



Duration of carriage of multidrug-resistant bacteria in dogs and cats in veterinary care and co-carriage with their owners

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ABSTRACT

Background: The emergence and spread of multidrug-resistant organisms (MDROs) represent a threat to human and animal health.

Objectives: To assess duration of carriage of MDROs in dogs and cats presented to veterinary clinics/hospitals in Switzerland. To estimate prevalence, duration of and risk factors for MDRO carriage in their owners and the occurrence of co-carriage in owner-pet pairs.

Methods: Prospective, longitudinal, observational study. Nasal swabs and fecal samples were collected from 50 owners of dogs and cats presented to 3 large veterinary hospitals, 1 medium-sized clinic and 1 practice. If pet or owner tested positive for a MDRO, follow-up samples were collected for up to 8 months. Methicillin-resistant (MR) *Staphylococcus aureus*, MR *S. pseudintermedius*, MR coagulase-negative staphylococci (MRCoNS), MR *Macrococcus* spp., cephalosporinase- and carbapenemase-producing (CP) Enterobacterales were isolated and further characterized by MALDI-TOF MS, microdilution, β -lactam resistance gene detection, REP/ERIC-PCR, multilocus sequence typing or whole-genome sequencing. Risk factors for MDRO carriage in owners were explored based on questionnaire-derived data.

Results: Five out of 50 owners carried 3rd generation cephalosporin-resistant Enterobacterales (3GC-R-Ent.), and 5/50 MRCoNS. In 3 dogs and 4 cats carriage of 3GC-R-Ent. persisted for up to 136 days after discharge (median 99 days, IQR 83 days, range 36–136 days), in two cats isolates were carbapenem-resistant. Owner-pet co-carriage was not observed. No specific risk factors for MDRO carriage in owners were identified.

Conclusions: After discharge from veterinary care, dogs and cats may carry 3GC-R-Ent. for prolonged time periods. Carriage of MDROs was common in owners, but pet-owner co-carriage of the same MDRO was not observed.

Abbreviations: AMR, Antimicrobial resistance; 3GC-R, Third Generation Cephalosporin-resistant; 3GC-R-Ent., Third Generation Cephalosporin-resistant Enterobacterales; CI, Confidence interval; COL-R, Colistin-resistant; CP, Carbapenemase-producing; CR, Carbapenem-resistant; CRE, Carbapenem-resistant Enterobacterales; CLSI, Clinical and Laboratory Standards Institute; ERIC-PCR, Enterobacterial repetitive intergenic consensus polymerase chain reaction; ESBL, Extended spectrum β -lactamase; ESBL-*E. coli*, ESBL-producing *Escherichia coli*; ESBL-KP, ESBL-producing *Klebsiella pneumoniae*; EUCAST, European Committee on Antimicrobial Susceptibility Testing; IQR, Interquartile range; KP, *Klebsiella pneumoniae*; MALDI-TOF MS, Matrix-assisted laser desorption/ionization time of flight mass spectrometry; MDR, Multidrug-resistant; MDROs, Multidrug-resistant organisms; MICs, Minimal inhibitory concentrations; MLST, Multilocus sequence typing; MR, Methicillin-resistant; MRCoNS, Methicillin-resistant coagulase-negative staphylococci; MRSA, Methicillin-resistant *Staphylococcus aureus*; MRSP, Methicillin-resistant *Staphylococcus pseudintermedius*; pAmpC, Plasmid-encoded AmpC; REP-PCR, Repetitive element palindromic polymerase chain reaction; ST, Sequence type; TMP-S, Trimethoprim/sulfamethoxazole; WGS, Whole-genome sequencing.

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1. Introduction

Antimicrobial resistance (AMR) is a threat to both human and animal health. Bacteria are considered multi-drug resistant if they have acquired non-susceptibility to at least one agent in three or more antimicrobial categories [1]. Dogs and cats are considered family members in many households and may be a source of multidrug-resistant organisms (MDROs) [2]. Dogs and cats share the owner's living space and outdoor environment and exchange affectionate behaviours with their owners which commonly involve very close physical contact or contact with saliva [3]. In a recent study from Belgium, 98.3% of respondents indicated that highly intense interactions such as licking of the owner's face or hands and eating from the owner's plate occurred at least sometimes [4]. While a close emotional connection with their pet benefits the owner's physical and mental wellbeing [5,6], such high intensity interactions may lead to the transmission of microorganisms, in particular those residing on the skin, on mucosal surfaces [7] or in the intestinal tract [8].

Due to the close human-animal bond, pets also benefit from advanced hospital care in case of illness or accident. Like human hospitals, veterinary hospital environments favor the selection and transmission of MDROs due to a high density of susceptible patients and the selective pressure exerted by the use of broad acting antimicrobials [9]. During their hospitalization, small animal patients may therefore acquire MDROs [10,11]. Understanding the risk of cross-species transfer of MDROs or relevant resistance genes between MDRO carrying pets and their owners is a central element when tackling the One Health aspects of AMR [8,12–14].

As part of a large multicenter project to assess the role of companion animal medicine in the selection and dissemination of MDROs, we recently reported a high rate of acquisition of 3rd generation cephalosporin-resistant Enterobacterales (3GC-R-Ent.) by cats and dogs in veterinary hospitals in Switzerland [11]. Acquisition of genetically closely related 3GC-R-Ent. and carbapenem-resistant (CR) *Escherichia coli* (Sequence type [ST]410; *bla*_{OXA181}-positive) in a subset of cats and dogs included in that investigation was recently reported [10].

In this part of the project, we explored the duration of carriage of MDROs in dogs and cats in veterinary care and determined potential co-carriage of the same MDROs as well as the prevalence, duration and risk factors of MDRO carriage in their owners.

2. Materials and methods

2.1. Ethics statement

Ethical approval for collection of samples and data from humans, cats and dogs was obtained from the regional Ethics Committees on research involving humans (Ref. 2018–00866) and the Veterinary Office (Ref. BE 16/18) as required. Written consent for collection of oronasal and rectal swabs/stool samples and the use of patient and pseudonymized questionnaire-derived data was obtained from owners before enrolment in the study. For pseudonymization, the names of animals and owners were replaced by enrolment codes. Study data were collected and managed using the Research Electronic Data Capture system (REDCap) hosted at the University of Bern [15,16].

2.2. Study design and setting

This investigation was part of a prospective multi-center longitudinal observational study to elucidate the role of veterinary hospitals in the selection and spread of MDROs. The study was conducted in Switzerland between May 2018 and March 2019 at 3 large referral hospitals (clinics 1–3), 1 medium-sized clinic (clinic 4) and a small practice delivering predominantly outpatient care (practice 1).

At presentation of their animal to a veterinary clinic or practice owners were invited to participate in the study by filling in a pet-

centered and owner-centered risk-factor questionnaire and by submitting nasal swabs and fecal samples for culture. In addition, their dogs and cats were sampled via oro-nasal and rectal swabs at presentation and discharge from the veterinary hospitals as previously described [11]. If either owner or pet carried a MDRO, owners were invited to participate in a follow-up study, which foresaw monthly resampling until at least two negative results had been obtained. The three parts of the study presented here and the populations of dogs, cats and owners enrolled in the different parts of the study are shown in Fig. 1 (Study design).

2.3. Data collection

2.3.1. Questionnaire

Participating owners were given a hardcopy and a link to the questionnaire [11]. The owner-centered questionnaire retrieved demographic, lifestyle and general health data, dietary habits, current or previous medical treatments, travel history and exposure to farm animals as well as questions assessing the closeness of the contact between owners and their animals, such as sharing the same bed. The results of the animal-centered questionnaire and their association with MDRO carriage have been previously reported [11].

2.3.2. Sampling

Owners were given detailed instructions and written information on how to perform their own nasal swabbing and stool sample collection. They also performed the follow-up oronasal and rectal sampling of their pet as previously described [11]. Rectal and oronasal swabs were collected with dry sterile swabs with Amies transport medium (Sarstedt AG & Co. KG, Nümbrecht, Germany). Swabs and fecal samples were sent to the laboratory (IFIK Bern) in padded envelopes with pre-paid priority shipping.

2.3.3. Isolation and identification of bacteria

Bacteria were isolated and identified as previously described [10,17]. Briefly, rectal swabs and fecal samples were tested for the presence of Gram-negative MDROs. Swabs were placed into 5 ml of non-selective Luria-Bertani (LB) enrichment broth at 37 °C for 24 h. A loopful of the culture was then streaked onto ChromID® extended spectrum β-lactamase (ESBL) plates to select for 3GC-R, or ChromID® OXA-48 and ChromID® CARBA plates (bioMérieux SA, Marcy-l'Étoile, France) to select for CR bacteria. Plates were incubated at 37 °C for 24 h under aerobic conditions. Colonies were sub-cultivated onto tryptone soy agar plates containing 5% sheep blood (TSA-S; Becton & Dickinson Company, Franklin Lakes, USA). Carbapenemase production was assessed using the Blue-Carba test [18].

Oro-nasal swabs were tested for the presence of methicillin-resistant (MR) staphylococci and macrococci using a two-step selective enrichment followed by selection on MR *Staphylococcus* (*S.*) *aureus* (MRSA) selective agar (BBL CHROMagar MRSA II, Becton, Dickinson and Company) at 37 °C for 24 h.

Isolates were identified to the species level using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) analysis (Bruker Daltonics GmbH, Bremen, Germany). Further characterization of human MR staphylococci including antimicrobial susceptibility testing, sequence typing and identification of *mecA* was only performed for 2/6 isolates.

2.3.4. Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) of a panel of antimicrobials were determined by broth microdilution using Sensititre EUST, EUVSEC, EUVSEC2 and GNX2F plates (Thermo Fisher Scientific, Waltham, USA) following guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) [19,20].

Extended spectrum β-lactamase and carbapenemase genes were

identified using the new CT103XL microarray (Check-Points, Wageningen, The Netherlands [21]). Prior to whole genome sequencing (WGS), carbapenemase-producing (CP) isolates were tested for the presence of *bla*_{OXA-48-like} genes by PCR [22]. The methicillin resistance genes *mecA*, *mecB*, and *mecD* were identified by PCR as previously described [23–25].

2.3.5. Rep-PCR and whole genome sequencing

Genetic relationships and clonality between isolates of the same species were determined by Repetitive element palindromic/enterobacterial repetitive intergenic consensus polymerase chain reaction (REP/ERIC-PCR) [26,27] and multilocus sequence typing (MLST) for MRSA, MR *Staphylococcus pseudintermedius* (MRSP), MR coagulase-negative staphylococci (MRCoNS), MR *Macrococcus* spp. using the corresponding schemes published in the pubMLST database (<https://pubmlst.org/databases/>) and for colistin-resistant (COL-R), ESBL-producing and CP Enterobacterales using the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>). Fifteen Gram-negative *E. coli* strains from dogs and cats included in this part of the study and six *E. coli* isolates from owners were further characterized via WGS. Whole genome sequencing was used to confirm the identity of selected isolates and the presence of specific resistance genes.

2.4. Statistical methods

Statistical analysis was performed using the NCSS® program (NCSS11 Statistical Software. 2016. NCSS, LLC, Kaysville, Utah, USA. [ncss.com/software/ncss](http://www.ncss.com/software/ncss)).

The overall prevalence of MDRO carriage in owners was calculated with a 95% confidence interval (CI). Acquisition was defined as the presence of a genetically unrelated MDRO species or strain in follow-up samples. Persistence was defined as isolation of MDROs with matching molecular profiles (REP-PCR, WGS) and antimicrobial resistance profiles from repeated samples of the same individual.

Associations between MDRO carriage in owners and specific questionnaire-derived variables were examined using univariable regression analysis. As no variables with a *p*-value of <0.1 were identified, multivariable models could not be built.

If either owner or her/his pet carried MDRO, the total number of contact days (days after hospital release of the animal until the last sampling time point) was calculated in order to estimate opportunities for transmission.

3. Results

3.1. Study populations

3.1.1. Pet owners

A total of 50 owners belonging to 46 households provided nasal and stool samples and 38 (76%; 95%CI, 61.8–86.9) of them filled in the questionnaire.

Demographic and questionnaire-derived owner data are shown in Supplemental Table 1. The median age of participating owners was 50 years (interquartile range [IQR] 17; range 25–79 days); 21 (55.3%; 95% CI, 28.2–56.8) owners were women and 17 (44.7%; 95%CI, 21.2–48.8) were men. There was an equal distribution of owners living in cities, small towns and the countryside. The majority of owners (73.7%) lived in the proximity (<1 km) of at least one farm and 71.1% had received antimicrobial therapy in the past two years. Of the 38 respondents, 2 (5.3%) worked in the human healthcare system and 30 (79%) had been in contact with the healthcare system (as patients) in the past 12 months. None of the respondents had been diagnosed with MDRO infections in the past two years.

3.1.2. Dogs and cats

A total of 271 animals including 183 (67.5%) dogs and 88 (32.5%) cats were enrolled in the original screening study. Of these, 34 MDRO-positive animals (25 dogs, 9 cats) were included in the study on

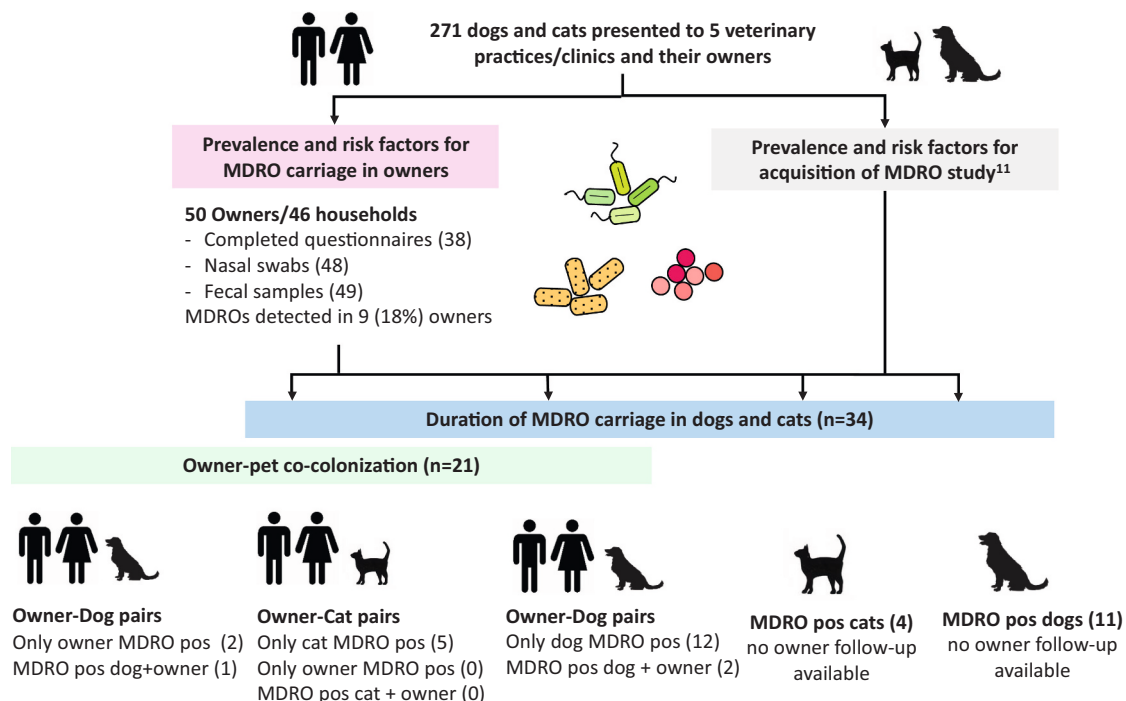


Fig. 1. Study design. Out of a population of 271 animals enrolled in a large prevalence study, 25 dogs and 9 cats, which had tested positive for MDRO carriage and 2 dogs belonging to a MDRO positive owner were followed up by nasal and rectal swabbing for up to 8 months. 50 owners of animals participating in this study submitted nasal swabs and/or fecal samples, 38 submitted questionnaires. Five owner-cat pairs and 16 owner-dog pairs were followed to monitor for co-colonization. The part of this study in the grey box has been previously reported [11]. Abbreviations: MDROs, multidrug-resistant organisms.

duration of carriage and 21 pet-owner pairs were included in the co-carriage study (Fig. 1).

Demographic, hospitalization and treatment data of the 37 animals included in the follow-up and co-carriage study are shown in Table 1. Of the 28 dogs, 6 dogs were mixed breeds and 22 pure breeds, belonging to 19 different breeds, the most common being cotton de Tuléar ($n = 2$), Jack Russell Terrier ($n = 2$) and French Bulldog ($n = 2$). All dogs were family dogs and slept in the owner's bed. Of the 9 cats, 4 were European shorthaired, and one each of the following, Maine Coon, Persian, Siberian, Exotic long haired and Bengal. All cats slept in or on their owner's beds.

A subgroup of 7/34 (21%; 95%CI, 8.7–37.9) pets included in the duration of carriage study showed long-term carriage of 3GC-R-Ent. (>30 days; Fig. 2). These animals had a broad range of underlying clinical problems. Five animals had been treated with potentiated aminopenicillins, one with fluoroquinolone and 1 had not been treated with antibiotics. There was no significant association between duration of antimicrobial treatments, antimicrobial class and MDRO persistence.

Table 1

Demographic and treatment data of dogs and cats included in the follow-up and the co-carriage study.

Parameter	Dogs ($n = 28$)	Cats ($n = 9$)
Median age; years (IQR)	6.5 (4.8–8)	9 (4.3–12.2)
Median weight; kg (IQR)	15.2 (16–28.2)	4.8 (4–5.4)
Sex		
Female (entire/neutered)	8/6	0/2
Male (entire/neutered)	5/9	0/7
Diagnoses		
Gastrointestinal disease	6	2
Neurological disease	7	1
Urinary tract disease	1	3
Orthopedic/traumatic disease	5	1
Endocrine disease	1	0
Dermatologic disease	1	0
Infectious disease	2	0
Immune-mediated disease	2	0
Unknown	2	1
Other	1	1
MDRO status at presentation (pos/neg; n)	8/20	2/7
Hospitalized (yes/no)	27/1	8/1
Hospitalization days; median (IQR)	3 (2.5–3.7)	3.5 (1.6–6.4)
Admitted to ICU (yes/no)	15/9	4/4
ICU days; median (IQR)	2 (1.6–3.3)	4.5 (0.3–9.9)
Antimicrobial treatment during hospitalization (yes/no)	21/6	6/2
Antimicrobials used during hospitalisation ^a		
Amoxicillin/clavulanic acid	2	3
Ampicillin/sulbactam	6	3
Cefazolin	7	0
Cephalexin	1	0
Clindamycin	2	0
Doxycycline	1	0
Enrofloxacin	4	0
Marbofloxacin	0	1
Metronidazole	1	0
TMP-S	1	0
Antimicrobials used after discharge (yes/no)	11/16	6/2
Amoxicillin/clavulanic acid	5	6
Cephalexin	2	0
Clindamycin	1	0
Doxycycline	1	0
Enrofloxacin	2	0
Median duration of antimicrobial treatment post discharge; days (IQR)	7 (4.1–10.9)	8 (3.8–12.2)

ICU: intensive care unit; IQR: interquartile range; TMP-S: Trimethoprim/sulfamethoxazole.

^a Includes mono- and combination therapy.

3.1.3. Prevalence, MDRO isolates and risk factors for MDRO carriage in owners

MDROs were isolated in 9/50 owners (18%; 95%CI, 8.6–31.4) at any sampling time point during the study period.

ESBL-producing *E. coli* (ESBL-*E. coli*) (producing CTX-M-55, $n = 2$; CTX-M-15, $n = 2$) were isolated from fecal samples of four owners. A further 4 owners showed nasal colonization with MRCoNS (*S. epidermidis* $n = 2$; *S. haemolyticus* $n = 2$). In one family member (149 B), MRSA (ST97) and a fecal CTX-M-1-producing *E. coli* were isolated. At follow up 3 months later, the owner carried a nasal MR *S. warneri* and a different fecal *E. coli* strain (producing a CTX-M-14/–24-like). The person was healthy, did not work in the healthcare sector and had no history of international travel. A second person in the same household tested negative for MDRO on a single sample.

The full list of variables included in the risk factor analysis are shown in Supplemental Table 1. None of the variables were significantly associated with MDRO carriage in owners. Details and MICs of isolates are shown in Supplemental Tables 2 and 3.

3.1.4. Duration of MDRO carriage in dogs and cats

A subgroup of 34 MDRO-positive pets were followed up for a median duration of 150 days (IQR 58; range 45–247 days). Of these, 7 dogs and 3 cats carried MR Gram-positive bacteria (8 MR staphylococci and 1 MR *Macrococcus (M.) canis*) oro-nasally. In 12 dogs and 4 cats, one 3GC-R-Ent. strain was isolated from a rectal swab. In 8 dogs and 2 cats, 3GC-R-*Klebsiella pneumoniae (KP)* and *E. coli* were co-isolated. MR staphylococci and 3GC-R-Ent. were isolated simultaneously in 1 cat. The details of the 3GC-R-Ent. isolates from animals included in this study are shown in Supplemental Tables 4 and 5.

The median time between discharge and first follow-up was 81 days (IQR 32, range 34–164 days). No persistence was found in any of the 10 animals carrying MR *Staphylococcus* spp. or MR-*M. canis* when resampled between 60 and 164 days after discharge.

Of the 27 animals carrying 3GC-R-Ent. at discharge, 7 were still carrying one ($n = 6$) or two ($n = 1$) 3GC-R-Ent. isolates at their first recheck 36 to 99 days after discharge: 3 of them carried ESBL-producing *KP* (ESBL-*KP*) (26, 59, 148), 3 an ESBL-*E. coli* (69, 76, 253) and 1 cat both microorganisms (96). The second follow-up was obtained from 22 of the 27 animals, and only 3 animals still carried one 3GC-R-Ent. isolates (26, 59, 69). In the third and the fourth follow-up, no MDROs were isolated anymore. Sampling time points, duration of carriage and isolates of dogs and cats carrying MDRO beyond 30 days are shown in Fig. 2. Details of all animals included in the study are shown in Supplemental Figs. 1–3.

3.1.5. Antimicrobial resistance profiles

MICs for all isolates included in the study are shown in Supplemental Tables 2–5. Human *E. coli* isolates showed an ESBL phenotype and besides their resistance to 3GC (6/6), were resistant to β -lactam antibiotics (4/6), trimethoprim/sulfamethoxazole (4/6), aminoglycosides (2/6), fluoroquinolones (2/6) and tetracyclines (2/6). None of the isolates showed resistance to carbapenems or colistin.

The resistance profiles of the two MR *Staphylococcus* isolates that were further characterized are shown in Supplemental Table 3.

Antimicrobial resistance profiles of the Gram-negative MDROs isolated at discharge from dogs and cats have been previously reported [11]. These are shown again in Supplemental Tables 4 and 5 as starting point for the follow-up study.

3.1.6. Molecular epidemiology/relatedness of the MDROs

The bacteriologic profiles of all Gram-negative isolates found in pets included in this investigation are listed in Supplemental Tables 4 and 5.

In 4 animals 3GC-R-Ent. were repeatedly isolated. In one animal (26) the *KP* isolate was initially typed as DHA-1, CTX-M-1/–3/–5 and as DHA-1 in later samples. All 4 *KP* isolates from this animal belonged to the same REP-PCR group (A). In three further animals, *KP* isolates (DHA-1, $n = 2$; CTX-M-1/–3/–5, $n = 1$) with the same REP-PCR profiles were

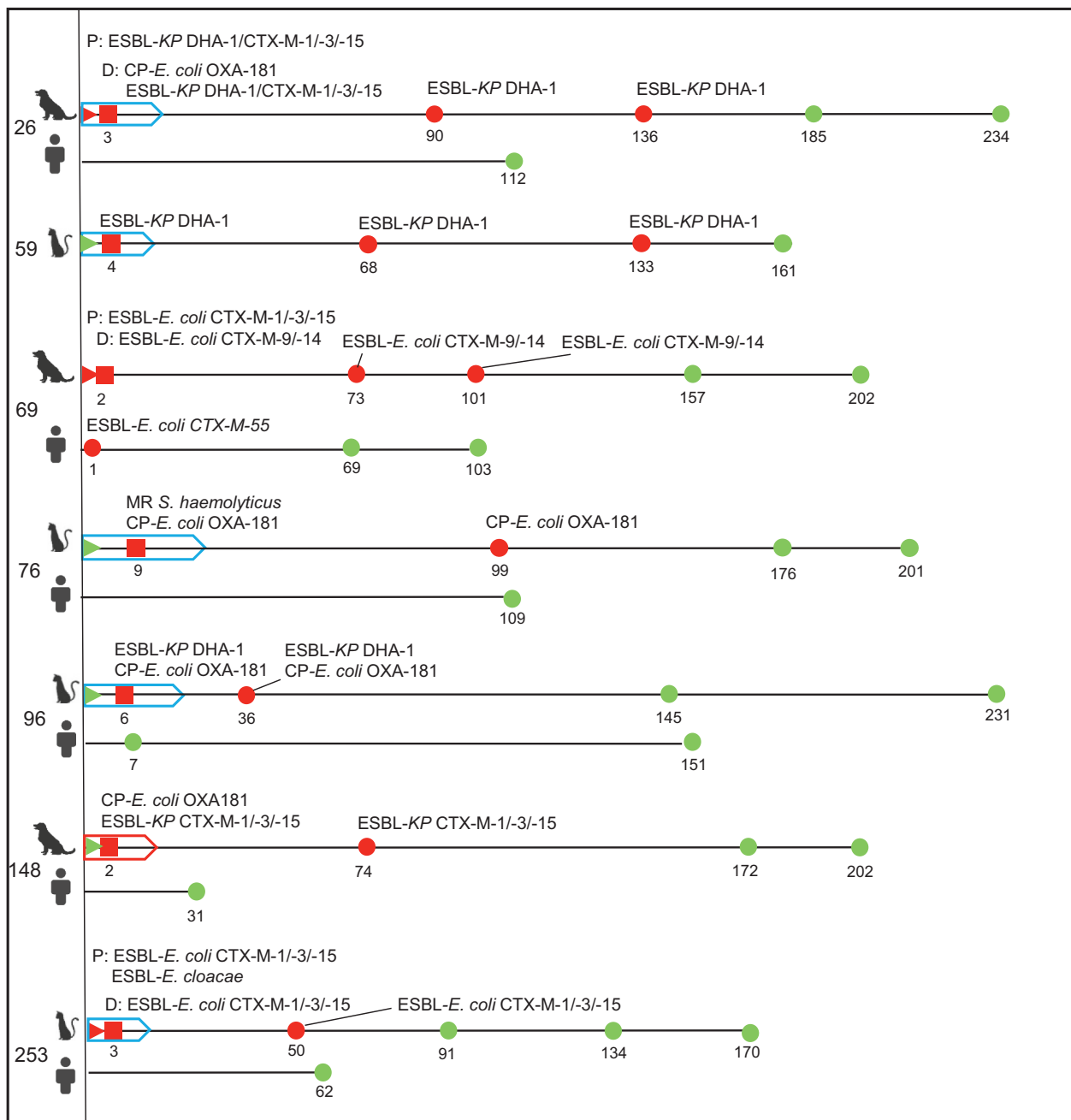


Fig. 2. Duration of carriage of multidrug-resistant organisms in dogs, cats and their respective owners. Only dogs and cats which were colonized with genetically related isolates beyond 30 days are shown. For a graphical overview of all cases included in the follow-up study see Supplemental Figs. 1–3.

P: Presentation; D: Discharge.

Red symbols: MDRO isolated.

Green symbols: no MDRO isolated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

▷ Presentation

◻ Discharge

○ Follow-up

Antimicrobial treatments

◻ Potentiated aminopenicillins

◻ Fluoroquinolones

Abbreviations: CP: carbapenemase-producing; ESBL: extended spectrum β -lactamase-producing; *E. coli*: *Escherichia coli*; KP: *Klebsiella pneumoniae*; *E. cloacae*: *Enterobacter cloacae*; MR: Methicillin-resistant; *S.*: *Staphylococcus*

repeatedly isolated.

3GC-R-*E. coli* were isolated repeatedly in 6 animals. In two animals (67; 255) repeated isolates showed different REP-PCR profiles. In the remaining 4 animals, hospital-acquired isolates that were repeatedly isolated from the same individual after discharge shared common REP-PCR profiles and ESBL/Plasmid-encoded AmpC (pAmpC) genes (CTX-M-9/-14; CMY-42, CTX-M-1/-3/-15). In 2 animals hospital-acquired OXA-181-producing *E. coli* was repeatedly isolated (76; 96) [10].

3.1.7. Co-carriage of humans and pets

16/50 (32%; 95%CI, 19.5–46.7) owners sampled themselves at the time of presentation of their animal to a veterinary practice or clinic and 34/50 (68%; 95%CI, 53.3–80.5) after discharge of their pet. Twelve owners decided to submit samples only after their own animal had tested positive for MDRO on one or several occasions leading to delayed submissions.

Twenty-one owner-pet pairs with owner and/or pet carrying MDRO were followed for up to 152 days (median 79; range 0–152 days, IQR 49) accumulating 1622 days of contact between MDRO carrying pets and/or owners. No co-carriage of MDRO was documented in repeated samples. In two cases (69 and 94), owner and dog carried both an ESBL-*E. coli* but those isolates were not genetically related (69, owner: CTX-M-55, ST95; dog: CTX-M-1/-3/-15 at presentation and CTX-M-9/-14 at discharge and follow-up. 94, owner: CTX-M-55, ST1193; dog: CMY-42, ST410). Representative cases from the co-colonization study are shown in Figs. 2 and 3. A graphical overview of all cases included in the co-colonization study are shown in Supplemental Figs. 1 and 2.

4. Discussion

We previously reported a very high rate of acquisition of MDROs by dogs and cats treated in veterinary hospitals [11] and documented an outbreak of OXA-181-producing *E. coli* in one of the participating hospitals [10]. In this part of the study, we described the duration of MDRO carriage in a subset of dogs and cats from the original study and assessed their owners for potential MDRO co-carriage at the time of admission of their pet and during a follow-up period in order to document potential transmission events.

The results of this study document enteric colonization of dogs and cats with 3GC-R-KP for up to 136 days and for 3GC-R-*E. coli* for up to 101 days. While 18% of participating owners carried MDROs, co-carriage or interspecies transmission of MDRO between animals and their owners was not demonstrated in the 21 pet-owner pairs that were followed up.

In this cohort of pet owners, the proportion of MDRO positivity and in particular the proportion of owners with enteric carriage of 3GC-R-*E. coli* (10%) was in the upper range of what has been reported. Previous studies conducted in Switzerland demonstrated a prevalence of carriage of 5.6–10% for 3GC-R-Ent. in healthy volunteers [28], 1.5% for COL-R Enterobacterales in healthy and primary care patients [29] and 4.2% for 3GC-R-*E. coli* in owners of healthy pets [12]. While the number of participating owners was small, the relatively high positivity rate might reflect the worldwide rise in the prevalence of colonization of healthy humans with MDR *E. coli* and other Enterobacterales [28,30].

All human *E. coli* isolates had an ESBL/AmpC-positive genotype (*bla*_{CTX-M-55}, *bla*_{CTX-M-15}, *bla*_{CTX-M-1}). In addition to their extended-spectrum cephalosporin resistance, two thirds of the isolates were resistant to β -lactam antimicrobials, two thirds to trimethoprim/sulfamethoxazole (TMP–S) and one third each to aminoglycosides, fluoroquinolones and tetracyclines. While the resistance to many first line antimicrobials is problematic, none of the isolates showed resistance to carbapenems or colistin. Since their first isolation in the 1980s, isolates of the *bla*_{CTM-X} ESBL genotype have become widespread internationally accounting for over 90% of ESBL-producing strains from community individuals [31]. CTX-M-producing *E. coli* have been commonly identified in Switzerland from various sources including from water of rivers

and lakes [29], poultry, cattle and pigs at slaughter [32].

No specific risk factors for MDRO carriage in owners were identified, but the power of this analysis is low due to a small number of participants. Review of individual questionnaires of owners carrying MDRO did not confirm any of the known risk factors such as previous antimicrobial use [30], recent hospitalization (≤ 12 months) [33], or international travel [30]. We assume that due to the widespread dissemination of ESBL-*E. coli*, there are now many routes for humans to get in contact with MDROs and the absence of risk factors no longer suggests absence of colonization.

Based on the previous findings of a very high acquisition rate of MDROs in dogs and cats in veterinary care and the clonal dissemination of OXA-181-producing *E. coli* in one clinic, we aimed to follow their persistence and possible co-colonization in pet-owner pairs. While in the majority of pets MDRO carriage was no longer demonstrated at recheck, 3 dogs and 4 cats showed prolonged enteric carriage of ESBL-KP for up to 139 days and ESBL-*E. coli* for up to 101 days. No persistence was shown for Gram-positive MDROs.

The durations of colonization in pets in this study are similar to what has been described in humans in long-term care (median 144 days (range, 41–349 days)) [34]. Studies in human new-borns (12.5 months (IQR 9.5–17.5)) [35] and adult patients post hospitalization report much longer durations of carriage (median of 58 months (range 41–59 months)) [36]. In contrast, colonization in healthy returning travellers appeared to be much shorter (<3 months) [37,38].

The long-term enteric colonization of pets is problematic, as pets typically use litter boxes or defecate in gardens, parks and walking areas, and thus directly contaminate the close environment of humans [39–41]. Furthermore, cleaning of the cat litter boxes or picking up of faeces from the ground, may directly expose owners to MDROs. Grooming behaviours may lead to colonization of the oral cavity, fur and skin, from where MDROs can be transmitted to owners via petting, cuddling, licking of the owner's face or hands or eating from the owner's plate. In a recent questionnaire-based study such highly intense contact was reported to occur at least occasionally in over 90% of households [4].

All dogs and cats included in this study were family animals living in close contact with their owners as they shared living quarters and the large majority also the owner's bed. Despite this close relationship, there was no evidence of any co-colonization in this study population. Transmission of MDROs between companion animals and their owners has previously been reported to occur in 1.5–5.5% of pet-owning households [8,12,42]. In contrast, intra-household transmission between hospitalized patients and their family members (40% [43] and 18.5% [44]) and between infants and adults (32% [35]) has been found to be much higher presumably due to a more intense contact between humans as between pets and humans.

While no pet-owner co-colonization was identified, we recently reported the co-colonization of pets and veterinary staff by CR-*E. coli*, suggesting that interspecies transmission at the respective clinics is possible [45].

Limitations of this study include the relatively small number of pet-owner pairs, the irregular sampling intervals and the fact that owners were only sampled 1–2 times if negative. Given the low sensitivity of bacterial culture, more samples would be necessary to achieve a higher sensitivity of the methods for detecting MDROs [46]. For the follow-up, owners were sampling their own pets and, despite detailed instructions, it is possible that the sampling technique in particular with regards to the nasal sampling retrieved insufficient material to demonstrate carriage.

In conclusion, dogs and cats requiring hospitalization may carry 3GC-R-Ent. for several months. While MDRO carriage was common in pet owners, co-carriage or interspecies transmission of MDROs between animals and their owners was not demonstrated, despite close owner-pet relationships.

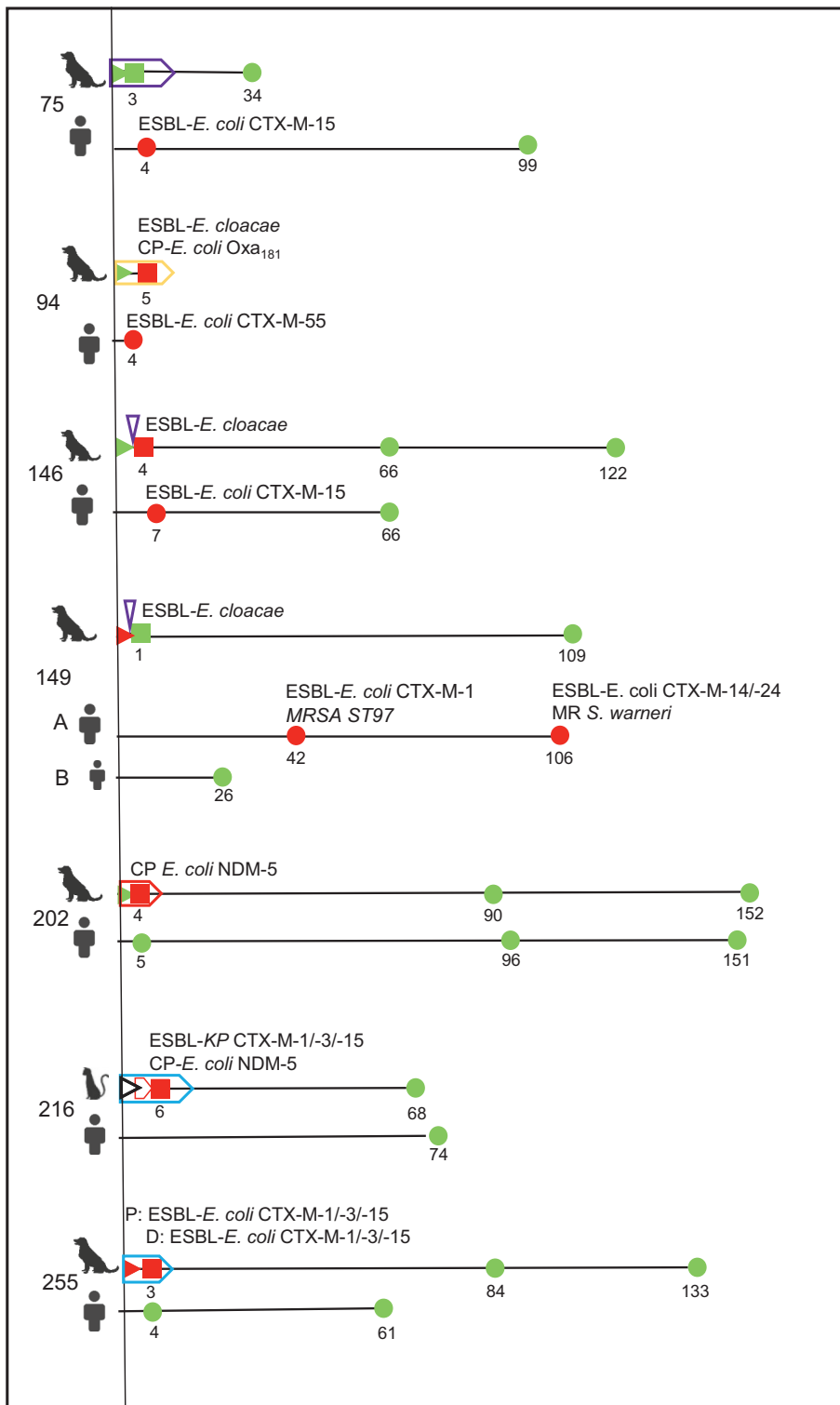


Fig. 3. Representative cases from the co-colonization study. While several owner-pet pairs showed colonization with multidrug-resistant organisms, co-colonization with genetically related organisms was not detected in any of the 21 owner-pet pairs in the study.

For a graphical overview of all cases included in the co-colonization study see supplemental Figs. 1 and 2. P: Presentation; D: Discharge.

Red symbols: MDRO isolated.

Green symbols: no MDRO isolated.

Open symbol: no sample analyzed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

▷ Presentation

◻ Discharge

○ Follow-up

Antimicrobial treatments

◁ First generation cephalosporin

◁ Potentiated aminopenicillin

◁ Fluoroquinolone

◁ Clindamycin + trimethoprim-sulphamethoxazole

▽ Perioperative ceftazolin

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2021.100322>.

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