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When cat rescue fails — Part 1. Lion-clip shaving, enilconazole bathing and oral itraconazole treatment failed to control an iatrogenic *Microsporum canis* outbreak in a shelter: but a novel ophytrium-chlorhexidine shampoo and mousse treatment succeeded in the home

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Abstract

Objective: To identify the reason(s) for failure to prevent repeated nosocomial dermatophyte infection of shelter cats and to present alternative strategies which are less stressful to the cats and humans involved.

Animals: The Dorset branch of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) seized 30 of 32 Persian cats from a cat breeder on the 2nd of August 2019 and put them into Ashley Heath RSPCA shelter. Two cats and three dogs were left at home. All 30 cats were returned to their guardian in July 2020. Three cats died between 2020 and 2022, leaving 29 cats which were seized on the 8th of March 2022 and put into two RSPCA shelters. The dogs were not seized. Four cats were later euthanased.

Methods: RSPCA Animal Treatment Reports along with photographs used for evidence in the court case against the breeder and the veterinary records of their primary veterinary surgeon were retrospectively reviewed.

Results: Clinical signs of dermatophytosis occurred on the indexcase one week after dematting with clippers in August 2019. *Microsporum canis* (*M. canis*) was cultured from 27 of 30 (90%) cats on the 39th day of the Ashley Heath shelter stay in 2019. *M. canis* infection could only have been acquired while in RSPCA care because the incubation period from infection to development of clinical signs for *M. canis* is one to three weeks, and there was no history of ringworm in their home.

Efforts to control the ringworm outbreak in the shelter included lion clipping, weekly enilconazole bathing and oral itraconazole (EI treatment) for five or seven weeks until culture negative. Six cats became culture positive again in 2020. Cats were housed in pairs in 2019 to 2020 and singly in 2022, therefore transmission was indirect (i.e. via fomites).

Two cats remained positive when the cats were returned to their guardian in 2020, all 30 were treated with a single ophytrium-chlorhexidine (Douxo S3 Pyo) shampoo followed by ophytrium-chlorhexidine mousse rubbed into their fur for three weeks. It was reasonable to conclude that the ophytrium-chlorhexidine treatment had cleared the *M. canis*

infection for the following six reasons: first no cat developed clinical signs of dermatophytosis during the 20 months in their home. Second, no obvious ringworm lesions were seen on the photographs taken for evidence and third, no veterinary surgeon noted in the records any lesions suspicious of dermatophytosis during the examination of the cats within days of them being seized again in March 2022. Fourth: no positive dermatophyte results were produced as evidence during the trial (ringworm charges against the owner were dropped). Fifth: three of four dermatophyte tests performed on EI untreated cats were negative and samples from the positive cat were after three months in the shelter. Sixth: no EI treatment was administered until the cats had been in the shelters again for two and a half months. Nevertheless, shelter staff assumed ringworm infection from the arrival of the cats in 2022 and again subjected them to the stressful shaving/bathing/itraconazole protocol.

Clinical relevance and conclusions: This is the first report of ophytrium-chlorhexidine for treatment and prevention of *M. canis* in cats. We recommend shelter staff be trained in basic barrier nursing and be educated regarding the difference between true dermatophyte infection versus fomite carriage.

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Introduction

Dermatophytosis is an important shelter-associated infection^{Moriello, 2014; Moriello et al, 2020; Mozes et al, 2017; Newbury et al, 2007; Newbury et al, 2011; Newbury et al, 2015} and outbreaks can lead to widespread infection of animals (especially cats), human health risk, and disruption of shelter activities, including shelter closure. Although around 41 species of fungus have been reported in cats,^{Frymus et al, 2013} *Microsporum canis* (*M. canis*) is responsible for the majority of cases of feline dermatophytosis.^{Mancianti et al, 2003} *M. canis* usually causes a mild, self-limiting infection in cats, with multifocal alopecia and scaling, typically on the ears, face, head and legs.^{Nuttall et al, 2008; Moriello, 2020a} *M. canis* is a zoonotic infection.

The environment can be contaminated by *M. canis* arthrospores both by symptomatic animals and through asymptomatic *M. canis* carriage;^{Mancianti et al, 2003} arthrospores are highly resistant and can survive in dry environments for up to between nine and thirteen months, although in high humidity they lose infectivity within days.^{Moriello, 2020a} Fomite carriage is important in feline dermatophytosis because it can lead to fungal cultures which are falsely positive in the sense that,

although a dermatophyte was accurately cultured, the spores were present on the fur due to contamination from the environment, not because the cat was genuinely infected. ^{Moriello, 2014} This misinformation can, in turn, lead to misdiagnosis and/or unnecessary prolonged treatment, or even euthanasia. ^{Moriello, 2014; Moriello, 2020a} Environmental decontamination is an essential part of the treatment of cats with *M. canis*. ^{Moriello, 2020a} The removal of naturally infective material minimises the risk of disease transmission via fomite contamination, and eradication of environmental spores minimises problems with positive fungal culture mis-interpretation. ^{Moriello, 2014; Moriello, 2020a} Successful dermatophyte prevention and decontamination of shelters ^{Moriello, 2014; Moriello *et al*, 2020; Mozes *et al*, 2017; Newbury *et al*, 2007; Newbury *et al*, 2011; Newbury *et al*, 2015} and household environments ^{Moriello, 2019} is well documented.

Ringworm is more common in Persian cats than other breeds of cat, ^{Hnilica & Medleau, 2002} possibly due to ineffective grooming of their dense and long-haired coat, immunological deficits ^{Nuttall *et al*, 2008; O'Neill *et al*, 2019} or hampered ability to groom ^{O'Neill *et al*, 2019} related to brachycephalic (i.e. flattened face) conformation. Brachycephaly causes upper jaw dental malformations, mandibular prognathism and aberrant occlusal patterns of the canines and incisors ^{Schmidt *et al*, 2017; Schlueter *et al*, 2009} all of which make grooming more difficult. The flattened face also leads to chronic ocular problems, especially chronic epiphora, conjunctivitis, and facial dermatitis. ^{Schlueter *et al*, 2009} The Persian cat is one of the oldest and most popular cat breeds in the world and has a characteristic phenotype that was unchanged for centuries. In the last century, cat fanciers began to modify the Persian into its present-day appearance, ^{Schmidt *et al*, 2017} which is very different from the original cat who had a normal nose length. ^{Engberg, 2010} Selective breeding has exaggerated their brachycephaly: ^{Schlueter *et al*, 2009} the “Peke-faced” or “Ultra-type” Persians represent the more extreme degree of brachycephalism. ^{Schmidt *et al*, 2017; Berteselli *et al*, 2023} Currently the Cat Fanciers’ Association considers the “Peke-face” to be the modern Persian standard despite the serious health and welfare problems such conformation causes. ^{Berteselli *et al*, 2023}

Methods

Data sources

This retrospective study was based solely on historical data and we had full written consent by the owner of the cats for use of her data, therefore no ethical approval was required. The RSPCA Animal Treatment Reports for August 2019 to July 2020 and March to November 2022, the case histories of their attending veterinary surgeon, and the evidence bundle presented by the RSPCA prosecution lawyers were the sources of the data presented here.

Cats

The RSPCA seized 30 of 32 Persian cats on the 2nd of August 2019 from a breeder, accidentally missing two cats who remained at home. They intentionally left the lady’s three dogs. The 30 cats were taken to Ashley Heath RSPCA shelter, and they were returned to their home in batches from mid to late July 2020.

Three of the 32 cats died between 2020 and 2022, leaving 29 cats which were seized again on the 8th of March 2022

(including this time one of the two cats which were left behind in 2019). Thirteen cats were taken to Blackberry Farm RSPCA shelter; nine cats to Ashley Heath RSPCA shelter and seven cats were taken to Lynwood Veterinary Surgery then transferred to Ashley Heath shelter, except for Cat PJB/18 who was euthanased on the 16th of March at the veterinary practice. Cat PJB/4 was subsequently transferred from Blackberry Farm to Ashley Heath shelter. The RSPCA reference numbers for the cats began with PJB/.

Microsporum canis detection

Plucked hair samples and toothbrush combings were sent to Synlab Veterinary Pathology Group (VPG) laboratory (formerly known as TDDS), Exeter, England, for dermatophyte culture on Sabouraud Dextrose agar with chloramphenicol and actidione. The plates were incubated for up to 14 days at 30°C and *M. canis* was identified by the morphology of the colonies. After the positive culture of samples from the index case in 2019, the other 29 cats were sampled for dermatophyte culture. In June 2022, samples from PJB/2 were tested by culture (for four weeks) and histopathology at the Royal Veterinary College, London, England.

At the end of each five or seven-week treatment protocol, all treated cats were screened for *M. canis* spores by the standard technique of brushing the coat daily for 30 days with a brand-new toothbrush assigned to each cat. A cat was deemed ringworm negative after two negative dermatophyte cultures at the VPG laboratory, at least one week apart.

Following the initial outbreak, shelter staff were vigilant about lesions which might be attributable to *M. canis*, and they monitored ringworm by a combination of scanning suspicious lesions on the coat with a Wood's lamp, hair plucking and in-house culture tests Kruuse Dermatophyte (Kruuse, Langeskov, Denmark), or RapidVet-D Companion Animal (dms laboratories inc, Flemington, NJ 08822, USA) in addition to sending samples to VPG laboratory.

Statistical analysis

Statistical analysis was by Fisher exact test, using the online calculator:

<https://www.socscistatistics.com/tests/fisher/default2.aspx> with significance set to $p < 0.05$.

Results

Microsporum canis outbreaks in the Ashley Heath shelter in 2019-2020

All 30 cats were put into the Ashley Heath shelter in 2019. The index dermatophyte case in this group of cats was Cat PJB/27 where on the 15th of August 2019 the first clinical sign of ringworm—a small area of alopecia on the right side of his face—was noticed one week after a general anaesthetic, dental and de-matt by the RSPCA veterinary surgeon in their Bournemouth clinic on the 7th of August 2019. The first dermatophyte test was conducted on Cat PJB/27 on the 1st of September: Wood's lamp was negative, but culture was positive. On the 10th of September 2019, 39 days after the cats were seized on the 2nd of August, the other cats were sampled for culture: *M. canis* was isolated at the VPG laboratory

from the plucked fur of 27 of the 30 cats. The cats had no previous history of ringworm. Since the incubation period from infection to development of clinical signs for *M. canis* is seven to 21 days,^{Frymus *et al*, 2013} the infection was most likely from the clippers used in the de-matting or acquired in the shelter.

In early 2020, six cats became re-infected. The records state that Cat PJB/9 was located in the RSPCA “grooming room” on January 21st and on the 23rd of January 2020 positive fluorescence was seen on hair shafts under Wood’s lamp: the cat had been culture negative in October 2019 therefore the source of re-infection was likely the grooming room. The cats were returned to their guardian in July 2020: two cats (PJB/12 and PJB/29) were still known to be infected with *M. canis* but were returned despite it being a zoonotic infection.

The cats were housed in groups of two in the 2019-2020 seizure, and in solitary pens in 2022; therefore *M. canis* transmission in the shelter was indirect, via fomites.

Microsporium canis outbreaks within the shelters in 2022

There were no positive culture test results in the records prior to samples taken in the second half of June 2022 (by which time many of the cats had been EI treated) when Cat PJB/4 tested positive on the 30th of June and Cat PJB/10 was positive on either the 16th or 30th of June (the records did not specify which). One of the two test cultures of PJB/20 became overgrown by bacteria and a new sample was taken on 21st of July, 2022, but the results were not recorded. Results of other cats tested in May or June were either negative or not recorded (Table 1).

Ashley Heath shelter. By the time they were first tested for *M. canis* in June 2022, 12 of the 16 cats at Ashley Heath shelter had already had EI treatment beginning in May 2022. Only one of the four cats (PJB/4) which had not been treated prior to testing was positive (he had been moved to Ashley Heath from the Blackberry Farm shelter).

Cat PJB/3 tested negative in June 2022, despite not having been treated for dermatophytes according to his records, but he was positive on the 30th of September 2022. However, from the 22nd of July to the 14th of September Cat PJB/3 was in Magnolia House Veterinary Clinic being treated for a respiratory condition and feline coronavirus, so theoretically he could have become infected with *M. canis* there, rather than in the two weeks back at the shelter.

Blackberry Farm Shelter. The Animal Treatment Records for the cats taken to this shelter were either very poorly kept or heavily redacted. In March 2022, the records of the 13 cats taken to the Blackberry Farm shelter stated that samples in dermatophyte transport medium had already been taken and they were awaiting results, but no results were ever recorded in the Animal Treatment Reports, nor were any presented as evidence in court, therefore these record entries seem to be based on either an assumption made by the attending veterinary surgeon or on misinformation given to her.

Samples from Cat PJB/1 were sent for culture on the 6th of April 2022 despite being Wood’s lamp negative, but the result was not recorded. Cat PJB/4 was transferred from Blackberry Farm to Ashley Heath shelter where he first tested positive on the 30th of June 2022. Eight cats were tested by culture on the 1st or 18th of May 2022, but the results were either not recorded or were later redacted (Table 1). The other three cats were not tested until June 2022.

All 12 remaining cats were tested on the 16th and/or 30th of June 2022: PJB/10 was recorded as being positive on one of those tests (which one was not specified) and an itraconazole course was started on the 6th of July 2022. It was impossible to determine from the records whether or not all the cats at Blackberry Heath were treated with the full EI course (see below).

Table 1. *Microsporium canis* tests and treatment dates in 2022

Shelter	Cat	<i>M. canis</i> testing						<i>M. canis</i> treatment			
		Date	Culture	Date	Culture	Date	Culture	Lion clip date	Weekly enilconazole start date	Itraconazole start date	
BF	PJB/1	6.4.22	NR	16.6.22	NR	30.6.22	NR	6.4.22	5.5.22 only recorded once	5.5.22 one dose recorded	
L AH	PJB/2	27.6.22	Negative after 4 weeks culture (RVC), post-mortem						29.3.22	17.5.22 - 22.6.22	25.5.22 - 22.6.22
L AH	PJB/3	30.6.22	Negative	30.9.22	Positive			29.3.22	None recorded		
BF to AH	PJB/4*	30.6.22	Positive					8.4.22	ND due to PKD		
AH	PJB/5	30.6.22	Negative	2.11.22	Negative			3.5.22	17.5.22 (a single wash)	ND (PKD wrongly suspected)	
AH	PJB/6	29.6.22	Negative	13.7.22	NR			3.5.22	17.5.22	25.5.22	
AH	PJB/7	29.6.22	Negative	13.7.22	NR			29.3.22	17.5.22	25.5.22	
AH	PJB/8	29.6.22	Negative					3.5.22	ND due to PKD		
BF	PJB/9	18.5.22	NR	16.6.22	NR	30.6.22	NR	8.4.22	None recorded **		
BF	PJB/10*	16.6.22 & 30.6.22		One of these two tests was positive: but which one was not recorded				12.4.22	5.5.22 & 21.7.22-18.8.22	5.5.22 & 6.7.22 - 18.8.22	
BF	PJB/11*	11.5.22	NR	16.6.22	NR	30.6.22	NR	11.4.22	None recorded		
BF	PJB/12*	18.5.22	NR	24.5.22	NR	30.6.22	NR	12.4.22	None recorded	5.5.22 one dose recorded	
L AH	PJB/13	29.6.22	Negative	13.7.22	NR	10.11.22	NR	3.5.22	17.5.22	25.5.22	
BF	PJB/14*	18.5.22	NR	16.6.22	NR	30.6.22	NR	12.4.22	5.5.22 only recorded once	5.5.22 one dose recorded	
BF	PJB/15	16.6.22	NR	30.6.22	NR			12.4.22	5.5.22 only recorded once	5.5.22 one dose recorded	
AH	PJB/16	29.6.22	Negative	13.7.22	NR			3.5.22	19.5.22	25.5.22	
L AH	PJB/17	29.6.22	Negative	13.7.22	NR			10.3.22	23.5.22 - 8.7.22	25.5.22	
L	PJB/18	17.3.22	No dermatophyte lesions seen on gross post-mortem by RVC							ND	ND
L AH	PJB/19	29.6.22	Negative	13.7.22	NR			29.3.22 & 3.5.22	19.5.22	25.5.22	
BF	PJB/20*	18.5.22	NR	16.6.22 & 30.6.22	bacterial overgrowth of earlier plate, so repeat test: results NR	21.7.22	NR	11.4.22	5.5.22 only recorded once	5.5.22 one dose recorded	
AH	PJB/21	29.6.22	Negative	13.7.22	NR			29.3.22	19.5.22	25.5.22	
AH	PJB/22	29.6.22	Negative	13.7.22	NR			3.5.22	19.5.22	25.5.22	

L AH	PJB/ 23	29.6.22	Negative	13.7.22	NR			10.3.22	19.5.22	25.5.22
BF	PJB/24*	16.6.22	NR	30.6.22	NR			11.4.22	5.5.22 only recorded once	5.5.22 one dose recorded
AH	PJB/ 25	29.6.22	Negative	13.7.22	NR			29.3.22	19.5.22	25.5.22
AH	PJB/ 26	29.6.22	NR	13.7.22	NR			29.3.22	19.5.22	25.5.22
BF	PJB/ 27*	18.5.22	NR	16.6.22	NR	30.6.22	NR	11.4.22	5.5.22 only recorded once	5.5.22 one dose
BF	PJB/ 28*	18.5.22	NR	16.6.22	NR	30.6.22	NR	12.4.22	5.5.22 only recorded once	5.5.22 one dose
BF	PJB/29*	18.5.22	NR	16.6.22	NR	30.6.22	NR	6.4.22	None recorded ***	None recorded

This table shows the *M. canis* culture test dates and results (when recorded), and such treatment details as were available in 2022. Wood's lamp examinations and 2019 culture results are described in the text.

* Blackberry Farm records on the 9th or 11th of March 2022 claimed that samples had already been sent for dermatophyte culture from those cats and that they were awaiting results, possibly the attending veterinary surgeon was mistaken because no results were ever recorded, nor was any dermatophyte treatment instituted in March or April of 2022. A freedom of information request to the Blackberry Farm shelter for the test results was ignored.

** 12.5.22 record said "*Permission to restart ringworm treatment*" yet no treatment was documented.

*** Although no Imaverol baths were recorded the 15.6.22 records said "... *now no longer being bathed in case allergy component* " which implies that bathing had previously been taking place.

- AH: Ashley Heath shelter
- BF: Blackberry Farm shelter
- L: taken to Lynwood Veterinary Surgery on 8.3.22: PJB/18 was euthanased there
- L AH: taken to Lynwood Veterinary Surgery on 8.3.22. By 10.3.22 only Cats PJB/13, PJB/17, PJB/18 and PJB/23 remained there; the others had already been transferred to Ashley Heath shelter, and by 14.3.22 all except PJB/18 were also in the shelter.
- ND: not done
- NR: not recorded
- PKD: polycystic kidney disease
- RVC: Royal Veterinary College

Cat references in bold show the two cats which were returned to their owner in 2020 still known to be positive for *Microsporium canis* (*M. canis*).

The grey shaded boxes on cat reference numbers indicate which cats the owner was initially charged with "*failing to*

provide veterinary treatment in respect of ringworm.”

Microsporium canis management in the shelter was by shaving, enilconazole bathing and itraconazole dosing

Efforts to control the dermatophytosis outbreak included extensive shaving (i.e. lion-clipping, Figure 1), weekly bathing with enilconazole 100 mg/ml (Imaverol, Audevarde, France), diluted 1 in 50, (Imaverol was used off-label, although a chlorhexidine and miconazole shampoo licenced for cats was available in the UK), and Itraconazole (Itrafungol 10 mg/ml oral solution, Virbac, France) at a dose of 5 mg/kg q24h (EI protocol). All of the cats were lion-clipped / shaved at least once in both 2019 and 2022, often under sedation, sometimes under general anaesthetic, occasionally fully conscious. The EI protocol was administered for five weeks in 2019 and 2020 and for up to seven weeks in 2022.



Figure 1. A lion-clipped Persian cat (PJB/13) in the RSPCA Ashley Heath shelter in 2022.

Twenty-seven of the 30 cats tested positive in 2019 and were treated with the full EI protocol. In early 2020, six cats (which had tested negative on two consecutive cultures after treatment in 2019), became re-infected and were EI treated again. Cats PJB/12 and PJB/29 were returned to their guardian in July 2020 still infected with *M. canis*. The RSPCA record sheets of those two cats finish with the words “*highly contagious.*”

It was impossible to ascertain the exact number of re-infected cats and exactly how many had been treated because the

2022 RSPCA Animal Treatment records were heavily redacted, most laboratory test results and full records were not provided despite several freedom of information requests by their owner and an obligation to provide such information under UK data protection legislation and the Royal College of Veterinary Surgeons (RCVS) Code of Conduct (paragraphs 13.12 and 13.14). Such information as was made available is presented in Table 1.

On the 8th of March 2022 the RSPCA re-seized the remaining 29 cats (which included PJB/6 whom they had previously missed). Although no dermatophyte positive laboratory results were presented during the prosecution trial of the owner, RSPCA staff assumed that the cats were infected: the veterinary records frequently said “*Known M. canis positive*,” and so they lion-clipped all the cats (except PJB/18) in early April, but did not begin the EI protocol until late in May 2022.

Cat PJB/4 was initially at Blackberry Farm but subsequently sent to Ashley Heath shelter (due to him having polycystic kidney disease (PKD)), leaving 12 cats at that shelter. Blackberry Farm records suggest that EI treatment began on the 5th of May 2022 (58 days post-seizure), but apart from a single treatment recorded for most of the cats, it is unclear whether or not EI treatment was continued at that shelter (except for Cat PJB/10 who received a six week EI course beginning July 2022), although the cats were double tested in June 2022, similarly to the cats at Ashley Heath.

Cat PJB/18 was euthanased at Lynwood Veterinary Surgery, thus there remained 16 cats in the Ashley Heath shelter in 2022: PJB/4 had already been clipped at Blackberry Farm, and the other 15 were lion-clipped in March (n = 9) or May (n = 7) (PJB/19 was clipped in both March and May), but no cat was tested for dermatophytes prior to the end of June 2022.

Wood’s lamp examinations were performed whenever dermatophytosis was suspected: but in 2022 on only 15 occasions were samples taken for culture at the same time. Fourteen cats were examined by both Wood’s lamp and culture at the same time (Cat PJB/3 twice, giving 15 pairs of Wood’s and culture results). Two of four Wood’s lamp fluorescence positive cats were also positive by culture; none of the 11 Wood’s lamp negative results was a false negative, therefore in the hands of these operators the Wood’s lamp test was 100% sensitive but only 50% specific.

After dermatophyte treatment ended in June 2022, 12 cats in the Ashley Heath shelter were examined by Wood’s lamp on the 29th or 30th of June 2022: Cats PJB/3 and PJB/4 (both of which had not been EI treated) were noted to have fluorescence, but only Cat PJB/4 was culture positive. Cat PJB/3, who was negative in June, was subsequently positive by both culture and Wood’s lamp when tested on the 30th of September 2022, despite being housed singly. The fur of Cat PJB/5 fluoresced under Wood’s lamp on the 2nd of November 2022, but culture was negative.

Notably, one Blackberry Farm veterinary surgeon said she saw lesions that were suspicious of mycosis on eight of the cats when she lion-clipped them, but none of the other veterinary surgeons made similar comments when lion-clipping the other cats. Cat PJB/1 was negative by Wood’s lamp on the 6th of April 2022 which was after the cat was lion-clipped and the Blackberry Farm veterinary surgeon noted that there were lesions which she suspected might be due to mycosis; a sample was sent for culture, but the result was not recorded. This was the only time that both a Wood’s lamp and a culture test were recorded to have been performed for any of the 13 cats in the Blackberry Farm shelter: when the cats were tested by culture in May and June the records give no indication of a Wood’s lamp examination having been performed.

Microsporium canis management using ophytrium-chlorhexidine in the home

All 30 cats were returned to their guardian in July 2020, including two cats (PJB/12 and PJB/29) still culture positive for *M. canis*. When returning from cat shows, the cat breeder had routinely used an ophytrium-chlorhexidine containing mousse (DOUXO[®] S3 PYO Mousse, Ceva Santé Animale, Libourne, France), as recommended by her attending veterinary surgeon (JGL): this had prevented introduction of dermatophytes into her cattery in the past. Therefore, on return from the shelter, the cats' guardian treated all the cats (and her three dogs) topically by shampooing once with ophytrium and 3% chlorhexidine digluconate shampoo (DOUXO[®] S3 PYO Shampoo, Ceva Santé Animale, Libourne, France) followed by gently rubbing into the fur the ophytrium-chlorhexidine containing mousse three times a week for three weeks.

She also vacuumed her premises, disinfected the cat carriers and floor with a commercially available disinfectant and cleaner containing didecyldimethylammonium chloride 17.48 g/kg, alkyl (C12-16) dimethylbenzyl ammonium chloride 17.48 g/kg, and N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine 14.99 g/kg (Anigene HLD4V Professional Surface Disinfectant Cleaner, Byotrol, Chester, UK), and ran a Levoit HEPA air purifier.

Evidence for the success of *M. canis* prevention using ophytrium-chlorhexidine treatment and environmental hygiene

It was extremely difficult to ascertain the *M. canis* infection status of the cats from the RSPCA records for 2022 because either the RSPCA had failed to keep adequate records or the records had been deliberately redacted to remove information on this, and other, subjects. Nevertheless, the balance of evidence supports a conclusion that the cats were negative for *M. canis* when seized again in March 2022 for the following six reasons: first, in over one year following the ophytrium-chlorhexidine treatment described above which was administered on return to their home in July 2020 no cat or dog required further ringworm treatment until the cats had been in the shelters again for over two months in 2022 (by which time they had opportunity to become infected again by the shelter): this was the first evidence that the ophytrium-chlorhexidine treatment had eliminated *M. canis* spores from this cohort of cats.

Second, although all 29 cats and three dogs were examined by a Lynwood veterinary surgeon on the day of being seized on the 8th of March 2022 (to assess which required immediate veterinary attention), and examined in detail by RSPCA veterinary surgeons within three days of being seized, none of the veterinary surgeons noted lesions suspicious of dermatophytosis until after lion-clipping over one month later. Third, all 29 cats were photographed as court evidence for the prosecution with emphasis on photographs which supported evidence for the charges against the cat owner: no photograph purported to show ringworm lesions, and no photograph showed typical dermatophytosis lesions. Fourth, no positive culture results were in the records prior to June 2022: the two cats which tested positive had spent almost three months within the shelter during which they had time to become infected. No positive laboratory result was presented by the prosecution lawyers; indeed, three charges of “*failing to provide veterinary treatment in respect of ringworm*” were dropped. Fifth, dermatophytosis treatment was not begun before the 5th (Blackberry Farm) and the 19th (Ashley Heath) of May 2022 which was two months after the cats were seized.

Sixth: three of four cats were culture negative without full EI treatment. As shown in Table 1, the records clearly showed that Cats PJB/3, PJB/4, and PJB/8 were only shaved but not EI treated (due to ill health), and Cat PJB/5 was bathed only once in addition to being shaved. Samples from these four cats were taken for culture: three cats were negative, Cat PJB/4 was positive (sample taken on the 30th of June 2022). A fifth cat—Cat PJB/1—was tested on the 6th of April: the culture result wasn't recorded but a Wood's lamp examination was negative.

Two more cats were possibly uninfected: the bodies of Cats PJB/18 and PJB/2 were presented to the Royal Veterinary College (London, England) for post mortem in March and June of 2022 respectively; on both occasions the RSPCA warned the pathologist that the cats were infected with *M. canis*. The gross post mortem of PJB/18 recorded no ringworm lesions on careful examination of the skin (the RSPCA refused to share the histopathology and culture reports with the cat's owner). The full post mortem report for PJB/2 stated, "*there was no evidence of fungal organisms either on microscopic examination or culture, therefore the reported ringworm had likely resolved following treatment.*" Due to his deteriorating health PJB/2 had received only 26 days of itraconazole, not a full five to seven week course.

In summary: untreated cats PJB/3 and PJB/8 were culture negative. Cat PJB/5 was also culture negative and therefore can be counted as uninfected (unless a single enilconazole wash effected a cure, which is unlikely). Given the 100% sensitivity of Wood's lamp examinations established above it is reasonable to include Cat PJB/1 amongst the negative cats: thus we have good evidence that at least four of the ophytrium-chlorhexidine treated cats were uninfected when seized in 2022. Zero of four ophytrium-chlorhexidine treated cats (for which we know the infection status with reasonable certainty) were re-infected, compared with six of 27 EI treated cats that were re-infected in the shelter in 2020: unsurprisingly the difference was not statistically significant ($p > 0.05$), given the low numbers. However, if we assume for a moment that none of the 29 cats became re-infected in their home after the ophytrium-chlorhexidine protocol (as there was good evidence that they had not been re-infected while in their home prior to March 2022, as detailed above), and compare them with the six of 27 EI treated cats that were re-infected in the shelter in 2020, the difference would be statistically significant ($p = 0.009$).

Discussion

A retrospective study such as the one presented here is not ideal for discovering whether the ophytrium-chlorhexidine product is more efficacious than lion-clipping, enilconazole, and itraconazole for treating *M. canis* infection. Ideally, a prospective controlled study should have been done in 2019: the 27 infected cats should have been divided into two groups treated with each of the treatments while in the same environment. The next best option would have been to test all 29 cats in March 2022, to see if infection had persisted in the household since the ophytrium-chlorhexidine treatment in July 2020, but we only had convincing culture evidence for three uninfected cats in 2022 (the positive EI-untreated cat had had almost three months in the shelter in which to become infected).

"*Absence of Evidence is not Evidence of Absence*" Carl Sagan cited by Feres & Feres, 2023 is a quotation which encapsulates the difficulties presented in this paper. We endeavoured to establish whether the ophytrium-chlorhexidine protocol had

eliminated *M. canis* infection in this cohort of cats but were unable to do so to our satisfaction due to our inability to present a complete set of dermatophyte culture results for all 29 cats in March 2022, (i.e. before they could become re-infected in the shelters). The records claimed that dermatophyte tests were done by the RSPCA in early 2022, but the results were not presented. Either the RSPCA had failed to keep adequate records or the records had been deliberately redacted: given the excellent records kept in 2019-2020 the latter explanation appears to be more likely. Furthermore, we were stymied by the refusal of the RSPCA and of the Lynwood veterinary surgeon to release the full dermatophyte test results in contravention of the Data Protection Act and of the RCVS Code of Conduct, paragraph 13.14 which states that, at the request of a client, veterinary surgeons must provide copies of any relevant clinical and client records. This includes relevant records which have come from other practices, if they relate to the same animal and the same client. Withholding and redaction of records was detrimental to the defence case of the cats' caretaker.

What the written evidence did show was that there was no conclusively positive *M. canis* result prior to the end of June 2022; this was after the cats had spent almost three months in the shelters, during which they had time to become infected, and after many of the cats had been EI treated. Yet, the records indicated that the shelter staff believed all cats to be infected without any objective evidence that we could find to support this: no typical dermatophyte lesions and no positive test results. The origin of their belief may have been a witness statement by the attending veterinary surgeon expert for the prosecution who stated "*Pooled samples from all cats in each group were submitted for fungal culture. These were all positive for Microsporum Canis, a form of Ringworm, [sic] indicating that all cats were likely to be carriers for this disease.*" He had signed a statement which read, "*I shall be liable to prosecution if I have willfully stated anything which I know to be false or do not believe to be true*" which means he risked prosecution by the state and disciplinary action by the RCVS if his statement was untrue. However, if the claim of positive culture tests was true, why were the laboratory results not presented as evidence? The veterinary surgeon might have been mistaken: the statement was made over one month after he had dealt with those cats and he may have been thinking of another case. The prosecution also charged the owner for not obtaining veterinary treatment for a ruptured corneal ulcer: a charge which was also proven to be untrue by examination of her veterinary surgeon's records and yet they proceeded to include a dramatic photograph of the healed, but scarred, eye in the jury bundle.

A negative result is harder to prove than a positive one and just one positive result taken shortly after the cats were re-seized would disprove that the short ophytrium-chloride course had eliminated *M. canis* from the household. Was it possible that ringworm lesions were hidden under matted fur? In April 2022, after one month in the shelter, the cats were lion-clipped and the Blackberry Farm veterinary surgeon noted that she saw lesions suspicious of mycosis on almost all of the cats she shaved: PJB/1; PJB/4; PJB/9; PJB/10; PJB/12; PJB/14; PJB/15; PJB/28 and PJB/29. However, she examined only one (PJB/1) by Wood's lamp but saw no fluorescence. She sent samples from only PJB/1 for dermatophyte culture on that occasion, but the results were not presented in the records. However, all of the cats in the other shelter were lion-clipped (except PJB/18) and none of the other veterinary surgeons documented seeing lesions they suspected might be dermatophytosis (they did record fleas though).

The balance of evidence presented in this paper supports a conclusion that the cats seized in 2022 were free of *M. canis* infection. However, the statement of the attending veterinary surgeon does introduce a small credible doubt and it is to be

hoped that in future a prospective, blinded, controlled study of ophytrium-chlorhexidine can be conducted in a cattery with endemic *M. canis* to establish whether it is more effective than EI. Ideally, in the present study, a cross-over ophytrium-chlorhexidine protocol trial should have been done while the cohort of cats was present in the rescue shelter, rather than in the different conditions of their home environment, because it could be argued that *M. canis* infection is generally self-limiting, so that the two cats who were returned infected might have eliminated the fungus anyway; however Persians cats are notoriously difficult to treat for dermatophytosis^{Hnilica & Medleau, 2002} and may carry spores subclinically for a very long time.

The other major difference between the two EI treatment episodes in the shelter and the ophytrium-chlorhexidine protocol in their home which could have accounted for the cessation of the infection, was that the owner intentionally cleaned and disinfected the environment with the aim of eliminating *M. canis* spores when the cats were returned from the shelter; this is a step which the RSPCA did not appear to take. The RSPCA Animal Treatment reports did not contain any indication of what—if any—environmental decontamination was being performed. Another study of recurrent infection of Persian cats, where enilconazole was also used, appeared to have the same failing of not addressing the environmental persistence of *M. canis* spores. Hnilica & Medleau, 2002 described a breeding cattery of 22 Persian cats infected with *M. canis*: they were treated with topical 0.2% enilconazole repeated every three days for a total of eight applications and became culture negative by day 28. However, six months later, four cats had developed clinical dermatophytosis and all cats had positive fungal cultures. Similarly, in our study, records showed that six previously culture-negative cats became re-infected in the Ashley Heath shelter in 2020 about three months after EI treatment, and that at least one cat become culture positive in Blackberry Farm shelter in 2022, even though the cats were housed in pairs in 2019-2020 and singly in 2022, showing that there was either indirect transmission of *M. canis* within the shelters and/or that the shaving and EI protocol had failed to properly clear the cats of infection.

The reasons for repeated infections of the cats in the shelters was not established, but had to involve failure of regular disinfection of the premises along with poor barrier nursing hygiene training of the staff. Decontamination of premises is essential for dermatophytosis control and is not as difficult as people often imagine: Moriello 2020a^{seventy foster family homes where *M. canis* infected cats had lived were successfully decontaminated by cleaning with over-the-counter household detergents.} Moriello, 2019^{Hard surfaces were disinfected with 1:100 concentration household bleach or accelerated hydrogen peroxide, then rinsed.} Moriello, 2019^{A dermatophytosis outbreak in a large USA shelter was successfully eradicated within five months by removal of organic material, especially cat hair, via sweeping or vacuuming.} Newbury *et al*, 2015^{Surfaces were washed with a detergent until visibly clean and rinsed with water. Sodium hypochlorite 5.25% diluted at 1:32 was used as a disinfectant. This protocol was repeated at least twice weekly. Bedding was changed daily. Litter boxes and bowls were changed daily, washed with hot soapy water and then disinfected.} Newbury *et al*, 2015

The evidence showed that it was the RSPCA who introduced *M. canis* into this multicat household in 2019. The first clinical signs of ringworm were in Cat PJB/27 one week after a general anaesthetic, dental and de-matt by an RSPCA veterinary surgeon: this is how the cat was likely infected, and the clippers were the probable source of the infection. While in the shelter, the cats were subjected to repeated clipping, usually requiring a sedative or general anesthetic for

the procedure. Any kind of skin trauma—such as clipping—facilitates ringworm infection since the fungus cannot penetrate healthy skin.^{Frymus *et al.*, 2013} Clipping is a controversial measure that can facilitate topical therapy and remove infected hairs,^{Nuttall *et al.*, 2020} but it can also result in skin trauma, disseminate infection on the cat,^{Moriello, 2020a} and increase environmental contamination. In her 2020 review, Dr Karen Moriello stated that in her experience clipping the hair coat is not necessary: if fur has to be removed for any reason, it should be done with round-tipped metal scissors, not clippers.^{Moriello, 2020a} Matting of the fur (already a problem in Persian cats) was exacerbated due to the repeated clipping and shampooing, and—more seriously—the stress of clipping, weekly bathing and daily oral dosing was associated with many episodes of lower urinary tract disease.^{Addie & Livy, 2024} There are no controlled studies assessing the efficacy of clipping for treatment or prevention of dermatophytosis,^{Moriello, 2020a} but in the cohort of cats reported here, the ophytrium-chlorhexidine protocol used by their owner without clipping was far more effective in eradicating *M. canis* from the cats and premises than that used by the shelter.

Ophytrium is a natural ingredient extracted from the root of the *Ophiopogon japonicus* plant: topical application of ophytrium is believed to prevent the adhesion of pathogens to the skin,^{Gatellet *et al.*, 2021} although no studies have specifically shown prevention of *M. canis* spore adhesion to skin. Our study was unable to differentiate whether it was the ophytrium or the chlorhexidine component of the product that was effective. Most studies on the efficacy of topical chlorhexidine to treat *M. canis* have used chlorhexidine combinations—usually with miconazole—rather than chlorhexidine alone.^{Moriello 2020b; Moriello & Verbrugge, 2007; Hnilica & Medleau, 2002} The efficacy of chlorhexidine products has been questioned.^{Moriello 2020b; Hnilica & Medleau, 2002} However, an *in vitro* study showed that chlorhexidine alone had good efficacy against avian *Microsporium* arthrospores,^{Thongkham *et al.*, 2022} and an *in vitro* comparison of various dermatophyte products showed that chlorhexidine products required three treatments to eliminate *M. canis* spores but that they had little or no residual antifungal activity.^{Moriello, 2020b} It may have been either the chlorhexidine or the ophytrium, or a synergistic effect of both, which eliminated the spores from the infected cats the RSPCA returned to their guardian, and prevented re-infection of the cats while they were in their home. When returning from cat shows the cat breeder had routinely used a topical ophytrium/chlorhexidine antifungal to prevent ringworm introduction into her household.

A gentler and less expensive approach to treat *M. canis* than that used by Dorset RSPCA was employed in a shelter in the Czech Republic utilising the mycoparasitic fungus *Pythium oligandrum*,^{Načeradská *et al.*, 2021} which could have been obtained by the RSPCA when the cats were re-infected in 2022. A solution of *Pythium oligandrum* (Ecosin, BARD) was applied gently by stroking the cats with a glove soaked in the product two days on, two days off, for six weeks and succeeded.^{Načeradská *et al.*, 2021} Materials soaked in the product were also placed in the entrance to the outdoor areas in order to apply the product containing *P. oligandrum*. In this way, the cats were in contact with the product when passing through the entrance, including non-socialized cats for which normal handling was impossible. Therefore, timid cats did not have to be bathed in the product,^{Načeradská *et al.*, 2021} since bathing is a very stressful procedure for cats.^{Rand *et al.*, 2002}

In a retrospective study of 5,644 shelter cats 584 were dermatophyte culture positive, but only 94 culture-positive cats had lesions; the remaining 490 culture-positive cats were fomite carriers (lesion-free and negative on repeat fungal culture).^{Moriello, 2014} In the present retrospective study, the RSPCA failed to differentiate actual *M. canis* infected cats from fomite carrier cats: the former have lesions and systemic itraconazole is warranted, the latter simply have spores on their

fur and systemic treatment is unnecessary, but in 2019 all culture-positive cats were subjected to the full clipping/bathing/dosing protocol regardless of absence of lesions. In 2022 after the second seizure, the RSPCA staff and attending veterinary surgeons assumed that all the cats were infected with *M. canis* and lion-clipped them all: their notes frequently stated, “*Known M. canis positive*” even though their records provided no positive test results to verify the claim (indeed the charges the RSPCA had brought against the owner for having ringworm-infected cats were later dropped). When Cats PJB/18 and PJB/2 were submitted for post-mortem the RSPCA warned the pathologists that the cats were infected with *M. canis*, but a negative culture and histopathology showed that Cat PJB/2 was not infected and grossly no lesions were noted on the skin of PJB/18 (the RSPCA refused to release the full histopathology report in contravention of the data protection act and of the RCVS Code of Conduct).

The RSPCA records reveal that the staff became almost obsessed with ringworm to the exclusion of attending to other conditions, for example, many of the cats lost a considerable amount of weight (hundreds of grams) in the spring of 2020 which was not investigated. Cat PJB/8 was lion-clipped preparatory to being bathed with enilconazole and dosed with itraconazole but those treatments were postponed due to abdominal masses having been palpated; nevertheless a thorough toothbrush coat sample was sent to the VPG laboratory for culture because the other cats were being sampled at the end of their seven weeks therapy: it was negative even after two weeks of culture. Cat PJB/8 would have been unnecessarily subjected to the chronic stress of a seven-week course of weekly bathing and daily oral dosing if the abdominal masses had not been detected. Cat PJB/3 was subjected to two courses of treatment in 2019 even though his dermatophyte culture results were negative, as RSPCA staff thought they were seeing positive Wood’s lamp fluorescence and microscopic hair changes typical of dermatophytosis. Fomite carriage of dermatophytosis spores on cat fur is an important cause of mis-diagnosis of dermatophytosis. ^{Moriello, 2014} We do not know how many of the cats were subjected to stressful treatments for ringworm they did not have.

The heavy-handed approach of the Dorset RSPCA branch to the situation was catastrophic for the cats because they were kept in a stressful shelter environment as prosecution evidence for many years (the survivors remain in the shelters at time of writing in April 2024). The repeated failure of the shelter’s diagnostic and environmental hygiene practices for dermatophytosis resulted in unnecessary stress and suffering for the cats, incurred enormous unnecessary expense, and put their staff at constant risk of being infected with *M. canis*. Ringworm was not the only infectious disease caught in the Ashley Heath shelter.

Conclusions

So far as we are aware, this is the first report of using ophytrium-chlorhexidine to treat and prevent *M. canis* infection. Failure to eradicate ringworm spores from shelter premises, train shelter staff in basic barrier nursing techniques and to differentiate true infection from fomite carriage caused unnecessary suffering to cats seized by the RSPCA. We recommend that more gentle approaches to dermatophytosis control be used in shelters, with an emphasis on prevention, and thorough disinfection of spores in the shelter environment, rather than on the animal. Shelter workers need to become aware of the difference between culture-positive dermatophyte spore fomite carriers and truly infected cats, and to

recognise that the former do not require systemic treatment.

This report demonstrates the failure of a seizure approach to the problem of animal welfare in a multicat environment: seized animals are considered evidence in law and can be kept caged up for years, subjected to repeated infections and other stressors all of which are severely detrimental to their welfare. We recommend this approach be reserved for cases of deliberate cruelty, but animal guardians who are unable to cope for some reason should be given help, rather than prosecuted, as exemplified by the approach of another RSPCA branch documented by Hill *et al.*, 2019.

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Statements and Declarations

Disclosures: The authors are the veterinarians of the cats in this study, but we declare that we have no conflicts of interest in regard to any of the products mentioned in this paper. DDA was paid by Legal Aid as an expert witness in the cat owner's trial.

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