Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-20-00109: Kathrin Jeske, Duygu Emirhar, Jesús T. García, David González-Barrio, Pedro P. Olea, Francisco Ruiz Fons, Jana Schulz, Anne Mayer-Scholl, Gerald Heckel, and Rainer G. Ulrich. Frequent *Leptospira* spp. detection but absence of Tula orthohantavirus in *Microtus* spp. voles, northwestern Spain.

MATERIAL AND METHODS

Between 2011 and 2014, voles were trapped at 26 sampling sites within the western part of the Duero river basin, northwestern Spain (Fig. 1 and Table S1). These sites were selected as they cover a significant part of the area newly colonized by common voles (*Microtus arvalis*) during the last 50 years (Garcia et al. 2020). The region was traditionally dominated by extensive cultivation of cereal crops (mainly wheat and barley), but the recent introduction of different irrigation crops (mainly alfalfa, corn and some winter cereals) has created a landscape composed of discrete irrigated and non-irrigated areas in which different farming methods are mixed. Since our main objective was to maximize the collection of samples to guarantee a sufficient number of captures at each site, we used different trapping protocols in this study: some sites were sampled by eight 7 x 7 trapping grids (392 traps/site), others by 3-10 trap lines, consisting of 12 traps with 5 m between traps in each line, and some other sites by distributing traps in 15–80 capture points per study site, with 10 traps per point (150–800 traps per study site). In all cases, we used LFATDG Sherman Live Traps (7.62 cm × 8.89 cm × 22.86 cm, H. B. Sherman Traps, Inc., Tallahassee, Florida, USA) without or with bait (carrot or apple slices). We tried to ensure that trap locations encompassed the diversity of habitats at each site (e.g., crop fields, fallows, field margins, boundaries of roads and rural tracks, ditches). To maximize trapping effectiveness, we placed traps on active burrow systems whenever present, on inactive ones or randomly within the fields and margins when no burrows were found at all. Traps were

open for 24 h, or until captures reached a minimum of at least 10 individuals per site, which usually took no more than 48 h. We georeferenced all the capture points in the field with a GPS device. Captures were made in different months throughout the study period, covering all seasons of the year. Individuals were captured and sedated with an intramuscular injection of a solution containing ketamine (10 mg/kg; Imalgene; Boehringer Ingelheim, Barcelona, Spain) and medetomidine (1 mg/kg; Medetor; CP-Pharma Handelsges., Burgdorf, Germany) and thereafter humanely euthanised by cervical dislocation.

Carcasses of trapped animals were transported refrigerated to our labs where they were weighed and age class (juvenile (less than 14.5 g), subadult (14.5 up to 19.5 g), adult (more than 19.5 g; Morris 1972), sex and different biometric measurements were recorded. A detailed necropsy was performed under biosafety 2 containment in cabinets, and tissue samples were collected and preserved frozen at -20 C. For each animal, trapping date and site, and species were recorded. Additionally, the phase of the population cycle, as well as the distance to the nearest water point (in m) were recorded (see Table S2). Weather data were obtained during the 90 d prior to rodent capture (Agencia Estatal de Meteorología 2020, InfoRiego 2020; see Table S2).

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Table S1. Results of *Leptospira* spp. and Tula orthohantavirus detection depending on province, trapping location, year, and species for common voles (*Microtus arvalis*) and Lusitanian pine voles (*Microtus lusitanicus*) in northwestern Spain, 2011-2014. MLST, multi locus sequence typing; n.d., not determined; n.a., not applicable; RT-PCR, reverse-transcription PCR; RT-rtPCR, reverse-transcription real-time PCR.

Province	Site	Year	Species	Number of trapped voles	*Leptospira* spp.					Tula orthohantavirus
					PCR positive/tested	Prevalence in %	95% confidence interval	*secY*-based genomospecies identification	MLST	RT-PCR or RT-rtPCR positive/tested
León	Burón	2012	*M. arvalis*	7	2/7	28.6	3.7 – 71	n.d.	n.a.	0/7
	Polvoredo	2012	*M. lusitanicus*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
	Riaño	2012	*M. lusitanicus*	3	2/3	66.7	9.4 - 99.2	n.d.	n.a.	0/3
	San Emiliano	2012	*M. arvalis*	6	0/6	0	0 - 45.9	n.a.	n.a.	0/0
	Villargusan	2012	*M. arvalis*	4	1/4	25.0	0.6 - 80.60	n.d.	n.a.	0/0
	Subtotal			21	5/21	23.8	8.2 - 47.2	n.d.	n.a.	0/11
Palencia	Arbejal	2012	*M. arvalis*	32	0/32	0	0 - 10.9	n.a.	n.a.	0/18
	Autillo de campos	2012	*M. arvalis*	2	0/2	0	0 - 84.2	n.a.	n.a.	0/2
	Boada de campos	2012	*M. arvalis*	26	0/26	0	0 - 13.2	n.a.	n.a.	0/19
		2013	*M. arvalis*	2	1/2	50	1.3 - 98.7	n.d.	n.a.	0/1
		2014	*M. arvalis*	102	20/100	20	12.7 - 29.2	13x *L. kirschneri*	2x 110	0/65
	Boadilla del Camino	2011	*M. arvalis*	5	2/5	40	5.3 - 85.3	1x *L. kirschneri*	1x 110	0/2
	Castromocho	2012	*M. arvalis*	29	5/29	17.2	5.9 - 35.8	3x *L. kirschneri*	1x 110	0/23
	Cervera de Pisuerga	2012	*M. arvalis*	5	0/5	0	0 - 52.2	n.a.	n.a.	0/3
	Frechilla	2012	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/0
	Frómista	2011	*M. arvalis*	9	0/9	0	0 - 33.6	n.a.	n.a.	0/4
		2012	*M. arvalis*	2	0/2	0	0 - 84.2	n.a.	n.a.	0/1
	Fuentes de Nava	2012	*M. arvalis*	11	0/7	0	0 – 41.0	n.a.	n.a.	0/8
	Paredes de Nava	2012	*M. arvalis*	42	0/42	0	0 - 8.4	n.a.	n.a.	0/21
	Revilla de campos	2012	*M. arvalis*	5	0/5	0	0 - 52.2	n.a.	n.a.	0/5
		2013	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
	Villaluenga de la vega	2012	*M. arvalis*	15	0/15	0	0 - 21.8	n.a.	n.a.	0/14
			M. lusitanicus	1	0/1	0	0 - 97.5	n.a.	n.a.	0/0
	Villanueva de la Torre	2012	*M. arvalis*	23	0/13	0	0 - 24.7	n.a.	n.a.	0/23
	Villarramiel	2014	*M. arvalis*	13	1/13	7.7	0.2 - 36.0	1x *L. kirschneri*	n.d.	0/13
	Villoldo	2012	*M. arvalis*	36	1/34	2.9	0.1 - 15.3	n.d.	n.a.	0/20
	Villorquite del Páramo	2012	*M. arvalis*	28	1/28	3.6	0.1 - 18.4	n.d.	n.a.	0/27
			M. lusitanicus	1	0/1	0	0 - 97.5	n.a.	n.a.	0/0
	Subtotal	391	31/383	8.1	5.6 - 11.3	n.d.	n.d.	0/260	n.a.	0/270

Table S1 (continued)

Province	Town	Year	Species	Number of trapped voles	*Leptospira* spp.					Tula orthohantavirus
					PCR positive/ tested	Prevalence in %	95 confidence interval	*secY*-based genomospecies identification	MLST	RT-PCR and/ or RT-qPCR positive/ tested
Valladolid	Villalar de los Comuneros	2013	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
		2014	*M. arvalis*	38	2/38	5.3	0.64- 17.8	1x *L. kirschneri* 1x *L. borgptersenii*	n.d.	0/24
	Subtotal			39	2/39	5.1	0.6 - 17.3	n.d.	n.a.	0/25
Zamora	Bretó	2012	*M. arvalis*	8	0/6	0	0 - 45.9	n.a.	n.a.	0/2
	Milles de la Polvorosa	2012	*M. arvalis*	19	1/16	6.3	0.2 - 30.2	1x *L. kirschneri*	1x 110	0/6
	San Martin de Valderaduey	2012	*M. arvalis*	19	1/19	5.3	0.1 - 26.0	n.d.	n.a.	0/15
		2014	*M. arvalis*	11	0/11	0	0.0 – 28.5	n.a.	n.a.	0/7
	Villafáfila	2012	*M. arvalis*	16	0/16	0	0 – 20.6	n.a.	n.a.	0/13
		2013	*M. arvalis*	1	0/1	0	0 - 97.5	n.a.	n.a.	0/1
		2014	*M. arvalis*	72	8/72	11.1	4.9 - 20.7	4x *L. kirschneri* 1x *L. borgpetersenii*	2x 110	0/49
	Subtotal			146	10/141	7.1	3.5 – 12.75	n.a.	n.a.	0/93
Total			*M. arvalis*	591	46/580	7.9	5.9 - 10.4	24x *L. kirschneri* 2x *L. borgptersenii*	6x 110	0/384
			M. lusitanicus	6	2/6	33.3	4.3 - 77.7	n.d.	n.a.	0/4
			*M*icrotus spp.	597	48/586	8.2	6.1 - 10.7	24x *L. kirschneri* 2x *L. borgptersenii*	6x 110	0/388

Table S2. Explanatory variables that were analyzed using univariate and generalized linear mixed-effects model (GLMM) to investigate the prevalence of *Leptospira* spp. in individuals of the common vole (*Microtus arvalis*) trapped in northwestern Spain, 2011-2014.

Variable	Description of variables
Weight	in grams, to the nearest 0.1 gram
Age class	juvenile, subadult and adult
Sex	male, female
Distance to the next water body	used as a proxy for soil moisture, logarithm of distance in meter
Maximum temperature	in C, the single maximum temperature registered throughout the 90 days prior to capture (InfoRiego 2020, Agencia Estatal de Meteorología)
Rainfall	in mm, accumulated rainfall during the 90 days prior to capture (InfoRiego 2020, Agencia Estatal de Meteorología)
Relative humidity	in %, mean of the relative humidity registered per day in the 90 days prior to capture (InfoRiego 2020, Agencia Estatal de Meteorología)
Phases of the population cycle in which the common voles were at the capture date	high abundance (peak phase)* versus low abundance** (Luque-Larena et al. 2013, Paz et al., 2013, Jareño 2014, Mougeot et al. 2019, Santamaría et al. 2019). The different population cycle phases (increasing, decreasing, peak and crash or low abundance) were established over long temporal series of abundance data in the study area after Jareño (2014), Mougeot et al. (2019) and Planillo et al. (submitted).
Year	2012, 2013, and 2014

*27.7 vole captures per 100 traps; **0.23 vole captures per 100 traps