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## Serological survey of rodent-borne viruses in Finnish field voles

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Cowpox virus, Finland, Hantavirus, Ljungan virus, Lymphocytic choriomeningitis virus, Orthopoxvirus, Rodent, Vole, Zoonotic

### Abstract

In northern Europe, rodent populations display cyclic density fluctuations, which can be correlated with the human incidence of zoonotic diseases they spread. During density peaks, field voles (*Microtus agrestis*) become one of the most abundant rodent species in northern Europe, yet little is known of the viruses they host. We screened 709 field voles, trapped from 14 sites over three years, for antibodies against four rodent-borne, potentially zoonotic viruses or virus groups: hantaviruses, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV) and orthopoxviruses (OPV). Antibodies against all four viruses were detected. However, seroprevalence of hantaviruses, LV and LCMV was low. OPV antibodies (most likely cowpox) were more common but restricted geographically to southeast Finland. Within these sites, antibody prevalence showed delayed density-dependence in spring and direct density-dependence in fall. Higher seroprevalence was found in spring than fall. These results substantially increase knowledge of the presence and distribution of viruses of field voles in Finland, as well as CPXV infection dynamics.

## Introduction

To control zoonotic diseases, the identification of reservoir hosts and study of transmission dynamics in their populations, is essential (Mills & Childs 1998; Luis et al. 2013). Of taxonomic groups, rodents are considered one of the largest sources of zoonotic agents (Luis et al. 2013). In northern Europe, rodent populations display multiannual, high-amplitude cyclic density fluctuations (Hansson & Henttonen 1985; Norrdahl 1995, Korpela et al. 2013), which can be correlated with the human incidence of zoonotic diseases they spread (for example Kallio et al. 2009; Olsson et al. 2009). During density peaks, field voles (*Microtus agrestis*) become one of the most abundant rodent species in northern Europe (Hanski & Henttonen 1996), yet very little is known of the viruses they carry.

The purpose of this study was to evaluate zoonotic and potentially zoonotic viruses circulating in field vole populations in Finland. The best-known rodent-borne zoonotic viruses in Europe include hantaviruses, lymphocytic choriomeningitis virus (LCMV), Ljungan virus (LV) and cowpox virus (CPXV) (Vaehri et al. 2013; Kallio-Kokko et al. 2005; Jääskeläinen et al. 2013; Kinnunen et al. 2011). No human-to-human transmission has been identified for these viruses (except for LCMV infections associated with organ transplantation; Fischer et al. 2006). European hantaviruses and LCMV are naturally transmitted solely from rodents (Vapalahti et al. 2003; Charrel & de Lamballerie 2010). Human CPXV infections have emerged from pet rats and cats (Ninove et al. 2009), with wild rodents as the reservoir. The ultimately important role of rodents makes infection epidemiology in rodent populations especially germane to human risk assessment.

In Europe, several hantavirus species circulate in populations of their rodent and insectivore hosts (Olsson, Leirs & Henttonen 2010, Vaehri et al. 2013). Puumala virus (PUUV) is widely distributed in bank vole (*Myodes glareolus*) populations (Brummer-Korvenkontio, Henttonen & Vaehri 1982; Vapalahti et al. 2003; Olsson, Leirs & Henttonen 2010), and Tula (TULV) and Tatenale (TATV, proposed name) viruses in *Microtus* voles. TULV has been mainly associated with common and sibling voles (*Microtus arvalis* and *Microtus levis*, respectively) (Plyusnin et al. 1994). However, more recent detection of TULV in other *Microtus* species (Scharninghausen et al. 2002, Plyusnina et al. 2008; Schmidt-Chanasit et al. 2010), including field voles regionally separate of other carrier species in Germany (Schmidt-Chanasit et al. 2010), indicates a wider host range. TULV is not currently considered pathogenic to humans, although a suspected case has emerged (Schultze et al. 2002, Klempa et al. 2003). TATV was recently isolated from field voles in the UK (Pounder et al. 2013).

LCMV was thought to be the only arenavirus in Europe and to reside primarily in the house mouse (*Mus musculus*) (Blasdell et al. 2008). However, high seroprevalence in other mice and vole species (including field voles) (Kallio-Kokko et al. 2006; Laakkonen et al. 2006; Blasdell et al. 2008; Tagliapietra et al. 2009), and the identification of an independent genetic lineage in wood mice (Ledesma et al. 2009), has led to the suggestion of spillover and/or the circulation of multiple related and cross-reactive arenaviruses.

LV was first isolated from bank voles in Sweden (Niklasson et al. 1999), and has since been detected in several mouse and vole species (Hauffe et al. 2010; Jääskeläinen et al. 2013), including most recently, field voles in the UK (Salisbury et al. 2013). This parechovirus has attracted research interest due to its alleged, though highly debated, association with severe human conditions (Niklasson et al. 2007; Nilsson et al. 2009). Notably, high seroprevalence to LV or LV-like virus has been detected in humans in Finland (Jääskeläinen et al. 2013).

High CPXV seroprevalence has been found in field and bank voles, and wood mice (Crouch et al. 1995; Chantrey et al. 1999; Kinnunen et al. 2011). Human infection with this orthopoxvirus (OPV) is uncommon, although suggested to be increasing following the cessation of cross-reactive smallpox vaccinations (Vorou, Papavassiliou & Pierrousakos 2008). As all OPV antibodies are cross-reactive, OPVs other than CPXV may induce some of the serological findings. CPXV is, nevertheless, the only known wildlife-borne OPV in Europe (Kinnunen et al. 2011) and is therefore used in this article to describe OPV antibody presence in field voles.

While all of the described viruses have been reported in multiple rodent species, comprehensive surveys are required to understand occurrence patterns and draw inferences regarding the host role in virus maintenance. Here we use widespread sampling of field vole populations in Finland to evaluate the spatial and temporal distribution of antibodies to selected rodent-borne viruses, as well as factors that influence infection dynamics within their populations.

## Methods

### *Vole trapping and abundance*

Field voles were trapped from 14 open grassland fields, each > 1 ha, across central-eastern Finland (Figure 1). Trapping was conducted at each site in spring (late April – May) and fall (late September – October) for three consecutive years to include each phase of a vole cycle (fall 2008 - spring 2011). The fall 2010 trapping occasion at Tohmajärvi was not possible due to unavailability of the site. These sites are included in the long-term national vole monitoring program (see Korpela et al. 2013), and past abundance data are available for most.

For each sampling occasion, 100 standard metal mouse snap-traps were set in clusters of three along a line with an intercluster distance of 10 - 20 m. Traps were baited with a small piece of bread, and left for one night. The following morning captured voles were measured, sexed, aged (overwintered or not overwintered) and frozen at < -20°C.

### *Dissection and serology*

Voles were thawed, and the heart removed and placed into a tube with phosphate-buffered saline (Sironen et al. 2002). Lung and liver samples were also collected from each individual and refrozen for potential PCR analyses. Occasionally voles were damaged during the trapping process or by scavengers, thereby preventing the ascertainment of organ samples. Antibodies reactive to PUUV/hantaviruses, LCMV,

LV and CPXV/OPV were detected from heart extracts using immunofluorescent antibody tests (IFAT), as described previously (Kallio-Kokko et al. 2006, Kinnunen et al. 2011; Jääskeläinen et al. 2013).

### *Statistical analyses*

Only CPXV prevalence was sufficient to permit further enquiry. Variation in CPXV seroprevalence was studied using data from sites where CPXV antibodies were detected on at least one trapping occasion. Throughout the sampling period, only four early spring-borne juvenile voles were captured in spring and no overwintered voles in fall. Juveniles were therefore removed from spring data, and generalized linear models with binomial error distributions and a logit link function used to separately evaluate CPXV seroprevalence in spring and fall. The full models for each season included site, year, current density, density on the previous trapping occasion, weight, sex, and the interaction of weight and sex. Weight and densities were centered by mean. A model set constituting 95% of Akaike weights of all models nested within the full model was then averaged (Grueber et al. 2011) using the MuMIn package (2011) in R software (R Development Core Team 2012). A generalized linear model, including the main effects of site, year and season, was used to compare seroprevalence between spring and fall.

## **Results**

A total of 715 field voles were captured from fall 2008 to spring 2010, of which 709 were tested for antibodies against the four rodent-borne viruses. Ten (1.4 %), 17 (2.4 %) and four (0.6 %) voles were seropositive to hantaviruses, LCMV and LV, respectively (results are summarized in Table 1). RT-PCRs specific for respective viruses were conducted on lung samples from individuals seropositive to hantavirus and LCMV (also some samples from seronegative voles within the same sites) (Klempa et al. 2006; Vieth et al. 2007), and on liver samples from individuals seropositive to LV (Donoso Mantke et al. 2006). All results were negative (data not shown).

One hundred and seventeen voles were seropositive to CPXV. All seropositive individuals were captured from four sites (Figure 1), where seroprevalence on a sampling occasion ranged from 0 to 93 % (Figure 2). The likelihood of an individual field vole to be seropositive in spring was positively associated with vole density in the previous fall (Table 2, Figure 3), and in fall, positively associated with current density. Seroprevalence to CPXV was higher in spring than fall (Estimate =  $1.27 \pm 0.47$ ,  $Z = 2.7$ ,  $P = 0.007$ ).

## **Discussion**

This is the first study to evaluate the occurrence of zoonotic viruses in this highly abundant and widely fluctuating rodent species, the field vole, in northern Europe. Research on viruses of field voles has been neglected, largely due to emphasis on Puumala hantavirus in bank voles (*Myodes glareolus*), the causative agent of

nephropathia epidemica (hemorrhagic fever with renal syndrome) and a common zoonosis in Finland (Vaehri et al. 2013). As such, we present new results on the distribution of zoonotic viruses, and the first description of cowpox virus dynamics in field vole populations of northern Europe.

Hantaviruses in arvicoline rodents (voles and lemmings) are highly cross-reactive (Vaehri, Vapalahti & Plyusnin 2008). In our earlier unpublished smaller surveys, hantavirus antibodies were regularly detected in field voles in Finland (prevalence 3-5%), but no antigen was found. Therefore, spillover of PUUV from sympatric bank voles was considered the source. TULV has been found in field voles in central Europe (Scharninghausen et al. 2002; Schmidt-Chanasit et al. 2010), and TATV virus in England (Pounder et al. 2013). The employed RT-PCR detects all hantaviruses (PUUV, TULV and TATV). Due to the lack of PCR positive field vole samples (hantavirus infections are chronic with presence of RNA and antigen in tissues of reservoir hosts: Easterbrook & Klein 2008), PUUV spillover remains the probable cause of antibodies in the sampled field voles.

Antibodies against LV (or a LV-like virus) were recently identified in Finland for the first time; in both bank voles and humans (Jääskeläinen et al. 2013). While the virus presence is now known, the search for animal reservoirs, and identification of the circulating strain(s), remains. In this study, LV seroprevalence was low and viral RNA non-detectable, which is indicative of spillover from sympatric species - probably bank voles (Hauffe et al. 2010; Jääskeläinen et al. 2013). Our finding contrasts those from the UK where high prevalence was reported in field vole populations through PCR (Salisbury et al. 2013).

The relationship between LCMV and field voles is unclear. While seroprevalence was mostly low, at some sites it was within the range reported for house mouse populations (Childs et al. 1992). In particular, 4 of 45 (8.9 %) voles tested from Viitasaari (G in Figure 1) in fall 2008 were seropositive. Our results are in line with the earlier findings of LCMV-like antibodies in a number of rodent species in Europe without sympatric *Mus* species (Laakkonen et al. 2006, Tagliapietra et al. 2009), and thus support the circulation of multiple LCMV-like strains (see Ledesma et al. 2009).

Contrary to the other viruses, seroprevalence to CPXV was high in certain populations. A clear spatial distribution was identified along the south-east of Finland (Figure 1). Moreover, this geographical area corresponds to OPV antibody findings in other rodent species, cats, dogs, horses, and lynxes (Pelkonen et al 2003, Kinnunen 2011), and importantly, to a severe human cowpox case in a 4-year-old girl from 2000 (Pelkonen et al. 2003). Of note, bank voles were also captured in the two sites with highest field vole CPXV seroprevalence (Figure 1), suggesting potential interspecific transmission. Pelkonen et al. (2003) found high seroprevalence in bank voles in Southern Finland.

At CPXV positive sites, antibody prevalence showed delayed density-dependence in spring and direct density-dependence in fall. Density dependence, along with the finding that CPXV reduces field vole survival (Burthe et al. 2008), indicates that CPXV may contribute to the cyclic regulation of vole populations. While the identified spatial distribution precludes any widespread regulatory effect

in Finland, elsewhere and at local scales the potential contribution of cowpox virus to vole density fluctuations warrants further investigation.

CPXV seroprevalence was higher in spring than fall. The relatively high proportion of seropositive voles in spring is probably diluted towards fall by recruitment of naïve juvenile voles during spring and summer. Reproduction essentially ceases during winter (Myllymäki 1977), while transmission continues to occur. It is worth noting that CPXV is a DNA virus with a short viremia of 2-3 weeks, while hanta- and arenaviruses cause chronic infection. Therefore the transmission dynamics of these viruses differ. For the same reason, PCR identification of CPXV positive individuals is more difficult (Kinnunen et al. 2011).

In summary, serological evidence of hantavirus, LCMV, LV and CPXV was found in field vole populations of Finland. These are the first published results on viral pathogens based on comprehensive field vole sampling. Though seropositivity to hantavirus was shown, no PCR positive field voles were found, supporting the idea of spillover from sympatric species. LV has been associated with bank voles (Hauffe et al. 2010; Jääskeläinen et al. 2013), and the host role of field voles may be minor. The evidence is less clear for LCMV. CPXV antibodies were locally common, and antibody prevalence was most influenced by population density and season. However, the influence of sympatric species, particularly bank voles, deserves further attention.

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## Author disclosure statement

No competing financial interests exist.

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Table 1. Site-based seroprevalence to hantaviruses, lymphocytic choriomeningitis virus and Ljungan virus.

<b>Code</b>	<b>Site</b>	<b>No. sampled</b>	<b>Hantavirus</b>	<b>LCMV</b>	<b>LV</b>
E	Kannus	38	1 (2.6 %)	1 (2.6 %)	0
J	Karvia	23	0	1 (4.3 %)	0
F	Koli	35	0	2 (5.7 %)	0
A	Kuusamo	13	1 (7.7 %)	0	0
M	Luumäki	73	1 (1.4 %)	1 (1.4 %)	0
K	Mikkeli	78	0	0	2 (2.6 %)
C	Muhos	27	0	1 (3.7 %)	0
L	Punkaharju	79	0	1 (1.3 %)	1 (1.3 %)
B	Puolanka	3	0	0	0
D	Sotkamo	57	1 (1.8 %)	1 (1.8 %)	0
H	Suonenjoki	100	1 (1.0 %)	2 (2.0 %)	0
I	Tohmajärvi	62	2 (3.2 %)	2 (3.2 %)	0
G	Viitasaari	110	3 (2.7 %)	5 (4.5 %)	1 (0.9 %)
N	Violahti	11	0	0	0
	<b>Total</b>	<b>709</b>	<b>10 (1.4 %)</b>	<b>17 (2.4 %)</b>	<b>4 (0.6 %)</b>

\*The number of sampled voles and seropositive individuals is summed for the six trapping occasions (five for Tohmajärvi). Lymphocytic choriomeningitis virus is abbreviated to LCMV, and Ljungan virus to LV. The percentage of seropositive voles is shown in brackets alongside the number seropositive. Codes correspond to the map in figure 1.

Table 2. Averaged coefficients of generalized linear model sets with binomial error distributions used to examine the likelihood of a field vole being cowpox antibody positive on a site where cowpox antibodies were detected at least once.

Parameter	Spring			Fall		
	Estimate (s.e)	Z	P	Estimate (s.e)	Z	P
Intercept	-0.70 (0.70)	1.0	0.326	0.51 (0.69)	0.7	0.469
Current abundance	0.12 (0.16)	0.7	0.460	<b>0.19 (0.08)</b>	<b>2.6</b>	<b>0.011</b>
Past abundance	<b>0.16 (0.05)</b>	<b>2.8</b>	<b>0.004</b>	0.25 (0.15)	1.7	0.095
Male sex	0.06 (0.81)	0.1	0.943	0.17 (0.53)	0.3	0.752
Body mass	-0.12 (0.10)	1.2	0.234	0.06 (0.05)	1.3	0.180
Male sex × body mass	-0.24 (0.16)	1.5	0.142	0.02 (0.09)	0.2	0.867
Site (Punkaharju)	1.37 (0.91)	1.5	0.142	1.04 (0.76)	1.4	0.175
Site (Tohmajärvi)	-1.26 (1.24)	1.0	0.320	-3.15 (1.74)	1.8	0.070
Site (Virolahti)	19.51 (1696.28)	0.0	0.991	4.14 (1.64)	2.5	0.012
Year (2010/2009)	-0.29 (1.13)	0.3	0.800	-2.97 (1.52)	1.9	0.052
Year (2011/2011)	-0.19 (1.06)	0.2	0.861	-2.32 (1.71)	1.4	0.177

\* Intercepts represent a female of average body mass at site M (Luumäki) in year 2008 (spring) or 2009 (fall) in a population of average current and past densities. Estimates are given on a logit scale, statistically significant coefficients are written in bold.

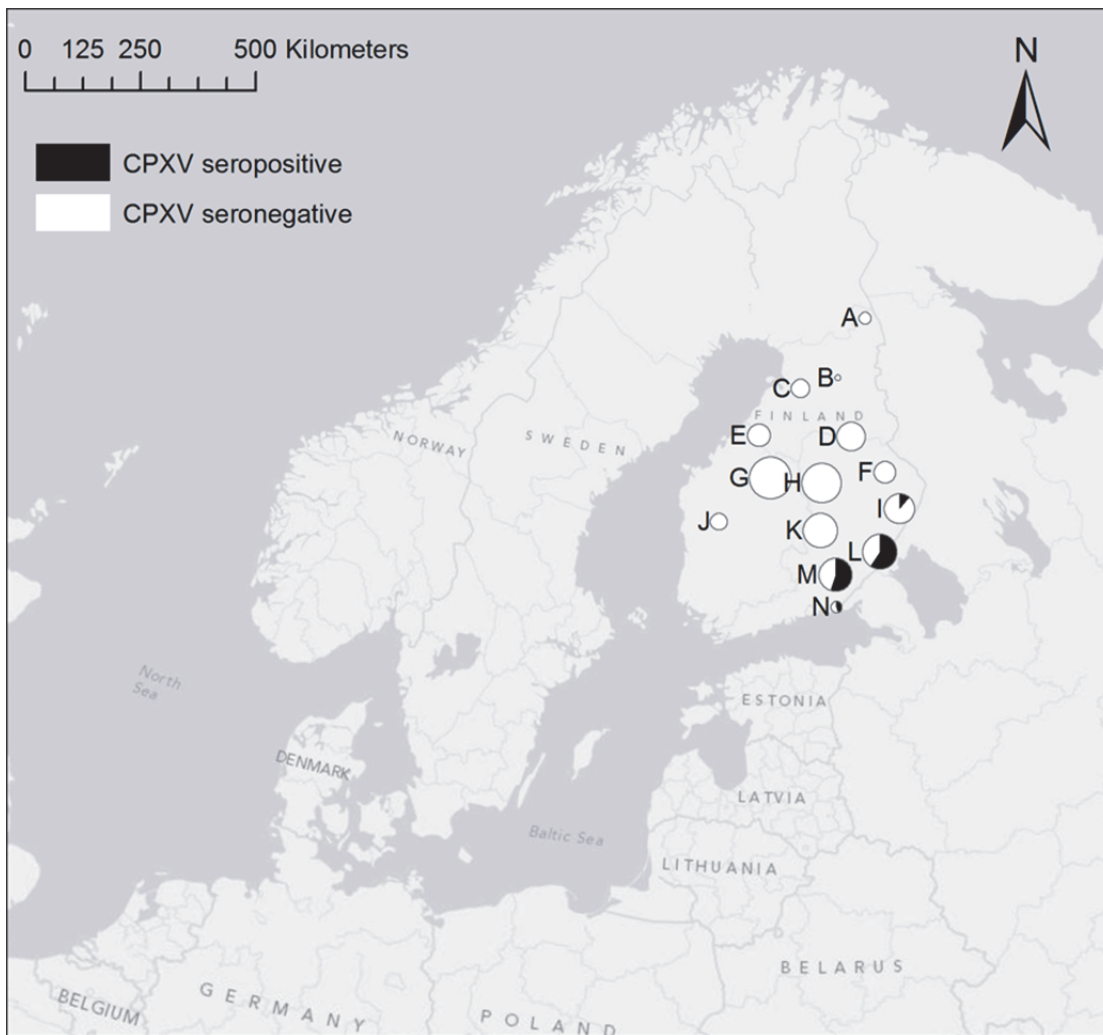


Figure 1. Trapping sites in Finland. Circle size denotes the total number tested (3 [B] to 110 [G] individuals) for antibodies against the four viruses. Black and white colors refer to CPXV seropositive and seronegative individuals, respectively.

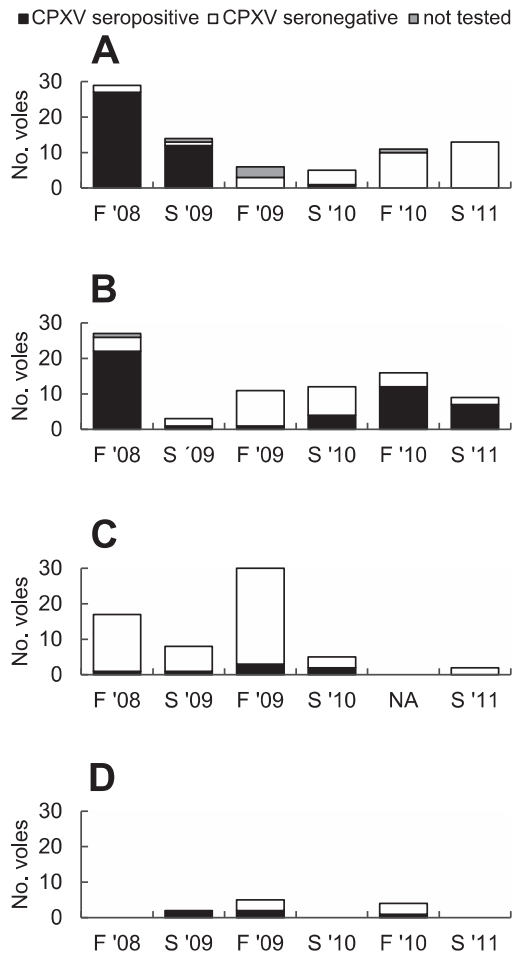


Figure 2. Summary information of CPXV seropositive sites (S = spring, F = fall); (A) Luumäki [M in Figure 1], (B) Punkaharju [L], (C) Tohmajärvi [I] and (D) Virolahti [N]

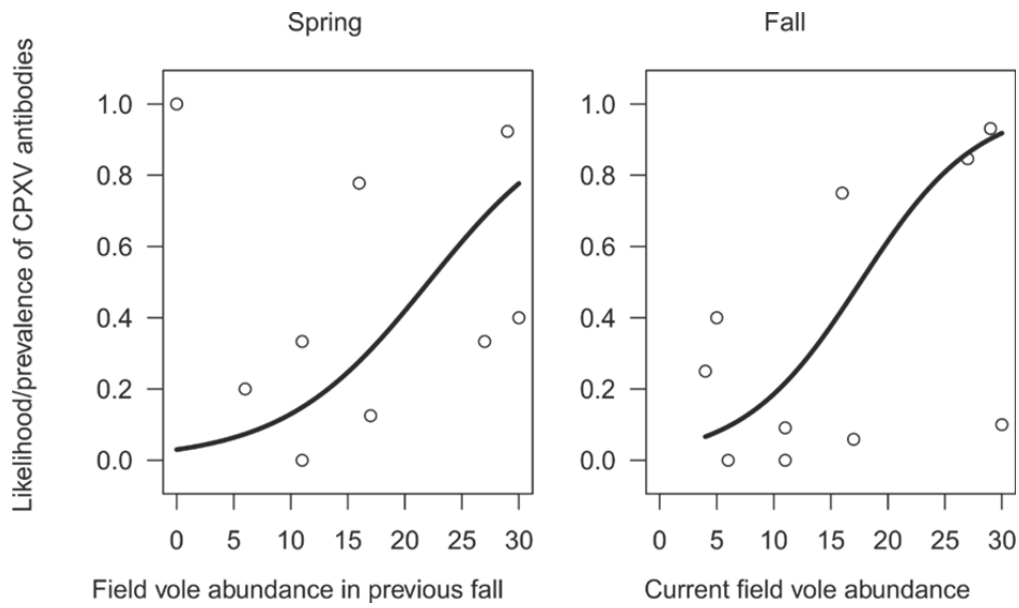


Figure 3. The predicted probability of a field vole being CPXV antibody positive (A) in spring in relation to field vole density the previous fall and (B) in fall in relation to current density based on averaged model coefficients in Table 2. Circles denote observed prevalence.