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Long-term follow up of the effect of a pheromone therapy on feline spraying behaviour

D. S. MILLS, J. C. WHITE

FOLLOWING neutering, 10 per cent of male domestic cats and up to 5 per cent of females have been reported to urine spray (Hart and Cooper 1984) and this can pose a major problem to the owner. A variety of treatments have been proposed for the control of this behaviour, ranging from environmental manipulation to eliminate the stimuli eliciting the behaviour (Borchelt and Voith 1986), behaviour therapy to reduce the impact of perceived stressors in the environment (Landsberg and others 1997), anxiolytic pharmacotherapy (Overall 1997) and surgical lesioning of the hypothalamus (Hart and Voith 1978) or olfactory tract (Hart 1981). More recently, the use of a synthetic analogue of the feline facial pheromone F3 (Feliway; CEVA Animal Health) to control the behaviour has been described (Pageat 1996). It is applied daily to specific areas of the home environment and has been effective in reducing urine spraying in between 74 and 97 per cent of cases (Pageat 1996, Frank and others 1999). However, all reports published to date on the efficacy of this treatment relate to the effects during initial application of the product. This short communication describes the long-term follow up of 43 cats originally involved in a study by White and Mills (1997) examining the efficacy of F3, without behaviour therapy, on chronic urine spraying problems (range four to 120 months). In this group, it was found that urine spraying was reduced in 91 per cent of cases during the first five weeks of treatment. All cats involved in the follow up had shown some improvement during this original study.

A telephone interview was conducted with owners approximately 10 months after they had completed the original trial (White and Mills 1997). At this time they were asked when they had last used the pheromone treatment, how frequently they used it, how frequently their cat sprayed and when it had resumed spraying if appropriate.

Six cats were still not spraying at this time; in the remaining 37 cases, 27 were still spraying at a lower rate than they had at the commencement of the trial, seven were spraying at the same rate and three had deteriorated relative to this time. Twenty-one owners had not used the treatment in the previous seven months and 13 owners were still using the pheromone treatment in the home, but no owners were continuing to use it on a daily basis. Nine owners used it only when the cat sprayed and four on an occasional basis between one and three times a week. Eleven owners reported that the urine spraying had increased slightly between one and two months after they had stopped using the spray, and a further 10 reported a similar change some time later than this. None of the three owners whose cats were spraying more than at the start of the trial, and five of the seven who reported no change in spraying frequency, had continued to use the pheromone spray, the other two were using it on an occasional basis.

These results compare favourably with those from other treatments. There are no reports on the short- or long-term efficacy of behaviour therapy alone, known to the authors, but buspirone, a serotonin-1 agonist, has been found to produce an improvement in 52 per cent (32/62) of cats. However, a relapse rate following the cessation of treatment was found in 50 per cent of cases (Hart and others 1993).

While approximately 55 per cent of cats prescribed the gamma-aminobutyric acid agonist diazepam for this condition improve initially, 90 per cent reportedly relapse when medication is stopped (Marder 1991, Cooper and Hart 1992) and progestogens, such as medroxyprogesterone acetate and megestrol acetate are reportedly effective in only about 30 per cent of cases (Hart 1980). In addition, there are many potential adverse effects associated with the use of these drugs (Overall 1997). By contrast, there are no reported medical complications associated with the use of pheromone therapy and in this study, 77 per cent of cases were still under adequate control 10 months following treatment. While only six (14 per cent) cases were reported not to spray at this time, the continued lower incidence of spraying in approximately 63 per cent of cases, combined with a low overall use of the treatment which had previously been effective, would suggest that spraying was now at an acceptable level to these clients. Since the aim of behaviour therapy is to resolve the problem for the client rather than eliminate the behaviour per se (Askew 1997), these improved cases should not be considered a failure.

The results of this study suggest that treatment with the F3 analogue results in a long-term change in spraying behaviour by some cats. This treatment appears to be more effective in both the short and long term than other physical or behavioural therapies reported for the control of non-sexual urine spraying in the cat, and with no known medical complications or contraindications and a relatively easy application procedure, should be considered a first line of treatment.

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Prevalence of shiga toxin-producing *Escherichia coli* in healthy slaughter pigs in Switzerland

R. STEPHAN

THE importance of the shiga toxin (Stx)-producing *Escherichia coli* (STEC) group has increased since a foodborne infection caused by STEC was first reported in 1983 (Riley and others 1983). The pathogenicity of STEC in human beings is associated with diverse virulence factors. The main factors are the ability to form cytotoxic exotoxins (Stxs), which can be subdivided into a shiga toxin 1 (Stx1) and a shiga toxin 2 group (Stx2 group), as well as producing attachment-effacement lesions and the presence of an enterohaemolysin gene (EHEC-*hlyA*) (Boerlin and others 1999).

The source of STEC foodborne infection has often been found to be foods of bovine origin or other faecally cross-contaminated foods. Cattle are currently considered the main reservoir of STEC pathogenic to human beings. The results of a Swiss study of faeces of healthy beef cattle at slaughter showed a STEC carriage rate ranging from 2.3 to 23.7 per cent (Stephan and others 2000).

In addition, there are some reports on the isolation of Stx-positive *E. coli* from pigs; however, the strains that were tested often originated from pigs with diarrhoea or oedema disease (Moon and others 1999, Osek 1999). Although *E. coli* of the serogroups O138, O139 and O141 producing the Stx2e variant can cause oedema disease in pigs, they are generally considered to be non-pathogenic to human beings. However, Beutin and others (1993) and Caprioli and others (1993) reported a STEC prevalence of 7.5 per cent and 7.8 per cent, respectively, in healthy pigs. Heuvelink and others (1999) found O157:H7 strains in two of 145 healthy pigs. Moreover, since a high

degree of genetic relatedness between O101 strains harbouring *stx2e* genes of human and porcine origin was demonstrated (Franke and others 1995), the role of pigs as asymptomatic carriers of STEC in the epidemiology of human disease needs further research. The aim of this study was to investigate the prevalence of STEC in healthy pigs at slaughter in Switzerland and to characterise such strains by subtyping the toxins and by determining additional virulence factors.

In a prospective study, 216 faecal samples from fattened pigs at slaughter were screened by PCR to detect Stx-encoding genes. The population consisted of pigs from nine different farms. After enrichment for six hours in brilliant green bile broth (BBL), the samples were subcultured on MacConkey's agar (Oxoid) and incubated at 37°C for 18 hours. The colonies were washed off in 0.85 per cent saline. This plate eluate was then evaluated by PCR with primers based on sequences targeting a region conserved between the *stx1* and *stx2* genes. Sequences of primers and cycling conditions have been described by Burnens and others (1995). Bacterial DNA was prepared by incubating 2 µl of washed-off cultures in 42 µl aqua bidest for 10 minutes at 100°C. Amplifications were performed in a total volume of 50 µl containing 200 µM deoxyribonucleoside-5'-triphosphate, 30 pmol of each primer, 5 µl of 10-fold concentrated polymerase synthesis buffer and 2.5 U of Taq-DNA-polymerase (Promega). The amplified products were visualised by gel electrophoresis in 1.8 per cent agarose agar stained with ethidium bromide. *E. coli* EDL 933 was used in each run as a positive control, and *E. coli* U4-41 was used as a negative control. In 13 selected positive samples, the eluate was cultured again on sheep's blood agar, and at least 10 single colonies each were tested by the same PCR protocol in order to obtain STEC isolates. Only one Stx-producing isolate per sample was then subjected to further typing.

The isolates were biochemically confirmed as *E. coli*, on the basis of acid production from mannitol, o-nitrophenyl-β-D-galactopyranoside test, hydrogen sulphide and indol production, proof of urease and lysine decarboxylase, and were tested for sorbitol fermentation and β-D-glucuronidase activity on Fluorocult agar (Merck). The genotype of the Stx B subunit and the EHEC-*hlyA*, *eae* and *astA* (heat-stable enterotoxin of enteroaggregative *E. coli*) genes were determined by separate PCRs, using primers and cycling conditions described previously (Schmidt and others 1994, Rüssmann and others 1995, Yamamoto and Nakazawa 1997). Serotyping of somatic antigens was performed with polyvalent antisera to O8, O9, O20, O64, O101, O138, O139, O141, O147, O149 and O157.

The PCR product of Stx-encoding genes was detected in 42 (19.4 per cent) of the 216 samples analysed in this study. A clustering of positive samples could be found in some herds with prevalences ranging from 0 per cent (one herd) to 26.3 per cent.

Thirteen STEC isolates from faecal samples of 13 different pigs of seven of the nine herds were further characterised. Serotypes O138, O139 and O141, which are commonly associated with oedema disease or diarrhoea in the pig, were not found. Moreover, none of the tested isolates belonged to serogroups O101 or O157. Further results of strain characterisation are summarised in Table 1. Phenotypically, 12 of the 13 isolates were both sorbitol and β-D-glucuronidase positive. Only one isolate was α-haemolysin positive. Subtyping of the *stx* genes of the isolates by using Stx1- and Stx2-specific primers showed that one isolate possessed *stx1*, 11 isolates *stx2e* and one isolate possessed *stx2* and *stx2e* genes. Osek (1999) found only the Stx2e variant in 78 Stx-PCR positive isolates, where the isolates in that study originated from 76 pigs with diarrhoea and two healthy pigs. The *eae* and the EHEC-*hlyA* genes, which are strongly correlated with symptomatic disease in human beings, were present in the isolate harbouring the *stx1* gene. None of the other isolates was posi-

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TABLE 1: Characterisation of 13 shiga toxin-producing *Escherichia coli* isolates from faecal samples of healthy pigs at slaughter

Number of isolates	α-Hly phenotype	S/G	Stx-type	Serotyping*	Additional virulence factors <i>eae</i>	EHEC- <i>hlyA</i>	<i>astA</i>
1	-	+/+	Stx1	-	+	+	-
5	-	+/+	Stx2e	-	-	-	-
1	-	-/-	Stx2e	-	-	-	-
4	-	+/+	Stx2e	-	-	-	+
1	-	+/+	Stx2/Stx2e	-	-	-	-
1	+	+/+	Stx2e	-	-	-	-

* Tested with polyvalent antisera I, II, III (O8, O9, O20, O64, O101, O138, O139, O141, O147, O149 and O157)

S Sorbitol fermentation, G β-D-glucuronidase, Stx Shiga toxin, EHEC-*hlyA* Enterohaemolysin gene, α-Hly α-haemolysin, + Positive, - Negative

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