

***Campylobacter* spp. in Finnish small mammals**

Meeri Ylänen

Licentiate Thesis in Veterinary Medicine

University of Helsinki

Faculty of Veterinary Medicine

Department of Food Hygiene and Environmental Health

Environmental Hygiene

2020



Tiedekunta - Fakultet – Faculty Eläinlääketieteellinen tiedekunta		Osasto - Avdelning – Department Elintarvikehygienian ja ympäristöterveyden osasto	
Tekijä - Författare - Author Meeri Ylänen			
Työn nimi - Arbetets titel - Title <i>Campylobacter</i> spp.in Finnish small mammals			
Oppiaine - Läroämne – Subject Ympäristöhygienia			
Työn laji - Arbetets art - Level Lisensiaatin tutkielma		Aika - Datum - Month and year Huhtikuu 2020	Sivumäärä - Sidoantal - Number of pages 39 + 2 liitettä (18 ja 2 sivua)
Tiivistelmä - Referat – Abstract			
<p>Kampylobakteerit ovat gramnegatiivisia, pääosin mikroaerobisia bakteereja, joita esiintyy monien eläinten suolistossa. Ihmisille kampylobakteerit ovat erityisesti suoliston taudinaiheuttajia ja kampylobakteerit ovatkin yleisimpiä bakteeriperäisiä ihmisten suolistotulehduksen aiheuttajia kehittyneissä maissa, myös Suomessa. Suolistotulehduksen lisäksi kampylobakteerit voivat aiheuttaa ihmisille esimerkiksi Guillain-Barré -hermostosairautta ja pitkäkestoisiakin niveloireita aiheuttavaa reaktiivista niveltulehdusta. Ihmisen tyypillisimmät taudinaiheuttajat kampylobakteereista ovat <i>Campylobacter jejuni</i> ja <i>Campylobacter coli</i>. Näistä kahdesta selvästi yleisempi taudinaiheuttaja on <i>C. jejuni</i>. Sekä <i>C. jejuni</i> että <i>C. coli</i> ovat termofiilisiä kampylobakteereja. Kampylobakteeritartunta on yleensä ulosteperäinen ja erityisesti ulosteella saastunut broilerinliha on tärkeä kampylobakterioosin lähde. Maatilojen ympäristössä olevien jyrtsijöiden on todettu olevan yksi kampylobakterioosin riskitekijöistä ja kampylobakteereja on havaittu jyrtsijälajeilla, esimerkiksi metsähiirillä ja vesimyrillä. Suomessa jyrtsijöiden kantamia kampylobakteereja ei ollut tutkittu ennen tämän tutkielman aloittamista. Päästäisiä koskevia tutkimuksia kampylobakteerien osalta on maailmanlaajuisestikin hyvin vähän.</p> <p>Tutkimusosiossa tutkittiin termofiilisten kampylobakteerien yleisyyttä suomalaisissa pienjyrtsijöissä ja päästäisissä. Tutkimuksen hypoteesina oli, että suomalaisista piennisäkkäistä löytyy kampylobakteereja. Tutkimuksessa tutkittiin yhteensä 372 eläintä 12:sta eri lajista, jotka oli pyydystetty 24:ltä paikalta eri puolilta Suomea. Tutkituista eläimistä 342 oli jyrtsijöitä ja 40 päästäisiä. Tutkimuksen tarkoituksena oli selvittää isäntälajien välisiä eroja kampylobakteerien yleisyyden suhteen sekä esimerkiksi kampylobakteerien yleisyyttä eri puolilla Suomea. 76 metsämyyrää oli pyydetty keväällä ja kesällä vuonna 2017, kun taas vuodelta 2015 oli 82 metsämyyrää. Näitä kahta vuotta verrattiin toisiinsa mahdollisten vuosittaisten kampylobakteerierojen havaitsemiseksi metsämyyrissä.</p> <p>Tutkimus suoritettiin viljelemällä eläinten ulostetta selektiivisille kasvatustaljoille, joita inkuboitiin termofiilille kampylobakteereille otollisissa olosuhteissa. Bakteerikasvustoa viljeltiin tämän jälkeen epäselektiivisille taljoille puhdasviljelmiksi, joista tehtiin DNA-eristys. DNA:ta monistettiin PCR-menetelmän avulla ja PCR-tuotteet tutkittiin geielektroforeesin avulla bakteerilajien tunnistamiseksi.</p> <p>Tulokset olivat hypoteesin mukaiset. Tutkimuksessa todettiin <i>C. jejuni</i> 17,7 %-lla tutkituista eläimistä. Kampylobakteereja havaittiin neljällä jyrtsijälajilla: metsähiirillä, punamyrillä, metsämyyrillä ja peltomyyrillä. Suurinta esiintyvyyttä oli punamyrillä, joista 63,6 % oli kampylobakteeriposiitivisia. Kaikki tutkitut päästäisnäytteet olivat kampylobakteerinegatiivisia. Metsämyyrillä ja metsähiirillä todettiin paikkakunnan vaikuttavan kampylobakteerien yleisyyteen. Metsämyyrillä oli kampylobakteereja merkittävästi enemmän vuonna 2017 kuin 2015, mikä voi liittyä metsämyyrien vuosittaisiin kannanvaihteluihin.</p> <p>Tutkimustulosten perusteella jyrtsijät voivat mahdollisesti toimia kampylobakteerien reservuaarina ja mahdollisesti myös ihmisten kampylobakterioosin lähteenä. Jyrtsijät voivat mahdollisesti esimerkiksi tartuttaa ihmisiä tai maatilan eläimiä tai saastuttaa toimintatiloja tai vesilähteitä. Jatkotutkimuksia kuitenkin tarvitaan esimerkiksi selvittämään sitä, ovatko suomalaisten jyrtsijöiden kantamat kampylobakteerit ihmiselle patogeenisia kantoja.</p>			
Avainsanat - Nyckelord - Keywords <i>Campylobacter</i> , <i>Campylobacter jejuni</i> , campylobacteriosis, rodent, shrew, small mammal, Finland, kampylobakterioosi, jyrtsijä, päästäinen, piennisäkkäs, Suomi			
Säilytyspaikka - Förvaringställe - Where deposited E-thesis			
Työn johtaja (tiedekunnan professori tai dosentti) ja ohjaaja(t) - Instruktor och ledare - Director and Supervisor(s) Johtaja: Rauni Kivistö Ohjaaja: Rauni Kivistö ja Mirko Rossi			



Tiedekunta - Fakultet – Faculty Faculty of Veterinary Medicine		Osasto - Avdelning – Department Department of Food Hygiene and Environmental Health	
Tekijä - Författare - Author Meeri Ylänen			
Työn nimi - Arbetets titel - Title <i>Campylobacter</i> spp.in Finnish small mammals			
Oppiaine - Läroämne – Subject Environmental Hygiene			
Työn laji - Arbetets art - Level Licentiate thesis		Aika - Datum - Month and year April 2020	Sivumäärä - Sidoantal - Number of pages 39 + 2 appendixes (18 and 2 pages)
Tiivistelmä - Referat – Abstract			
<p>Campylobacters are gram negative, mostly microaerobic bacteria that live in the intestines of many animal species. Campylobacters are the most common cause of human bacterial enteritis in developed countries, including Finland. The most common human pathogens of <i>Campylobacter</i> spp. are <i>Campylobacter jejuni</i> and <i>Campylobacter coli</i>.</p> <p><i>Campylobacter</i> infection happens usually via fecal contamination. Especially contaminated, undercooked broiler meat is an important source of campylobacteriosis. Rodents near farms are noted as a risk factor for campylobacteriosis, and <i>Campylobacter</i> spp. have been detected in some rodent species. Studies regarding campylobacters in shrews are minimal worldwide.</p> <p>In this thesis, the <i>Campylobacter</i> occurrence in Finnish small rodents and shrews was studied. The hypothesis was that Finnish small mammals do carry <i>Campylobacter</i> spp. 342 rodents and 40 shrews from 12 species trapped from 24 locations throughout Finland were studied. The purpose of the study was to detect differences in <i>Campylobacter</i> occurrence between host species and for example to compare the occurrence in different parts of Finland. Possible annual differences in bank voles were studied with 76 animals from 2017 and 82 animals from 2015.</p> <p>The study was performed by cultivating fecal samples to selective agars that were incubated in conditions suitable for thermophilic <i>Campylobacter</i> spp. Bacterial growth was cultivated to non-selective agars for pure growth and DNA isolation was performed. DNA was multiplied using PCR and gel electrophoresis was used to determine the <i>Campylobacter</i> species.</p> <p>The results were consistent with the hypothesis. Four rodent species were <i>Campylobacter</i> positive: yellow-necked mouse, northern red-backed vole, bank vole and field vole. The occurrence was highest in northern red-backed voles, where 63,6 % of the animals were <i>Campylobacter</i> positive. All the studied shrews were <i>Campylobacter</i> negative. Bank voles had campylobacters significantly more in 2017 than in 2015, which may relate to the annual population changes in bank voles.</p> <p>According to the results it is possible that rodents could act as <i>Campylobacter</i> reservoir and source of human campylobacteriosis. Rodents could possibly infect humans or farm animals or contaminate estates or water sources. Further studies are needed to determine for example if the campylobacters in Finnish rodents' strains are pathogenic for humans.</p>			
Avainsanat - Nyckelord - Keywords <i>Campylobacter</i> , <i>Campylobacter jejuni</i> , campylobacteriosis, rodent, shrew, small mammal, Finland, campylobakterioosi, jyrtsijä, päästäinen, piennisäkäs, Suomi			
Säilytyspaikka - Förvaringställe - Where deposited E-thesis			
Työn johtaja (tiedekunnan professori tai dosentti) ja ohjaaja(t) - Instruktor och ledare - Director and Supervisor(s) Director: Rauni Kivistö Supervisor: Rauni Kivistö and Mirko Rossi			

CONTENTS

- 1 INTRODUCTION 1**
- 2 LITERATURE REVIEW 3**
 - 2.1. Characteristics of *Campylobacter* spp. 3
 - 2.2 Campylobacteriosis in humans..... 4
 - 2.2.1 Source of infection 4
 - 2.2.2 Risk factors 4
 - 2.2.3 Gastroenteritis..... 5
 - 2.2.4 Reactive arthritis 6
 - 2.2.5 Guillain-Barré syndrome 7
 - 2.2.6 Other diseases 7
 - 2.3 *Campylobacter* spp. in other animals..... 8
 - 2.3.1 *Campylobacter* spp. in rodents and shrews 8
 - 2.3.2 *Campylobacter* spp. in domesticated mammals 12
 - 2.3.3 *Campylobacter* spp. in birds..... 13
 - 2.4 *Campylobacter* spp. in water..... 14
 - 2.5 Detecting *Campylobacter* spp. from feces 15
 - 2.5.1 Cultivation methods 15
 - 2.5.2 Polymerase chain reactions (PCRs) 17
 - 2.5.3 Additional laboratory tests..... 17
 - 2.6 Discussion of the literature review 17
- 3 AIMS OF THE STUDY..... 19**
- 4 MATERIALS AND METHODS..... 20**
 - 4.1 Animals 20
 - 4.2 Cultivating *Campylobacter* spp..... 20
 - 4.3 Isolating *Campylobacter* DNA..... 21
 - 4.4 Analysing DNA with PCR 21
 - 4.5 Statistical analysis..... 23
- 5 RESULTS 24**
 - 5.1 Occurrence in different species..... 24
 - 5.2 Differences between sexes..... 25
 - 5.3 Differences between animals’ origins 26
 - 5.4 Differences between age groups..... 29
 - 5.5 Differences in weights 29
 - 5.6 Annual differences in bank voles 31
- 6 DISCUSSION 33**

7 ACKNOWLEDGEMENTS	39
REFERENCES	40
Appendix 1. The study material.....	47
Appendix 2. Animals by the sampling site.....	65

1 INTRODUCTION

Campylobacter spp. are the most common zoonotic cause of gastrointestinal disease in the European Union with over 240 000 reported cases in 2018 (European Food Safety Authority 2019). In Finland there were over 5000 reported cases of campylobacteriosis in 2018, vast majority of these being caused by *C. jejuni*. The overall incidence of campylobacteriosis in Finland was 93/100 000. 14 % of the reported infections were domestic, though the originating country was not known in 45 % of the cases (National Institute for Health and Welfare 2019).

Human campylobacteriosis is usually a foodborne infection and especially poultry meat is a common source of campylobacteriosis (Wilson et al. 2008). *Campylobacter* spp. are known to occur through fecal contamination also in the environment, for example in surface waters also in Finland (Hörman et al. 2004). There have also been reported water related *Campylobacter* outbreaks in Finland with up to thousands of patients (Hänninen et al. 2003, Laine et al. 2011).

There are several animal species, for example dogs and chickens, that may have campylobacteriosis even without visible symptoms (Shanker et al. 1988, Chaban et al. 2010) and these animals are known to be risk factors of human campylobacteriosis (Neimann et al. 2003). Several rodent species have also been detected carrying *Campylobacter* spp. (Gelling et al. 2012, Backhans et al. 2013) and they are considered as a risk factor especially for *Campylobacter* infections in poultry on farms (Ellis-Iversen et al. 2012).

Seasonal variation is typical in the campylobacteriosis incidence both in humans and broilers in Finland. The incidence rates start to rise during spring, reach the seasonal peak in late summer and descend during autumn. Similar seasonal variations are known to occur in other Northern European countries as well (Jore et al. 2010).

The literature review of this licentiate thesis describes the basic information of *Campylobacter* spp, their role as human pathogens, their commonness in other animals and some of the laboratory methods for detecting them. The experimental part studies the occurrence of

Campylobacter spp. in different wild rodent and shrew species in Finland. The aim of the study was to determine whether Finnish small mammals carry *Campylobacter* spp. in their intestinal tract and if they could act as reservoir for them. A Swedish study discovered *Campylobacter* spp. from 16 % of tested rodents (Backhans et al. 2013) and the hypothesis of this study is to find *Campylobacter* spp. in Finnish rodents with similar occurrence. Previous studies of *Campylobacter* spp. in shrews have been minimal, but both Healing et al. (1991) and Meerburg et al. (2006) discovered that none of the studied shrews carried *Campylobacter* spp. in their digestive tract. Based on that information, the expectation was to find very low *Campylobacter* occurrence in shrews.

2 LITERATURE REVIEW

2.1. Characteristics of *Campylobacter* spp.

Campylobacters are zoonotic Gram-negative, typically oxidase-positive bacteria that grow mostly in microaerobic conditions. There are at least 17 *Campylobacter* species and their growth requirements vary between species (Debruyne et al. 2008). For many species the optimal growth temperature is 30-37 °C but thermophilic species such as *C. jejuni* and *C. coli* have the optimal growth temperature of 42 °C (Doyle et al. 1981, Debruyne et al. 2008).

Campylobacter morphology is usually helical or straight rod, but the cells may transform into coccoid form for example in temperatures lower than the growth temperature (Rollins and Colwell 1986). The cells are typically 0,2-0,8 µm wide and 0,5-5 µm long (Debruyne et al. 2008). *Campylobacter* spp. have one or multiple flagella which are essential for the colonization of mucosa and causes most of the *Campylobacter* spp. being motile (Pavlovskis et al. 1991, Debruyne et al. 2008).

The occurrence of *Campylobacter* spp. varies in different host species. Clinically healthy hosts, for example chickens and dogs, may carry *Campylobacter* spp. in their gastrointestinal tract (Shanker et al. 1988, Chaban et al. 2010), but *Campylobacter* spp. are mostly known as pathogens that cause especially gastroenteritis. The most common human pathogens of *Campylobacter* species are *C. jejuni* and *C. coli*, but other species, for example *C. fetus*, *C. hyointestinalis* and *C. upsaliensis*, are also known to cause gastroenteritis and other diseases (Gillespie et al. 2002, Blaser et al. 2008).

2.2 Campylobacteriosis in humans

2.2.1 Source of infection

Campylobacter infection is fecal-oral and the primary transmission route is the food chain. Most of the diagnosed sporadic *Campylobacter* infections have been associated with eating undercooked poultry meat. Besides poultry, contaminated meat of other animals such as cattle and sheep have also been associated with sporadic campylobacteriosis (Wilson et al. 2008). Other food products such as vegetables may also act as vehicles for campylobacters through cross-contamination (De Boer and Hahné 1990).

There have also been outbreaks of campylobacteriosis that have originated from drinking contaminated water or milk (Laine et al. 2011, Fernandes et al. 2015). Gardner et al. (2011) associated a campylobacteriosis outbreak with eating raw peas that had been contaminated with wild bird feces. In the European Union in 2018, the most common sources of campylobacteriosis in the reported outbreaks were milk and broiler meat. There were also two reported waterborne outbreaks (European Food Safety Authority 2019).

The most common causes of waterborne outbreaks in Finland are campylobacters and noroviruses. Between years 1998 and 2018 there were 21 reported waterborne outbreaks that were associated with *Campylobacter* spp. (National Institute for Health and Welfare 2020). In 2007 in the town of Nokia, approximately 6500 residents developed gastroenteritis because of drinking water contamination with sewage water (Laine et al. 2011). *Campylobacter* spp. were the most commonly isolated pathogens from the patients. 27,5 % of the tested patients were positive of *Campylobacter* spp. and most of these were *C. jejuni*. *Campylobacter* spp. were also isolated from water samples (Laine et al. 2011).

2.2.2 Risk factors

There have been multiple studies of risk factors of campylobacteriosis with slightly different aspects and results. Kapperud et al. (2003), Neimann et al. (2003) and Domingues et al. (2012)

all noted in their studies eating at barbecue, consuming unpasteurized dairy products and having contact with animals as significant risk factors. Two of the studies concluded eating undercooked poultry also as one of the main risk factors (Neimann et al. 2003, Domingues et al. 2012). Conversely, Kapperud et al. (2003) did not notice this as a risk factor but this may be due to the relatively small amount of cases and controls having that kind of exposure. However, eating poultry that had been bought raw was identified as a risk factor in this study (Kapperud et al. 2003).

Neimann et al. (2003) and Domingues et al. (2012) noted contact with both farm animals and pets as risk factors, whereas Kapperud et al. (2003) identified only contact with farm animals as a risk factor. Neimann et al. (2003) associated contact with cat with diarrhea or daily contact with a kitten with increased risk of *Campylobacter* infection.

In their case-control study, Schönberg-Norio et al. (2004) studied risk factors in domestic cases of *C. jejuni* or *C. coli* infections in Finland. They concluded that additionally to eating undercooked meat, also drinking dug well water and swimming in natural waters were risk factors during the seasonal peak of campylobacteriosis.

Other campylobacteriosis risk factors include for example drinking untreated water, eating grapes and having contact with environmental sources, for example play grounds with wild bird feces (Kapperud et al. 2003, Neimann et al. 2003, Domingues et al. 2012).

In the overall risk factors, travelling abroad is an important one (Neimann et al. 2003, Domingues et al. 2012). This is consistent with the fact that the majority of campylobacteriosis especially in Nordic countries, including Finland, are travel-associated (European Food Safety Authority 2019).

2.2.3 Gastroenteritis

Gastroenteritis is the most common form of campylobacteriosis in humans. The infective dose of orally administered *C. jejuni* is low, for 500 *Campylobacter* organisms is known to be enough

to cause the infection (Robinson 1981). The incubation period is usually between one and four days but there have been cases where the symptoms have started even ten days after the infection (Horn and Lake 2013).

The most common symptoms of *Campylobacter* related gastroenteritis include diarrhea, abdominal pain, nausea, vomiting and fever (Robinson 1981, Lochter et al. 2002). Usually the acute gastroenteritis lasts from a few days to a week (Hannu et al. 2002, Lochter et al. 2002).

2.2.4 Reactive arthritis

Reactive arthritis is an inflammatory joint disease that is often preceded by bacterial gastroenteritis. There are multiple bacteria that can cause reactive arthritis and *Campylobacter* spp. are one of the known main causes (Ajene et al. 2013). Both *C. jejuni* and *C. coli* have been associated with reactive arthritis (Hannu et al. 2002, Lochter et al. 2002). Some studies indicate that patients with reactive arthritis have a longer duration of diarrhea than patients without joint symptoms (Hannu et al. 2002, Lochter et al. 2002).

The incidence rate of reactive arthritis following *Campylobacter* gastroenteritis varies in different studies. In their systematic review with 14 cohort studies, Ajene et al. (2013) concluded the weighted mean incidence being 9/1000. In a Danish study, 16 % of 173 patients developed reactive arthritis after *Campylobacter* infection (Lochter et al. 2002) whereas in a Finnish study with 609 patients the occurrence was 7 % (Hannu et al. 2002). The incidence is usually higher in adults than in children (Hannu et al. 2002, Ajene et al. 2013).

The symptoms typically start in a few weeks after the original infection (Lochter et al. 2002, Ajene et al. 2013). *Campylobacter* related reactive arthritis is usually mild with the most usual symptoms being pain and swelling of multiple joints, limitation of joint movement and pain in lower back (Hannu et al. 2002). The symptoms usually last for a couple months but there have also been patients who have had symptoms for over a year (Lochter et al. 2002).

2.2.5 Guillain-Barré syndrome

Guillain-Barré syndrome is an acute polyneuropathy that causes neurodegeneration (Campbell 1957). Multiple studies indicate that *Campylobacter* spp. are the cause of Guillain-Barré syndrome usually in 20-50 % of the diagnosed cases (Jacobs et al. 2008). The severity of the syndrome varies, and campylobacters have been associated with both mild and severe cases (van Koningsveld et al. 2000). The *Campylobacter* associated Guillain-Barré syndrome develops usually after gastroenteritis, but the syndrome is not common since it is detected in less than 1 % of the diagnosed *Campylobacter* gastroenteritis patients (van Koningsveld et al. 2000, McCarthy et al. 2001, Baker et al. 2012). Guillain-Barré syndrome evolves usually two weeks after the gastroenteritis, but the time varies from a week to a month (de Jager et al. 1991, Baker et al. 2012).

The usual symptoms of Guillain-Barré syndrome include evolving muscle weakness, difficulties with moving and pain. Some patients have difficulties with swallowing or breathing or changes in blood pressure or heart rate (Constant et al. 1983, de Jager et al. 1991). In severe cases patients may need assistance with breathing (Constant et al. 1983). Most of the patients recover in two years but in some cases the recovery is not perfect (de Jager et al. 1991). The fatality rate is low, around 2 % (Cheng et al. 2000).

2.2.6 Other diseases

Besides gastroenteritis, reactive arthritis and Guillain-Barré syndrome, *Campylobacter* spp. have also been associated for example with bacteremia. Unlike with gastroenteritis, *C. fetus* is noted as an important cause of *Campylobacter* bacteremia and in some studies *C. fetus* has even been detected as the main cause of the disease (Pacanowski et al. 2008, Fernández-Cruz et al. 2010). In a Finnish study executed in 1998-2007, 0,3 % of the diagnosed *Campylobacter* infections were discovered as bacteremia with a mortality rate of 3 % (Feodoroff et al. 2011). There have also been rare cases of *Campylobacter* spp. associated for example with hepatitis, abortion, pancreatitis and meningitis in humans (Blaser et al. 2008).

2.3 *Campylobacter* spp. in other animals

2.3.1 *Campylobacter* spp. in rodents and shrews

Previous studies have typically discovered relatively low *Campylobacter* occurrences in most wild rodents. The occurrence rates in four studies are described in Table 1.

Table 1. The number of positive samples of *C. jejuni* and *C. coli* and the number of animals studied in some wild rodent species in the studies of Rosef et al. 1983, Healing et al. 1991, Meerburg et al. 2006 and Backhans et al. 2012.

Species	N:o of positive samples / N:o of animals studied							
	<i>C. jejuni</i>				<i>C. coli</i>			
	Rosef et al. (1983)	Healing et al. (1991)	Meerburg et al. (2006)	Backhans et al. (2012)	Rosef et al. (1983)	Healing et al. (1991)	Meerburg et al. (2006)	Backhans et al. (2012)
Harvest mouse <i>Micromys minutus</i>	-	-	0/6	-	-	-	0/6	-
House mouse <i>Mus musculus</i>	-	0/23	3/83	2/125	-	0/23	3/83	15/125
Wood mouse <i>Apodemus sylvaticus</i>	0/20	0/29	0/19	-	0/20	0/29	0/19	-
Yellow-necked mouse <i>Apodemus flavicollis</i>	-	0/3	-	5/18	-	0/3	-	0/1
Brown rat <i>Rattus norvegicus</i>	-	-	0/8	2/58	-	-	1/8	5/58
Bank vole <i>Myodes glareolus</i>	0/24	1/33	0/3	-	0/24	0/33	0/3	-

Table 1 continues.

Species	N:o of positive samples / N:o of animals studied							
	<i>C. jejuni</i>				<i>C. coli</i>			
	Rosef et al. (1983)	Healing et al. (1991)	Meerburg et al. (2006)	Backhans et al. (2012)	Rosef et al. (1983)	Healing et al. (1991)	Meerburg et al. (2006)	Backhans et al. (2012)
Common vole <i>Microtus arvalis</i>	-	-	0/31	-	-	-	0/31	-
Field vole <i>Microtus agrestis</i>	-	0/23	0/3	-	-	0/23	0/3	-
Tundra vole <i>Microtus oeconomus</i>	-	-	0/1	-	-	-	0/1	-

Most of the identified *Campylobacter* spp. in rodents have been *C. jejuni* or *C. coli*, but there have been positive results for other species as well. Meerburg et al. (2006) found 1,2 % of the house mice positive for *C. hyointestinalis* and Backhans et al. (2012) found 1,6 % of the house mice and 11,1 % of the yellow-necked mice carrying *C. upsaliensis*.

Campylobacteriosis is usually asymptomatic in rodents. For example, Gelling et al. (2012) examined 74 clinically healthy water voles and found 8,1 % of them *Campylobacter* positive. Lone et al. (2013) infected mice with *C. jejuni* and observed physical changes in the animals as well as the *Campylobacter* occurrence in the mice's feces. Even high doses of *C. jejuni* didn't induce weight loss or other symptoms of illness, even though the high bacterial density was associated with cecal inflammation and dysbiosis of cecal microbiota. All the infected mice secreted *C. jejuni* in their feces over the experimental period of 21 days (Lone et al. 2013).

There are only few studies of *Campylobacter* spp. in shrews. A British study examined 30 shrews from three different species and found none of them carrying *Campylobacter* spp. in their intestinal tract. Instead, *C. jejuni* was found from the spleens of one water shrew and one common shrew (Healing et al. 1991). The study of Meerburg et al. (2006) included 129 shrews from two species and all of them were *Campylobacter* negative.

In his master's thesis, Tikkanen (2019) studied *C. jejuni* and *C. coli* in small mammals in Finnish cattle and swine farm environments and found six rodent species positive for *C. jejuni*. The positive species were yellow-necked mouse (66,3 %), bank vole (63,9 %), field vole (25 %), brown rat (20 %), harvest mouse (70 %) and southern vole (8,3 %). The sample sizes were limited in some of the studied species, including harvest mouse (n = 10) and field vole (n = 4). All the studied 31 shrews from four different species were *Campylobacter* negative. In contrast to some of the previous studies, all the 89 house mice were *Campylobacter* negative. All the samples were negative for *C. coli*.

2.3.2 *Campylobacter* spp. in domesticated mammals

Campylobacter spp. are known to occur in many domesticated animals. The bacteria can be part of the animals' normal intestine flora and so occur in animals that are clinically healthy. *Campylobacter* spp. have been detected for example from asymptomatic dogs, cats and bovines (Andrzejewska et al. 2013, Ramonaitė et al. 2013). However, *Campylobacter* spp. are considered as possible intestinal pathogens for these animals. Olson and Sandstedt (1987) infected six dogs with either *C. jejuni* or *C. upsaliensis*. One of the dogs infected with *C. jejuni* developed diarrhea four days after the infection and one of the dogs infected with *C. upsaliensis* developed soft feces. Chaban et al. (2010) noted that dogs with diarrhea had higher *Campylobacter* prevalence, species richness and levels than clinically healthy dogs. 58 % of the clinically healthy dogs were *Campylobacter* positive whereas the share in diarrheic dogs was 97 %. In both groups, the most common species was *C. upsaliensis* followed by *C. jejuni*. *C. coli* was detected only in diarrheic dogs (Chaban et al. 2010). Table 2 describes the occurrence ranges of some *Campylobacter* species in clinically healthy dogs and cats according to three studies.

Table 2. The occurrence ranges of some *Campylobacter* species in clinically healthy dogs and cats. The information is based on the following studies: Rossi et al. 2008, Chaban et al. 2010, Andrzejewska et al. 2013.

Animal	Occurrence of <i>Campylobacter</i> species				
	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. upsaliensis</i>	<i>C. helveticus</i>	<i>C. lari</i>
Dog	2,4-11,5 %	0-2,4 %	30,8-42,9 %	1,9-10 %	0-1,9 %
Cat	4,8-8,5 %	0-1,4 %	14,3 %	19 %	0 %

Out of farm animals, pigs are typical *Campylobacter* hosts especially for *C. coli*. Rosef et al. (1983) studied 114 pigs and found 100 % of them positive for *C. coli*. In the same study, 8,1 % of the studied 197 sheep and 0,8 % of the 254 cows were positive for *C. jejuni*. All the studied goats and horses were *Campylobacter* negative.

Sproston et al. (2011) studied *C. jejuni* and *C. coli* in a farm that had both cattle and sheep and noted that cattle had significantly higher *Campylobacter* prevalence than the sheep. 21,9 % of the cattle were *Campylobacter* positive and vast majority of these cases were *C. jejuni*. Conversely, 59,4 % of the 14 % of *Campylobacter* positive sheep had *C. coli*.

Ramonaité et al. (2013) studied three dairy farms and noted that younger bovines had overall higher *Campylobacter* prevalence and levels than milking cows. Calves had *Campylobacter* spp. prevalence of 86,5 % whereas cows had the prevalence of 60,6 %. Overall, 66,2 % of the positive samples were *C. jejuni*, 24,2 % were *C. coli* and the rest were *C. lari* and *C. fetus* (Ramonaité et al. 2013). *C. fetus* may be a significant pathogen in cattle since it can cause spontaneous abortions. Morrell et al. (2010) studied 150 aborted bovine foetuses and found 5,3 % of them positive with *C. fetus*. These foetuses had pneumonia lesions but also for example enteritis, hepatitis or myocarditis.

2.3.3 *Campylobacter* spp. in birds

Campylobacter occurrence in chickens has been studied multiple times. Ellis-Iversen et al. (2012) took over 2000 samples from 75 broiler flocks on six farms in the United Kingdom and discovered 58 % of the samples *Campylobacter* positive. Nadeau et al. (2002) examined over 2000 individual broilers in Canada and found *Campylobacter* prevalence of 40,7 %. 95,2 % of these cases were *C. jejuni*, whereas *C. coli* prevalence was 4,8 % (Nadeau et al. 2002).

Campylobacteriosis is typically asymptomatic in birds. Shanker et al. (1988) infected broiler chicks with *C. jejuni* and noted that none of the 380 birds developed diarrhea even though they secreted the bacteria in their feces. The colonization took place when the bacteria was presented through the oral route as well as straight to the cloaca. The colonization was rapid for the cloacal samples of 88 % of the *Campylobacter* positive chicks were *Campylobacter* positive three days after the infection. All of the colonized chicks remained *Campylobacter* positive throughout the experimental time of 14 days. Shanker et al. (1988) also noticed that giving the broiler chicks cecal content of older, *Campylobacter* free broilers prior to infection of *C. jejuni* did not prevent the *C. jejuni* colonization.

There are also wild avian species that are known to be *Campylobacter* hosts. The studied species have included for example geese, gulls and pigeons (Waldenström et al. 2007, Keller et al. 2014, Konicek et al. 2016). Keller et al. (2014) and Konicek et al. (2016) found *C. jejuni* being the most common *Campylobacter* species in wild birds followed by *C. coli*, *C. lari* and *C. helveticus*. The overall *Campylobacter* prevalences in wild bird in these studies were 9,2 % and 12,5 %. The studies also noted that juvenile animals had higher *Campylobacter* prevalence than older ones (Keller et al. 2014, Konicek et al. 2016). Waldenström et al. (2007) studied shorebirds and geese and noted that over 80 % of redshanks were positive for *C. lari*. In this study, overall 48,2 % of the studied wild birds were positive for *C. jejuni* or *C. lari*.

Waldenström et al. (2002) studied over 1700 wild birds from 107 different species in Sweden and noticed that the species' feeding habits had significant influence on the *Campylobacter* prevalence. For example, raptors and shoreline-foraging invertebrate eaters had typically relatively high *Campylobacter* prevalence whereas insectivores and granivores had low *Campylobacter* prevalence. Especially shorebirds had high prevalence, since 76,9 % of the 382 individuals from 19 species were *Campylobacter* positive. In the overall results, 5,6 % of the birds had *C. lari*, 5,0 % had *C. jejuni* and 0,9 % had *C. coli* (Waldenström et al. 2002).

2.4 *Campylobacter* spp. in water

Temperature has significant influence on the survival time of *Campylobacter* spp. in water. Rollins and Colwell (1986) studied the viable but nonculturable form of *C. jejuni* in aquatic environment and noted that the bacteria survived in stream water at 4 °C for over 4 months whereas the survival at 37 °C was 10 days. Buswell et al. (1998) studied the survival of culturable *Campylobacter* spp. in water and noted that the survival time was significantly longer in lower temperatures. Overall, *Campylobacter* spp. survived longer in 4 and 10 °C than in 22 or 37 °C. In the higher temperatures the maximum survival time was 48 hours whereas in the lower temperatures *Campylobacter* spp. survived even over 250 hours. The survival time was even longer, up to 700 hours, when the water contained microflora or biofilm

(Buswell et al. 1998). Joshua et al. (2006) noted that *C. jejuni* can form three forms of biofilms that increase the bacteria's resistance to environmental stress.

In 2000 and 2001, Hörman et al. (2004) analyzed 139 surface water samples from lakes and rivers in Finland to study the occurrence of *Campylobacter* spp, *Giardia* spp, *Cryptosporidium* spp. and noroviruses. The most frequently isolated pathogens were *Campylobacter* spp. with the occurrence of 17,3 %. Most of the samples were *C. jejuni*, followed by *C. lari* and *C. coli*. 25 % of the *Campylobacter* positive samples were undetermined *Campylobacter* spp. The samples from May were most frequently *Campylobacter* positive whereas all the samples from winter were *Campylobacter* negative (Hörman et al. 2004).

2.5 Detecting *Campylobacter* spp. from feces

2.5.1 Cultivation methods

In the previous studies examining *Campylobacter* spp. in small mammals' guts, swabs have been commonly used for cultivating stool samples (Rosef et al. 1983, Meerburg et al. 2006, Backhans et al. 2013). The used agars for *Campylobacter* selection have varied between studies but for further analysis some studies have included cultivation on blood agar plates. The selected agars, cultivation conditions and the function of the methods' in five studies are described in table 3.

Table 3. Cultivating conditions in previous studies considering *Campylobacter* spp. in small mammals' guts. Used abbreviations: modified charcoal cefoperazone deoxycholate agar (mCCDA), colistin-amphotericin-keflin agar (CAK), albimi Brucella broth (ABB), vancomycin-polymyxin-trimethoprim agar (VPT).

Study	Plate type	Temperature	Time	Oxygen level	Method's purpose
Tikkanen (2019)	mCCDA	41,5 ± 1 °C	48-72 h	Microaerobic	Primary culture
	Blood agar	41,5 ± 1 °C	24-72 h	Microaerobic	Pure culture
	Blood agar	37 ± 1 °C	48-72 h	Microaerobic	Further examinations.
Backhans et al. (2013)	mCCDA	41,5 °C	48 h	Microaerobic	Primary culture
Meerburg et al. (2006)	mCCDA	41,5 °C	48 h	Microaerobic	Primary culture
Rosef et al. (1983)	CAK	42-43 °C	24 & 48 h	Microaerobic	Primary culture
	CAK	37 °C	48 h	Aerobic	To assess the ability to grow in aerobic conditions.
	CAK	37 °C	48 h	Anaerobic	To assess the ability to grow in anaerobic conditions.
	CAK	25 °C	48 h	Microaerobic	To assess the ability to grow in lower temperature.
	CAK	37 °C	24 h	Microaerobic	Further examinations.
	Blood agar	37 °C	18-24 h	Microaerobic	Further examinations.
Healing et al. (1991)	VPT	43 °C	72 h	Microaerobic	Primary culture
	Preston agar	43 °C	72 h	Microaerobic	Primary culture
	Blood agar	43 °C	72 h	Microaerobic	Further examinations and to ensure purity of bacterial growth.

2.5.2 Polymerase chain reactions (PCRs)

PCR methods have been used to identify different *Campylobacter* species. Gelling et al. (2012) and Backhans et al. (2013) used multiplex PCR, which is used for duplicating multiple genes at the same time. By using primers for different *Campylobacter* specific genes, this method enables determination of different *Campylobacter* species (Inglis and Kalischuk 2003). Meerburg et al. (2006) used amplified fragment length polymorphism (AFLP), which also reveals the bacteria's genotypes (Johnsen et al. 2007).

2.5.3 Additional laboratory tests

Gram staining or other morphology analysis have been used in many previous studies for determining whether the bacteria growth has had typical *Campylobacter* morphology (Rosef et al. 1983, Meerburg et al. 2006). Other previously used tests have been for example hippurate, catalase, oxidase and H₂S production tests (Rosef et al. 1983, Backhans et al. 2013).

2.6 Discussion of the literature review

Multiple rodent species have been detected carrying *Campylobacter* spp, but studies in Finland are very limited. In some studies and species the sample sizes have been low and the results between studies have varied. Studies including shrews are minimal, so even though these animals are not considered as typical *Campylobacter* hosts, their role has not been studied properly. Since *Campylobacter* spp. are important human pathogens that can cause even large epidemics of gastroenteritis and also more severe diseases like Guillain-Barré syndrome it is important to study these potential *Campylobacter* hosts.

Most of the previous studies have used *Campylobacter* selective agars that have been incubated in temperatures over 41 °C. Selectivity is required when cultivation is done from stool samples, because feces contain multiple bacteria. The relatively high cultivation temperature in most studies does not induce growth in non-thermophilic *Campylobacter*

species like *C. fetus*. However, *C. jejuni* and *C. coli* are the most common causes of human campylobacteriosis and these higher temperatures have been optimal for their detection.

3 AIMS OF THE STUDY

The aim of this study was to determine the occurrence of *Campylobacter* spp. in Finnish small rodents and shrews from different aspects. One interest was the overall occurrence, but the main focus was differences between host species. The aim was to determine whether some species have higher occurrence of *Campylobacter* spp. than others and if there are differences what *Campylobacter* spp. they have.

One aspect of this study was to examine possible differences between years considering the *Campylobacter* occurrences in bank voles. Differences related to the animals' origin, sex, weight and age were studied as well.

Overall, the aim of the study was to estimate small mammals' role as *Campylobacter* hosts in Finland and their possible connection to human campylobacteriosis.

4 MATERIALS AND METHODS

4.1 Animals

The sample animals were captured, killed and prepared by Natural Resources Institute Finland. The animals were collected from different parts of Finland using snap traps. The animals or their colon and feces were delivered frozen to the laboratory and melted before further laboratory examination.

All the studied animals and the related information are in appendix 1. In total, 342 rodents and 30 shrews from 12 different species were included in the study.

4.2 Cultivating *Campylobacter* spp.

The feces were cultured directly on mCCDA plates with cotton swab that was dipped in peptone water. After cultivation, agars were incubated in microaerophilic conditions at 41,5 °C. With the first 207 samples, the agars were inspected two, four and seven days after cultivation. The rest 165 samples were incubated only for two days.

When typical *Campylobacter* growth was noticed, bacteria were cultured on two Nutrient Broth 2 plates. One of the plates was incubated in aerobic conditions at 25 °C and the other in microaerophilic conditions at 37 °C. After one day of incubation the plates were inspected for growth.

Bacteria that grew in microaerophilic but not in aerobic conditions were selected for DNA isolation. These bacteria were also frozen and stored in -72 °C for long-term preservation. For freezing, bacterial mass was moved into freezing tubes that included 1,5 ml of Nutrient Broth 2 and 15 % glycerol.

Gram staining was used for bacteria that didn't look typical *Campylobacter* growth or if mixed growth was suspected on either mCCDA or Nutrient Broth 2 plates. If the Gram stain showed typical Gram-negative rods, the isolate was included for further examinations.

4.3 Isolating *Campylobacter* DNA

Campylobacter DNA was extracted from the the growth on Nutrient Broth 2 plates. The isolation was performed with PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, California, USA) according to manufacturer's instructions. The concentration of the DNA was tested using NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The DNA was stored in - 20 °C for further examination, or if examined shortly after the extraction, in 4 °C.

4.4 Analysing DNA with PCR

The samples and positive controls were analysed with *C. jejuni* and *C. coli* specific multiplex PCR (Denis et al. 1999). Six primers were used to ensure *Campylobacter* DNA replication, and these primers have been described in table 4. MD16S primer pair was used for duplicating *16S* gene of *C. jejuni* and *C. coli*, whereas MDmapA primer pair was used for duplicating *mapA* gene of *C. jejuni*. The last primer pair (MDCOL2 and COL3) was used for duplication of *COL* gene of *C. coli*.

Positive controls were DNA isolated from *C. jejuni* (strain ATCC 33560) and *C. coli* (stain CCUG 11283). Negative control template was water. Master mix for the multiplex PCR has been described in table 5 and the PCR cycle is described in table 6.

Table 4. Primers used in multiplex PCR.

Primer name	Primer sequence
MD16S1	5'-ATC TAA TGG CTT AAC CAT TAA AC-3'
MD16S2	5'-GGA CGG TAA CTA GTT TAG TAT T-3'
MDmapA1	5'-CTA TTT TAT TTT TGA GTG CTT GTG-3'
MDmapA2	5'-GCT TTA TTT GCC ATT TGT TTT ATT-3'
COL3	5'-AAT TGA AAA TTG CTC CAA CTA TG-3'
MDCOL2	5'-TGA TTT TAT TAT TTG TAG CAG CG-3'

Table 5. The multiplex PCR master mix for one sample.

Ingredient (concentration)	Amount
Dynazyme polymerase (2 U/ μ l)	0,5 μ l
Buffer (10x)	2,5 μ l
dNTPs (10 mM)	0,5 μ l
Primer MD16S1 (5 μ M)	0,5 μ l
Primer MD16S2 (5 μ M)	0,5 μ l
Primer MDmapA1 (10 μ M)	1 μ l
Primer MDmapA2 (10 μ M)	1 μ l
Primer COL3 (10 μ M)	1 μ l
Primer MDCOL2 (10 μ M)	1 μ l
Water	15 μ l
DNA template	1,5 μ l
Total	25 μ l

Table 6. The multiplex PCR cycle.

Number of cycles	Temperature	Time
1	95 °C	10 min
35	95 °C	30 s
	59 °C	1 min 30 s
	72 °C	1 min
1	72 °C	10 min
1	4 °C	Forever

4.5 Statistical analysis

The results were analysed using SPSS Statistics (IBM SPSS version 24, Chicago, USA). The chi-square test was used for testing the correlation between *Campylobacter* occurrence and the sample animals' features including sex, origin, age and weight. Annual differences in bank voles were also examined. Variables with p-value lower than 0,05 were considered significant.

5 RESULTS

5.1 Occurrence in different species

All the species' sample sizes and the occurrence of *Campylobacter* positive samples in the studied species are shown in table 7. *Campylobacter* spp. were found from four rodent species. All the studied shrews were *Campylobacter* negative. Overall, the highest prevalence was in northern red-backed vole, followed by yellow-necked mouse, bank vole and field vole. All the positive samples were identified as *C. jejuni*.

Table 7. *Campylobacter* positive samples in each species.

Species	Latin name	N:o of positive samples / N:o of samples	Percentage of positive samples
Northern red-backed vole	<i>Myodes rutilus</i>	7/11	63,6 %
Yellow-necked mouse	<i>Apodemus flavicollis</i>	27/65	41,5 %
Bank vole	<i>Myodes glareolus</i>	26/158	16,5 %
Field vole	<i>Microtus agrestis</i>	6/37	16,2 %
Tundra vole	<i>Microtus oeconomus</i>	0/49	0 %
Common shrew	<i>Sorex araneus</i>	0/26	0 %
Grey red-backed vole	<i>Myodes rufocanus</i>	0/17	0 %
Wood lemming	<i>Myopus schisticolor</i>	0/4	0 %
Eurasian pygmy shrew	<i>Sorex minutus</i>	0/2	0 %
Laxmann's shrew	<i>Sorex caecutiens</i>	0/1	0 %
Water shrew	<i>Neomys fodiens</i>	0/1	0 %
Water vole	<i>Arvicola amphibius</i>	0/1	0 %
Total		66/372	17,7 %

5.2 Differences between sexes

Overall, 43 out of 219 males and 23 out of 153 females were *Campylobacter* positive. The proportions of *Campylobacter* positive animals in each sex of each species are shown in figure 1.

Species-specific chi-square testing showed that sex had influence on the *Campylobacter* occurrence in bank voles (p-value = 0,019). Bank vole males had campylobacteriosis more often than the females. The correlation was not statistically significant in yellow-necked mice (p-value = 0,486), field voles (p-value = 0,920) or northern red-backed voles (p-value = 0,898).

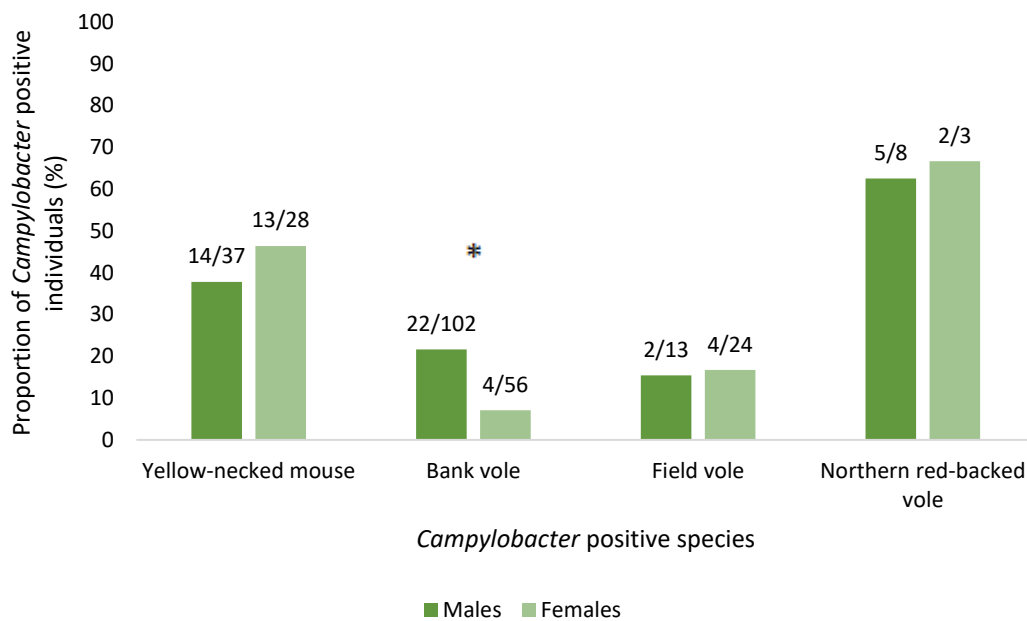


Figure 1. The proportions of *Campylobacter* positive males and females in each species. An asterisk (*) means that the difference between sexes is statistically significant.

5.3 Differences between animals' origins

In order to investigate the possible differences between *Campylobacter* occurrence in different locations, each animal's origin was noted. The locations are shown in figure 2. Appendix 2 describes the sample animals from each location. Out of 24 locations, 14 had *Campylobacter* positive animals. The proportions of *Campylobacter* positive animals from the sampling sites are shown in table 8.

Chi-square testing showed no significant correlation between the animal's *Campylobacter* status and origin in field voles (p-value = 0,320). Conversely, the location did have statistically significant correlation with the *Campylobacter* status in bank voles (p-value = 0,047) and yellow-necked mice (p-value = 0,022). All the 30 bank voles from Pallasjärvi were *Campylobacter* negative although the overall occurrence in the species was 16,5 %. The occurrence in all the six other locations with more than five captured bank voles varied from 7,1 % to 37,5 %. The occurrence was highest in Korpilahti. Similarly, all the 11 yellow-necked mice from Vantaa were negative of *Campylobacter* spp. even though the occurrence in the overall study was 41,5 % and the occurrence range in the other three origins was 47,1-57,1 %.

The locational differences in northern red-backed voles could not be properly statistically studied because of the small sample sizes.



Figure 2. The sample animals' origins.

Table 8. The proportion of *Campylobacter* positive animals from each origin considering the *Campylobacter* positive species.

Location	N:o of <i>Campylobacter</i> positive samples / N:o of samples			
	Bank vole	Yellow-necked mouse	Field vole	Northern red-backed vole
Kilpisjärvi	-	-	0/1	0/2
Pallasjärvi	0/30	-	-	0/2
Muonio	10/39	-	-	7/7
Kolari	1/9	-	-	-
Pyhätunturi	2/28	-	-	-
Pisavaara	4/19	-	-	-
Muhos	1/2	-	-	-
Sotkamo	0/1	-	-	-
Toholampi	0/1	-	-	-
Koli	1/1	-	1/1	-
Viitasaari	1/6	-	1/12	-
Suonenjoki	1/2	-	3/10	-
Tohmajärvi	0/1	-	0/1	-
Karvia	1/3	-	-	-
Korpilahti	3/8	-	-	-
Punkaharju	0/1	-	0/4	-
Mikkeli	1/1	-	1/4	-
Vammala	0/3	-	-	-
Lammi	-	8/17	-	-
Hämeenkoski	-	4/7	-	-
Luumäki	0/1	-	0/1	-
Violahti	0/2	-	0/3	-
Kerava	-	15/30	-	-
Vantaa	-	0/11	-	-

5.4 Differences between age groups

Table 9 describes the proportions of *Campylobacter* positive animals from different age groups in the *Campylobacter* positive species.

Statistical analysis showed that the correlation between animal's age group and the *Campylobacter* status was not significant in bank voles (p-value = 0,074) or yellow-necked mice (p-value = 0,401). The statistical analysis could not be properly performed in field voles and northern red-backed voles because of the sample sizes.

Table 9. The proportion of *Campylobacter* positive animals in different age groups in the *Campylobacter* positive species.

Species	N:o of <i>Campylobacter</i> positive animals / N:o of studied animals		
	Juvenile	Overwintered	Adult
Bank vole	-	18/129	8/29
Yellow-necked mouse	3/5	-	24/59
Field vole	-	0/1	6/36
Northern red-backed vole	-	7/11	-

5.5 Differences in weights

In order to evaluate the correlation between the animal's weight and *Campylobacter* status, the animals' weights were combined into five groups separately in each *Campylobacter* positive species. The weight groups are described in table 10. The proportions of *Campylobacter* positive animals in the weight groups are shown in table 11.

The weight group did not have significant correlation with the animal's *Campylobacter* status in yellow-necked mice (p-value = 0,383) or bank voles (p-value = 0,418). The correlation was statistically significant in field voles (p-value = 0,029) where both of the two studied animals that weighted 20-25 g were *Campylobacter* positive and the only animal that was under 20 g

was *Campylobacter* negative. When the analysis was performed only with the three weight groups including more than five field voles, there were no significant differences between the weight groups (p-value = 0,924).

Statistical analysis was also performed to examine weight differences between the *Campylobacter* positive and negative animals in the same age groups but no significant differences were observed. The only notable difference was in adult field voles (p = 0,029). This was due to the fact that all but one field voles were included in this age group and so the results were similar with the overall correlation between the weight groups.

Table 10. The weight ranges in the weight groups in the *Campylobacter* positive species.

Species	Weight group 1	Weight group 2	Weight group 3	Weight group 4	Weight group 5
Bank vole	<20 g	20-25 g	25,1-30 g	30,1-35 g	>35 g
Yellow-necked mouse	<15 g	15-25 g	25,1-35 g	35,1-45 g	>45 g
Field vole	<20 g	20-25 g	25,1-30 g	30,1-35 g	>35 g

Table 11. The proportion of *Campylobacter* positive animals in different weight groups in the *Campylobacter* positive species.

Species	N:o of <i>Campylobacter</i> positive animals / N:o of studied animals				
	Weight group 1	Weight group 2	Weight group 3	Weight group 4	Weight group 5
Bank vole	3/13	6/37	5/42	0/9	0/5
Yellow-necked mouse	1/3	3/8	15/27	6/22	2/5
Field vole	0/1	2/2	1/8	1/11	2/14

5.6 Annual differences in bank voles

76 bank voles (55 males and 21 females) were from year 2017 whereas 82 bank voles (47 males and 35 females) were captured in 2015. The bank voles from 2017 were captured from 17 sites throughout Finland whereas the bank voles from 2015 were from four locations in northern Finland. Pyhätunturi and Kolari were the only shared bank vole origins between the years, but the sample sizes from 2017 were limited (Pyhätunturi n = 3, Kolari n = 1).

The bank voles from 2015 and 2017 were compared to study possible differences between years. Chi-square testing showed that the sampling year had significant correlation with the bank vole's *Campylobacter* status (p-value = 0,001) for the *Campylobacter* occurrence in the bank voles from 2015 was 7,3 % and 26,3 % in the bank voles from 2017.

When the years were studied separately there were differences in the correlation of different features and the *Campylobacter* status between years. The p-values of the correlation analysis with the studied features from different years are shown in table 14.

Table 14. The p-values of different features analysed for the correlation with the *Campylobacter* status of the animals from 2015 and 2017. An asterisk (*) means that the correlation was statistically significant.

Feature analysed for the correlation with the <i>Campylobacter</i> status	p-value	
	2015	2017
Sex	0,028 (*)	0,374
Origin	0,039 (*)	0,768
Weight	0,558	0,278

In the bank voles from 2015, 12,8 % of the males and 0 % of the females were *Campylobacter* positive, and the statistical analysis showed a significant correlation between the sex and the *Campylobacter* status. In the bank voles from 2017, 29,1 % of the males and 19 % of the females were *Campylobacter* positive, and in these animals the correlation was statistically insignificant.

Origin did have significant correlation with *Campylobacter* status in the bank voles from 2015 for the *Campylobacter* occurrence varied from 0 % to 21,1 % between the four locations. Pallasjärvi (n = 30) had the lowest occurrence whereas the highest occurrence was in Pisavaara. There were no significant locational differences in bank voles from 2017. The correlation between the animal's weight and the *Campylobacter* status was not significant in neither year.

Annual differences in the correlation of animal's age and *Campylobacter* status could not be analysed because all the bank voles from 2015 were the same age group. In the bank voles from 2017, age did not have significant correlation with the *Campylobacter* status (p-value = 0,843).

6 DISCUSSION

The results support the hypothesis of this study. There were *Campylobacter* positive rodent species but the *Campylobacter* occurrence varied widely between them. None of the shrews were *Campylobacter* positive, which is also consistent with previous studies (Healing et al. 1991, Meerburg et al. 2006, Tikkanen 2019).

Because of the small sample sizes in some of the studied species, consideration should be used before applying these results to a greater population. For example, the only studied water vole was *Campylobacter* negative, but previous studies have discovered this species with *Campylobacter* spp. (Gelling et al. 2012). Determining the true occurrence in Finnish animals in the species with low sample sizes requires more studies with more animals. In our study, northern red-backed voles had the highest *Campylobacter* occurrence in the studied host species, but the relatively small sample size (n = 11) should be minded.

Since the primary mCCDA plates were incubated in 41,5 °C, this method could reveal only thermophilic *Campylobacter* spp, so the results can't be generalized to concern all *Campylobacter* spp. For example, *C. fetus* may not grow in temperature this high (Debruyne et al. 2008). However, the thermophilic species such as *C. jejuni* and *C. coli* are the most common human pathogens and the main cause of human campylobacteriosis also in Finland (Blaser et al. 2008, National Institute for Health and Welfare 2019).

During the analysis, the incubation time of mCCDA plates was shortened from seven to two days. This was done after noticing that very few bacteria (2 samples out of 207 examined) started growing if there was no growth after two days of incubation. Shorter incubation time may have reduced the amount of positively identified samples. Still, based on the first 207 examined samples, this change probably didn't have significant effect on the results

Freezing is known to reduce the amount of living *Campylobacter* cells but the decrease decelerates by time. Sampers et al. (2010) noted that the reduction of *Campylobacter* spp. in chicken skin and minced chicken meat in – 22 °C was approximately one log₁₀ cfu/g after one day of freezing. However, prolonged storage did not result in further significant reduce. By

this, it is likely that the freezing of the sample animals did not have significant influence on the results in our study.

There were notable differences between *Campylobacter* occurrence in different hosts, since all the positive samples were from rodents. Still, there were also rodent species where *Campylobacter* spp. was not detected, for example tundra vole (n = 49). There is no clear explanation on what causes the differences between rodents, since many species live on same areas and have similar diets. For example, grey red-backed voles live in a wide range of different areas including birch and coniferous forests where also northern red-backed voles usually live (Valste and Halkka 2007). Still, out of these two species, only northern red-backed vole was noted as *Campylobacter* positive even though the animals were captured from the same sampling sites in the same years. However, in both of these species the sample sizes were relatively low (n = 17 and n = 11).

Our results were somewhat similar with the ones from the master's thesis of Tikkanen (2019). There were six small mammal species (yellow-necked mouse, bank vole, field vole, common shrew, Eurasian pygmy shrew and water vole) that were included in both studies. In both studies all the shrews were *Campylobacter* negative and all the positive samples were from rodents. When comparing the shared rodent species, both studies revealed the same species being *Campylobacter* positive even though the occurrences varied. The occurrences were higher in the master's thesis from Tikkanen (2019) especially in bank voles (63,9 % compared to 16,5 %). The animals were captured in different locations at different times and therefore the results should not be compared without caution. It is however possible that animals in farm environments have higher *Campylobacter* occurrences than the animals further away from human habitats. In both studies, all the identified campylobacters were *C. jejuni*.

According to diet, there are no big differences between *Campylobacter* positive and negative rodent species. For example, both bank voles and grey red-backed voles have mixed diet where they eat mostly plant parts, for example berries and seeds, but also invertebrates. Field voles and wood lemmings are both more strictly herbivores who have simpler diet that includes for example hay. All the studied shrew species are insectivores but water shrews are also known to eat frogs and small fishes (Valste and Halkka 2007). There is no obvious reason

for what causes some species having higher *Campylobacter* occurrences than others. Further investigations are needed for determining the differences between species.

There is no clear explanation on why bank vole males had significantly higher *Campylobacter* occurrence than the females even though the correlation between sex and the *Campylobacter* occurrence was not significant in any other species. The difference between sexes could relate to possible differences in the sexes' behaviour, if bank vole males for example tend to move on wider areas than the females and that way be more likely to get exposed to *Campylobacter* spp. Statistical analysis revealed that the difference between sexes in bank voles from 2017 was not significant, but also in these animals *C. jejuni* was more common in males than females. Most of the previous studies considering *Campylobacter* spp. in rodents have not studied the differences between sexes. Gelling et al. (2012) however noted that the sex did not have significant correlation with the *Campylobacter* occurrence in water voles.

In our study, age did not have significant correlation with the *Campylobacter* occurrence in any of the *Campylobacter* positive species. However, only five animals of these species were juvenile and they all were yellow-necked mice. Thus, the differences between older animals and juveniles in all the studied species can not be generalized from these results. Also, the age's influence on the *Campylobacter* occurrence in field voles and northern red-backed voles could not be studied because of the sample sizes in different age groups. Previous studies of *Campylobacter* spp. in rodents usually haven't studied the correlation between age and *Campylobacter* occurrence, so in order to evaluate these differences further studies are needed.

The correlation between the animal's weight and *Campylobacter* status was not significant in bank voles and yellow-necked mice but was so in field voles. However, in field voles the result is due to that the weight groups 1 and 2 had only three animals in total and because of this the occurrence differences between groups are seemingly significant. There were no significant weight differences between the *Campylobacter* positive and negative animals from the same age groups, which indicates that the *Campylobacter* status does not have significant effect on the animal's weight. The weight analysis in northern red-backed voles were not executed because the weight information was known only from two individuals.

In yellow-necked mice the origin's correlation with the *Campylobacter* occurrence was statistically significant. This is due to the fact that all the studied yellow-necked mice from Vantaa (n = 11) were *Campylobacter* negative whereas all the other locations had relatively high *Campylobacter* occurrence (combined mean 50 %). It is uncertain why the animals from Vantaa were *Campylobacter* negative. The animals were trapped inside office buildings in a park like area that is surrounded by fields. The yellow-necked mice from Lammi and Hämeenkoski were trapped inside outbuildings. It is likely that the animals from all of these three locations had at least some kind of indirect contact with humans but still there were significant differences in the *Campylobacter* occurrences in these places.

In bank voles the correlation between the animal's origin and the *Campylobacter* status was significant because all the animals from Pallasjärvi (n = 30) were *Campylobacter* negative whereas other locations with relatively high sample sizes all had bank voles that were positive of *C. jejuni*. When the statistical analysis was done separately to the bank voles from 2017, the origin did not have significant correlation with the *Campylobacter* occurrence conversely to the animals from 2015. This is due to that all the bank voles from Pallasjärvi were from the year 2015. The animals from Pallasjärvi were therefore not included in the analysis of the bank voles from 2017 and because of that the occurrences in different location in 2017 were more even.

Curiously, Pallasjärvi is partly located inside Muonio and the sampling sites in both locations were around the same areas. *Campylobacter* occurrence in bank voles from Muonio was 25,6 % (n = 39) and all these bank voles were from the year 2017. Also in the overall study, *Campylobacter* occurrence in bank voles was significantly higher in the animals from 2017 than in the animals from 2015. One possible reason for these annual differences in *Campylobacter* occurrence is the differences in the bank vole numbers between years. In 2015 the vole populations, including bank voles, in northern Finland were high whereas the populations in 2017 were low in most parts of the country (National Resources Institute Finland 2015, National Resources Institute Finland 2017). Vole populations typically have cyclic changes where the population grows for around two years until predators and shortage of food in winter cause the population to collapse (Huitu and Henttonen 2011). It is possible

that the shortage of food causes the voles to move in larger areas or eat food they otherwise wouldn't eat. Since the *Campylobacter* spp. can survive in freezing conditions for prolonged times (Sampers et al. 2010), the survival in the environment during wintertime could also be prolonged and that way the bank voles could be more likely to encounter living *Campylobacter* spp. and end up as *Campylobacter* hosts.

In this study, four rodent species were detected carrying *C. jejuni*, which is the main cause of human campylobacteriosis also in Finland (National Institute for Health and Welfare 2019). Out of these species especially bank voles and yellow-necked mice may live close to humans and are known to even enter buildings. Field voles are also common around human habitat especially on the countryside since they often live in fields and may eat bark in gardens (Valste and Halkka 2007). Our results indicate that these rodent species could be *Campylobacter* reservoir and possible origins of human campylobacteriosis. They could possibly cause infections straight through their feces by infecting humans and farm animals or by contaminating farm estates or water sources. Also northern red-backed voles that typically don't live as near humans as the other species could contaminate water sources especially in forest areas where humans may hike or camp. Hörman et al. (2004) found surface waters in Finland positive of *C. jejuni* and Rollins and Colwell (1986) noted that *C. jejuni* could survive in cold water for up to several months. By this, if these rodent species contaminated for example cool streams with *C. jejuni*, the water sources could stay *Campylobacter* positive for notable times and that way increase the chance of human exposure. This could happen for example through swimming which is a known campylobacteriosis risk factor in Finland (Schönberg-Norio et al. 2004).

Still, further studies are needed for better risk analysis. There are multiple *C. jejuni* strains and there are differences in the strains' occurrence in different host species. Scarcelli et al. (2005) found primates and poultry having similar *C. jejuni* strains as human patients but no strains were shared between humans and for example canines. Broman et al. (2004) studied migrating wild birds and found most of the *C. jejuni* strains being different than the human isolates, but some of the strains from bird species that tend to live close to humans did have similar strains with humans.

In his master's thesis, Tikkanen (2019) studied *C. jejuni* isolates from Finnish small mammals captured in farm environments by using multilocus sequence typing (MLST). Most of the sequence types were new and not compatible with known types from farm animals. These results do not indicate that farm animals and small mammals share same *Campylobacter* strains, but rather that small mammals have their own *Campylobacter* populations. Further studies are needed to compare the *C. jejuni* types from animals living in close proximity of humans to ones living further away from human contact. One interesting aspect could also be comparing the *Campylobacter* types in farm animals and the small animals in the same farm environment. The isolated *C. jejuni* strains from our study should also be typed and compared to human strains in order to evaluate the rodents' possible impact on human campylobacteriosis. If the strains are similar, the possibility of human campylobacteriosis originating from rodents is higher whereas if the strains have significant differences, the rodents' strains could be less likely to act as human pathogens. In any case it is still recommendable for especially farms to consider their pest control, for besides *C. jejuni* rodents may also carry other human pathogens, for example *Salmonella* or *Yersinia* spp. (Meerburg et al. 2006, Backhans et al. 2013).

7 ACKNOWLEDGEMENTS

The Walter Ehrström Foundation and Finnish Foundation of Veterinary Research are acknowledged for financial support.

I thank Natural Resources Institute Finland, especially Jukka Niemimaa, Otso Huitu and Heikki Henttonen, for trapping and preparing animals for our studies. I thank my supervisors Rauni Kivistö and Mirko Rossi for their support and help during the laboratory work and writing process. I also want to thank Urszula Hirvi for teaching me all the different techniques in the laboratory and for her patience during my learning process.

I also want to thank the people and students of summer school for their help, peer support and opposing. Working and writing was easier, when I knew there were other people in the same position as me. Lastly, thanks to my family and friends for all their support.

REFERENCES

- Ajene A, Fischer Walker C, Black R. Enteric Pathogens and Reactive Arthritis: A Systematic Review of *Campylobacter*, *Salmonella* and *Shigella*-associated Reactive Arthritis. *J Health Popul Nutr* 2013, 31: 299-307.
- Andrzejewska M, Szczepańska B, Klawe J, Śpica D, Chudzińska M. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* species in cats and dogs from Bydgoszcz (Poland) region. *Pol J Vet Sci* 2013, 16: 115-120.
- Backhans A, Jacobson M, Hansson I, Lebbad M, Thisted Lambertz S, Gammelgård E, Saager M, Akande O, Fellström C. Occurrence of pathogens in wild rodents caught on Swedish pig and chicken farms. *Epidemiol Infect* 2013, 141: 1885-1891.
- Baker M, Kvalsvig A, Zhang J, Lake R, Sears A, Wilson N. Declining Guillain-Barré Syndrome after Campylobacteriosis Control, New Zealand, 1988–2010. *Emerg Infect Dis* 2012, 18: 226-233.
- Blaser M, Engberg J. Clinical Aspects of *Campylobacter jejuni* and *Campylobacter coli* Infections. In: Nachamkin I, Szymanski C, Blaser M, eds. *Campylobacter*, 3rd edition. Washington, DC: American Society for Microbiology 2008: 99-107.
- Broman T, Waldenström J, Dahlgren D, Carlsson I, Eliasson I, Olsen B. Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans. *J Appl Microbiol* 2004, 96: 834-843.
- Buswell C, Herlihy Y, Lawrence L, McGuiggan J, Marsh P, Keevil C, Leach S. Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. *Appl Environ Microb* 1998, 64: 733-741.
- Campbell A. The Aetiology of Polyneuritis. *Proc R Soc Med* 1957, 51: 157-159.
- Chaban B, Ngeleka, M, Hill J. Detection and quantification of 14 *Campylobacter* species in pet dogs reveals an increase in species richness in feces of diarrheic animals. *BMC Mikrobiol* 2010, 10: 73.
- Cheng Q, Jiang G.-X, Press R, Andersson M, Ekstedt B, Vrethem M, Liedholm L, Lindsten H, Brattström L, Fredrikson S, Link H, de Pedro-Cuesta J. Clinical epidemiology of Guillain–Barré syndrome in adults in Sweden 1996–97: a prospective study. *Eur J Neurol* 2000, 7: 685-692.

Constant O, Bentley C, Denman A, Lehane J, Larson H. The Guillain-Barré syndrome following *Campylobacter* enteritis with recovery after plasmapheresis. *J Infection* 1983, 6: 89-91.

De Boer E, Hahné M. Cross-contamination with *Campylobacter jejuni* and *Salmonella* spp. from raw chicken products during food preparation. *J Food Protect* 1990, 53: 1067-1068.

Debruyne L, Gevers D, Vandamme P. Taxonomy of the Family *Campylobacteraceae*. In: Nachamkin I, Szymanski C, Blaser M, eds. *Campylobacter*, 3rd edition. Washington, DC: American Society for Microbiology 2008: 3-11.

de Jager A, Sluiter H. Clinical signs in severe Guillain-Barré syndrome: analysis of 63 patients. *J Neurol Sci* 1991, 104: 143-150.

Denis M, Soumet C, Rivoal K, Ermel G, Blivet D, Salvat G, Colin P. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett Appl Microbiol* 1999, 29: 406-410.

Domingues A, Pires S, Halasa T, Hald T. Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. *Epidemiol Infect* 2012, 140: 970-981.

Doyle M, Roman D. Growth and Survival of *Campylobacter fetus* subsp. *jejuni* as a Function of Temperature and pH. *J Food Protect* 1981, 44: 596-601.

Ellis-Iversen J, Ridley A, Morris V, Sowa A, Harris J, Atterbury R, Sparks N, Allen V. Persistent environmental reservoirs on farms as risk factors for *Campylobacter* in commercial poultry. *Epidemiol Infect* 2012, 140: 916-924.

European Food Safety Authority. The European Union One Health 2018 Zoonoses Report. *EFSA Journal* 2019, 17: 5926.

Feodoroff B, Lauhio A, Ellström P, Rautelin H. A Nationwide Study of *Campylobacter jejuni* and *Campylobacter coli* Bacteremia in Finland Over a 10-Year Period, 1998–2007, With Special Reference to Clinical Characteristics and Antimicrobial Susceptibility. *Clin Infect Dis* 2011, 53: e99-e106.

Fernandes A, Balasegaram S, Willis C, Wimalarathna H, Maiden M, McCarthy N. Partial Failure of Milk Pasteurization as a Risk for the Transmission of *Campylobacter* from Cattle to Humans. *Clin Infect Dis* 2015, 61: 903-909.

Fernández-Cruz A, Muñoz P, Mohedano R, Valerio M, Marín M, Alcalá L, Rodríguez-Créixems M, Cercenado E, Bouza E. *Campylobacter* bacteremia: clinical characteristics, incidence, and outcome over 23 years. *Medicine* 2010, 89: 319-330.

Gardner T, Fitzgerald C, Xavier C, Klein R, Pruckler J, Stroika S, McLaughlin J. Outbreak of Campylobacteriosis Associated with Consumption of Raw Peas. *Clin Infect Dis* 2011, 53: 26-32

Gelling M, Macdonald D, Telfer S, Jones T, Bown K, Birtles R, Mathews F. Parasites and pathogens in wild populations of water voles (*Arvicola amphibius*) in the UK. *Eur J Wildlife Res* 2012, 58: 615-619.

Gillespie I, O'Brien S, Frost J, Adak G, Horby P, Swan A, Painter M, Neal K. A Case-Case Comparison of *Campylobacter coli* and *Campylobacter jejuni* Infection: A Tool for Generating Hypotheses. *Emerg Infect Dis* 2002, 8: 937-942.

Hannu T, Mattila L, Rautelin H, Pelkonen P, Lahdenne P, Siitonen A, Leirisalo-Repo M. *Campylobacter*-triggered reactive arthritis: a population-based study. *Rheumatology* 2002, 41: 312-318.

Healing T, Greenwood M. Frequency of isolation of *Campylobacter* spp., *Yersinia* spp. and *Salmonella* spp. from small mammals from two sites in southern Britain. *Int J Environ Heal R* 1991, 1: 54-62.

Horn B, Lake R. Incubation period for campylobacteriosis and its importance in the estimation of incidence related to travel. *Eurosurveillance* 2013, 18: 17-22.

Huitu O, Henttonen H. Myyrien kannanvaihtelut aikojen ja ilmojen saatossa. *Metsätieteen aikakauskirja* 2011, 4: 281-284.

Hänninen M.-L, Haajanen H, Pummi T, Wermundsen K, Katila M.-L, Sarkkinen H, Miettinen I, Rautelin H. Detection and Typing of *Campylobacter jejuni* and *Campylobacter coli* and Analysis of Indicator Organisms in Three Waterborne Outbreaks in Finland. *Appl Environ Microb* 2003, 69: 1391-1396.

Hörman A, Rimhanen-Finne R, Maunula L, von Bonsdorff C.-H, Torvela N, Heikinheimo A, Hänninen M.-L. *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and Indicator Organisms in Surface Water in Southwestern Finland, 2000-2001. *Appl Environ Microb* 2004, 70: 87-95.

Inglis G, Kalischuk L. Use of PCR for Direct Detection of *Campylobacter* Species in Bovine Feces. *Appl Environ Microb* 2003, 69: 3435-3447.

Jacobs B, van Belkum A, Endtz H. Guillain-Barré Syndrome and *Campylobacter* Infection. In: Nachamkin I, Szymanski C, Blaser M, eds. *Campylobacter*, 3rd edition. Washington, DC: American Society for Microbiology 2008: 245-261.

Johnsen G, Kruse H, Hofshagen M. Genotyping of thermotolerant *Campylobacter* from poultry slaughterhouse by amplified fragment length polymorphism. *J Appl Microbiol* 2007, 103: 271-279.

Jore S, Viljugrein H, Brun E, Heier B.T, Borck B, Ethelberg S, Häkkinen M, Kuusi M, Reiersen J, Hansson I, Olsson Engvall E, Løfdahl M, Wagenaar J.A, van Pelt W, Hofshagen M. Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007. *Prev Vet Med* 2010, 93: 33-41.

Joshua G, Guthrie-Irons C, Karlyshev A, Wren B. Biofilm formation in *Campylobacter jejuni*. *Microbiology+* 2006, 152: 387-396.

Kapperud G, Espeland G, Wahl E, Walde A, Herikstad H, Gustavsen S, Tveit I, Natås O, Bevanger L, Digranes A. Factors Associated with Increased and Decreased Risk of *Campylobacter* Infection: A Prospective Case-Control Study in Norway. *Am J Epidemiol* 2003, 158: 234-242.

Keller J, Shriver W. Prevalence of Three *Campylobacter* Species, *C. jejuni*, *C. coli* and *C. lari*, Using Multilocus Sequence Typing in Wild Birds of the Mid-Atlantic Region, USA. *J Wildlife Dis* 2014, 50: 31-41.

Konicek C, Vodrážka P, Barták P, Knotek Z, Hess C, Račka K, Hess M, Troxler S. Detection of Zoonotic Pathogens in Wild Birds in the Cross-Border Region Austria – Czech Republic. *J Wildlife Dis* 2016, 52: 850-861.

Laine J, Huovinen E, Virtanen M, Snellman M, Lumio J, Ruutu P, Kujansuu E, Vuento R, Pitkänen T, Miettinen I, Herrala J, Lepistö O, Anttonen J, Helenius J, Hänninen M.-L, Maunula L, Mustonen J, Kuusi M. An extensive gastroenteritis outbreak after drinking-water contamination by sewage effluent, Finland. *Epidemiol Infect* 2011, 139: 1105-1113.

Locht H, Krogfelt K. Comparison of rheumatological and gastrointestinal symptoms after infection with *Campylobacter jejuni/coli* and enterotoxigenic *Escherichia coli*. *Ann Rheum Dis* 2002, 61: 448-452.

Lone A, Selinger L, Uwiera R, Xu Y, Inglis G. *Campylobacter jejuni* Colonization Is Associated with a Dysbiosis in the Cecal Microbiota of Mice in the Absence of Prominent Inflammation. *Plos One* 2013, 8: e75325.

McCarthy N, Giesecke J. Incidence of Guillain-Barré Syndrome following Infection with *Campylobacter jejuni*. *Am J Epidemiol* 2001, 153: 610-614.

Meerburg B, Jacobs-Reitsma W, Wagenaar J, Kijlstra A. Presence of *Salmonella* and *Campylobacter* spp. in Wild Small Mammals on Organic Farms. *Appl Environ Microb* 2006, 72: 960-962.

Morrell E, Barbeito C, Odeón C, Gimeno E, Campero C. Histopathological, Immunohistochemical, Lectin histochemical and Molecular Findings in Spontaneous Bovine Abortions by *Campylobacter fetus*. *Reprod Domest Anim* 2010, 46: 309-315.

Nadeau É, Messier S, Quessy S. Prevalence and Comparison of Genetic Profiles of *Campylobacter* Strains Isolated from Poultry and Sporadic Cases of Campylobacteriosis in Humans. *J Food Protect* 2002, 65: 73-78.

National Institute for Health and Welfare. *Kampylobakteerin esiintyvyys Suomessa*. <https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/taudit-ja-torjunta/taudit-ja-taudinaiheuttajat-a-o/kampylobakteeri/kampylobakteerin-esiintyvyys-suomessa>, accessed 19 December 2019, updated 12 September 2019.

National Institute for Health and Welfare. *Vesiepidemiat*. <https://thl.fi/fi/web/ymparistoterveys/vesi/vesiepidemiat>, accessed 18 February 2020, updated 6 February 2020.

National Resources Institute Finland 2015. *Pohjois-Suomessa myyrähuippu – Etelä- ja Itä-Suomessa myyräkannat kasvussa*. <https://www.luke.fi/uutinen/pohjois-suomessa-myyrahuippu-etela-ja-ita-suomessa-myyrakannat-kasvussa/>, accessed 24.2.2020.

National Resources Institute Finland 2017. *Myyräkannat alhaiset valtaosassa maata*. <https://www.luke.fi/uutinen/myyrakannat-alhaiset-valtaosassa-maata/>, accessed 24.2.2020.

Neimann J, Engberg J, Mølbak K, Wegener H. A case–control study of risk factors for sporadic campylobacter infections in Denmark. *Epidemiol Infect* 2003, 130: 353-366.

Olson P, Sandstedt K. *Campylobacter* in the dog: A clinical and experimental study. *Vet Rec* 1987, 121: 99-101.

Pacanowski J, Lalande V, Lacombe K, Boudraa C, Lesprit P, Legrand P, Trystram D, Kassis N, Arlet G, Mainardi J, Doucet-Populaire F, Girard P, Meynard J. *Campylobacter* Bacteremia: Clinical Features and Factors Associated with Fatal Outcome. *Clin Infect Dis* 2008, 47: 790-796.

Parsons B, Williams N, Pinchbeck G, Christley R, Hart C, Gaskell R, Dawson S. Prevalence and shedding patterns of *Campylobacter* spp. in longitudinal studies of kennelled dogs. *Vet J* 2011, 190: 249-254.

Pavlovskis O, Rollins D, Haberberger R, Green A, Habash L, Strocko S, Walker R. Significance of Flagella in Colonization Resistance of Rabbits Immunized with *Campylobacter* Spp. *Infect Immun* 1991, 59: 2259-2264.

Ramonaitė S, Rokaitytė A, Tamulevičienė E, Malakauskas A, Alter T, Malakauskas M. Prevalence, quantitative load and genetic diversity of *Campylobacter* spp. in dairy cattle herds in Lithuania. *Acta Vet Scand* 2013, 55: 87.

Robinson D. Infective dose of *Campylobacter jejuni* in milk. *Brit Med J* 1981, 282: 1584.

Rollins D, Colwell R. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Appl Environ Microb* 1986, 52: 531-538.

Rosef O, Gondrosen B, Kapperud G, Underdal B. Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Appl Environ Microb* 1983, 46: 855-859.

Rossi M, Hänninen M.L, Revez J, Hannula M, Zanoni R.G. Occurrence and species level diagnostics of *Campylobacter* spp., enteric *Helicobacter* spp. and *Anaerobiospirillum* spp. in healthy and diarrheic dogs and cats. *Vet Microbiol* 2008, 129: 304-314.

Sampers I, Habib I, De Zutter L, Dumoulin A, Uyttendaele M. Survival of *Campylobacter* spp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration, and heat treatment. *Int J Food Microbiol* 2010, 137: 147-153.

Scarcelli E, Piatti R, Harakava R, Miyashiro S, de Campos Fernandes F, Campos F, Francisco W, Genovez M, Richtzenhain L. Molecular Subtyping of *Campylobacter jejuni* subsp. *jejuni* Strains Isolated from Different Animal Species in the State of São Paulo, Brazil. *Braz J Microbiol* 2005, 36: 378-382.

Schönberg-Norio D, Takkinen J, Hänninen M.-L, Katila M.-L, Kaukoranta S.-S, Mattila L, Rautelin H. Swimming and *Campylobacter* infections. *Emerg Infect Dis* 2004, 10: 1474-1477.

Shanker S, Lee A, Sorrell T. Experimental colonization of broiler chicks with *Campylobacter jejuni*. *Epidemiol Infect* 1988, 100: 27-34.

Sproston E, Ogden I, MacRae M, Dallas J, Sheppard S, Cody A, Colles F, Wilson M, Forbes K, Strachan N. Temporal Variation and Host Association in the *Campylobacter* Population in a Longitudinal Ruminant Farm Study. *Appl Environ Microb* 2011, 77: 6579-6586.

Tikkanen J. Haittaeläimet maatilaympäristössä ja haittaeläimissä esiintyvät lämpökestoiset *Campylobacter jejuni* -bakteerit. Master's thesis, Helsinki, University of Helsinki, 2019.

Valste J, Halkka A. Nisäkkäät Suomen luonnossa. Otava, Helsinki, Suomi 2007.

Van Koningsveld R, Van Doorn P, Schmitz P, Ang C, Van der Meché F. Mild forms of Guillain-Barré syndrome in an epidemiologic survey in the Netherlands. *Neurology* 2000, 54: 620-625.

Waldenström J, On S, Ottvall R, Hasselquist D, Olsen B. Species diversity of campylobacteria in a wild bird community in Sweden. *J Appl Microbiol* 2007, 102: 424-432.

Wilson D, Gabriel E, Leatherbarrow A, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA, Diggle P. Tracing the Source of Campylobacteriosis. *Plos Genet* 2008, 9: e1000203.

Appendix 1. The study material. Used abbreviations: Ow: overwintered, Juv: juvenile, Ad: adult, Sel: postjuvenile with incomplete coat change reaching the back.

Number	Species	Sex	Age	Capture date	Location	Habitat	Weight (g)	Length (mm, body+tail)	<i>Campylobacter</i> status
1	Bank vole	Male	Ow	4.6.2015	Kolari	Forest	23,3	100+40	Positive
2	Bank vole	Female	Ow	4.6.2015	Kolari	Forest	26,4	100+48	Negative
3	Bank vole	Female	Ow	4.6.2015	Kolari	Forest	24,2	95+49	Negative
4	Bank vole	Male	Ow	4.6.2015	Kolari	Forest	24,2	96+39	Negative
5	Bank vole	Male	Ow	4.6.2015	Kolari	Forest	23,5	96+42	Negative
6	Bank vole	Male	Ow	4.6.2015	Kolari	Forest	23,3	97+41	Negative
7	Bank vole	Female	Ow	4.6.2015	Kolari	Forest	27,0	97+42	Negative
8	Bank vole	Female	Ow	4.6.2015	Kolari	Forest	28,0	95+48	Negative
9	Tundra vole	Female	Ow	4.6.2015	Kolari	Field	37,3	108+35	Negative
10	Tundra vole	Male	Ow	4.6.2015	Kolari	Field	39,1	110+40	Negative
11	Tundra vole	Male	Ow	4.6.2015	Kolari	Field	40,7	104+39	Negative
12	Bank vole	Female	Ow	2.6.2015	Pisavaara	Field	26,4	100+43	Negative
13	Tundra vole	Female	Ow	2.6.2015	Pisavaara	Field	41,1	111+41	Negative
14	Tundra vole	Male	Ow	2.6.2015	Pisavaara	Field	50,3	117+40	Negative
15	Tundra vole	Male	Ow	2.6.2015	Pisavaara	Field	43,9	119+37	Negative
16	Common shrew	Male	Ow	2.6.2015	Pisavaara	Forest	9,73	65+40	Negative

17	Wood lemming	Male	Ow	2.6.2015	Pisavaara	Forest	27,0	95+14	Negative
18	Wood lemming	Female	Ow	2.6.2015	Pisavaara	Forest	33,3	95+17	Negative
19	Tundra vole	Female	Ow	2.6.2015	Pisavaara	Forest	41,2	114+37	Negative
20	Tundra vole	Female	Ow	2.6.2015	Pisavaara	Forest	52,3	118+43	Negative
21	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	33,1	102+53	Negative
22	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	26,5	106+45	Negative
23	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	25,9	96+44	Negative
24	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	24,8	96+47	Negative
25	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	31,7	96+49	Negative
26	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	21,0	88+44	Negative
27	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	27,6	99+48	Negative
28	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	25,0	91+46	Positive
29	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	29,1	90+43	Negative
30	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	24,7	97+46	Negative
31	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	30,0	97+46	Negative
32	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	27,6	93+48	Negative
33	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	25,2	97+46	Negative
34	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	25,8	98+47	Positive
35	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	27,8	99+46	Negative
36	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	24,4	93+44	Positive
37	Bank vole	Female	Ow	2.6.2015	Pisavaara	Forest	29,7	103+47	Negative
38	Bank vole	Male	Ow	2.6.2015	Pisavaara	Forest	27,7	93+45	Positive

39	Tundra vole	Male	Juv	13.6.2015	Pallasjärvi	-	18,8	85+26	Negative
40	Tundra vole	Male	Juv	13.6.2015	Pallasjärvi	-	18,8	89+27	Negative
41	Tundra vole	Female	Sel	13.6.2015	Pallasjärvi	-	26,6	100+38	Negative
42	Tundra vole	Male	Sel	13.6.2015	Pallasjärvi	-	26,8	94+35	Negative
43	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	54,0	125+46	Negative
44	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	59,6	126+43	Negative
45	Tundra vole	Male	Ow	13.6.2015	Pallasjärvi	-	53,2	122+39	Negative
46	Tundra vole	Male	Ow	13.6.2015	Pallasjärvi	-	61,2	133+48	Negative
47	Tundra vole	Male	Ow	13.6.2015	Pallasjärvi	-	74,2	134+45	Negative
48	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	62,6	131+47	Negative
49	Tundra vole	Female	Ad	13.6.2015	Pallasjärvi	-	27,9	95+34	Negative
50	Tundra vole	Male	Ad	13.6.2015	Pallasjärvi	-	29,0	99+38	Negative
51	Bank vole	Male	Ow	13.6.2015	Pallasjärvi	-	29,5	102+47	Negative
52	Bank vole	Male	Ow	13.6.2015	Pallasjärvi	-	30,8	106+49	Negative
53	Bank vole	Female	Ow	13.6.2015	Pallasjärvi	-	37,0	106+53	Negative
54	Bank vole	Female	Ow	13.6.2015	Pallasjärvi	-	28,6	95+45	Negative
55	Tundra vole	Female	Sel	13.6.2015	Pallasjärvi	-	27,1	91+35	Negative
56	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	52,9	112+48	Negative
57	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	41,0	112+37	Negative
58	Tundra vole	Male	Ow	13.6.2015	Pallasjärvi	-	62,5	120+42	Negative
59	Tundra vole	Male	Ow	13.6.2015	Pallasjärvi	-	69,9	134+47	Negative
60	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	59,7	122+48	Negative

61	Grey red-backed vole	Female	Ow	13.6.2015	Pallasjärvi	-	50,3	113+33	Negative
62	Bank vole	Male	Ow	13.6.2015	Pallasjärvi	-	31,1	103+54	Negative
63	Bank vole	Male	Ow	13.6.2015	Pallasjärvi	-	28,0	102	Negative
64	Northern red-backed vole	Female	Ow	13.6.2015	Pallasjärvi	-	21,9	93+31	Negative
65	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	50,6	118+48	Negative
66	Tundra vole	Female	Ow	13.6.2015	Pallasjärvi	-	54,6	133+42	Negative
67	Tundra vole	Male	Ow	13.6.2015	Pallasjärvi	-	71,4	133+41	Negative
68	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	25,8	97+44	Negative
69	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	29,9	103+43	Negative
70	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	28,8	96+45	Negative
71	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	28,8	102+48	Negative
72	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	28,5	106+50	Negative
73	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	28,1	102+43	Negative
74	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	21,5	89+41	Negative
75	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	26,3	96+46	Negative
76	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	23,5	97+48	Negative
77	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	37,4	96(+37)	Negative
78	Grey red-backed vole	Male	Ow	10.6.2015	Pallasjärvi	-	54,0	129+28	Negative
79	Common shrew	Female	Ow	10.6.2015	Pallasjärvi	-	11,5	69+37	Negative
80	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	34,1	108+44	Negative
81	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	22,6	99+49	Negative
82	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	28,2	101+52	Negative

83	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	32,9	110+48	Negative
84	Grey red-backed vole	Female	Juv	10.6.2015	Pallasjärvi	-	(8,3)	(70+)23	Negative
85	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	30,8	108+49	Negative
86	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	29,4	97+46	Negative
87	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	25,6	95+44	Negative
88	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	24,8	89+44	Negative
89	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	26,8	103+47	Negative
90	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	30,2	102+46	Negative
91	Bank vole	Female	Ow	10.6.2015	Pallasjärvi	-	22,9	90+47	Negative
92	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	23,3	94+44	Negative
93	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	24,0	98+45	Negative
94	Bank vole	Male	Ow	10.6.2015	Pallasjärvi	-	24,6	96+43	Negative
95	Northern red-backed vole	Male	Ow	10.6.2015	Pallasjärvi	-	32,4	111+33	Negative
96	Tundra vole	Male	Sel	10.6.2015	Pallasjärvi	-	31,5	101+35	Negative
97	Grey red-backed vole	Female	Ow	10.6.2015	Pallasjärvi	-	53,4	123+32	Negative
98	Tundra vole	Female	Ow	3.6.2015	Pyhätunturi	-	79,1	117+43	Negative
99	Tundra vole	Male	Ow	3.6.2015	Pyhätunturi	-	66,5	122+53	Negative
100	Tundra vole	Male	Ow	3.6.2015	Pyhätunturi	-	69,5	133+50	Negative
101	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	23,1	92+52	Negative
102	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	(24,9)	100(+12)	Negative
103	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	27,4	104+50	Negative
104	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	27,7	105+44	Negative

105	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	36,8	100+42	Negative
106	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	32,1	101+45	Negative
107	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	29,5	99+46	Positive
108	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	36,6	98+48	Negative
109	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	25,7	97+47	Negative
110	Wood lemming	Male	Ow	3.6.2015	Pyhätunturi	-	29,0	105+15	Negative
111	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	29,4	102+45	Negative
112	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	39,5	100+48	Negative
113	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	28,9	86+48	Negative
114	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	29,9	102+51	Negative
115	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	26,8	95+44	Negative
116	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	24,1	94+46	Negative
117	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	26,7	102+42	Negative
118	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	24,9	101+39	Negative
119	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	26,4	96+54	Negative
120	Water shrew	Male	Ow	3.6.2015	Pyhätunturi	-	14,9	78+66	Negative
121	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	29,2	92+45	Negative
122	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	25,0	96+44	Negative
123	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	25,9	97+52	Negative
124	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	26,2	99+48	Negative
125	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	23,5	91+38	Negative
126	Bank vole	Male	Ow	3.6.2015	Pyhätunturi	-	25,4	96+47	Negative

127	Bank vole	Female	Ow	3.6.2015	Pyhätunturi	-	25,9	92+47	Negative
128	Wood lemming	Male	Ow	3.6.2015	Pyhätunturi	-	28,6	97+14	Negative
129	Bank vole	Male	Ad	9.5.2017	Vammala	Forest	18,8	98+45	Negative
130	Bank vole	Male	Ad	9.5.2017	Vammala	Forest	19,9	96+41	Negative
131	Bank vole	Female	Ad	9.5.2017	Vammala	Forest	21,3	101+46	Negative
132	Bank vole	Male	Ad	10.5.2017	Karvia	Field	19,7	98+40	Positive
133	Bank vole	Male	Ad	10.5.2017	Karvia	Forest	18,1	100+35	Negative
134	Bank vole	Female	Ad	10.5.2017	Karvia	Forest	23,0	97+46	Negative
135	Field vole	Female	Ad	11.5.2017	Viitasaari	Forest	29,5	114+27	Negative
136	Bank vole	Male	Ad	11.5.2017	Viitasaari	Forest	24,0	106+42	Negative
137	Bank vole	Male	Ad	11.5.2017	Viitasaari	Forest	26,8	108+39	Positive
138	Bank vole	Male	Ad	11.5.2017	Viitasaari	Forest	26,7	112+42	Negative
139	Bank vole	Female	Ad	11.5.2017	Viitasaari	Forest	16,4	93+41	Negative
140	Bank vole	Female	Ad	11.5.2017	Viitasaari	Forest	24,5	103+44	Negative
141	Bank vole	Female	Ad	11.5.2017	Viitasaari	Forest	17,7	92+41	Negative
142	Field vole	Male	Ad	11.5.2017	Viitasaari	Field	40,4	123+27	Negative
143	Field vole	Male	Ad	11.5.2017	Viitasaari	Field	40,3	123	Positive
144	Field vole	Male	Ad	11.5.2017	Viitasaari	Field	36,7	117+29	Negative
145	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	30,4	116+26	Negative
146	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	46,6	123+27	Negative
147	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	46,4	125+27	Negative
148	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	46,4	122+25	Negative

149	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	42,7	120+28	Negative
150	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	30,4	116+25	Negative
151	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	48,6	120+28	Negative
152	Field vole	Female	Ad	11.5.2017	Viitasaari	Field	37,6	121+28	Negative
153	Bank vole	Male	Ad	12.5.2017	Korpilahti	Forest	24,2	105+46	Negative
154	Bank vole	Male	Ad	12.5.2017	Korpilahti	Forest	21,1	99+42	Negative
155	Bank vole	Male	Ad	12.5.2017	Korpilahti	Forest	25,8	106+48	Positive
156	Bank vole	Male	Ad	12.5.2017	Korpilahti	Forest	21,6	104+40	Positive
157	Bank vole	Male	Ad	12.5.2017	Korpilahti	Forest	21,0	102+45	Negative
158	Bank vole	Female	Ad	12.5.2017	Korpilahti	Forest	24,7	105+47	Negative
159	Bank vole	Female	Ad	12.5.2017	Korpilahti	Forest	15,9	97	Negative
160	Bank vole	Female	Ad	12.5.2017	Korpilahti	Forest	19,0	99+44	Positive
161	Field vole	Male	Ad	13.5.2017	Mikkeli	Field	26,9	115+27	Negative
162	Field vole	Male	Ad	13.5.2017	Mikkeli	Field	29,5	115+26	Negative
163	Field vole	Female	Ad	13.5.2017	Mikkeli	Field	24,6	110+26	Positive
164	Field vole	Female	Ad	13.5.2017	Mikkeli	Field	28,5	112+28	Negative
165	Bank vole	Male	Ad	13.5.2017	Mikkeli	Forest	17,4	99+41	Positive
166	Field vole	Male	Ad	14.5.2017	Virolahti	Field	27,9	112+25	Negative
167	Field vole	Male	Ad	14.5.2017	Virolahti	Field	35,9	119+27	Negative
168	Field vole	Female	Ad	14.5.2017	Virolahti	Field	37,7	124+26	Negative
169	Bank vole	Male	Ad	14.5.2017	Virolahti	Forest	17,7	100+40	Negative
170	Bank vole	Female	Ad	14.5.2017	Virolahti	Forest	19,9	101+43	Negative

171	Bank vole	Male	Ad	15.5.2017	Luumäki	Forest	21,8	101+40	Negative
172	Field vole	Male	Ad	15.5.2017	Luumäki	Field	26,6	105+22	Negative
173	Field vole	Female	Ad	16.5.2017	Punkaharju	Field	31,2	117+27	Negative
174	Field vole	Female	Ad	16.5.2017	Punkaharju	Field	29,9	107+23	Negative
175	Field vole	Female	Ad	16.5.2017	Punkaharju	Field	30,4	112+24	Negative
176	Field vole	Female	Ad	16.5.2017	Punkaharju	Field	34,9	117+27	Negative
177	Bank vole	Male	Ad	16.5.2017	Punkaharju	Forest	20,7	105+46	Negative
178	Bank vole	Male	Ad	17.5.2017	Tohmajärvi	Forest	18,2	97+40	Negative
179	Field vole	Female	Ad	17.5.2017	Tohmajärvi	Field	18,0	103+21	Negative
180	Field vole	Female	Ad	18.5.2017	Koli	Field	25,7	115+28	Positive
181	Bank vole	Male	Ad	18.5.2017	Koli	Forest	23,3	106+44	Positive
182	Field vole	Male	Ad	19.5.2017	Suonenjoki	Field	33,5	117+27	Positive
183	Field vole	Male	Ad	19.5.2017	Suonenjoki	Field	32,7	122+27	Negative
184	Field vole	Male	Ad	19.5.2017	Suonenjoki	Field	34,9	118+27	Negative
185	Field vole	Male	Ad	19.5.2017	Suonenjoki	Field	39,6	122+27	Negative
186	Field vole	Female	Ad	19.5.2017	Suonenjoki	Field	35,1	115+23	Negative
187	Field vole	Female	Ad	19.5.2017	Suonenjoki	Field	30,4	109+28	Negative
188	Field vole	Female	Ad	19.5.2017	Suonenjoki	Field	30,7	112+27	Negative
189	Field vole	Female	Ad	19.5.2017	Suonenjoki	Field	32,7	119+25	Negative
190	Field vole	Female	Ad	19.5.2017	Suonenjoki	Field	24,8	108+25	Positive
191	Field vole	Female	Ad	19.5.2017	Suonenjoki	Field	43,0	115+27	Positive
192	Bank vole	Male	Ad	19.5.2017	Suonenjoki	Forest	23,0	104+43	Positive

193	Bank vole	Male	Ad	19.5.2017	Suonenjoki	Forest	18,7	105+36	Negative
194	Bank vole	Male	Ow	23.5.2017	Toholampi	-	-	-	Negative
195	Bank vole	Male	Ow	24.5.2017	Muhos	-	-	-	Positive
196	Bank vole	Male	Ow	24.5.2017	Muhos	-	-	-	Negative
197	Bank vole	Male	Ow	25.5.2017	Sotkamo	-	-	-	Negative
198	Bank vole	Male	Ow	3.6.2017	Pyhätunturi	-	-	-	Positive
199	Bank vole	Male	Ow	3.6.2017	Pyhätunturi	-	-	-	Negative
200	Common shrew	Female	Ow	3.6.2017	Pyhätunturi	-	-	-	Negative
201	Bank vole	Male	Ow	3.6.2017	Pyhätunturi	-	-	-	Negative
202	Tundra vole	Female	Ow	3.6.2017	Pyhätunturi	-	-	-	Negative
203	Common shrew	Male	Ow	4.6.2017	Pisavaara	-	-	-	Negative
204	Bank vole	Male	Ow	5.6.2017	Kolari	-	-	-	Negative
205	Grey red-backed vole	Female	Ow	7.6.2017	Muonio	-	-	-	Negative
206	Grey red-backed vole	Male	Ow	7.6.2017	Muonio	-	-	-	Negative
207	Common shrew	Female	Ow	7.6.2017	Muonio	-	-	-	Negative
208	Tundra vole	Male	Ow	8.6.2017	Muonio	-	-	-	Negative
209	Grey red-backed vole	Female	Ow	8.6.2017	Muonio	-	-	-	Negative
210	Bank vole	Female	Ow	8.6.2017	Muonio	-	-	-	Positive
211	Bank vole	Male	Ow	8.6.2017	Muonio	-	-	-	Negative
212	Bank vole	Female	Ow	8.6.2017	Muonio	-	-	-	Negative
213	Grey red-backed vole	Male	Ow	8.6.2017	Muonio	-	-	-	Negative
214	Grey red-backed vole	Male	Ow	8.6.2017	Muonio	-	-	-	Negative

215	Bank vole	Male	Ow	8.6.2017	Muonio	-	-	-	Negative
216	Bank vole	Male	Ow	8.6.2017	Muonio	-	-	-	Positive
217	Bank vole	Male	Ow	8.6.2017	Muonio	-	-	-	Negative
218	Common shrew	Male	Ow	8.6.2017	Muonio	-	-	-	Negative
219	Grey red-backed vole	Male	Ow	9.6.2017	Muonio	-	-	-	Negative
220	Bank vole	Female	Ow	9.6.2017	Muonio	-	-	-	Positive
221	Bank vole	Male	Ow	9.6.2017	Muonio	-	-	-	Negative
222	Bank vole	Female	Ow	9.6.2017	Muonio	-	-	-	Positive
223	Bank vole	Male	Ow	9.6.2017	Muonio	-	-	-	Negative
224	Bank vole	Female	Ow	9.6.2017	Muonio	-	-	-	Negative
225	Northern red-backed vole	Male	Ow	9.6.2017	Muonio	-	-	-	Positive
226	Bank vole	Male	Ow	9.6.2017	Muonio	-	-	-	Negative
227	Bank vole	Male	Ow	9.6.2017	Muonio	-	-	-	Negative
228	Bank vole	Male	Ow	9.6.2017	Muonio	-	-	-	Negative
229	Bank vole	Female	Ow	9.6.2017	Muonio	-	-	-	Negative
230	Bank vole	Male	Ow	10.6.2017	Muonio	-	-	-	Negative
231	Northern red-backed vole	Male	Ow	10.6.2017	Muonio	-	-	-	Positive
232	Northern red-backed vole	Female	Ow	11.6.2017	Muonio	-	-	-	Positive
233	Northern red-backed vole	Male	Ow	11.6.2017	Muonio	-	-	-	Positive
234	Bank vole	Male	Ow	11.6.2017	Muonio	-	-	-	Positive
235	Bank vole	Male	Ow	11.6.2017	Muonio	-	-	-	Positive
236	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative

237	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
238	Tundra vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
239	Tundra vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
240	Bank vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
241	Bank vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
242	Bank vole	Male	Ow	11.6.2017	Muonio	-	-	-	Positive
243	Common shrew	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
244	Common shrew	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
245	Common shrew	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
246	Common shrew	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
247	Tundra vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
248	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
249	Bank vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
250	Bank vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
251	Grey red-backed vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
252	Common shrew	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
253	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
254	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
255	Tundra vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
256	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
257	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
258	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative

259	Tundra vole	Female	Ow	11.6.2017	Muonio	-	-	-	Negative
260	Grey red-backed vole	Male	Ow	11.6.2017	Muonio	-	-	-	Negative
261	Northern red-backed vole	Male	Ow	12.6.2017	Muonio	-	-	-	Positive
262	Bank vole	Male	Ow	12.6.2017	Muonio	-	-	-	Negative
263	Grey red-backed vole	Male	Ow	12.6.2017	Muonio	-	-	-	Negative
264	Common shrew	Male	Ow	12.6.2017	Muonio	-	-	-	Negative
265	Common shrew	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
266	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
267	Northern red-backed vole	Female	Ow	13.6.2017	Muonio	-	-	-	Positive
268	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Positive
269	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
270	Bank vole	Female	Ow	13.6.2017	Muonio	-	-	-	Negative
271	Common shrew	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
272	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
273	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
274	Bank vole	Female	Ow	13.6.2017	Muonio	-	-	-	Negative
275	Common shrew	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
276	Common shrew	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
277	Common shrew	Female	Ow	13.6.2017	Muonio	-	-	-	Negative
278	Bank vole	Female	Ow	13.6.2017	Muonio	-	-	-	Negative
279	Common shrew	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
280	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Negative

281	Bank vole	Male	Ow	13.6.2017	Muonio	-	-	-	Positive
282	Common shrew	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
283	Grey red-backed vole	Male	Ow	13.6.2017	Muonio	-	-	-	Negative
284	Tundra vole	Female	Ow	13.6.2017	Muonio	-	-	-	Negative
285	Bank vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
286	Northern red-backed vole	Male	Ow	14.6.2017	Muonio	-	-	-	Positive
287	Bank vole	Male	Ow	14.6.2017	Muonio	-	-	-	Positive
288	Grey red-backed vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
289	Bank vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
290	Bank vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
291	Grey red-backed vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
292	Grey red-backed vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
293	Bank vole	Male	Ow	14.6.2017	Muonio	-	-	-	Negative
294	Field vole	Male	Ow	17.6.2017	Kilpisjärvi	-	-	-	Negative
295	Common shrew	Male	Ow	17.6.2017	Kilpisjärvi	-	-	-	Negative
296	Common shrew	Male	Ow	17.6.2017	Kilpisjärvi	-	-	-	Negative
297	Common shrew	Male	Ow	17.6.2017	Kilpisjärvi	-	-	-	Negative
298	Northern red-backed vole	Male	Ow	18.6.2017	Kilpisjärvi	-	-	-	Negative
299	Eurasian pygmy shrew	Female	Ow	18.6.2017	Kilpisjärvi	-	-	-	Negative
300	Common shrew	Male	Ow	18.6.2017	Kilpisjärvi	-	-	-	Negative
301	Common shrew	Male	Ow	18.6.2017	Kilpisjärvi	-	-	-	Negative
302	Common shrew	Male	Ow	19.6.2017	Kilpisjärvi	-	-	-	Negative

303	Northern red-backed vole	Male	Ow	19.6.2017	Kilpisjärvi	-	-	-	Negative
304	Eurasian pygmy shrew	Male	Ow	19.6.2017	Kilpisjärvi	-	-	-	Negative
305	Common shrew	Female	Ow	19.6.2017	Kilpisjärvi	-	-	-	Negative
306	Laxmann's shrew	Male	Ow	19.6.2017	Kilpisjärvi	-	-	-	Negative
307	Yellow-necked mouse	Female	Ad	2015	Lammi	-	31,3	105+92	Positive
308	Yellow-necked mouse	Female	Ad	2015	Lammi	-	27,5	98+99	Negative
309	Yellow-necked mouse	Female	Ad	2015	Lammi	-	24,9	96+93	Positive
310	Yellow-necked mouse	Male	Ad	2015	Lammi	-	38,8	112+114	Positive
311	Yellow-necked mouse	Male	Ad	2015	Lammi	-	40,3	109+101	Positive
312	Yellow-necked mouse	Male	Ad	2015	Lammi	-	44,0	107+102	Positive
313	Yellow-necked mouse	Female	Ad	2015	Lammi	-	34,8	105+108	Positive
314	Yellow-necked mouse	Female	Ad	2013-2014	Lammi	-	26,1	88+102	Positive
315	Yellow-necked mouse	Female	Ad	2013-2014	Lammi	-	32,6	112+104	Positive
316	Yellow-necked mouse	Female	Ad	2013-2014	Lammi	-	38,2	105+107	Negative
317	Yellow-necked mouse	Female	Ad	2013-2014	Lammi	-	27,8	102+94	Negative
318	Yellow-necked mouse	Female	Ad	2013-2014	Lammi	-	40,0	103+115	Negative
319	Yellow-necked mouse	Female	Ad	2013-2014	Lammi	-	23,5	93+104	Negative
320	Yellow-necked mouse	Male	Ad	2013-2014	Lammi	-	45,8	121+115	Negative
321	Yellow-necked mouse	Male	Ad	2013-2014	Lammi	-	42,4	111+107	Negative
322	Yellow-necked mouse	Male	Ad	2013-2014	Lammi	-	43,0	113(+88)	Negative
323	Yellow-necked mouse	Male	Ad	2013-2014	Lammi	-	38,6	120+112	Negative
324	Yellow-necked mouse	Male	Ad	19.12.2014	Kerava	-	28,2	96+99	Positive

325	Yellow-necked mouse	Female	Ad	15.3.2015	Kerava	-	34,3	101+97	Positive
326	Yellow-necked mouse	Male	Ad	21.3.2015	Kerava	-	49,3	116+112	Positive
327	Yellow-necked mouse	Female	Ad	21.3.2015	Kerava	-	26,7	91+96	Positive
328	Yellow-necked mouse	Female	Ad	25.3.2015	Kerava	-	27,8	96+102	Positive
329	Yellow-necked mouse	Male	Ad	1.4.2015	Kerava	-	36,2	102+102	Negative
330	Yellow-necked mouse	Male	Ad	7.11.2011	Vantaa	-	27,5	94+111	Negative
331	Yellow-necked mouse	Male	Ad	21.12.2011	Vantaa	-	27,8	99+97	Negative
332	Yellow-necked mouse	Female	Ad	12.10.2012	Vantaa	-	42,5	105+113	Negative
333	Yellow-necked mouse	Female	Ad	15.10.2012	Vantaa	-	30,2	103+109	Negative
334	Yellow-necked mouse	Male	Ad	15.10.2012	Vantaa	-	41,9	100+109	Negative
335	Yellow-necked mouse	Male	Ad	7.11.2012	Vantaa	-	35,4	105+101	Negative
336	Yellow-necked mouse	Female	Ad	8.11.2012	Vantaa	-	25,7	94+103	Negative
337	Yellow-necked mouse	Male	Ad	8.11.2012	Vantaa	-	44,4	101+103	Negative
338	Yellow-necked mouse	Male	Ad	15.11.2012	Vantaa	-	40,6	104+112	Negative
339	Yellow-necked mouse	Female	Ad	6.11.2013	Vantaa	-	25,0	98(+69)	Negative
340	Yellow-necked mouse	Male	Ad	19.11.2013	Vantaa	-	29,2	101+107	Negative
341	Yellow-necked mouse	Male	Ad	2013	Hämeenkoski	-	30,2	104+97	Positive
342	Yellow-necked mouse	Female	Ad	2013	Hämeenkoski	-	39,7	111+105	Positive
343	Yellow-necked mouse	Male	Ad	2013	Hämeenkoski	-	34,6	111+109	Negative
344	Yellow-necked mouse	Male	Ad	2013	Hämeenkoski	-	51,5	115+125	Negative
345	Yellow-necked mouse	Male	Ad	2013	Hämeenkoski	-	44,8	112+112	Positive
346	Yellow-necked mouse	Female	Juv	25.6.2014	Kerava	-	(14,6)	83+84	Negative

347	Yellow-necked mouse	Male	Ad	7.6.2014	Kerava	-	25,2	90+85	Positive
348	Yellow-necked mouse	Female	Ad	4.6.2014	Kerava	-	19,9	93+92	Negative
349	Yellow-necked mouse	Male	Ad	14.5.2014	Kerava	-	30,3	105(+92)	Negative
350	Yellow-necked mouse	Male	Ad	18.5.2014	Kerava	-	31,8	96+100	Positive
351	Yellow-necked mouse	Male	Ad	4.6.2014	Kerava	-	54,7	114(+75)	Positive
352	Yellow-necked mouse	Female	Ad	14.5.2014	Kerava	-	23,0	92+84	Negative
353	Yellow-necked mouse	Male	Juv	24.5.2014	Kerava	-	12,9	69+78	Positive
354	Yellow-necked mouse	Male	Ad	11.3.2014	Kerava	-	25,1	93+84	Positive
355	Yellow-necked mouse	Male	Juv	24.5.2014	Kerava	-	16,3	75+73	Positive
356	Yellow-necked mouse	Male	Ad	20.5.2014	Kerava	-	25,7	95+94	Negative
357	Yellow-necked mouse	Male	Ad	10.5.2014	Kerava	-	25,4	97+93	Negative
358	Yellow-necked mouse	Male	Ad	25.5.2014	Kerava	-	39,5	109+110	Negative
359	Yellow-necked mouse	Male	Ad	16.5.2014	Kerava	-	42,8	107+112	Positive
360	Yellow-necked mouse	Female	Ad	30.3.2014	Kerava	-	39,0	109+116	Negative
361	Yellow-necked mouse	Female	Ad	15.9.2011	Kerava	-	26,2	97+95	Positive
362	Yellow-necked mouse	Female	Juv	21.4.2010	Kerava	-	15,4	83+79	Positive
363	Yellow-necked mouse	Male	Juv	28.4.2010	Kerava	-	15,3	83+80	Negative
364	Yellow-necked mouse	Male	Ad	2010	Kerava	-	14,7	79+89	Negative
365	Yellow-necked mouse	Female	Ad	5.9.2011	Kerava	-	32,1	102+107	Positive
366	Yellow-necked mouse	Female	Ad	29.8.2011	Kerava	-	45,2	104+106	Negative
367	Yellow-necked mouse	Male	Ad	31.8.2011	Kerava	-	35,6	107+100	Negative
368	Yellow-necked mouse	Male	Ad	2011	Kerava	-	38,7	106+111	Negative

369	Yellow-necked mouse	Female	Ad	2011	Kerava	-	33,3	100+99	Negative
370	Water vole	Male	Ad	4.5.2014	Kerava	-	176,3	168+99	Negative
371	Yellow-necked mouse	Female	Ad	2013	Hämeenkoski	-	30,5	104+103	Positive
372	Yellow-necked mouse	Male	-	2013	Hämeenkoski	-	47,9	117+117	Negative

Appendix 2. Animals by the sampling site. Number of sample animals according to the sampling site.

Location	N:o of sample animals												
	Bank vole	Yellow-necked mouse	Tundra vole	Field vole	Common shrew	Grey backed vole	red-backed vole	Northern red-backed vole	Wood lemming	Eurasian pygmy shrew	Laxmann's shrew	Water shrew	Water vole
Kilpisjärvi	-	-	-	1	7	-	2	-	2	1	-	-	
Pallasjärvi	30	-	22	-	1	4	2	-	-	-	-	-	
Muonio	39	-	15	-	15	13	7	-	-	-	-	-	
Kolari	9	-	3	-	-	-	-	-	-	-	-	-	
Pyhätunturi	28	-	4	-	1	-	-	2	-	-	1	-	
Pisavaara	19	-	5	-	2	-	-	2	-	-	-	-	
Muhos	2	-	-	-	-	-	-	-	-	-	-	-	
Sotkamo	1	-	-	-	-	-	-	-	-	-	-	-	
Toholampi	1	-	-	-	-	-	-	-	-	-	-	-	
Koli	1	-	-	1	-	-	-	-	-	-	-	-	
Viitasaari	6	-	-	12	-	-	-	-	-	-	-	-	
Suonenjoki	2	-	-	10	-	-	-	-	-	-	-	-	
Tohmajärvi	1	-	-	1	-	-	-	-	-	-	-	-	
Karvia	3	-	-	-	-	-	-	-	-	-	-	-	
Korpilahti	8	-	-	-	-	-	-	-	-	-	-	-	

Location	N:o of sample animals												
	Bank vole	Yellow-necked mouse	Tundra vole	Field vole	Common shrew	Grey backed vole	red-backed vole	Northern red-backed vole	Wood lemming	Eurasian pygmy shrew	Laxmann's shrew	Water shrew	Water vole
Punkaharju	1	-	-	4	-	-	-	-	-	-	-	-	-
Mikkeli	1	-	-	4	-	-	-	-	-	-	-	-	-
Vammala	3	-	-	-	-	-	-	-	-	-	-	-	-
Lammi	-	17	-	-	-	-	-	-	-	-	-	-	-
Hämeenkoski	-	7	-	-	-	-	-	-	-	-	-	-	-
Luumäki	1	-	-	1	-	-	-	-	-	-	-	-	-
Violahti	2	-	-	3	-	-	-	-	-	-	-	-	-
Kerava	-	30	-	-	-	-	-	-	-	-	-	-	1
Vantaa	-	11	-	-	-	-	-	-	-	-	-	-	-