

# Increasing Incidence of Zoonotic Visceral Leishmaniasis on Crete, Greece

Maria Antoniou, Ippokratis Messaritakis, Vasiliki Christodoulou, Ioanna Ascoksilaki, Nikos Kanavakis, Andrew J. Sutton, Connor Carson, and Orin Courtenay

To determine whether the incidence of canine leishmaniasis has increased on Crete, Greece, we fitted infection models to serodiagnostic records of 8,848 dog samples for 1990–2006. Models predicted that seroprevalence has increased 2.4% (95% confidence interval 1.61%–3.51%) per year and that incidence has increased 2.2- to 3.8-fold over this 17-year period.

Zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum* is a disease of humans and domestic dogs (the reservoir) transmitted by phlebotomine sandflies. According to the World Health Organization (1), ZVL was first recorded on Crete in 1907, after which it featured prominently in medical literature as a serious public health problem. In Chania, Crete, the annual incidence in the 1930s was 50 cases/30,000 population (2), and 21% of 1,115 dogs were positive for ZVL by the formol-gel serologic test; 30% of those were symptomatic (1).

After World War II, use of DDT against malaria vectors and focal destruction of *Leishmania* spp.-infected dogs is thought to have reduced ZVL on Crete (3). Sandflies were not found in villages systematically sprayed during 1946–1949 compared with unsprayed villages (4). During 1951–1975, only 33 alleged human ZVL cases were recorded on Crete (5), and reports of canine ZVL were scanty. In 1983 a serosurvey of 72 stray dogs in Chania identified only 1 infected dog (1).

By the late 1980s, *Phlebotomus neglectus*, the putative vector of *L. infantum* on Crete (6), was abundant in stone walls inside and outside villages around Heraklion (7). During 1999–2004, *P. neglectus* was found in abundance

in human dwellings and rural locations (hollows in olive trees and near rodent burrows) (8). Since 1991, 38 persons who came to hospitals in Heraklion were confirmed as having cases of ZVL. Today, canine infection is confirmed throughout the island (M. Antoniou, unpub. data), and seroprevalences (30%–40%) are some of the highest reported in Europe. These accounts anecdotally suggest that the incidence of ZVL has increased on Crete during the past decade, which, if so, would be relevant to the public and veterinary health sectors.

## The Study

We statistically examined diagnostic records of 8,848 dogs sampled in the eastern Crete districts of Lasithi and Heraklion during 1990–2006. Data were supplied by the Faculty of Medicine, University of Crete (n = 1,205 dogs from 97 villages tested by using an indirect immunofluorescent antibody test [IFAT], cutoff titer 160, during 1999–2006, accompanied by demographic and geographic records); and by the Ministry of Agriculture Serology Laboratory, Heraklion (n = 7,643 dogs tested by using an IFAT, cutoff titer 200, during 1999–2005, but without accompanying records). Samples were collected by veterinarians in private practice from any dog initially brought to their clinic for any reason, or by government veterinarians for any dog encountered, irrespective of clinical condition, during prearranged visits to villages. Numbers of villages and dogs sampled in any year depended on available resources at the time.

To reduce potential sampling bias, we first tested serologic data from 6 villages (located 9–45 km apart) consistently sampled annually during 1999–2006 (n = 744 dogs). The age-adjusted annual prevalence increased significantly with sample year (odds ratio [OR] 1.18, 95% confidence interval [CI] 1.089–1.271, p < 0.001) and showed no significant differences in slope or intercept (by using robust standard errors) compared with data from 91 less consistently sampled villages with demographic records (n = 461 dogs; slope × village group interaction OR 0.91, 95% CI 0.800–1.023, p not significant; intercepts OR 5.35, 95% CI 0.902–3.688, p not significant).

These combined datasets were then compared with crude prevalence data for 1990–2005 (n = 7,643 dogs) calculated from the ministry records; no difference was detected in prevalence slopes (slope × data source interaction OR 1.06, 95% CI 0.995–1.30, p not significant; Figure). In univariable or multivariable logistic regression that controlled for dog age and clustering on villages (1999–2006, n = 1,205 dogs), no statistical confounding of the probability of a dog being seropositive was attributed to dog use (companion, guard, or hunting dog), sex, crude habitat type, or village altitude (p ≥ 0.215; see online Technical Appendix Table, available from [www.cdc.gov/EID/content/15/6/932-](http://www.cdc.gov/EID/content/15/6/932-)

Author affiliations: University of Crete, Heraklion, Greece (M. Antoniou, I. Messaritakis, V. Christodoulou); Veterinary Laboratory of Heraklion, Heraklion (I. Ascoksilaki); Veterinary Services, Sitia, Greece (N. Kanavakis); and University of Warwick, Coventry, UK (A.J. Sutton, C. Carson, O. Courtenay)

DOI: 10.3201/eid1506.071666

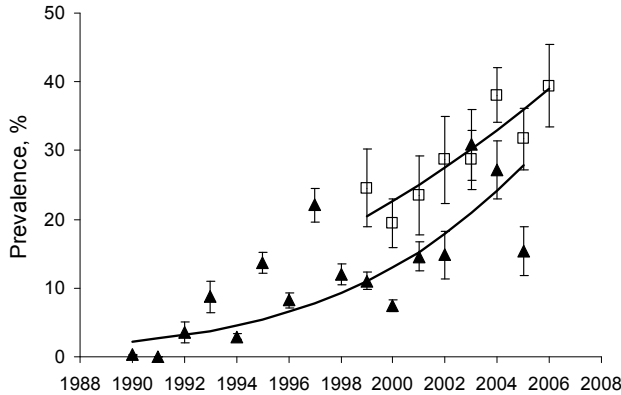


Figure. Annual seroprevalence of zoonotic visceral leishmaniasis in dogs on Crete, Greece, 1990–2006. Shown are logistic fits of age-adjusted prevalences (line and squares) for dogs from 97 villages (indirect immunofluorescent antibody test [IFAT] cutoff titer 160) and crude seroprevalences (line and triangles) calculated from records of the Veterinary Laboratory of Heraklion, Crete, Hellenic Ministry of Rural Development and Food ([www.minagric.gr](http://www.minagric.gr)) (IFAT cutoff titer 200). Binomial standard error bars are shown.

Techapp.pdf). The final fit of seroprevalence against time was significant (OR 1.24, 95% CI 1.221–1.264,  $p < 0.001$ ), and the linearized difference in model prevalence (and binomial confidence limits) over the 17-year study equated to a mean prevalence increase of  $-\ln(1 - 0.321)/16 = 2.4\%$  (95% CI 1.61%–3.51%) per year.

To assess the change in infection incidence, our principal aim, we used 3 standard epidemiologic models (9–11; online Technical Appendix) to calculate infection rates accounting for time, dog age, and potential loss of infection. The first method (model 1) used the cross-sectional age-prevalence data (IFAT cutoff titer 160), in which the proportion of seropositive dogs in each age class is fitted by varying the rates of infection and recovery. A second method (model 2) used these same data to describe the infection rate as it varied with both time and age until reaching the best fit. The third method (model 3) estimated the infection rate from longitudinal data of previously unexposed dogs  $\leq 12$  months of age that were followed up during 1 transmission season.

Results from the 3 models were consistent (Tables 1, 2) and showed a relative increase in the mean infection rate

estimated to be 2.20–3.78-fold higher during 2005–2006 than during 1999–2000. The models differed in approach and age of dogs considered by necessity of the model, number of estimated parameters, or model reduction. Inclusion of a parameter describing loss of infection (Table 1, model 1) did not significantly lower the infection rate estimates as might be expected compared with a single parameter (Table 1, model 2) or longitudinal (Table 2, model 3) model, both of which identified younger ( $\leq 2$  years of age) dogs to be at substantially greater risk for infection ( $p < 0.0001$ ).

**Conclusions**

The potential contribution of any improvements in diagnostic test sensitivity or vigilance to the increasing incidence of ZVL infection is unclear. The difference in cutoff titers between data sources minimally shifted the absolute prevalence values, but not relative prevalence slopes, with time (Figure). Any loss of infection with age (Table 1, model 1) could result from nonmutually exclusive biologic processes including recovery from infection, death, or reduced past exposure (9,11). The latter possibility is unlikely on Crete because of the higher risk identified in young dogs in all biannual periods. Disproportionate numbers of deaths of seropositive dogs is not suggested by a decline in ZVL clinical signs in older dogs in this study (data not shown) or elsewhere (11,12). Loss of detectable *Leishmania*-specific antibody is the more likely explanation because the observed rates of serorecovery are not dissimilar to those (e.g., 0.062/month) estimated by cohort studies elsewhere (12).

Actual infection rates are likely to be higher than those shown here because IFAT sensitivity is  $< 100\%$ . Similarly, absolute prevalences, particularly low values for 1990–1991, should be treated with caution because the official leishmaniasis control program on Crete (1984–1995) began before this period when infection was presumably sufficient on the island to warrant intervention. The intervention comprised elimination of IFAT-seropositive dogs (cutoff titer 400) but did not include insecticide spraying (DDT spraying ceased in 1950; V. Chatzistefanou, pers. comm.).

The continual increase in canine seroprevalence during the latter part of the intervention (Figure) suggests that the culling policy was unsuccessful in reducing transmission;

Table 1. Variation in incidence over time estimated from cross-sectional data for 1,205 dogs with accompanying demographic records, Crete, Greece, 1990–2006\*

Period	Model 1				Model 2		No. dogs
	Incidence/mo	95% CI	Loss of infection/mo	95% CI	Incidence/mo	95% CI	
1999–2000	0.016	0.0107–0.0206	0.045	0.0260–0.0645	0.015	0.0093–0.0213	237
2001–2002	0.029	0.0114–0.0455	0.071	0.0201–0.1211	0.023	0.0166–0.0298	219
2003–2004	0.030	0.0216–0.0381	0.049	0.0320–0.0667	0.029	0.0221–0.0367	401
2005–2006	0.059	0.0233–0.0946	0.106	0.0383–0.17430	0.032	0.0205–0.0477	348

\*Only model 1 is designed to estimate loss of infection (serorecovery). CI, confidence interval. A full description of these models is available in the online Technical Appendix (available from [www.cdc.gov/EID/content/15/6/932-Techapp.pdf](http://www.cdc.gov/EID/content/15/6/932-Techapp.pdf)).

Table 2. Variation in incidence over time estimated from longitudinal data for 179 dogs with accompanying demographic records, Crete, Greece, 1990–2006\*

Period	Model 3		
	No positive/ no. tested	Incidence/ mo	95% CI
1999–2000	6/56	0.014	0.0061–0.0280
2001–2002	7/30	0.033	0.0153–0.0612
2003–2004	12/56	0.030	0.0172–0.0490
2005–2006	10/37	0.039	0.0210–0.0670

\*CI, confidence interval. A full description of this model is available in the online Technical Appendix (available from [www.cdc.gov/EID/content/15/6/932-Techapp.pdf](http://www.cdc.gov/EID/content/15/6/932-Techapp.pdf)).

likely reasons for the low efficacy of dog culling in other leishmaniasis-endemic regions have been described (13). Officially, destruction of seropositive dogs is still required today unless the owner agrees to veterinary treatment of the dog or to keep the dog under sandfly-proof netting. However, there is no current policy on Crete to combat vectors. We conclude that the results of our study are consistent with a postwar reemergence and current increasing incidence of ZVL infection on Crete.

#### Acknowledgments

We thank E. Svirinaki for technical assistance; M. Spyridakis, G. Parasyris, C. Parasyris, and K. Milathianakis for contributing dog samples; R.J. Quinnell for comments on an earlier version of the manuscript; and P. Ready and J.P. Dedet for valuable discussions.

Dr Antoniou is an assistant professor of parasitology at the Medical School of the University of Crete. Her research interests are *Leishmania* and *Toxoplasma* parasites, the epidemiology of zoonoses, and development of strategies for disease control.

#### References

1. Chaniotis B. Leishmaniasis, sandfly fever and phlebotomine sandflies in Greece: an annotated bibliography. Geneva: World Health Organization; 1994. WHO/LEISH/94.34.
2. Adler S, Theodor O, Witenberg G. Investigations on Mediterranean kala azar. XI. A study of leishmaniasis in Canea (Crete). *Proc R Soc Lond B Biol Sci.* 1938;125:491–516. DOI: 10.1098/rspb.1938.0039
3. Hertig M. *Phlebotomus* and residual DDT in Greece and Italy. *Am J Trop Med Hyg.* 1949;29:773–809.
4. Hadjinicolaou J. Present status of *Phlebotomus* in certain areas of Greece. *Bull World Health Organ.* 1958;19:967–79.
5. Leger N, Saratsiotis A, Pesson B, Leger P. Leishmaniasis in Greece. Results of an entomological study during June 1977. *Ann Parasitol Hum Comp.* 1979;54:11–29.
6. Leger N, Gramiccia M, Gradoni L, Madulo-Leblond G, Pesson B, Ferte H, et al. Isolation and typing of *Leishmania infantum* from *Phlebotomus neglectus* on the island of Corfu, Greece. *Trans R Soc Trop Med Hyg.* 1988;82:419–20. DOI: 10.1016/0035-9203(88)90145-9
7. Leger N, Pesson B, Madulo-Leblond G, Ferte H, Tselentis I, Antoniou M. The phlebotomes of Crete [in French]. *Biologia Gallo-Hellenica.* 1993;20:135–43.
8. Ivovic V, Patakakis M, Tselentis Y, Chaniotis B. Faunistic study of sandflies in Greece. *Med Vet Entomol.* 2007;21:121–4. DOI: 10.1111/j.1365-2915.2006.00649.x
9. Williams BG, Dye C. Maximum likelihood for parasitologists. *Parasitol Today.* 1994;10:489–93. DOI: 10.1016/0169-4758(94)90163-5
10. Sutton AJ, Gay NJ, Edmunds WJ, Hope VD, Gill ON, Hickman M. Modelling the force of infection for hepatitis B and hepatitis C in injecting drug users in England and Wales. *BMC Infect Dis.* 2006;6:93. DOI: 10.1186/1471-2334-6-93
11. Courtenay O, MacDonald DW, Lainson R, Shaw JJ, Dye C. Epidemiology of canine leishmaniasis: a comparative serological study of dogs and foxes in Amazon Brazil. *Parasitology.* 1994;109:273–9.
12. Quinnell RJ, Courtenay O, Garcez L, Dye C. The epidemiology of canine leishmaniasis: transmission rates estimated from a cohort study in Amazonian Brazil. *Parasitology.* 1997;115:143–56. DOI: 10.1017/S0031182097001200
13. Courtenay O, Quinnell RJ, Garcez LM, Shaw JJ, Dye C. Infectiousness in a cohort of Brazilian dogs: why culling fails to control visceral leishmaniasis in areas of high transmission. *J Infect Dis.* 2002;186:1314–20. DOI: 10.1086/344312

Address for correspondence: Orin Courtenay, Populations and Disease Research Group, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK; email: [orin.courtenay@warwick.ac.uk](mailto:orin.courtenay@warwick.ac.uk)

# EMERGING INFECTIOUS DISEASES\*

SUBMIT MANUSCRIPTS - [HTTP://MC.MANUSCRIPTCENTRAL.COM/EID/](http://mc.manuscriptcentral.com/EID/)

<http://www.cdc.gov/ncidod/eid/instruct.htm>

# Increasing Incidence of Zoonotic Visceral Leishmaniasis on Crete, Greece

## Technical Appendix

### Analysis of Demographic and Environmental Associations with Canine Prevalence of Zoonotic Visceral Leishmaniasis

The multivariate full logistic model to test potential effects of demographic and environmental conditions on canine prevalence is shown in the Technical Appendix Table.

Technical Appendix Table. Demographic and environmental factors potentially affecting the likelihood of infection in dogs on Crete, Greece, 1990–2006\*

Variable	Odds ratio (95% confidence interval)	z statistic	p value	No.
Age, y†	0.03 (–0.004 to 0.065)	1.74	0.081	1,205
Dog use				
Hunter	Baseline			850
Pet	0.82 (0.579–1.171)	–1.08	0.279	183
Guard	0.79 (0.512–1.223)	–1.06	0.291	172
Sex				
Male	Baseline			485
Female	1.01 (0.756–1.338)	0.04	0.969	720
Vegetation type‡				
A	Baseline	–0.42	0.676	682
B	0.73 (0.165–3.222)	–0.42	0.676	24
C	1.40 (0.822–2.382)	1.24	0.215	159
D	1.13 (0.694–1.829)	0.48	0.630	114
E	0.95 (0.571–1.588)	–0.19	0.850	226
Elevation, m				
>0–50	Baseline			648
>50–100	0.72 (0.371–1.383)	–0.99	0.320	103
>100–500	1.23 (0.752–2.014)	0.83	0.408	325
>500–880	0.98 (0.561–1.724)	–0.06	0.954	129

\*Statistics for the full multivariate logistic regression model included values for baseline categorical levels, controlling for effects of dog age (also shown), and clustering observations by village (where dog lived).

†Noncategorical variable. Statistics represent regression coefficient, not odds ratio.

‡Vegetation types according to the Corine biotype and habitat classification scheme (<http://biodiversity-chm.eea.europa.eu/information/document/F1088156525>). A, urban fabrics; B, nonirrigated arable land; C, olive groves and fruit and berry plantations; D, complex cultivation patterns; E, land largely occupied by agriculture with areas of natural vegetation including natural grassland and *Sclerophyllous* spp.).

The goodness-of-fit of the full model was assessed by using the Pearson statistic ( $\chi^2_{419} = 426.95$ ,  $p > 0.384$ ) and the Hosmer-Lemeshow statistic on a collapsed number of groups ( $n = 10$ )

on the basis of the deciles of the predicted logits ( $\chi^2 = 7.28$ , df 8,  $p > 0.507$ ). Both statistics were in agreement that the number of infected dogs predicted by the model was not significantly different from the observed number. None of the variables tested were significant in univariable analysis.

## Epidemiologic Models Used to Calculate Infection Rates

### Model 1

Cross-sectional age-prevalence (indirect immunofluorescence antibody cutoff titer 160) was fitted to a 2-parameter model by finding the proportion of seropositive dogs  $p(a)$ , at mean age  $a$ , from

$$p(a) = \frac{\lambda}{\lambda + \rho} \left( 1 - e^{-(\lambda + \rho)a} \right)$$

by varying the cumulative rate of infection ( $\lambda$ ) and recovery rate ( $\rho$ ) where  $\lambda/(\lambda + \rho)$  is the asymptotic proportion of positive animals. The model was fitted by maximum likelihood methods according to Williams and Dye (1) (results in Table 1 in the main text, model 1). Age prevalences were calculated for biannual periods by using mean ages of standardized age-class intervals 0–12 >12–24, >24–36, >36–60, and >60 months selected to give approximately equal numbers of dogs per age class. The model assumes that rates of infection and recovery are constant with age, that the population is homogeneously exposed, and that seroconversion immediately follows exposure. Loss of seropositivity (infection) in this model may indicate a loss of detectable antibody titer with age (time), disproportionately high death rates among seropositive dogs, or changes in population exposure. Under the model assumptions, the average age (in months) of patent infection is equivalent to  $1/\lambda$ , and the average duration of seropositivity detected by the test is  $1/\rho$ .

### Model 2

According to methods previously described (2), the cumulative rate of infection in 1998 by age  $a$  is described by the function  $f(a)$

$$f(a) = z + \frac{(u - z)}{1 + (e^{(a-w)})^v}$$

where  $u$ ,  $z$ ,  $w$ , and  $v$  are model constants capturing changes in the cumulative rate of infection with age.  $f(a)$  describes all infections in previous years and is estimated for infection rates for more recent years (1999–2006). No attempt has been made to reduce the parameters describing this function because the priority is to ensure sufficient flexibility and a good fit to the data. The rate of infection from 1999 onwards is then modeled as the product of a function describing its trend over time  $g(t)$  and a function describing its trend with age  $h(a)$

$$\lambda(t, a) = g(t)h(a)$$

To standardize results  $h(0)$  is fixed to equal 1. Both  $g(t)$  and  $h(a)$  are parameterized on piecewise constant functions. The model was fitted to the binomial prevalence data (infected, not infected) by using maximum likelihood. The most parsimonious reduced model (results in Table 1 in the main text, model 2) was achieved by comparing model deviance ( $d_1 = 122.1$ ) with a nested model in which variation in the infection rate was held constant across ages (deviance  $d_2 = 180.3$ ), showing the extra parameter describing dog age (2 categories:  $\leq 2$  and  $> 2$  years) to be highly significant ( $d_2 - d_1 = 58.2$ ,  $df = 1$ ,  $p < 0.0001$ ). Thus, this parameter was retained in the final model.

### Model 3

As previously described (3), the rate of infection  $\lambda$  was estimated from the proportion ( $p$ ) of previously unexposed dogs  $\leq 12$  months of age ( $n = 179$ ) born before the transmission season that seroconverted to positive during a follow-up exposure period of  $t = 8$  months, where  $p = 1 - e^{(-\lambda t)}$  (results in Table 2, model 3). Incidence values were expressed in months.

### References

1. Williams BG, Dye C. Maximum likelihood for parasitologists. *Parasitol Today*. 1994;10:489–93. [PubMed DOI: 10.1016/0169-4758\(94\)90163-5](https://pubmed.ncbi.nlm.nih.gov/1010160169-4758(94)90163-5/)
2. Sutton AJ, Gay NJ, Edmunds WJ, Hope VD, Gill ON, Hickman M. Modelling the force of infection for hepatitis B and hepatitis C in injecting drug users in England and Wales. *BMC Infect Dis*. 2006;6:93. [PubMed DOI: 10.1186/1471-2334-6-93](https://pubmed.ncbi.nlm.nih.gov/101186/1471-2334-6-93/)
3. Courtenay O, MacDonald DW, Lainson R, Shaw JJ, Dye C. Epidemiology of canine leishmaniasis: a comparative serological study of dogs and foxes in Amazon Brazil. *Parasitology*. 1994;109:273–9. [PubMed](https://pubmed.ncbi.nlm.nih.gov/)