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SHORT REPORT
Owner-collected swabs of pets: a method fit for the purpose of zoonoses research

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SUMMARY

As part of the preparation of a large cohort study in the entire German population, this study examined the feasibility of cat and dog owners collecting nasal and oral swabs of their animals at home as a method of assessing exposure to zoonoses. In veterinary clinics in Hannover, Germany, 100 pet owners were recruited. Nasal and oral swabs of pets were taken by a veterinarian at the clinic and owners took swabs at home. Swabs were analysed regarding bacterial growth and compared (owner *vs.* vet) using Cohen's kappa and McNemar's test. The return rate of kits was 92%, and 77% of owners thought it unnecessary to have veterinarian assistance to swab the mouth. McNemar's test results: oral swabs 78% agreement with Gram-positive bacterial growth, 87% agreement with Gram-negative bacterial growth; with similar results for nasal swabs. Although sample quality differed, this method allowed the receipt of swabs from pets in order to obtain information about colonization with zoonotic pathogens.

Key words: Cats, dogs, feasibility, nasal/oral swabs, veterinary public health.

In Germany more than 20% of households own a pet, producing a total of about 8·2 million cats and 5·4 million dogs [1]. Many pets live in very close proximity to their owners or family members. However, there is an important aspect concerning this close relationship which should not be ignored. The health of animals and humans is closely related and numerous zoonotic pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) or *Toxoplasma* can be transmitted from animals to humans and vice versa.

Therefore, it is necessary to examine pets and their owners in parallel. This approach raises a number of problems, e.g. regarding transport of pets to a human recruitment centre, hygiene regulations in examination rooms or high costs for home visits by veterinarians. Thus, this investigation was set up as a feasibility study to test if pet owners are able to take oral and nasal swabs from their animals at home and if these samples are comparable to those samples taken by an experienced veterinarian.

A convenience sample of dogs ($n=82$) and cats ($n=18$) in the region of Hannover, Germany was

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collected from May 2011 to September 2011. Those cats and dogs selected were presented by their owners to the Small Animal Hospital of the University of Veterinary Medicine Hannover, Foundation (20 dogs, 3 cats) or one urban (24 dogs, 11 cats) or one suburban (38 dogs, 4 cats) veterinary clinic for surgery for routine examination or diagnosis of orthopaedic problems. These animals showed no signs of respiratory infection or other infections and were not taking a course of antibiotics. Three different recruiting sites were chosen in order to contact a wide range of pet owners.

Owners were informed about the study and asked for written consent to take part in the study including taking samples of their animal.

Participants were recruited in the morning hours from 09:00 to 13:00 hours during clinic opening hours. As an incentive, samples of dog and cat food were offered to increase the overall response of the pet owners. Only one pet from each owner was sampled and it was the owner's choice if they owned more than one animal.

Pet samples were taken by both a study investigator and the pet owner to allow direct comparison of results. Two nasal swabs and one oral swab were collected from each animal by the veterinarian (study investigator). The owner was present during the procedure and was shown how to take samples. At home, the owner was advised to take samples immediately after returning. A dispatch box was prepared for the owner, containing sampling materials and sampling instructions. The box was pre-stamped and pre-addressed for return to the collaborating laboratory.

One nasal swab and one oral swab were collected from the animal by the owner. The owner also collected a faecal sample and cat owners additionally took a hair sample from their cat. Owners were advised to keep samples in the refrigerator before dispatch. For collection of samples, sterile single-use sample collection packs (Copan® eSwab, Copan Italia Spa, Italy) were used, these contained polypropylene screw-cap tubes with an internal conical shape filled with 1 ml Liquid Amies Medium together with one regulation size applicator swab with flocked nylon fibre tip (colour code: orange, catalogue no. 481CE; colour code: pink, catalogue no. 480CE).

One of the nasal swabs from each animal taken by the veterinarian was personally delivered to the laboratory the same day. The other nasal swab and the oral swab were kept under refrigeration at ~4 °C and sent to the laboratory by surface mail the next day. The choice of which nasal swabs was to be delivered personally was made by random selection. Owner-taken samples were sent by the owner by surface mail to the laboratory. It was intended that this mailing should be performed on the same day as the veterinarian's mailing procedure.

If packages did not arrive at the laboratory within 3 days after recruitment, participants were reminded by telephone. If they were not contacted the first time, three additional calls (one per day) were made at different times during the day.

To obtain information on animals and owners and about the condition of samples, four questionnaires regarding data collection were used. These questionnaires were completed by the veterinarian at the recruitment site, by the owner at home and by the laboratory staff.

In addition, a set of questions was directed at the pet owners that refused to participate in order to obtain information on possible non-response bias.

For bacterial culture of swabs three different solid media were used: Gassner agar, Columbia agar with sheep blood and *Streptococcus*/*Staphylococcus* selective agar. These were incubated for 48 h at 35–37 °C. After 24 h and 48 h Gram-negative and Gram-positive bacterial growth was recorded and categorized as sparse growth, moderate growth, and heavy growth. Specific bacterial species were not analysed.

Data was transferred into a database and analysed using SAS *vs.* 9.2 (SAS Institute Inc., USA). Analysis was performed by quantifying the frequencies of collected data in different subgroups of interest.

Of 144 pet owners asked, 100 (69·4%) agreed to participate in the feasibility study. Of the 144 pet owners, 112 owned a dog and 32 owned a cat. Of the recruited 100 animals, 82% were dogs and 18% cats. The age of owners varied from 18 to 80 years (mean age 45 years). Of the participating pet owners, there were 26 (26%) men and 74 (74%) women, whereas for non-participants, there were relatively more men who refused to participate (28% men, 72% women).

This marginal difference could be due to the general willingness of men and women to participate voluntarily in studies, which is greater in women [2, 3]. In general, more women than men brought their animals to the veterinarian. Regarding dog or cat ownership, dog owners were more willing to participate. The main reason for owners refusing to participate was that they thought it impossible to swab their animal (38%, 57% of non-participating cat owners and 30% of non-participating dog owners). Other owners addressed

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Table 1. *Response and return rate of packages*

	Men	Women	Cats	Dogs
Participation	26 (26%)	74 (74%)	18 (18%)	82 (82%)
Refusal	12 (27%)	32 (73%)	14 (32%)	30 (68%)
Medium age of participants (years)	48	43	6	6
Medium weight of participants (kg)	—	—	5	24
Returned packages	21 (81%)	71 (96%)	17 (94%)	75 (91%)
Complete packages (questionnaire + swabs + faecal sample + hair)	16 (76%)	44 (62%)	8 (47%)	52 (69%)
Both swabs returned	21 (100%)	68 (96%)	16 (94%)	74 (99%)
Returned nasal swabs unsuitable	1 (5%)	1 (1%)	1 (6%)	1 (1%)
Returned oral swabs unsuitable	1 (5%)	0 (0%)	1 (6%)	0 (0%)
Difficulties with swabbing the nose	6 (29%)	22 (31%)	7 (41%)	21 (28%)
Difficulties with swabbing the mouth	3 (14%)	4 (6%)	2 (12%)	5 (7%)

Values given are absolute number (%).

did not have time (9%) or were not the legal owner of the animal (9%), while 27% did not give a reason for refusing. Only 4% feared hurting their animal or thought it to be too much effort (4%) or just did not feel like participating (6%). Personal communication (from recruited pet owners) suggests that pet owners know their pet and its behaviour really well, although this was not able to be evaluated by the study team.

Categorizing education level according to the German school system, 46% of owners had attended grammar school (Gymnasium), 39% had been to secondary school up to year 10 (Realschule), and 15% had been to lower level secondary school up to year 9 (Hauptschule). This is in line with other studies in Germany as well as in other countries, where people with a higher education level are more likely to take part in epidemiological studies [2–5].

Age characteristics of owners and animals and the weight of animals varied (see Table 1).

By this method a representative survey of the population was achieved. Although this was not the intention of the feasibility study, the (pet) study population of dogs and cats was close to a random sample of that particular population, but not with regard to the proportion of cats and dogs within the pet population. Only a small number of cat owners could be recruited although the cat population is 1½ times that of the dog population [6]. The reason for this is that cat owners were less willing to participate because they deemed it too difficult and also that sampling collection only took place during morning hours and not many cats were presented at the clinic at that time compared to the afternoon.

The return rate of distributed sample kits was 92%. Of these, 95% were complete, enclosing the nasal and oral swabs and the questionnaire. In three returned kits the questionnaire was missing and one dog owner forgot to return a nasal swab.

Of the 92 returned kits, 17 were from cat owners and 75 from dog owners. In total, 89 questionnaires were returned and 90 nasal and 91 oral swabs (90%). Only 62 owners sent a faecal sample.

Response rates in epidemiological studies have decreased during the last two decades [4]. For example, the recent Northern German Lymphoma and Leukemia Study (NLL) had a response rate of only 49% [7]. It should therefore be emphasized that the return rate of kits and the percentage of participation in our study was higher than usually expected in Germany.

This may be due to the assumption that pet owners who bring their animals regularly to a veterinarian are more interested in health and studies on health affairs than others.

More than half (58·5%) of the owners sampled their pet on the same day it was sampled by the veterinarian, 20·7% of owners sampled their animal the next day, so 79% of owners sampled their animal the same day or the next day (min 0 h, max 15 days, 75th percentile 1 day). Overall the mean time interval between swabbing the animal by the veterinarian and by the owner was 6·3 h (two extreme values of 10 days and 15 days were excluded from the analysis). Swabs sent by the veterinarian by post took 1 day from posting until arrival at the laboratory which was the same as with the owners' samples. There were no delivery delays on the part of the mail services. Therefore it appears to be a reliable way to transport swabs to the laboratory without any substantial loss of quality of the laboratory findings.

Ninety-one of 92 oral swabs (99%) and 89 of 91 nasal swabs (98%) taken by the owner were able be used for microbiological culture after arrival at the laboratory. It therefore seems that owners are generally able to take swabs themselves after seeing this performed once and reading the instructions later.

The comparability of bacteriological results was tested with regard to general agreement, Cohen's kappa value, and McNemar's test for asymmetry [8]. As a matter of sensitivity analysis these results were re-checked via logistic regression analyses. Owners' samples were compared to samples taken by the veterinarian and sent to the laboratory by surface mail the following day. Another comparison was conducted between samples taken by the veterinarian delivered directly to the laboratory and samples sent by surface mail to assess the effects of the mail service.

Results of Gram-positive bacteria for the comparison between veterinarian and owner showed an agreement of 71% (nasal swabs) to 78% (oral swabs) with no significant asymmetry ($P=0{\cdot}2393$) and a positive kappa value concerning the nasal swab. By contrast, a significant asymmetry ($P=0{\cdot}0073$) regarding the growth of Gram-positive bacteria in the mouth was observed. Similar results were also observed for Gram-negative bacteria, but on a different scale. Overall agreement rates ranged from 55% (nasal swabs) to 87% (oral swabs), with some of the swabs having statistically significant asymmetries and some having no significant asymmetries. The general agreement with regard to bacterial growth of nasal swabs taken by a veterinarian compared to those taken by the owner was smaller than the agreement for oral swabs. But these differences are mainly due to an increase of bacterial findings for pet owners which is much more prevalent in Gram-positive bacteria.

As expected there were different results between those swabs sent by mail and those delivered directly to the laboratory. Agreements ranged from 56% (Gram-positive) to 60% (Gram-negative). The amount of bacteria increased although swabs were kept under refrigeration prior to posting. It is known that for some bacteria the time between sampling and arrival at the laboratory needs to be very short, for example some species of *Streptococcus* or *Bordetella*, so that they do not become overgrown by other bacteria [9]. There could be various reasons for the differences in bacteriological results of veterinarian samples and owner samples, e.g. incorrect or unsterile swabbing or lack of refrigeration in storage of swabs.

As the cultured bacteria were not identified regarding genus or species, it was not possible to definitely state what the reason for more bacterial growth is in owner-collected swabs or mailed veterinarian swabs, since different bacteria show different growth characteristics.

The occasional phenomenon of no bacteria being able to be cultured from veterinarian swabs as well as from owner swabs could be explained by the fact that the swabs were dry and possibly bacteria were not able to adhere. Therefore, moistening swabs should be considered as an option in future studies. This would on the other hand carry the danger of contamination.

Nevertheless, sampling by the veterinarian may not be ideal. It can be seen that pets are more patient when sampled by their owners and more nervous when sampled by an unknown veterinarian. This topic is often discussed as a general problem in practice. Moreover, pets are more intimidated in the clinical setting and therefore more likely to keep still than at home. But there is no method of recognizing this in advance.

Questionnaire information was used for additional evaluation. Responses to questions in the owners' questionnaire concerning problems with swabbing indicated that a total of 28 owners had problems swabbing the nose (30·4%) and seven owners had problems swabbing the mouth (7·6%). Of these, seven were cat owners (41·1% of all kits returned by cat owners) and 21 dog owners (28% of all kits returned by dog owners) who had difficulties with nasal swabs (see Table 1). On the other hand, there were five dog owners and two cat owners who had problems with swabbing the mouth, indicating that the animal had not kept still.

Problems with collecting faecal samples only occurred with outdoor cats and cat owners never had problems with collecting hair samples from their cat. Seventy-seven percent of owners who completed the questionnaire at home agreed that a veterinarian is not necessary for swabbing the mouth, but only 56% of owners were of the same opinion regarding swabbing the nose. Overall, the satisfaction with the study was very good: 87% finding it completely acceptable and 72% of owners stating that they would participate in such a study again.

These results are comparable to a study where parents took swabs of their children [10]. The good acceptance could be due to the fact that demands on owners were manageable and the questionnaire was

very short, so participants did not have to invest too much time. Taking into account possible uncertainties, the method of self-swabbing can now be seen as a proof of principle which should be extended to research into specific bacteria. As the main intention of the study was to test for feasibility, the bacteriological analysis only resulted in information about sparse, moderate and heavy growth of Gram-negative and Gram-positive bacteria. There was no further identification of the bacteria performed. In future studies specific bacteria should be searched for and comparisons for specific diagnostic tests should be made. However, because of very low prevalences of some bacteria in dog or cat populations, large-scale epidemiological investigation or experimental settings would be needed to extend the technique described.

The great number of owners who thought a veterinarian unnecessary for swabbing the mouth can be seen as an indication that it is a relatively easy procedure whereas swabbing the nose appears to be more difficult. Moreover, nose swabbing is more unpleasant for cats and dogs because the nose is a very sensitive part of an animal [11, 12].

With the recent study it was shown that pet owners are reliable in following instructions, taking samples at home and returning study material, so it can be assumed that in large cohort studies behaviour will be similar. To ensure proper sampling techniques, written and illustrated instructions as well as videos (e.g. web-based on the study's internet page) need to be developed. Although in general there is no control regarding taking samples in a sterile way, it is possible to gain material with which both the correlation between animals and humans as well as the normal flora of cats and dogs can be examined.

This represents a good way of keeping costs to a minimum and increasing participation. Furthermore, it contributes to veterinary public health and the one-health approach.

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DECLARATION OF INTEREST

None.

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