Paw hygiene of assistance dogs and pet dogs compared to their users' and owners' shoe soles: equally clean or extra attention required?

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Abstract

Background and aim. In the Netherlands, many disabled people benefit from the daily use of an assistance dog (AD). Despite the European and Dutch laws that prohibit the denial of ADs to public places, this still occurs on a regular basis. The main argument is that dogs compromise the hygiene with their presence, which could be a health hazard. Meanwhile, people are allowed to walk into and out of public places freely wearing the same shoes they wear outside. The goal of this study is to quantify Enterobacteriaceae family colonies and to investigate the presence of *C. difficile* on the paws of ADs and pet dogs (PDs) and the shoe soles of their users and owners, respectively. These numbers may tell that additional measures are needed to protect the hygiene in important environments, like hospitals.
Materials and methods. 25 ADs and their 25 users, and 25 PDs and their 25 owners were acquired as participants for this study, that took place in February and March of 2020. Each participant was asked to walk their dogs for 15-30 minutes prior to sampling. The front paws and shoe soles were sampled using premoistened sponges and further processed for the demonstration and quantification of bacteria of the Enterobacteriaceae family as a measure of general hygiene, and the demonstration of *C. difficile.* Each PD owner or AD user filled out a general questionnaire about the care for their dogs, and AD users were asked to fill out an additional questionnaire on the experience of using an AD.
Results. Dog paws came back negative for bacteria of the Enterobacteriaceae family more often than shoe soles, and also had lower numbers of those bacteria. This was most distinct in the comparison of PDs and their owners; the numbers were similar between ADs and their users. *C. difficile* was only found on one AD user's shoe soles.
Conclusions. The general hygiene of dog paws is better than that of shoe soles, mostly caused by the better general hygiene of PD paws in comparison to their owners' shoe soles; ADs and their users had comparable levels of general hygiene. *C. difficile* is not a bacteria that is regularly found on dog paws or shoe soles. Possible hygiene measures for hospitals could be (wet) wipes, (sticky) mats, or overshoes for dog paws, and overshoes, brushes, (sticky) mats, and UV lights for shoe soles.

Introduction

In the Netherlands, many people with a mental or physical disability benefit from the daily use of an assistance dog (AD). Eleven accredited organizations[1] deliver hundreds of ADs a year, and the largest organization had over 700 active human-dog teams in 2018[2]. These dogs are able to guide the blind through the world, complete tasks for those who can't and offer support in case of post-traumatic stress disorder (PTSD) or autism, for example. However, the degree of acceptance differs per country and culture, which presents itself in the denial of access for these dogs to health care facilities, public transportation, shops, and so on. It is thought to be especially the case for invisible disabilities, like PTSD. Research conducted on behalf of Koninklijk Nederlands Geleidehondenfonds (KNGF, or The Royal Dutch Guide Dog Foundation) in 2019 showed that 40% of their AD users was denied at a public place in the past year[3]. The argument that is most frequently posited, is that dogs contaminate their environment which could be a health hazard. Meanwhile, people are allowed to walk into and out of – for example – hospitals freely wearing the same shoes they wear outside.

When it comes to ADs, several different terms are utilized. By Dutch law and the global authority Assistance Dogs International (ADI), 'assistance dog' is applied as the umbrella term. AD training foundations do not always follow that decision and sometimes create their own names for their ADs. Subgroups of ADs are guide dogs (for the guiding of visually impaired or blind people), hearing dogs (for the alerting of hearing impaired or deaf people to specific sounds), and service dogs. All of these three subgroups are represented in this study. Service dogs include mobility assistance dogs, alert service dogs (for the detection of the imminent onset of a medical condition), response service dogs (for the provision of safety during or after a medical episode), and psychiatric service dogs (for the mitigation of a mental health disability, such as autism or PTSD). Emotional support dogs, which are not trained like ADs but provide support by being present, or therapy dogs, which are trained pet dogs, are not included in this system, and therefore may not have the same rights as ADs regarding public access[4,5].

This study focuses on the presence of bacteria from the Enterobacteriaceae family as a measure of general hygiene, and *Clostridium difficile*, an important causative agent of diarrhea, on the foot pads of ADs and the shoe soles of their users or trainers. Other relevant bacteria for a hospital environment like methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and also extended-spectrum beta-lactamase (ESBL) *Escherichia coli* are rarely found on dogs. *Clostridium difficile*, on the other hand, are found on dogs on a more regular basis[6]. A study conducted in Slovenia on the presence of *C. difficile* showed that this bacteria is found in higher quantities on footwear than on foot pads of pet dogs[7].

Previous research has been conducted on the microbiological composition of dog fur and paws. A single sample taken from fur yielded a significantly lower prevalence of the Enterobacteriaceae family than a single sample taken from the foot pads; only one in 20 dogs carried members of the Enterobacteriaceae family on its fur[8]. Because the typical duration of a doctor's appointment is fairly short (10 to 20 minutes), the chances of contamination of the environment due to fur is rendered relatively low. The foot pads of dogs can be seen as the equivalent of a person's shoe soles, and that is why the foot pads were chosen to be the center of this research. A group of pet dogs (PDs) and their owners were used as a control group.

The goal of this case-control study is 1) to determine if and how many colonies of the Enterobacteriaceae family and if *Clostridium difficile* can be cultivated from the paws of ADs and PDs, 2) to compare those numbers to the samples taken from the shoes of respectively their users and their owners and 3) to investigate if measures must be taken to reduce the contamination of the environment (like a hospital) due to ADs. An additional goal is to get an overview of the current experiences of AD users, during their navigation through life with their AD, and possible improvements.

Materials and methods

Materials

Using previous research, the sample size was calculated and found to be a 100 individuals (50 dogs and 50 humans), to be able to obtain a power of 80%. Due to time limitations, those numbers were then divided in 25 ADs and their 25 users (or trainers), and 25 PDs and their 25 owners.

To acquire ADs and their users for this research, the networks of two Dutch official assistance dog foundations were used: Koninklijk Nederlands Geleidehondenfonds (KNGF, or The Royal Dutch Guide Dog Foundation; Amstelveen) and Bultersmekke Assistancedogs (BMA; Assendelft). Members of those foundations received a newsletter containing information about the research and they could then take action to enroll. Potential participants were sorted on date of enrolment and their geographical location, so multiple participants could be visited on one day. PDs and their owners were acquired via the network of the researcher. The dogs were of a wide variety, including breed, sex, and age. Figure 1 shows the geographical distribution of the participants.

Figure 1. *Map of the Netherlands: geographic distribution of the participants. A red marker indicates the home of an assistance dog (AD), a blue one indicates the home of a pet dog (PD), and a green one indicates a home where there is both an AD and a PD present. Map made with Google Maps ©.*

The visits took place in the morning, during the months February and March of 2020. Every participant was asked to wear shoes they would normally wear to shops, restaurants, hospitals, or in public transportation, for example. Samples from the front paws of the dogs and their owners' or users' shoe soles were collected. The front paws were preferred over the back paws, because the number of bacteria of the Enterobacteriaceae family is believed to be larger on the front paws[9] (possibly due to their use for digging, among others) and easier accessible, as dogs are often used to give paw.

In addition, the participants filled out a questionnaire during the visit (hereinafter referred to as ‘general questionnaire’), including questions about the dog’s vaccinations and other infection preventive measures, hygiene (how often do they bathe their dog, is the dog allowed in their beds), and diet (presence of raw meats). AD users were also asked to answer a few questions about their experiences of using an AD (hereinafter referred to as ‘experience questionnaire’).

Finally, ten (academic) Dutch hospitals were approached and asked about their opinion, protocols, and regulations regarding ADs (hereinafter referred to as ‘hospital questionnaire’).

Methods

Coding

Each human-dog couple was given a code, consisting of three parts: the first part indicated whether it is an AD-user couple (AD) or a PD-owner couple (PD), the middle part is a number from 1 to 25, and the last part indicates whether it is the dog (D; dog) or the human (O; owner) within the couple. For example: the first AD-user couple would get the code AD-01, with AD-01-D being the AD and AD-01-O being the AD user. The first PD-owner couple would get the code PD-01, with PD-01-D being the PD and PD-01-O being the PD owner.

Sampling and preservation

The participants were asked to walk their dogs for 15-30 minutes, prior to sampling, taking a route they would normally take. Within 30 minutes after this walk, both of their shoes and the pads and toe webbing of both of the front paws were sampled by the researcher, for which sterile gloves were worn. Their surfaces were swabbed using premoistened Polywipe™ (premoistened with 7 mL phosphate buffer; Medical Wire & Equipment) sponge swabs; four sponges per human-dog couple were needed. The sponges were stored in stomacher bags (BagPage, Interscience). The sponge used to swab the right shoe or paw was designated to be further processed for the demonstration and quantification of bacteria of the Enterobacteriaceae family, and the sponge used to swab the left shoe or paw was designated to be further processed for the demonstration of *Clostridium difficile*. The samples were placed in a cooler (approximately 5 to 10˚C) and were processed the same day, within one to six hours.

The Polywipe™ sponges were already shown to be effective for the recovery of *C. difficile* from the environment[7,10]. Prior to this research, a proof of principle was conducted for the recovery of bacteria of the Enterobacteriaceae family. This was done by swabbing the sole of a shoe, which was contaminated with a solution with a known concentration of *E. coli* bacteria. The method in the following paragraph was used and was effective for the recovery of bacteria of the Enterobacteriaceae family; the concentration of bacteria found was equivalent to that of the solution used to contaminate the shoe sole.

Processing of samples Enterobacteriaceae

93 mL of enrichment medium (peptone-saline solution) was added to the stomacher bags containing the sponges for demonstration and quantification of bacteria of the Enterobacteriaceae family. The contents were homogenized (Bagmixer®, Interscience) and a dilution series up to 10^{-6} was executed. 1 mL of every dilution step was placed on Enterobacteriaceae (EB) Petrifilms™ (3M). The Petrifilms were

incubated at a temperature of 37˚C for approximately 24 hours. The presence of colonies of the Enterobacteriaceae family was then determined, using colony morphology; colonies of the Enterobacteriaceae family are red and have a yellow halo and/or colony-associated gas bubbles. Those colonies were counted.

Processing of samples *Clostridium difficile*
50 mL of Ringer solution was added to the stomacher bags containing the sponges for demonstration of *C. difficile*. The contents were homogenized. 3 mL of fluid from the bags was transferred to tubes containing 5 mL enrichment medium (brain/heart infusion broth; produced in-house at IRAS-VPH lab). The tubes were incubated anaerobically at a temperature of 37˚C for approximately 48 hours.

Fluid from the tubes was inoculated on Brazier's agar plates (Oxoid/Thermo Fisher Scientific), using disposable inoculation loops. The plates were incubated anaerobically at a temperature of 37˚C for approximately 48 hours. The presence of *C. difficile* was then determined, using colony morphology; *C. difficile* colonies are gray or white, opaque and flat, have a ground glass appearance and rough, fimbriated edges. The colonies are lecithinase negative and, due to the production of p-cresol, emit a phenolic odor[11]. Further determination was performed by placing the plates under a UV light source (365 nm). *C. difficile* colonies fluoresce yellow to green. For confirmation, a latex agglutination test is needed, but that was not possible during this research considering the budget.

In one sample, colonies atypical in morphology for *C. difficile* were present and UV fluorescence occurred. Judging by the smell, these could be *Pseudomonas* spp. colonies. It was decided to do further investigation and inoculate some of these colonies onto cetrimide and nalidixic acid (CN) plates. Normally, *Pseudomonas* spp. are aerobic, but some species are able to convert nitrates (including *Pseudomonas aeruginosa*) and, as a result, do not need oxygen[12,13]. This explains why these colonies were able to grow under anaerobic circumstances.

Statistics
Data were collected in Excel spreadsheets (Microsoft) and processed using the desktop application of RStudio (Rstudio Inc.). Several tests were conducted. The mean amount of colony-forming units (CFUs) of bacteria of the Enterobacteriaceae family was compared between the following groups: human versus dog, PD owners versus AD users, and PDs versus ADs. These comparisons were done by means of a *t*-test. To directly compare the dog to its owner or user, paired *t*-tests were performed. The applying hypotheses are shown in Table 1.

Table 1. *Hypotheses of the executed* t*-tests and paired* t*-tests, for comparing the means of the amount of colony-forming units of bacteria of the Enterobacteriaceae family, recovered from dogs' right front paws and humans' right shoe soles.*

***T*-tests**	*Human vs. dog*	H_0 = there is no difference between the mean amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from the right front paws of dogs and the right shoe soles of humans. H_1 = there is a difference between the mean amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from the right front paws of dogs and the right shoe soles of humans.
	Pet dog owners vs. assistance dog users	H_0 = there is no difference between the mean amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from the right shoe soles of pet dog owners and the right shoe soles of assistance dog users. H_1 = there is a difference between the mean amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from the right shoe soles of pet dog owners and the right shoe soles of assistance dog users.
	Pet dogs vs. assistance dogs	H_0 = there is no difference between the mean amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from the right front paws of pet dogs and the right front paws of assistance dogs. H_1 = there is a difference between the mean amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from the right front paws of pet dogs and the right front paws of assistance dogs.
Paired *t*-tests	*Pet dog vs. pet dog owner*	H_0 = the mean of the differences, between the amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from pet dogs' right front paws and their owners' right shoe sole, is zero. H_1 = the mean of the differences, between the amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from pet dogs' right front paws and their owners' right shoe sole, is not zero.
	Assistance dog vs. assistance dog user	H_0 = the mean of the differences, between the amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from assistance dogs' right front paws and their users' right shoe sole, is zero. H_1 = the mean of the differences, between the amount of colony-forming units of bacteria of the Enterobacteriaceae family recovered from assistance dogs' right front paws and their users' right shoe sole, is not zero.

An additional goal of this research was to investigate the existence of a factor or factors that could be linked to the amount or presence or absence of recovered CFUs of the Enterobacteriaceae family from the right front paws of dogs. For this purpose, (logistic) regression models were set up and executed. Among the eighteen possible factors were age, vaccination status, time spend outside, and weather. Information about these factors was acquired from the general questionnaires.

Finally, the results of the experience questionnaires were reviewed. Chi-squared and proportion tests were performed to determine if there were any differences in the answers to the multiple choice questions between AD users with a visible handicap, and users with an invisible handicap, and also between AD users who were in a wheelchair and users who were not. A visible handicap would be blindness (as the blind often use a red and white cane) or being in a wheelchair, and an invisible handicap would be deafness, a psychological handicap, or using a walker or normal cane (as the elderly also use such aids). Experience shows that, as there are many different AD harnesses, ADs are often mistaken for normal PD when their user is not visibly handicapped, or uses an aid that is not typically linked to being handicapped. The AD users in wheelchairs did not participate in the sampling, as their shoes are unable to collect contamination from the environment, so they were only asked to fill out the experience questionnaire.

Literature search

PubMed, Scopus, and Google Scholar were used as literature sources, to the date of April 15, 2020. Key words in different combinations were used; an overview can be found in Appendix A. Studies were only included when they were written in their original language, which were English and Dutch. Other relevant citations were found by reviewing reference lists of found studies and lists of suggestions given by the search engine.

Based on title and abstract, studies were evaluated on relevance. Relevant ones were saved using RefWorks software (ProQuest®) for full assessment later on. Inclusion criteria for articles or studies comprised they had to be published in a scientific journal or a scientifically supported book, or written by an expert in the field of ADs. Other exclusion or inclusion criteria were based on the questions that came up while writing of the discussion. 36 citations have been included. Appendix B shows a PRISMA flow diagram.

The researcher had no previous experience in the field of ADs and has done no previous research on this topic, so that kind of bias was not present.

Results

In Table 2, the sampling results are shown. The full table of results can be found in Appendix C. *C. difficile* was only found in one sample; however, based on morphology, suspected colonies were found in more samples.

Table 2. *Sampling results: data used for (paired)* t*-tests.*

	Recovered CFUs Enterobacteriaceae		***C. difficile presence***		***Pseudomonas* spp. presence**
	Mean (absolute numbers)	*Mean (logarithms)*	*Suspicion*	*UV light fluorescence*	
Dogs	3,444	0.9604	7	0	0
Humans	107,893	2.1286	9	1	1
Assistance dogs	1,228	1.2000	4	0	0
Assistance dog users	38,364	1.7496	6	1	0
Pet dogs	5,660	0.7208	3	0	0
Pet dog owners	177,422	2.5076	3	0	1

Enterobacteriaceae: comparing of groups

During sampling it was observed that sponges were generally dirtier coming from shoe soles than those coming from dog paws, macroscopically speaking. It seems like shoe soles are able to hold on to more material collected from the environment than dog paws can.

In the examination of the Petrifilms it was found that 72% of dogs in general came back clean, and 42% of humans in general, ‘clean’ meaning zero CFUs of the Enterobacteriaceae family were identified. When looking at the subgroups, 32% of PD owners, 52% of AD users, 80% of PDs, and 64% of ADs were clean.

Dog versus human. When comparing the mean recovered CFUs between human and dog, the two groups differed significantly ($P < 0.05$ and $P < 0.01$). **As shown in Table 1, the mean of dogs is lower than that of humans, and thus the general hygiene of dog paws can be considered to be better than that of shoe soles.**

PD owner versus AD user. When comparing the mean recovered CFUs between PD owners and AD users, the two groups did not differ significantly ($P > 0.05$). **The general hygiene of the shoe soles of PD owners and AD users can be considered to be equal.**

PD versus AD. When comparing the mean recovered CFUs between PDs and ADs, the two groups did not differ significantly ($P > 0.05$). **The general hygiene of the paws of PDs and ADs can be considered to be equal.**

Enterobacteriaceae: examination of couples

A paired *t*-test was conducted, because the observations of a dog and its owner or user are in fact not two separate observations, but dependent ones. A person may not frequent every spot a dog would, and a dog will not always follow its owner, but they take the same route on walks and spend time in the same type of environments. The means of the differences in recovered CFUs between a dog and its owner or user were 1.7868 for the PD group and 0.5496 for the AD group. To calculate these means for each group, the number of recovered CFUs of dogs was subtracted from the number of recovered CFUs of humans.

PD and PD owner couples. The *P*-value that was found for this comparison was very small ($P < 0.05$ and $P < 0.01$), which makes it very unlikely for the mean of the differences to be equal to zero. **The general hygiene of the paws of PDs is considered to be better than the shoe soles of their owners.**
AD and AD user couples. Although the mean recovered CFUs of ADs was found to be lower than that of their users, it was calculated that $P > 0.05$. **The general hygiene of the paws of ADs and the shoe soles of their users can be considered to be equal.**

Enterobacteriaceae: factors
The general questionnaire produced a lot of information and various possible factors that could be of influence on the amount of recovered CFUs of the Enterobacteriaceae family from dog paws. The factors that were taken into account were dog type (PD or AD), geological location (urban or rural), hypoallergenicity, coat type (long, short, or curly), age in years, sex, neutering (yes or no), vaccination status (per type of vaccination), flea and worm control status, bathing frequency (number of times a year), diet, sleeping place, prohibited areas at home (kitchen, bathroom, or bedroom), weather (wet or dry), and number of walks a day, including time spent outside and locations visited during walks. These locations comprised the own neighborhood, beaches, forests, designated dog walking areas, public parks, wastelands, and pastures.

Figure 2 shows the vaccination status of the participating dogs. 100% of ADs was vaccinated with the cocktail vaccine and against leptospirosis, as this is mandatory. 64% of PDs had received at least one vaccination; this was significantly different from ADs ($P < 0.05$ and $P < 0.01$). Kennel cough had the lowest vaccination coverage. As rabies is not endemic in the Netherlands, this vaccination is not required; except when the dog travels to a foreign country.

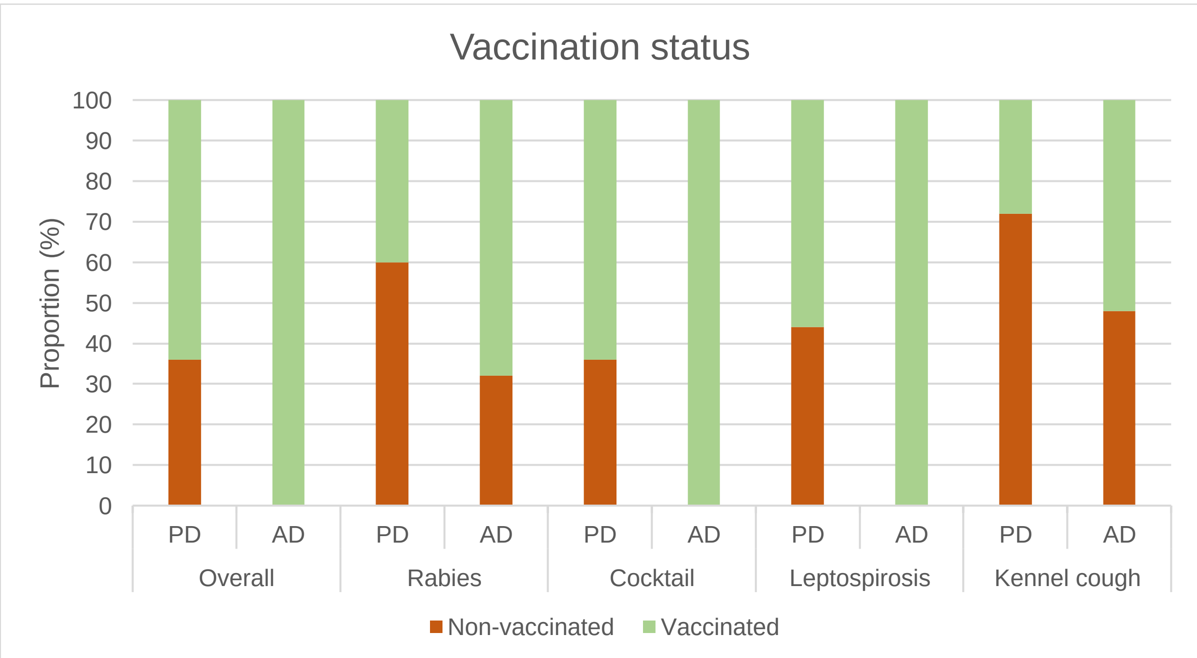

Figure 2. *The vaccination status of pet dogs (PD) and assistance dogs (AD): overall (vaccinated = received one or more vaccinations on recommended schedule), rabies vaccination, cocktail vaccination (canine parvovirus, canine distemper virus, and canine adenovirus causing infectious canine hepatitis), leptospirosis vaccination, and kennel cough (canine parainfluenza virus and/or* Bordetella bronchiseptica*).*

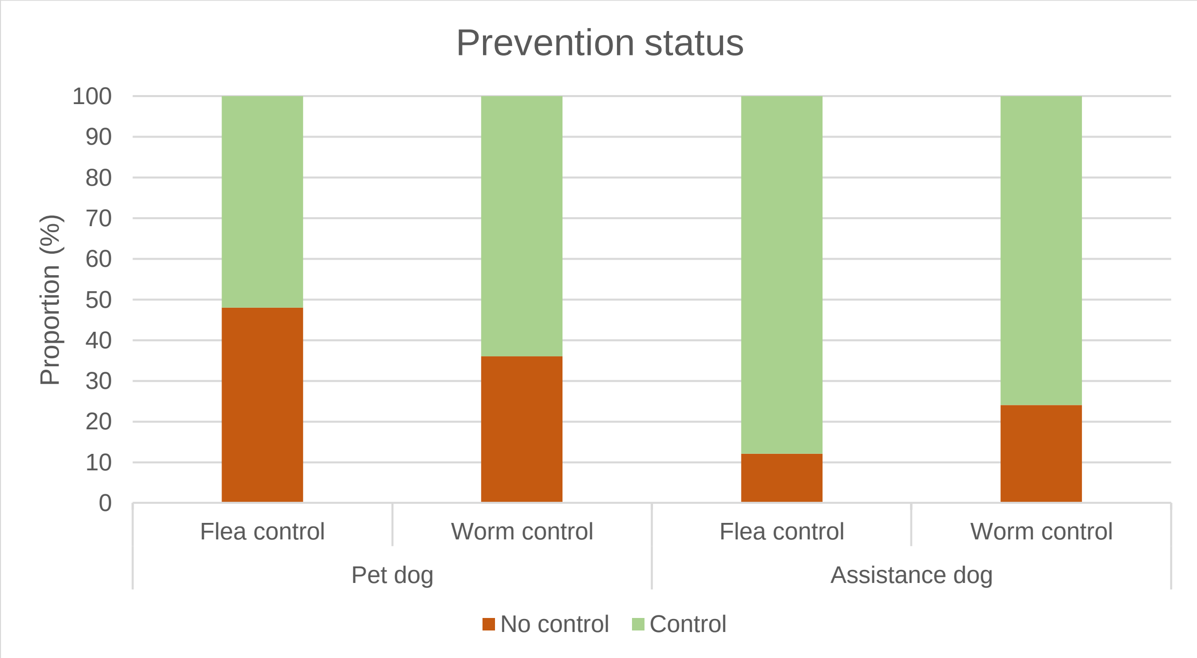

Figure 3. *The prevention status of pet dogs (PDs) and assistance dogs (ADs).*

The difference between the proportion of PDs receiving flea control and the proportion of ADs receiving flea control (Figure 3) was significant ($P < 0.05$ and $P < 0.01$). There was no difference in proportions concerning worm control ($P > 0.05$).

As neutering is mandatory for ADs, the proportion of neutered dogs in this group was high, being 84%. The reason why this number is not 100%, is because there were three young dogs which still had to be neutered, and one dog for which was made an exception. This was a Silken Windsprite, who are known for having a high risk of complications during or after anesthesia. In the PD group, 56% was neutered. This was significantly different from the AD group ($P < 0.05$).

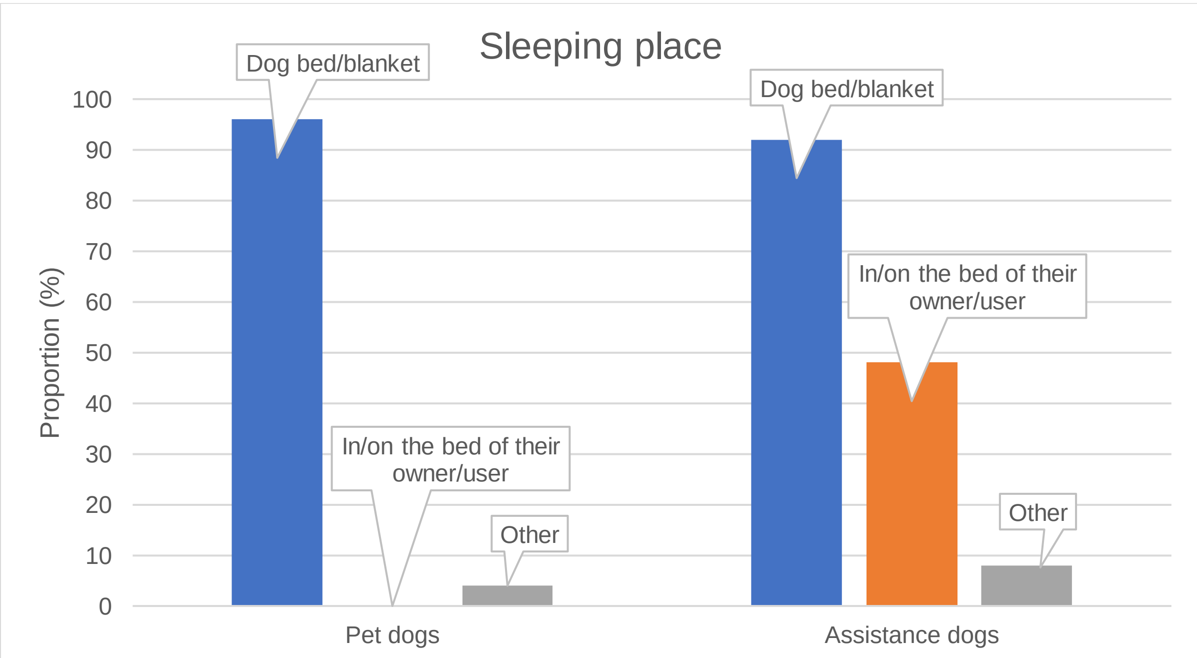

Figure 4. *The sleeping places of pet dogs and assistance dogs.*

Figure 4 shows that while PDs never sleep in or on the bed of their owners, almost half of ADs do. This is because a lot of ADs have to be near their users to do their job, such as detecting epileptic seizures, providing psychological support, or waking them up.

The information about whether or not a dog sleeps on a dog bed or blanket was taken into account, because these beds or blankets may act as an accumulation spot for dirt. Subsequently, dogs could gather this additional dirt on their paws when they sleep in such places.

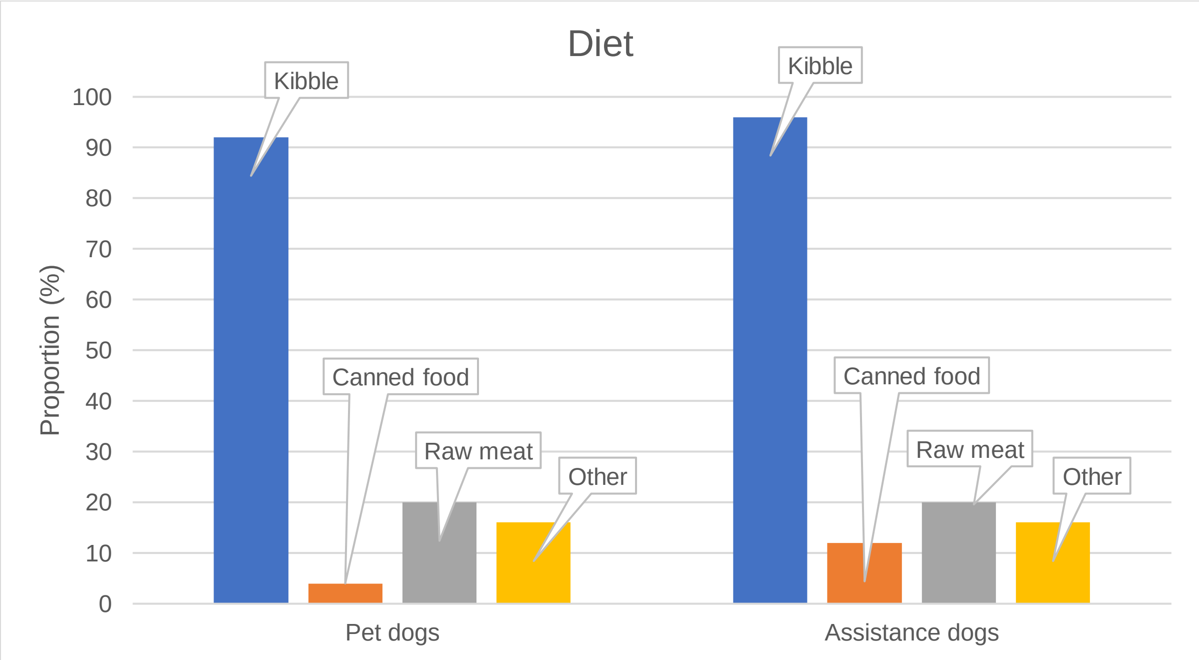

Figure 5. *The diet of pet dogs and assistance dogs.*

The main component of these dogs' diet was kibble (Figure 5). Canned food got offered to a small proportion, and raw meat to a slightly larger proportion. Other foods could be snacks or supplements.

A regression test was executed. A multivariable model on the amount of recovered CFUs from dog paws could not yield any factors, as too many CFU counts were zero (which resulted in heteroscedasticity). Therefore, a logistic regression test was performed, to see if any factors could be linked to either the presence or absence of CFUs recovered from dog paws. First, univariable models were set up for every of the 18 possible factors. None of the possible factors were significantly associated. For seven variables, $P < 0.25$ was found (see Table 3). One of those variables had too little individuals per subgroup, and so that variable was left out ('sleeping place'). The six other possible factors were added to a multivariable model. Association was found for three variables: worm control (not being on worm control), diet (not having 'other' as a part of the dog's diet), and locations visited during walks ('neighborhood' as a location that was not visited during walks) ($P < 0.05$). Table 4 shows the adjusted odds ratios (ORs) and their 95% confidence intervals (95% CIs) for these associations.

Table 3. *Unadjusted odds ratios (ORs) and their 95% confidence intervals (95% CIs) for the possible factors in the univariable models with* P*-values < 0.25, linked to the presence or absence of colony forming units (CFUs) of the Enterobacteriaceae family, recovered from dog paws.*

	Number of dogs			**ORs**	**95% CIs**	***P*-values**
	CFUs present (n = *14)*	*CFUs absent* (n = *36)*				
Dog type: assistance dog	9 (36%)	16 (64%)	*Being a pet dog*	0.4	0.1 – 1.6	0.2
Dog type: pet dog	5 (20%)	20 (80%)				
Worm control	13 (37%)	22 (63%)	*Not being on worm control*	0.1	0.006 – 0.7	0.05
No worm control	1 (7%)	14 (93%)				
Other elements present in diet	4 (50%)	4 (50%)	*Not having other elements in their diets*	0.3	0.06 – 1.5	0.1
No other elements present (other than kibble, canned food, or raw meat)	10 (24%)	32 (76%)				
Neighborhood as visited location during walks	11 (24%)	34 (76%)	*Not having 'neighborhood' as a location visited*	4.6	0.7 – 38.8	0.1
No neighborhood as visited location	3 (60%)	2 (40%)				
Age (in years): 0-1	2 (18%)	9 (82%)				
Age: 2-5	3 (20%)	12 (80%)	*Age: 2-5*	1.1	0.2 – 9.9	0.9
Age: 6-7	3 (25%)	9 (75%)	*Age: 6-7*	1.5	0.2 – 13.6	0.7
Age: 8-13	6 (50%)	6 (50%)	*Age: 8-13*	4.5	0.7 – 38.6	0.1
Vaccinated against rabies	10 (37%)	17 (63%)	*Not being vaccinated against rabies*	0.4	0.09 – 1.3	0.1
Not vaccinated against rabies	4 (17%)	19 (83%)				
Sleeping place: bench or dog bed/blanket	12 (26%)	35 (74%)	*Not having bench or dog bed/blanket as sleeping place*	5.8	0.5 – 132.4	0.2
Sleeping place: not bench or dog bed/blanket	2 (67%)	1 (33%)				

Table 4. *Adjusted odds ratios (ORs) and their 95% confidence intervals (95% CIs) for the identified factors in the multivariable model, linked to the presence or absence of colony forming units (CFUs) of the Enterobacteriaceae family, recovered from dog paws.*

	ORs	**95% CIs**	***P*-values**
Not being on worm control	0.04	0.001 – 0.4	0.007
Not having other elements in their diets (other than kibble, canned food, or raw meat)	0.06	0.002 – 0.5	0.007
Not having 'neighborhood' as a location visited during walks	15.8	1.4 – 339.0	0.04

However, the estimates of the variables differed substantially while omitting the variables one by one out of the multivariable model (> 15%).

Experience of having an AD

The experience questionnaire was conducted to get an impression of the situation of AD users in the Netherlands; 31 AD users entered (including the 25 who participated in the sampling). The first three questions were three statements with the following five options from which the participants could choose: fully agree, somewhat agree, neutral, somewhat disagree, or fully disagree. The first statement, outlined in Figure 6, was 'I feel free to go wherever I want to with my assistance dog'.

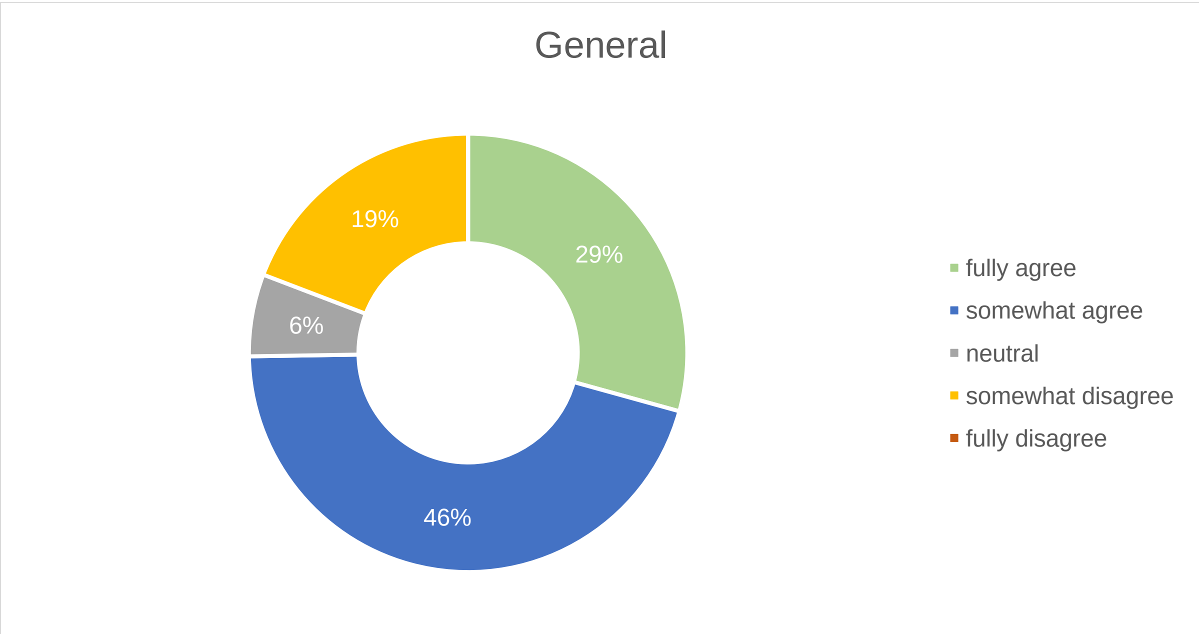

Figure 6a. *General opinion of assistance dog users on the statement: 'I feel free to go wherever I want to with my assistance dog.'*

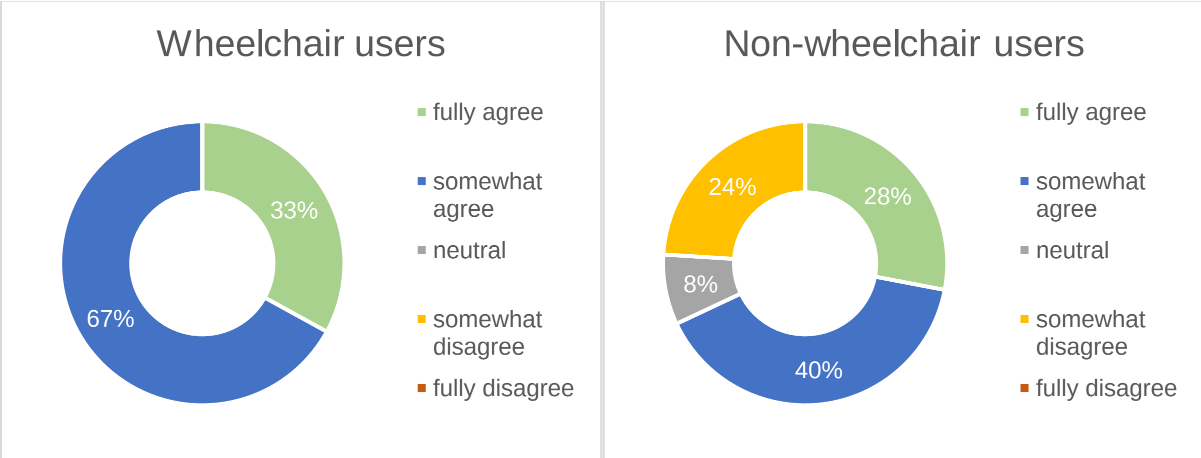

Figure 6b. *Opinion of assistance dog users on the statement: 'I feel free to go wherever I want to with my assistance dog', differentiated in wheelchair users and non-wheelchair users.*

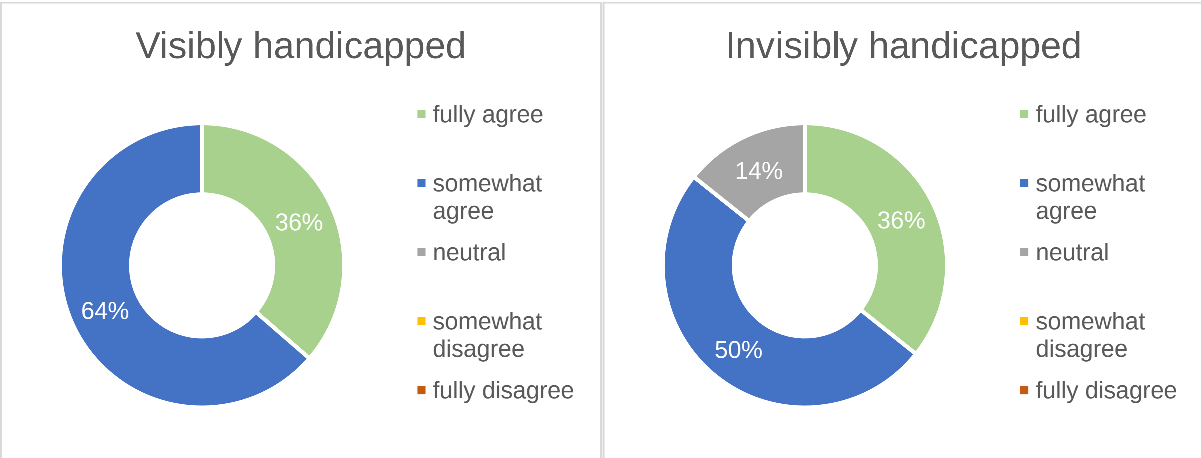

Figure 6c. *Opinion of assistance dog users on the statement: 'I feel free to go wherever I want to with my assistance dog', differentiated in the visibly handicapped and invisibly handicapped.*

Chi-squared tests were performed to compare the percentages of the chosen options between wheelchair users and non-wheelchair users, and between the visibly handicapped and invisibly handicapped. The differences were not significant ($P > 0.05$).

The second statement was 'I feel like society understands why I have an assistance dog'. The results are shown in Figure 7.

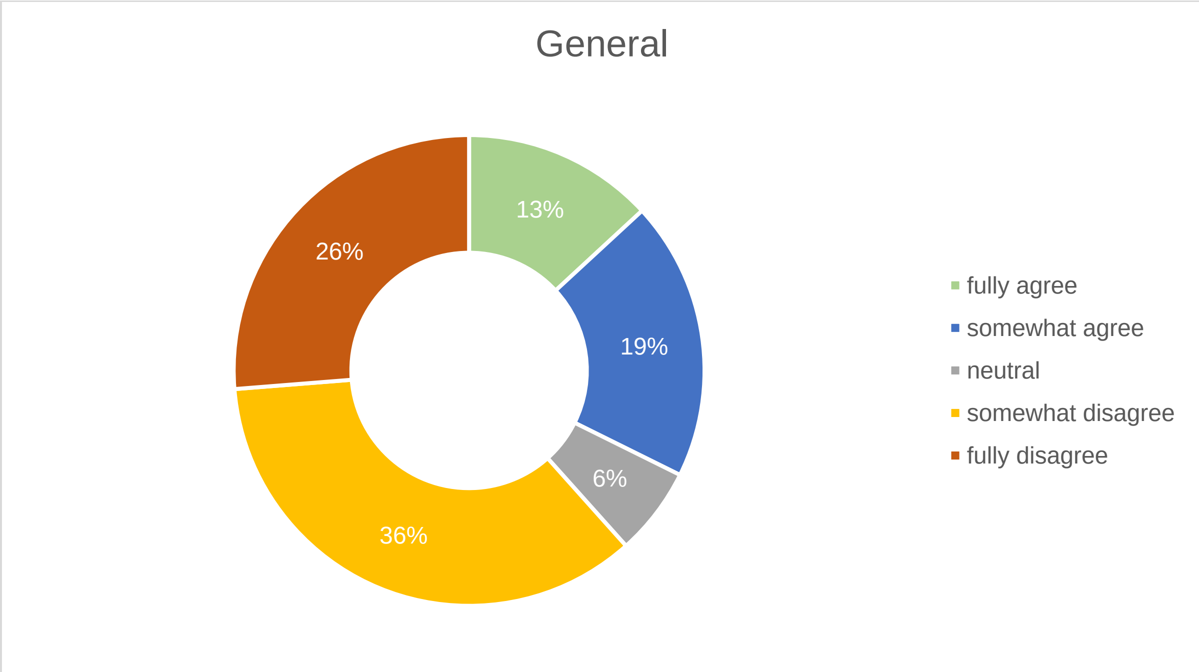

Figure 7a. *General opinion of assistance dog users on the statement: 'I feel like society understands why I have an assistance dog.'*

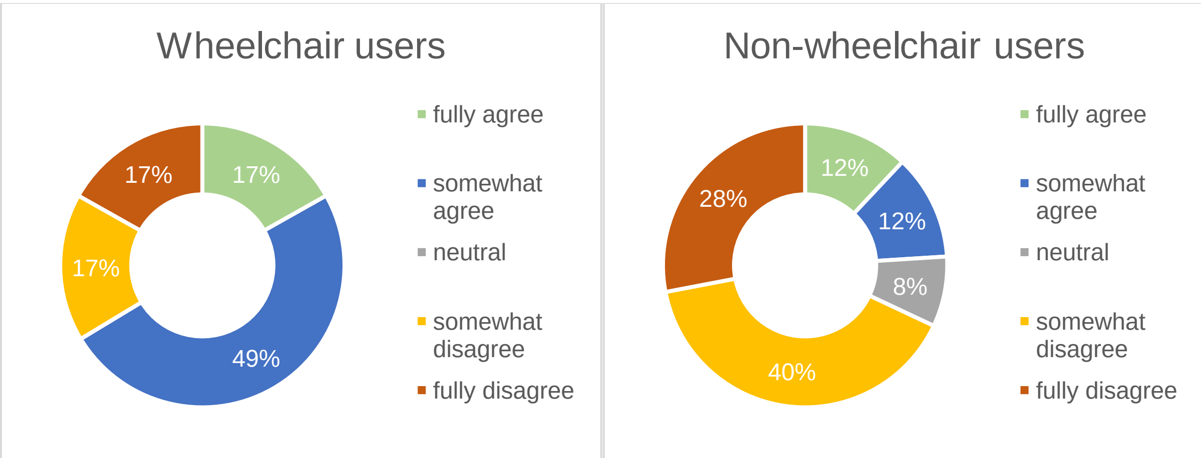

Figure 7b. *Opinion of assistance dog users on the statement: 'I feel like society understands why I have an assistance dog', differentiated in wheelchair users and non-wheelchair users.*

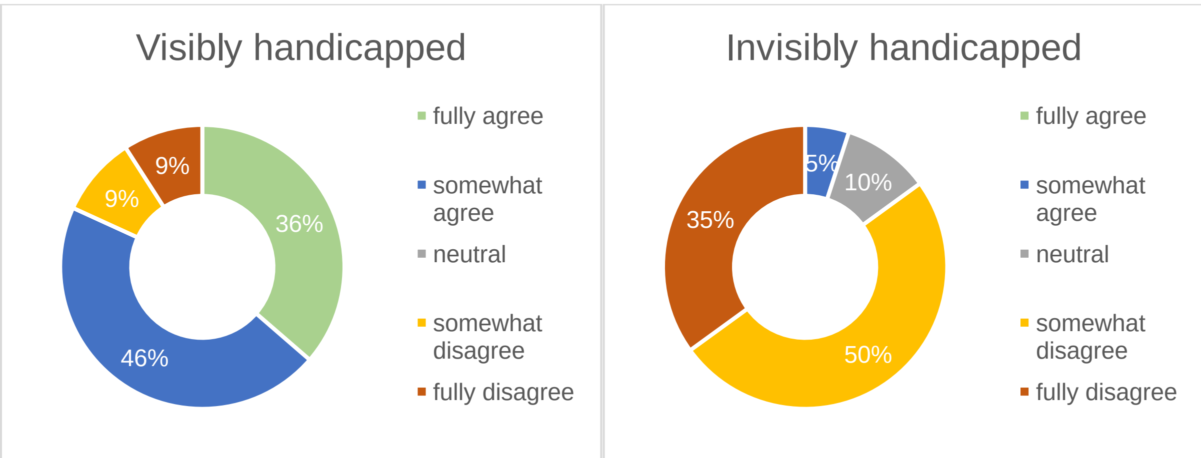

Figure 7c. *Opinion of assistance dog users on the statement: 'I feel like society understands why I have an assistance dog', differentiated in the visibly handicapped and invisibly handicapped.*

Again, chi-squared tests were executed. There were no differences between the answers of wheelchair users and non-wheelchair users ($P > 0.05$); they feel equally understood. However, the differences between the answers coming from the visibly handicapped and the invisibly handicapped were significant ($P < 0.05$ and $P < 0.01$).

Finally, the third statement was 'I feel like society accepts my assistance dog in public environments.' Figure 8 displays the results.

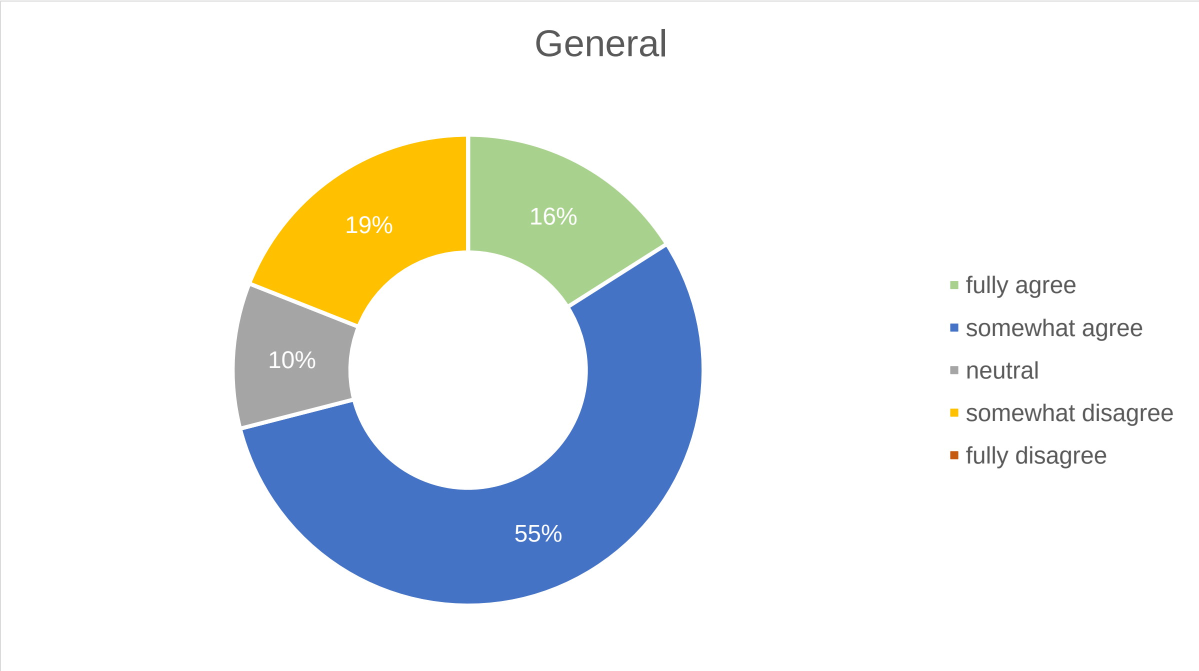

Figure 8a. *General opinion of assistance dog users on the statement: 'I feel like society accepts my assistance dog in public environments.'*

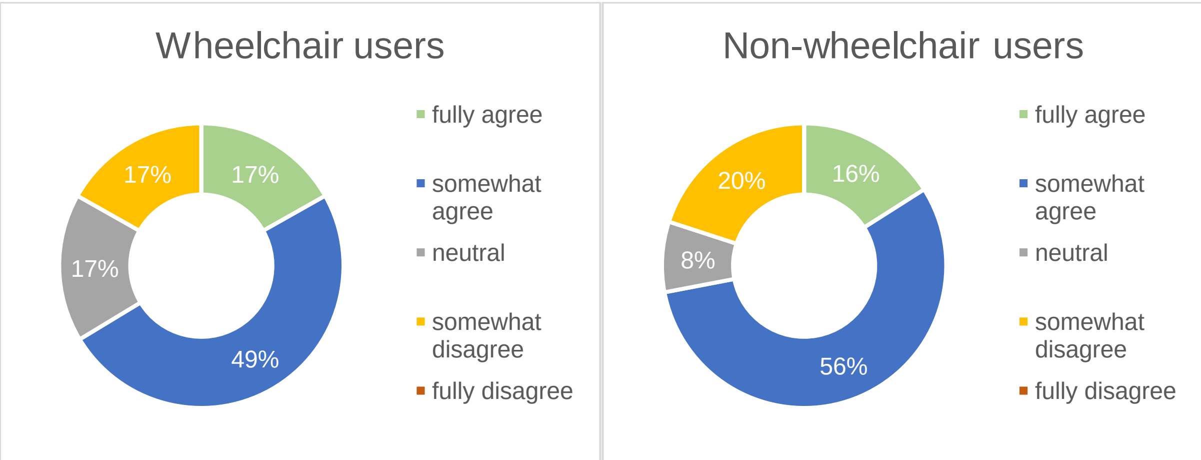

Figure 8b. *Opinion of assistance dog users on the statement: 'I feel like society accepts my assistance dog in public environments', differentiated in wheelchair users and non-wheelchair users.*

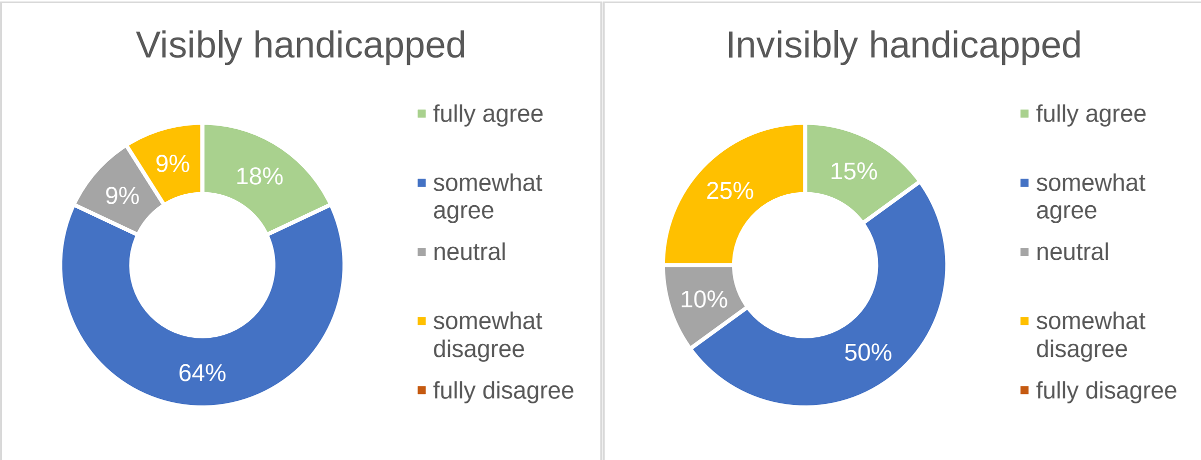

Figure 8c. *Opinion of assistance dog users on the statement: 'I feel like society accepts my assistance dog in public environments', differentiated in the visibly handicapped and invisibly handicapped.*

The results from the chi-squared tests were: no significant differences between neither the answers of wheelchair users and non-wheelchair users, nor those of the visibly handicapped and invisibly handicapped ($P > 0.05$).

In 2016, a UN agreement on the rights of the handicapped took effect. It states that the handicapped should be treated as any other person, that they should have access to mobility aids, and that facilities for the general population should be available to them, in a manner and at a time of their choice[14]. Subsequently, changes were made to Dutch legislation. It clearly states that effective adjustments should be embedded for the handicapped, including at least the admittance of ADs[15].

It became clear during this research that, despite the law, the participants were frequently halted at public places: almost 81% had experienced this once or several times with their current AD (see Figure 9 for this and more results).

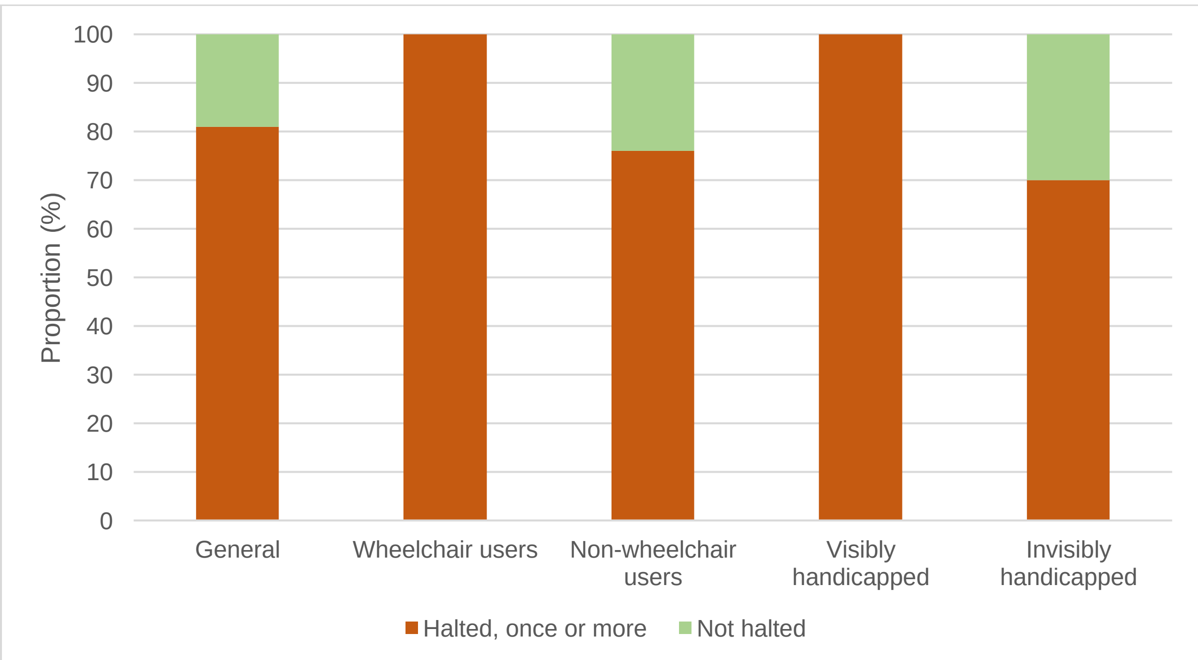

Figure 9. *Proportions of participants halted once or more with their current assistance dog at public places; in general and differentiated in subgroups.*

The answers shown in Figure 9 were not significantly different between wheelchair users and non-wheelchair users, but they were distinct between the visibly handicapped and invisibly handicapped ($P < 0.05$).

The public places where participants were halted once or more that were named the most, were health care facilities (thirteen times), such as hospitals (five times), dentists, doctor's offices (both three times), and pharmacies (two times). Restaurants (ten times) and other food stores (also seven times), such as ice cream parlors, a supermarket, a butcher shop, a bakery, a snack bar, and foreign food shops, came in second place. Next were other shops (ten times), such as drugstores, a hardware store, and clothing stores. In fourth place came public transportation (six times), such as taxis (four times). Other places that were mentioned, were hotels, playground, museum, and even the home of family and friends.

Hygiene was the main reason for these events (named thirteen times), followed by allergies (three times), and inconvenience for other visitors (three times). Another argument a participant heard, was that dogs in general were not allowed in, therefore they could not make an exception. This again shows that there is a serious lack of knowledge about this subject and the law. Other reasons were that the allowance of the AD would require extra cleaning, that the AD was wearing the wrong harness and was hence not recognized as an official AD (which it was), that they had experienced problems with previous dogs, that a sterile environment must be maintained to the best of their ability, and that dogs are not allowed in the vicinity of food.

To find out whether or not there were hospitals which generally allowed ADs without any trouble, this was also inquired in the experience questionnaire. 77% of participants had experience with hospitals which generally allowed ADs (Figure 10). They named 29 hospitals, of which 20 were unique.

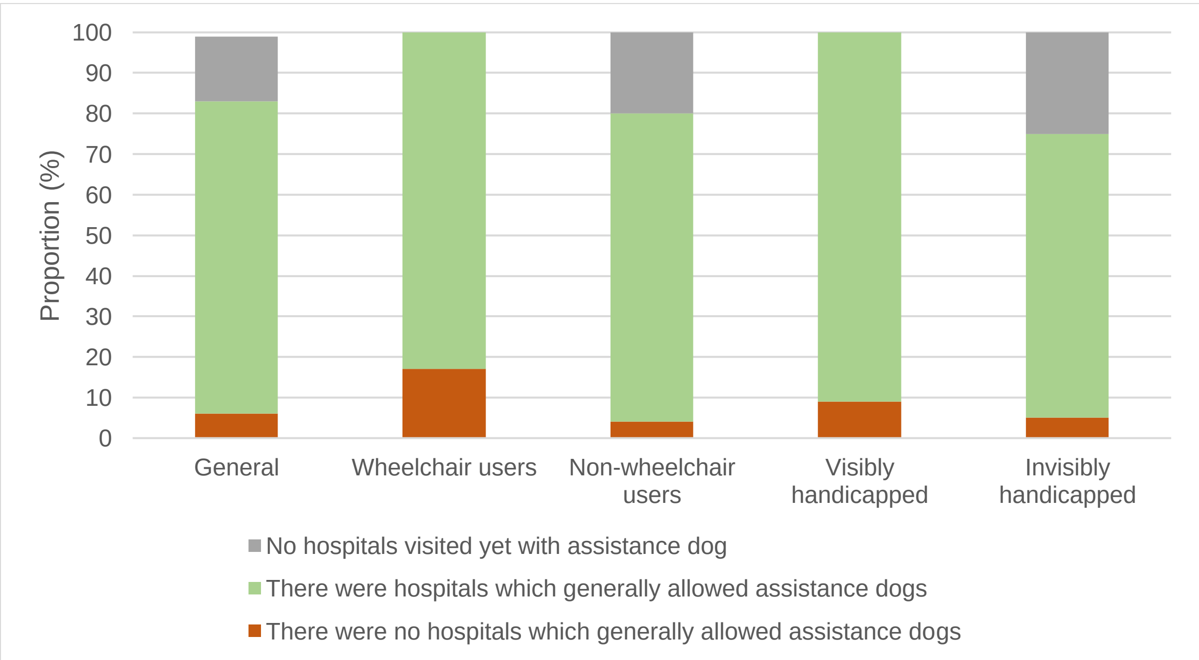

Figure 10. *Experiences of participants of hospital visits with their assistance dogs; in general and differentiated in subgroups.*

The differences in answers between wheelchair users and non-wheelchair users, and the visibly handicapped and invisibly handicapped were not significant ($P > 0.05$).

Having had experiences of being halted at the entrance of public places, it is imaginable that AD users feel like they cannot take their AD with them to certain places where there may be a low degree of acceptance for these dogs. They were asked if this was something they were confronted with. 58% of participants stated that they were. The results per subgroup can be found in Figure 11.

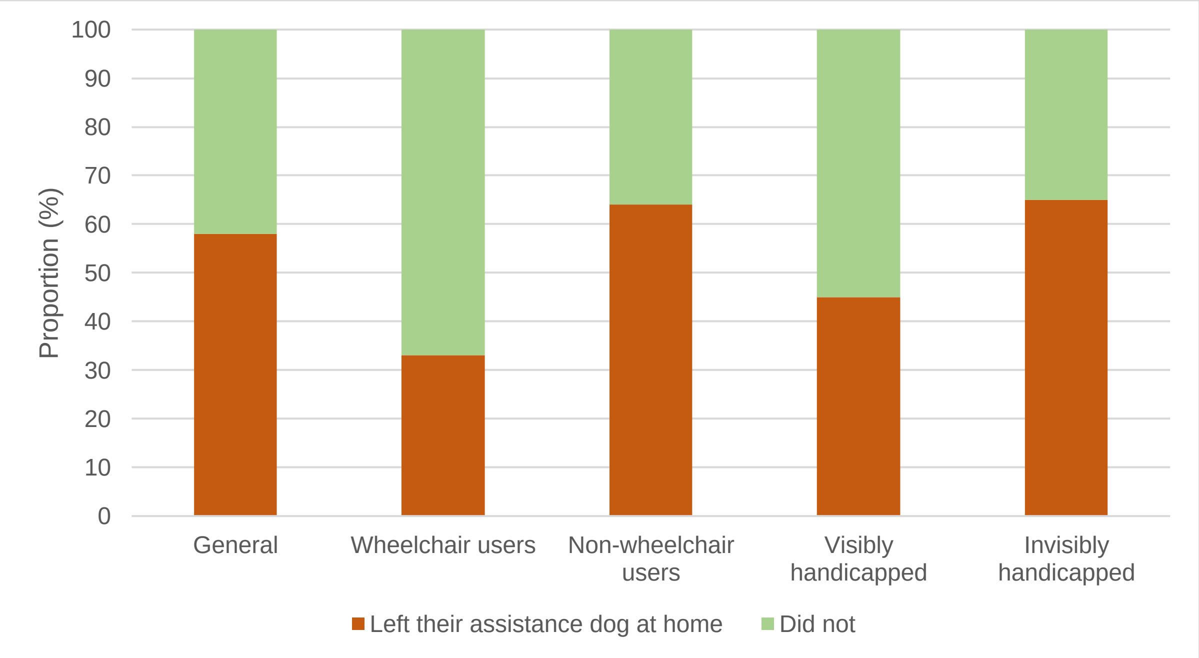

Figure 11. *Proportions of participants who left their assistance dog at home, because they felt like there was a low degree of acceptance for these dogs at certain places; in general and differentiated in subgroups.*

The proportion tests executed on these data showed that there were no significant differences in answers between wheelchair users and non-wheelchair users, and the visibly handicapped and invisibly handicapped ($P > 0.05$).

Participants said that leaving their AD at home because of a low degree of acceptance, could make them feel uncomfortable or uneasy, upset, insecure, and even stressed and not accepted. They feel it is tiresome to constantly wonder if their dogs were or were not allowed at a certain place. Others explained that it depends on the situation and location, whether it was logical or not that an AD was not allowed (for example, saunas or intensive care departments of hospitals). They said it is also a case of taking other visitors into account.

Participants were asked about possible improvements in public space, public knowledge, and other topics regarding ADs. Their answers are displayed in Table 5.

Table 5. *Possible improvements in the infrastructure of public space, public knowledge, and other topics regarding assistance dogs (ADs), mentioned by AD users.*

Public knowledge	*For whom?*	Civilians visiting public places.
		Security guards.
		Store personnel.
		People working in the hospitality industry.
		Company owners.
		Health care workers.
		Other organizations.
	About what?	The fact that every AD or AD in training has an identification card, that shows that it is a certified AD, coming from an official and licensed organization, and the name of its user.
		Education about hygiene and the impact of ADs on hygiene.
		Education about the reason and need for an AD, and the fact that an AD is something completely different than a pet dog.
		Education about the types of ADs, as people often only know the guide dog type.
		Dealing with ADs as a non-user: no touching, no talking, no eye contact, ignore the AD (even when it comes to you), keep yourself and your own dog at a distance.
		Education about invisible diseases, as people tend to not recognize these diseases and are often biased.
		Education about the used terms regarding ADs, and the use of standardized terms, set up by Assistance Dogs International (ADI).
		More research on the effect of ADs on their users and corresponding publicity.
		Overall understanding, clarity, and acceptance.
Infrastructure public space	*What?*	More space for ADs in public transportation; they often have to lie down in the aisle, which can make for dangerous situations, for both people and ADs.
		Not constructing shared places in traffic, especially for blind people; they have no orientation and they cannot make eye contact with motorists.
		The availability of an elevator at all times; this is obviously important for wheelchair users, but also when there are only escalators (the fur on dog paws can get caught between the steps).
		Facilitating of vacations of ADs and their users; they are often denied in dog-free accommodations. This limits the range of choice and makes the AD user very dependent on certain resorts, hotels, organizations, et cetera. This also applies to transportation by taxi.
		It should be noted that revolving doors are an issue for guide dog users.

Table 5 (continued). *Possible improvements in the infrastructure of public space, public knowledge, and other topics regarding assistance dogs (ADs), mentioned by AD users.*

Identification and welcoming of ADs	*What?*	Stickers near the entrance of public buildings, indicating that ADs are welcome. These stickers already exist, but they are only present in small numbers, and frequently targeted at a single type of ADs, most often guide dogs.
		Uniform AD harnesses, for every type of AD and regardless of which organization they are from, that can be recognized by any civilian.
		Education and publicity on these stickers and harnesses, plus information about imitation harnesses of non-official ADs (sometimes even pet dogs).
		Education about the communication with AD users: how the handle the situation correctly?

Participants noted that the largest gain can be made in the public knowledge area and communication between AD users and non-AD users.

Hospitals

Unfortunately, only three of the ten approached hospitals responded, and two of those follow common protocols (hereinafter referred to as hospitals A and B, the third hospital being hospital C). This research coincided with the SARS-CoV-2 (COVID-19) pandemic, so it is imaginable that these questionnaires were not a priority.

It is not clear how many hospital visits involve an AD per year. Hospital A said they receive around five requests or announcements from AD users regarding bringing their ADs with them.

All of the hospitals agreed on the requirements for an AD: vaccinations and flea and worm control has to be up-to-date. There were no specific cleaning protocols for after an AD visit, only regular or daily ones. Visible contamination, such as hairs, feces, and saliva is removed from the room and from used materials. Hand hygiene was considered very important. Hygiene checks, such as cleanliness on view or swabbing and culturing, were not being regularly executed; therefore, there are no hygiene limit values in place, such as the maximum allowed number of cultivable CFUs from a certain surface. ADs were not allowed on intensive care units, rooms for aseptic treatments, storage rooms for medical and sterile materials, and food preparation areas. The department of nursing was added to that list by hospitals A and B. Where ADs are allowed, the attending doctor has to give their permission on the admittance of the AD.

There were some differences in the strictness of the hospital protocols. Hospital C said to have no objections to ADs visiting their hospital, provided that protocols are followed, and the reason or need to bring their AD is not asked. Hospital A and B require the AD users to announce and register their ADs in advance. They permit ADs to come along to brief treatments (< four hours) or a visit to their outpatient clinic. However, ADs are not allowed to accompany their users when they are hospitalized. Moreover, during this period, an AD cannot visit their user. Hospital C does allow ADs during hospitalization, as long as there is permission from the attending doctor, and the room is cleaned daily.

Discussion

Sampling results

Contrary to many people's beliefs, this research showed that dog paws in general have a better general hygiene than shoe soles. The prejudice of dirty dog paws is not a strange one: the bottom of a dog paw has a large surface area because of their nails, toes, pads, and fur. This may create the possibility of a large buildup of bacteria.
Besides that, people are often used to take their shoes off when coming back home, while dogs cannot. However, when visiting a hospital, a shop, or traveling by public transportation, shoes are usually not taken off. This would make the feet of dogs and humans more equal in these kinds of situations. One potential explanation why dog paws are cleaner than shoe soles, is because dogs groom themselves, including their paws. This frequency is probably higher than the frequency of shoe cleaning. One study found a grooming frequency of nine to twelve times per hour in dogs kept in group housing[16]. This grooming comprises more than just paw grooming, but it gives a general impression. A fair percentage of participants of this research said they cleaned the paws of their dogs standardly after a walk. Because shoes are often off when at home, they do not need to be cleaned as much. Generally, they are only cleaned when they are visibly dirty.

Another feasible reason why dog paws are cleaner, that is also connected to grooming, are the possible antibacterial properties of dog saliva. Canine saliva contains immune[17] and non-immune antimicrobial factors, including lysozyme and salivary peroxidase. The levels of both of those substances were found to be three times as high as in human saliva[18]. Another study discovered that the saliva of male and female dogs acted bactericidal against the bacteria *E. coli* (which is a member of the Enterobacteriaceae family) and *Streptococcus canis*[19]. This could explain why there were fewer, and often zero CFUs recovered from dog paws, in comparison to shoe soles.

Apparently, PD owners and AD users, and PDs and ADs are equal in general hygiene, as significant differences were not found. The type of human or the type of dog is not of influence on the general hygiene of their shoe soles or paws, respectively.

To place the found numbers of recovered CFUs in perspective, one could compare these numbers to the number of recoverable CFUs of the Enterobacteriaceae family in dog feces. On average, these numbers are 10^{10} to 10^{11} CFUs per gram of dog feces[20]. This concentration was never found on either dog paws or shoe soles.

When looking at the dog-human couples, it became clear that, while PD paws have a better general hygiene than the shoe soles of their owners, the general hygiene of AD paws and the shoe soles of their users is equal. Thus, the conclusion 'dogs in general have better hygiene' is most likely caused by the better hygiene of PDs, and not so much by that of ADs. Why is it that ADs and their users are more equal in general hygiene than PDs and their owners? One theory behind this could be that ADs and their users spend more time together, because a user depends on their AD for support or completing tasks. Outside of their home, an AD accompanies its user wherever they go, for example to supermarkets and hospitals. This is especially the case for guide dogs, as they walk almost the same path as their user when assisting them. This is often not the case for PDs and their owners, as many places do not allow PDs. When at home, a PD and its owner will probably spend more time apart than an AD and its user. These events could explain why ADs and their users are more equal in general hygiene, than PDs and their owners.

C. difficile was only found in one of the samples, which was a sample from a shoe sole of an AD user. A study conducted in rural and urban Slovenia found 43% of shoe sole samples positive on *C. difficile*, and 24% of dog paw samples. The method of this research was mostly adopted into the current research; however, in the Slovenian study, the colonies suspicious for *C. difficile* are confirmed using PCR. In the current research, cultures of seven of the dog paw samples and nine of the shoe sole samples contained colonies that were suspicious of being *C. difficile*, because they had most or all characteristics based on colony morphology. Only one sample had colonies that fluoresced under UV light, the rest of the suspicious samples did not. Existing literature does not clarify what these colonies could be. It is also possible that some of the colonies were too small to detect fluorescence.

Pseudomonas spp. were also found in only one sample, being a shoe sole sample from a PD owner. In particular, *P. aeruginosa* is a major causative agent for nosocomial infections, for example urinary and respiratory tract infections, often linked to catheterization and intubation[21]. Polymicrobial biofilms containing *P. aeruginosa* form on catheters and ventilator tubes, and therefore this bacteria is linked to ventilator associated pneumonia (VAP)[22]. A chronic pneumonia caused by this bacteria is especially dangerous to cystic fibrosis patients[23]. Fortunately, this bacteria seems to be rare on dog paws and shoe soles.

Sampling methods and materials

The used sample size for this research was sufficiently large for the comparison of dog versus human. However, because of time and budget limits, this number was then divided into subgroups, instead of doubled to serve both dog types (AD and PD). Perhaps, when wielding a larger sample size, the non-significant differences in means of recovered Enterobacteriaceae CFUs would become significant.

The samples of dog paws came back negative (zero recovered CFUs of the Enterobacteriaceae family) more often than the samples of shoe soles. This could imply, again, that dog paws are cleaner than shoe soles. During this research, only diluted samples were inoculated. Therefore, the result ‘zero recovered CFUs’ does not mean there were not any bacteria of the Enterobacteriaceae family on the paw or shoe, it only shows that the number was below the detection limit (7×10^2). Because the method of Polywipe™ sponges and stomacher bags was used, the sponges needed to be suspended into a fluid, as to recover the bacteria sampled from the paws or shoes. A convenient amount of fluid was decided on, as to facilitate easy calculation of CFUs, and because the surface area of the sponges is fairly large (five by ten centimeters, double sided). This large surface ensures the recovery of as many bacteria as possible; the fact that the sponges are premoistened also contributes to this goal. This sampling method was therefore deemed to be most effective, also for the recovery of *C. difficile*, especially because the method was already shown to be effective for this bacteria[7,10].

One problem of the used methods is that the different surfaces of dog paws and shoe soles complicate their comparison. It was deemed very difficult to measure the surface of every dog paw, because this surface includes the foot pads, toe webbing and fur. Just measuring the bottom’s circumference would not be enough, and also not fair, as this would render a higher concentration of recovered CFUs per cm^2 than shoe soles. The left and right shoe soles of their owners or users are probably very similar, but the same cannot be said about dog paws. Hind paws are likely to be cleaner than front paws in terms of recoverable CFUs from the Enterobacteriaceae family[9], and so the CFU counts of front paws cannot simply be assumed to be the same for the hind paws. More research is needed in this area. Again, dog paws came back negative for Enterobacteriaceae more often than shoe soles, so it would seem that dog paws do indeed have better general hygiene than shoe soles.

At the start of this research, it was not yet clear whether the dogs would tolerate the sampling of their paws. The sponges feel wet and cold to the touch and although they do not have a scent, a dog could still get startled by the smell of the gloves, reminding them of a veterinarian's office. This was tested during the sampling of the first dog (a PD), and because she tolerated it very well, it was decided this was an appropriate sampling method. The dog was carefully approached form the side while talking to her, she was then allowed to sniff the gloves on the back of the researcher's hands and was asked to sit down. The researcher gave the commando to give paw and waited until the dog obeyed. The paw pads and toe webbing was then carefully swabbed. Afterwards, the dog was rewarded with a treat and petting. Only one dog, which was a PD, did not tolerate the sampling of its paws as well as the other dogs, because he was not used to his paws being handled. Virtually every AD was used to this, so the sampling of their paws did not pose any problems. PD owners were asked beforehand if they thought their PD would tolerate this method, which made for only successful visits.

Factors

Three factors could be linked to the presence or absence of recovered CFUs from dogs paws: worm control, diet, and locations visited during walks. The ORs from the multivariable model will be discussed in the following section, as they hold more value than ORs from univariable models. In this study, the odds of having CFUs recovered from their paws are lower for dogs that are not on worm control, as they are for dogs who are. The odds of having CFUs recovered from their paws are lower for dogs that do not have other elements in their diet, besides kibble, canned food, or raw meat, than those of dogs that do. The odds of having CFUs recovered from their paws are 15.8 times (or 1480%) higher for dogs who do not visit their neighborhood during walks, as they are for dogs who do. The first two factors seem hard to reconcile with the presence or absence of recovered CFUs. An explanation for the third one could be that dogs that do not visit their neighborhood during walks, spend more time in locations like beaches, forests, and parks, which may have higher contamination levels on their grounds than the sidewalks and streets in typical neighborhoods. However, the estimates of the variables differed too much (> 15%) while omitting them one by one from the multivariable model, which indicates confounding. It is not clear what the confounders are.

To fully identify factors linked to the amount or the presence or absence of recovered CFUs, it is wise to increase sample size. In addition, there should be an expansion of certain possible factors. For example, participants were asked how often they bathe their dog in a year. This question could be expanded by informing how long ago it was that they bathed their dog. Two other questions that popped up during the writing of this report were 'How often do participants clean their shoes, and when did they last clean them?', and 'Do participants standardly clean their dogs' paws after a walk?'. It is advised to ask at least these additional questions during future research on this topic.

Assigning a geological location to a human-dog couple, either urban or rural, was difficult, as a lot of areas are not clearly urban or rural. Some participants lived in the outskirts of a large city, which made their area very similar to the more urban parts of theoretical rural areas.

Popular literature considers a number of dog breeds to be hypoallergenic, like the labradoodle, poodle, and Airedale terrier. However, there is a lack of evidence proving the existence of such breeds[24]. Therefore, so-called hypoallergenicity would have no influence on the amount or presence of recovered CFUs, which it indeed did not.

Although raw meat being part of a dog's diet did not come up as a factor for the general hygiene of their paws, it could still pose a problem. A study found a significantly different fecal microbiome between dogs that were fed with Bones and Raw Food (BARF) diets, and dogs that were fed with commercial diets. The BARF diet group had higher abundances of, but not limited to, *E. coli* and *C. perfringens* in their feces, in comparison to commercially fed dogs[25]. Another study showed that raw-meat based diets (RMBDs) contain several zoonotic bacteria, such as *E. coli,* ESBL, *Listeria* spp., and *Salmonella* spp., and concerning parasites. These pathogens may pose a threat when transmitted to people[26]. A recent study confirmed that the levels of *Salmonella* spp. and *E. coli* in eight commercial RMBDs exceed the EU standards[27]. This could be especially dangerous to some AD users, as they are considered to be part of a high-risk group (for example, people with chronic diseases). PD owners may also be in this high-risk group, and young children and the elderly always are. In general, it is not advisable to feed BARF diets or RMBD's to dogs.

Experiences with ADs

The first statement on the experience questionnaire, 'I feel free to go wherever I want to with my assistance dog', did not harvest any significant differences between the answers from either the wheelchair users and non-wheelchair users, nor the visibly handicapped and invisibly handicapped. This suggests that those different groups did not choose differently; they feel evenly free to go wherever they want with their ADs. The answer option 'fully disagree' was not chosen by any participant, and the largest proportion (75%) of the answers was positive, either 'fully agree' or 'somewhat agree'. Fortunately, most participants feel fairly free to take their ADs to a place of their choice.

The second statement to which participants were asked to give their opinion, 'I feel like society understands why I have an assistance dog', did result in significantly different answers, between the visibly handicapped and the invisibly handicapped. Looking at Figure 7c, this denotes that the invisibly handicapped do not feel as understood about why they have an AD, as the visibly handicapped do. Moreover, the answer 'fully agree' was never chosen by an invisibly handicapped person. The participants of an investigation conducted among AD users showed that they felt like the invisibly handicapped received more negative reactions towards their use of an AD than the visibly handicapped. The cause was often a lack of knowledge. This is in line with the results to the second statement. Another conclusion of the study was that the negative reactions came from adults more frequently than they came from young people. The former were more rude, whereas the latter were more shy and awaiting[28].

The third statement was 'I feel like society accepts my assistance dog in public environments'. There were no significant differences between the answers of wheelchair users and non-wheelchair users, or the visibly handicapped and invisibly handicapped. This suggests that the feeling of acceptance by society of their ADs in public environments is not different between these groups. Just like the first statement, the answer 'fully disagree' was not chosen with this statement as well.

To the question if they have ever been halted at a public place because of their current AD, 81% of the AD users answered that they had. This is often due to a lack of information or knowledge, as an explanation about the situation by the AD user is usually enough to grant them access anyway; a hard denial is (fortunately) rarer. However, it was noted by a lot of AD users that it takes a lot of effort to keep educating others during their daily routines. The participants also had significantly different answers: the proportion that had never been halted was larger for the invisibly handicapped than for the visibly handicapped. This implies that a larger proportion of the invisibly handicapped has not been stopped (yet), when held next to that proportion of the visibly handicapped. Why is it that a larger percentage of the invisibly handicapped has never been halted at a public place, in comparison to the visibly handicapped? Looking at the data, this could be caused by the young age of the ADs that have never been halted. The first year of an AD's life is spent by learning the basics of obedience. It depends on the organization whether the dog already lives with its user, or stays at a foster family. Generally, during the second year of its life, the AD is taught the skills it needs for the aid of its user. Again, it varies per organization whether the AD already lives with its user, or still resides at the organization's facilities. Therefore, young ADs have not visited many public places yet. Note that ADs in training, wearing their harness, are also allowed to enter public places.

A number of participants said that they often or always call the place they want to visit with their AD in advance, to announce their stopover, or immediately walk up to an employee at a store, for example, to explain the situation. It is possible that the invisibly handicapped have a stronger tendency to do this, because they feel less understood about the reason they make use of an AD. This, and the young ages of the ADs that have never been halted, are possible explanations for the larger percentage of the invisibly handicapped that has never been halted, in comparison to the visibly handicapped.

The experience questionnaire showed that the most common reason for denying access to ADs and their users, is hygiene, or rather its violation. For example, restaurant owners may be afraid to lose their license based on hygiene infringement. They hide behind outdated laws and guidelines, but these are no longer apply to ADs. As explained before, the law states that ADs cannot be denied access to public places, including restaurants and hotels. In addition, the hygiene code of the Dutch hospitality industry states that ADs are always welcome to restaurants, among other places[29]. The Nederlandse Voedsel- en Warenautoriteit (NVWA; similar to the Food and Drug Administration) carries out checks in the hospitality industry. Naturally, they abide the law, and so their statement emphasizes that ADs are allowed to visit restaurants (and cafes and supermarkets), as long as they do not enter kitchens or storage rooms. The space in front of the counter at bakeries, butcher shops, and ice cream parlors is also approved for ADs[30].

This information must be distributed at least among most people in the hospitality industry. Still, ADs are denied access. Participants pointed out that their AD is an extension or even a part of themselves, so the denial of an AD is actually the indirect denial of their user. This is where AD organizations need to jump in. Fortunately, they are making good progress in this area. AD users can contact their organization about a situation in which they were denied access because of their AD.

Their organization will then discuss the situation with the public place in question, to push free access even further. The public places that need most attention in this process are health care facilities, and restaurants and other food stores.

The results of the experience questionnaire also show that hospitals do not discriminate between the types of AD users (wheelchair users and non-wheelchair users, and the visibly handicapped and the invisibly handicapped). The experiences participants had with the allowance of their ADs to hospitals was not significantly different. Moreover, most of the participants (77%) have been to a hospital that generally allowed ADs without any trouble. The Netherlands has a total of 120 hospitals. This research showed that at least 20 of those have admitted ADs without any trouble. A larger assessment would be needed to fully understand the current situation, as not all hospitals and geographical areas of the Netherlands were researched. Participants did note that the admittance of their AD was strongly dependent of the hospital employee they encountered on entering the hospital that day, which would mean that a lot of hospitals do not have clear protocols on the allowance ADs. The hospitals that completed the hospital questionnaire did have protocols, but they were not always as solid. The researcher advises hospitals to set up straightforward, unambiguous and complete protocols, which include the criteria for ADs (vaccination status, flea and worm control, identification), the range of allowance (departments, duration), and cleaning schedules. Naturally, these protocols should be known to all hospital staff and they should be in line with the law and current guidelines.

The tendency of participants to leave their AD at home, because of a possible low degree of acceptance for these dogs at certain public places, was similar between wheelchair users and non-wheelchair users, and the visibly handicapped and invisibly handicapped. 58% of participants said they left their AD at home once or several times, because of this possible low degree of acceptance. This is worrisome, as their ADs have important tasks to fulfill and they cannot do that if they are away from their users. Naturally, a proportion of users do not need their AD for every task or situation, but they should be allowed to decide on their own if it is needed that they take their AD with them, instead of deciding this based on a possible low degree of acceptance coming from society. This problem distresses some AD users. Again it is emphasized: access of ADs to public places needs to be claimed and more facilitated. This can be achieved with the help of organizations.

Improvements

A number of improvements were mentioned by the participants in the experience questionnaire. It became clear that most problems are caused by a lack of knowledge and poor communication. When ADs are denied access to a public place, this can often be changed by an explanation of the situation by the AD user. Civilians often do not know what the AD types are, or how to recognize an official AD. This is why there should be more education and publicity about ADs: about their identifications cards, what the AD types are, the wide array of breeds that is used, what their harnesses look like, why ADs are so important to their users, and what the law says about ADs. To facilitate the recognition of official ADs, it would be wise to design a uniform AD harness, that can be worn by any certified AD, regardless of which organization trained them. This harness should show their occupation (assistance dog) and a warning not to distract them, in Dutch and in English, accompanied by icons for people who cannot read (young children, for example). This is a topic that is already being discussed.

Stichting Gebruikers Assistentiehonden (Stichting GA; a foundation for AD users) is currently working on the uniform recognition of ADs[31].

As mentioned before, AD users can feel like there is a low degree of acceptance for their ADs. They do not always feel welcome in a public environment. To ameliorate this, stickers could be of great help. Stickers that welcome ADs into stores, for example, already exist, but there are only few of them and they are often targeted at only a single AD type. Participants indicated that these stickers are highly appreciated. So, it is advised to design a sticker that welcomes all types of ADs to come in, and to widely distribute them, along with informative flyers.

The distraction of an AD by a civilian can make for dangerous situations. An AD is still a dog, but with an important task to fulfill, for instance guiding its blind user. ADs are often trained to get into 'work mode' while wearing their harnesses, and are 'free' when they are not wearing their harnesses. A working AD should not be distracted, which is why a civilian should fully ignore them. This means no petting, no talking to them or making eye contact, even when it walks towards you. PDs can also be a distraction, which is why they should be kept at a distance, favorably on a leash. Potential ADs are carefully selected to be sure they can handle the job, and they get enough attention from their users[32]. In addition, they get the same amount of rest as a PD would[33]. So, being an AD is no case of animal cruelty and there is no need for civilians to make up for these assumed shortcomings.

No hygiene limit values for hospital environments could be found, for example allowed number of CFUs of the Enterobacteriaceae family on a certain surface; at least not for departments or areas which allow for the visit of ADs. However, one could think about possible hygiene measures, that may help in the decrease of nosocomial infections. Every hospital visitor could start wearing overshoes. A more environmentally friendly solution could be shoe brushes or longer doormats to reduce the amount of bacteria on shoe soles. These systems would have to be cleaned regularly, or else they would lose their function. Research was done on the usage of sticky mats in hospitals and a reduction of total isolated colonies from shoe soles was found[34]. Some types of sticky mats may also be helpful in decreasing the amount of dusts and *C. difficile* spores coming off shoe soles[35]. Another option could be the use of UV lights. Research suggests that these can be effective against several bacterial species found on shoe soles. It acquired the largest reduction in *E. coli* and *C. difficile*, among others, using a stand-on device with a UVC radiation session of eight seconds (HealthySole Plus, HealthySole®)[36]. Such a device could be placed in the entrance area of a hospital.

These options may also be applicable to ADs. Shoes are already available for dogs, and disposable ones could be easily designed. An objection to this product could be that a dog will not tolerate wearing it. One AD user said that they would not want to put their AD through this, so it debatable how many AD users would approve of this solution. Instinctively, doormats can easily be employed for ADs; however, the sticky mats should not hurt the AD by pulling its fur. UV lights cannot be used to decontaminate dog paws, as the light detrimental to the skin. Finally, dog paws could be cleaned with (wet) wipes on entering the hospital. Naturally, these wipes cannot contain harsh chemicals and should not give off a strong smell, as to not startle the AD. The current study showed that ADs allow their paws being handled very well, therefore this could be a good solution. Further research should decide how effective such wipes are.

Conclusions

This study showed that the general hygiene of dog paws is better than that of shoe soles. This result was mostly caused by the better general hygiene of PD paws in comparison to their owners' shoe soles, as ADs and their users had comparable levels of general hygiene. An explanation for this conclusion may be that ADs and their users spend more time together in the same environments. *C. difficile* was only found on one AD user's shoe soles, and *Pseudomonas* spp. were only found on one PD owner's shoe soles. Future research on dog paw hygiene should have a larger sample size to investigate possible factors that could be linked to the amount or presence or absence of recovered CFUs of the Enterobacteriaceae family.

The experience questionnaire pointed out that the invisibly handicapped feel less understood by society as to why they have an AD, as the visibly handicapped do. In addition, 81% of AD users have been denied access with their current AD once or several times, despite the law. Hygiene was one of the main reasons. The lack of knowledge of the general public on ADs and the law should be worked on, with the help of AD organizations. It would be wise to design a uniform harness for all AD types, regardless of the related organization. Hospitals should be encouraged to set up straightforward and unambiguous protocols on ADs. Possible hygiene measures could be (wet) wipes, (sticky) mats, or overshoes for dog paws, and overshoes, brushes, (sticky) mats, and UV lights for shoe soles.

Recommendations

Recommended future studies on this topic could be:

- A similar study, with a larger sample size and expanded questionnaires;
- Additional studies on the contamination dogs and humans add to their environment, regarding clothes, hair, fur, saliva, feces, et cetera;
- A similar study, which includes wheelchairs and walkers instead of shoe soles;
- A study on the effect of (wet) wipes on the general hygiene of dog paws, and on which solution on the wipes suits best;
- A study on the comparison of general hygiene between front paws and hind paws of dogs.

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Appendices

Appendix A. Search strategy.

<u>PubMed</u>

1. ((dog) AND grooming) AND frequency
2. ((antibacterial) AND saliva) AND dogs
3. ((((clostridium) AND colonies) AND properties)) AND agar
4. (brazier plates) AND clostridium
5. ((((((pseudomonas aeruginosa) AND hospital) AND infection diseases))) AND review)
6. ((disinfection) AND shoes) AND UV light

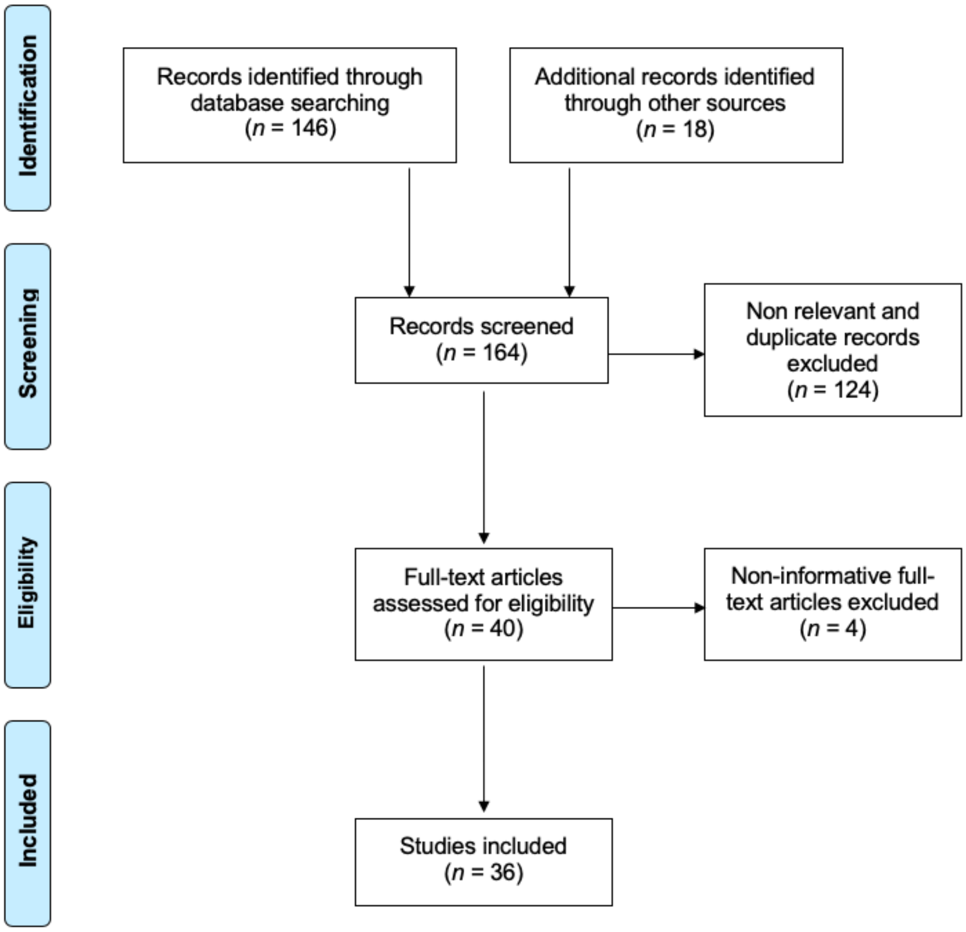
<u>Scopus</u>

1. (brazier's plates) AND clostridium

<u>Google Scholar</u>

1. (brazier's plates) AND clostridium

Appendix B. PRISMA flow diagram.



Modified from: Moher D, LIberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystemic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097.

Appendix C. Raw sampling data.

Assistance dogs.

Couple code	**Individual code**	**Recovered CFUs Enterobacteriaceae**	***C. difficile* presence**		***Pseudomonas* spp. presence**
			Suspected	*UV light fluorescence*	
AD-01	AD-01-D	0	-	-	-
	AD-01-O	0	-	-	-
AD-02	AD-02-D	1.5 x 10^3	-	-	-
	AD-02-O	9.0 x 10^2	-	-	-
AD-03	AD-03-D	0	-	-	-
	AD-03-O	0	yes	-	-
AD-04	AD-04-D	3.0 x 10^3	yes	-	-
	AD-04-O	8.7 x 10^5	yes	-	-
AD-05	AD-05-D	2.2 x 10^3	-	-	-
	AD-05-O	0 (<7 x 10^2)	-	-	-
AD-06	AD-06-D	1.9 x 10^3	-	-	-
	AD-06-O	0 (<7 x 10^2)	-	-	-
AD-07	AD-07-D	0	-	-	-
	AD-07-O	1.0 x 10^3	-	-	-
AD-08	AD-08-D	0	yes	-	-
	AD-08-O	0 (<7 x 10^2)	yes	-	-
AD-09	AD-09-D	7.0 x 10^2	-	-	-
	AD-09-O	1.0 x 10^3	-	-	-
AD-10	AD-10-D	0 (<7 x 10^2)	-	-	-
	AD-10-O	0	-	-	-
AD-11	AD-11-D	0 (<7 x 10^2)	yes	-	-
	AD-11-O	2.2 x 10^3	-	-	-
AD-12	AD-12-D	0	-	-	-
	AD-12-O	0	-	-	-
AD-13	AD-13-D	0	yes	-	-
	AD-13-O	0 (<7 x 10^2)	yes	-	-
AD-14	AD-14-D	0	-	-	-
	AD-14-O	6.0 x 10^3			-
AD-15	AD-15-D	0	-	-	-
	AD-15-O	0 (<7 x 10^2)	-	-	-
AD-16	AD-16-D	0 (<7 x 10^2)	-	-	-
	AD-16-O	0 (<7 x 10^2)	-	-	-
AD-17	AD-17-D	0	-	-	-
	AD-17-O	8.0 x 10^2	yes	-	-
AD-18	AD-18-D	0 (<7 x 10^2)	-	-	-
	AD-18-O	6.0 x 10^4	yes	yes	-
AD-19	AD-19-D	4.2 x 10^3	-	-	-
	AD-19-O	0	yes	-	-
AD-20	AD-20-D	1.2 x 10^3	-	-	-
	AD-20-O	7.1 x 10^3	-	-	-
AD-21	AD-21-D	0	-	-	-
	AD-21-O	0 (<7 x 10^2)	-	-	-
AD-22	AD-22-D	1.5 x 10^4	-	-	-
	AD-22-O	7.0 x 10^2	-	-	-
AD-23	AD-23-D	0	-	-	-
	AD-23-O	4.6 x 10^3	-	-	-
AD-24	AD-24-D	0	-	-	-
	AD-24-O	0	-	-	-
AD-25	AD-25-D	1.0 x 10^3	-	-	-
	AD-25-O	4.8 x 10^3	-	-	-

Pet dogs.

Couple code	**Individual code**	**Recovered CFUs Enterobacteriaceae**	***C. difficile* presence**		***Pseudomonas* spp. presence**
			Suspected	*UV light fluorescence*	
PD-01	PD-01-D	1.3×10^5	yes	-	-
	PD-01-O	4.3×10^6	yes	-	-
PD-02	PD-02-D	0	-	-	-
	PD-02-O	0	yes	-	-
PD-03	PD-03-D	0	yes	-	-
	PD-03-O	0	-	-	-
PD-04	PD-04-D	0	-	-	-
	PD-04-O	3.6×10^3	-	-	-
PD-05	PD-05-D	0 ($<7 \times 10^2$)	-	-	-
	PD-05-O	0 ($<7 \times 10^2$)	-	-	-
PD-06	PD-06-D	8.5×10^3	-	-	-
	PD-06-O	1.1×10^4	-	-	-
PD-07	PD-07-D	0 ($<7 \times 10^2$)	-	-	-
	PD-07-O	4.9×10^3	-	-	present
PD-08	PD-08-D	1.3×10^3	-	-	-
	PD-08-O	3.3×10^3	-	-	-
PD-09	PD-09-D	0 ($<7 \times 10^2$)	-	-	-
	PD-09-O	0	-	-	-
PD-10	PD-10-D	0 ($<7 \times 10^2$)	-	-	-
	PD-10-O	7.0×10^4	-	-	-
PD-11	PD-11-D	0	yes	-	-
	PD-11-O	1.0×10^3	-	-	-
PD-12	PD-12-D	0	-	-	-
	PD-12-O	0	-	-	-
PD-13	PD-13-D	0	-	-	-
	PD-13-O	7.0×10^2	-	-	-
PD-14	PD-14-D	0	-	-	-
	PD-14-O	2.7×10^3	-	-	-
PD-15	PD-15-D	0	-	-	-
	PD-15-O	1.4×10^3	-	-	-
PD-16	PD-16-D	0	-	-	-
	PD-16-O	3.0×10^3	-	-	-
PD-17	PD-17-D	0	-	-	-
	PD-17-O	3.0×10^3	-	-	-
PD-18	PD-18-D	0 ($<7 \times 10^2$)	-	-	-
	PD-18-O	0	-	-	-
PD-19	PD-19-D	8.0×10^2	-	-	-
	PD-19-O	2.7×10^3	-	-	-
PD-20	PD-20-D	0 ($<7 \times 10^2$)	-	-	-
	PD-20-O	7.7×10^3	-	-	-
PD-21	PD-21-D	0 ($<7 \times 10^2$)	-	-	-
	PD-21-O	7.1×10^3	-	-	-
PD-22	PD-22-D	9.0×10^2	-	-	-
	PD-22-O	1.7×10^3	-	-	-
PD-23	PD-23-D	0	-	-	-
	PD-23-O	0	yes	-	-
PD-24	PD-24-D	0 ($<7 \times 10^2$)	-	-	-
	PD-24-O	1.7×10^2	-	-	-
PD-25	PD-25-D	0 ($<7 \times 10^2$)	-	-	-
	PD-25-O	0	-	-	-

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