrubs.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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**Title: “Wedge meatoplasty as a treatment for stricture of the urethral meatus in a cat”**

Paper submitted in part fulfillment of the requirements for the Diploma in Small Animal Surgery (Soft Tissue) By Alistair Freeman.

Year: 2012

This manuscript was prepared by the author without outside assistance.

**Candidate:**

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I am happy to inform you that your revised manuscript The effect of silver impregnation of surgical scrub suits on surface bacterial contamination is accepted for publication in The Veterinary Journal. The Reviewer's comment on your revised manuscript appears below. I have performed further editing on the manuscript and the journal office will contact you in due course regarding the next steps towards publication.

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1 See: The Veterinary Journal (2001) 161, 101.  
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## Case report

### Wedge meatoplasty as a treatment for stricture of the urethral meatus in a cat

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(Accepted for publication in current form by The Journal of Feline Medicine and Surgery, 22<sup>nd</sup> February 2012)

#### Summary

A 3 year 6 month male neutered Siamese cat presented with idiopathic feline lower urinary tract inflammation (FLUTI) and dysuria, which appeared to be due to stricture of the urethral meatus. Wedge meatoplasty was performed which relieved the cat's dysuria and restored a normal urine stream. To the author's knowledge this is the first report of meatoplasty as a treatment for stricture of the urethral meatus in a cat.

#### Case report

A 3 year 6 month male neutered Siamese cat presented with a 2 month history of intermittent dysuria, pollakiuria and excessive licking of the prepuce/penis. Treatment with unspecified antibacterials, N-acetyl D-glucosamine, Royal Canin Urinary S/O diet and an unspecified homeopathic remedy had produced no improvement. Clinical examination revealed a full, non-painful bladder and pale mucosa at the tip of the penis.

Routine haematology and biochemistry screens were unremarkable. Expression of the bladder under general anaesthesia was possible but the urine stream was thin. Examination of the penis confirmed that the tissue surrounding the urethral meatus was hard, fibrous and pale and that the meatus was abnormally small (Figure 1). Analysis of a cystocentesis urine sample revealed slight haematuria, proteinuria, pyuria and hypersthenuria (specific gravity 1.050). Urine bacterial culture of this sample was negative. The urethral meatus was difficult to catheterise with a 4 French Jackson's cat catheter (Smiths Medical International) but the catheter passed easily along the rest of the urethra. A positive contrast retrograde urethrogram was unremarkable: a double contrast cystogram revealed mild thickening of the bladder wall. Abdominal ultrasonography confirmed the bladder wall thickening but was otherwise unremarkable. A diagnosis of idiopathic FLUTI was made on the basis of the history, clinical signs and diagnostic test results. Urethral muscle spasm or a stricture of the urethral meatus was suspected as the cause of the cat's dysuria.

Medical treatment was given with intramuscular buprenorphine 20µg/kg q8h (Vetergesic; Alstoe Animal Health), phenoxybenzamine 0.5mg/kg *per os* q12h (Dibenyline; Goldshield Pharmaceuticals), diazepam 2mg *per os* q8h (Valium; Roche) and intravenous Hartmann's solution 3ml/kg/h (Aquapharm No. 11; Animalcare). 24 hours later there was no improvement in the cat's dysuria so an



**Figure 1: photograph of the cat's penis at presentation showing small urethral meatus with surrounding pale discolouration of the penile mucosa.**

indwelling urinary catheter (Slippery Sam; Smiths Medical International) was placed and connected to a closed urine collection system (Infusion Concepts). The catheter was removed 24h later: the cat remained dysuric. Urinalysis of a cystocentesis sample was unremarkable apart from slight haematuria. Examination of the distal penis under general anaesthesia confirmed the previous findings and a stricture of the urethral meatus was strongly suspected. A wedge meatoplasty was performed to widen the urethral orifice: an approximately 3mm long triangular section of the dorsal wall of the distal urethra and overlying penile mucosa was removed, with the apex directed proximally and the base at the meatus (Figure 2). The urethral mucosa was sutured to the penile mucosa using simple interrupted sutures of 0.7 metric polyglactin 910 (Vicryl; Ethicon).

Postoperatively medical treatment with buprenorphine, phenoxybenzamine and Hartmann's solution was given as detailed above. An Elizabethan collar was used to prevent self-trauma. The cat showed no evidence of dysuria, produced a good urine stream and was discharged 48 hours postoperatively with medical treatment with meloxicam 0.05mg/kg *per os* q24h for 5 days (Metacam; Boehringer Ingelheim) and a further 7-day course of phenoxybenzamine. The owner was given management advice re: litter tray number and placement, minimising stress, feeding a commercial canned diet, encouraging water intake and encouraging exercise and outdoor activity.

At re-examination 7 days postoperatively the owner reported that the cat was urinating normally. Clinical examination revealed that the penis appeared to be healing uneventfully. Medical treatment was discontinued and the owner was asked to continue management of the cat at home as described above. At re-examination 21 days postoperatively the penis had completely healed and the

inflammation had resolved. The cat remained free of clinical signs 12 months later.



**Figure 2: intraoperative photograph of the cat's penis after resection of v-shaped wedge of tissue from the dorsal aspect.**

Penile urethral strictures in cats have been reported associated with damage from repeated or traumatic urethral catheterisation<sup>1</sup>. There was no history of previous urethral catheterisation in this case, however. The location of the stricture and history of excessive grooming of the area suggest that self-trauma may have caused the stricture here. The stricture was most likely not visible on a positive-contrast urethrogram because its location was distal to the tip of the catheter used to introduce the contrast material. The negative urine culture in the face of pyuria is consistent with previous reports that while pyuria is a common finding in cats with FLUTI associated with feline idiopathic cystitis (especially males that are obstructed)<sup>2</sup> only 1-22% of cats with FLUTI have a positive urine culture<sup>3-7</sup>.

Medical treatment was given initially despite the suspicion of meatal stricture to rule out urethral muscle spasm as a possible cause of the dysuria. An indwelling catheter was used for 24h to relieve the cat's dysuria while giving medical treatment more time to work. After 48h the complete lack of response to treatment and the consistent abnormal appearance of the meatus suggested stricture as a more likely cause and thus surgical treatment was pursued. Perineal urethrostomy<sup>8</sup> was considered for this case. However, meatoplasty was chosen as a first-line treatment due to the distal location of the stricture, potential complications of perineal urethrostomy<sup>9,10</sup> and the possibility of performing perineal urethrostomy at a later date if meatoplasty was unsuccessful.

In humans, stricture of the urethral meatus and/or fossa navicularis (the terminal portion of the penile urethra) has been reported due to recurrent

balanoposthitis<sup>11</sup>, balanitis xerotica obliterans<sup>12</sup>, junctional epidermolysis bullosa<sup>13</sup>, exposure to sulphur mustard<sup>14</sup> and as a complication of hypospadias repair<sup>15,16</sup>. Non-surgical therapy of meatal strictures in humans has been reported using repeated sounding of the meatus<sup>17-19</sup> and combined dilatation of the meatus and application of topical corticosteroid<sup>20</sup>. Sounding was considered inappropriate in this case as repeated sedation or general anaesthesia would be required and due to concern that the apparent fibrosis around the meatus would limit its effectiveness. Various meatoplasty techniques have been used for treatment of meatal stricture in humans including ventral transverse island fasciocutaneous penile flap (Jordan, 1987), dorsal and ventral meatotomy with a v-shaped relieving incision<sup>21</sup>, eversion meatoplasty<sup>22</sup>, resection and primary end-to-end anastomosis<sup>23</sup>, meatoplasty with double buccal mucosa grafts<sup>24</sup> or a dorsal preputial island flap<sup>25</sup> and meatoplasty with various other local skin flaps or free skin grafting<sup>11</sup>.

Here, a simple wedge meatoplasty similar to the eversion meatoplasty described by el-Kasaby *et al*<sup>22</sup> and the MAVIS modification of the Mathieu technique for hypospadias repair<sup>26</sup> was used: more complicated techniques involving flaps or grafts were considered inappropriate because of the small diameter of the cat's urethra. Multifilament absorbable suture material was chosen here for mucosal apposition on the basis of the described technique of eversion meatoplasty<sup>22</sup>, availability of the required size, because its softer nature might cause less postoperative irritation than a monofilament material and because it would not require removal and the consequent possibility of trauma to the delicate mucosal apposition. Use of a multifilament absorbable material did not appear to adversely affect healing.

Postoperative complications after meatoplasty in humans include abnormal urine stream<sup>22-25</sup>, glanular torsion<sup>23</sup>, abnormal appearance of the meatus<sup>26</sup>, wound dehiscence<sup>11,27</sup>, fistula formation<sup>26,27</sup> and stricture formation<sup>23</sup>. Complications were not seen in this case. Stricture formation and dehiscence were considered possible complications in this case as accurate mucosal apposition was technically challenging. Use of an operating microscope or loupes might allow use of a finer suture and more accurate suture placement, minimising this risk.

Assessment of the effect of meatoplasty in humans is often subjective, based on history and clinical examination findings including examination of the urine stream<sup>28</sup> and the same criteria were used to assess this case. Uroflowmetry has been proposed as a more objective measure of treatment effect<sup>28</sup> but to the author's knowledge it is not yet available to veterinary patients.

To the author's knowledge this is the first report of stricture of the urethral meatus and wedge meatoplasty in a cat. Wedge meatoplasty appeared to be an effective treatment in this case: further investigation is warranted to determine its efficacy and safety in a larger number of cases.

### Conflict of interest statement

The author has no financial or personal relationships with people or organisations that could inappropriately influence this work.

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