

Scotland's Rural College

Rabies outbreak in Greece during 2012-2014: use of Geographical Information System for analysis, risk assessment and control

Giannakopoulos, A; Valiakos, G; Papaspyropoulos, K; Dougas, G; Korou, LM; Tasioudi, KE; Fthenakis, GC; Hutchings, MR; Kaimaras, D; Tsokana, CN; Iliadou, P; Spyrou, V; Tzani, M; Birtsas, P; Kostoglou, P; Sokos, C; Doudounakis, S; Yon, L; Hannant, D; Artois, M; Tsiodras, S; Hadjichristodoulou, C; Billinis, C

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Rabies outbreak in Greece during 2012-2014: use of Geographical-Information-System for analysis, risk assessment and control

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4 1 **Rabies outbreak in Greece during 2012-2014: use of Geographical-Information-**
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58 31 **Short running head:** Investigation of rabies outbreak in Greece using GIS

SUMMARY

Objectives of this work were (i) geographic analysis of the 2012-2014 outbreak of rabies in Greece using GIS and (ii) comparative analysis of animal cases with data of potential human exposure to rabies together with environmental data, in order to provide information for risk assessment, effective monitoring and control. Most animal cases (40/48) involved red foxes, while domestic animals were also diagnosed with rabies. Overall, 80% of the cases were diagnosed in central northern Greece; 75% of the cases were diagnosed in low altitudes (<343.5 m), within a distance of 1 km from human settlements. Median distance from livestock farms was 201.25 m. Most people potentially exposed to rabies (889/1060) were presented with dog bite injuries. Maximum entropy analysis revealed that distance from farms contributed with the highest percent to define environmental niche profiles for rabid foxes. Oral vaccination programs were implemented in 24 administrative units of the country during 2013 and 2014, covering a total surface of approx. 60,000 km². Rabies reoccurrence in Greece emphasizes the need for ongoing surveillance in cross-border areas and in areas with intense human activity.

Key words: fox, geographical-information-system, Greece, rabies

INTRODUCTION

Rabies, an acute progressive disease of the central nervous system caused by neurotrophic viruses of the genus *Lyssavirus*, is almost always fatal in humans without timely medical care (e.g., post-exposure prophylaxis); every year, 55000 people around the world die of the disease. In Europe, rabies is found mainly in wild animals [1]. In Eastern Europe, at least four different variants of the causal virus have been described, which belong to the groups CE (Central Europe), EE (Eastern Europe), NEE (North Eastern Europe) or SF (Serbian fox) [2, 3].

Greece borders with Albania, Former Yugoslav Republic of Macedonia (FYROM), Bulgaria and Turkey. In the last three countries, rabies has been reported in wild and domestic animals and wildlife vaccination campaigns using oral live attenuated vaccines are being implemented most recently: in Bulgaria since 2009, in FYROM since 2011 and in Turkey since 2008 [4-6]. Despite successful oral vaccination campaigns of wildlife

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3 59 targeting to elimination of rabies in large parts of Europe [1], the disease still occurs in the continent and, in
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5 60 2012, 4884 cases were diagnosed in animals.
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8 61 Prior to 1950, rabies was endemic in Greece, although there were no official records regarding frequency of
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10 62 the disease. During the years 1953 and 1954, 995 and 1135, respectively, animal rabies cases were diagnosed, as
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12 63 well as six and four, respectively, rabies-related human deaths. In total, during the period 1951 to 1980, 11,472
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14 64 animal rabies and 53 human rabies cases had been diagnosed. The last case in humans was diagnosed in 1970.
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16 65 Eradication of the disease has been attributed to (i) widespread vaccination of 'ownerless free-roaming' dogs,
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18 66 purposefully implemented, (ii) widespread, free of charge vaccination of domestic dogs and (iii) increased
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20 67 public awareness. Thereafter, only one single case of rabies was diagnosed in a hunting dog in the Evros area,
21
22 68 near the border with Turkey, in 1987 [7, 8] (Fig. 1).
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25 69 After 1987, Greece had been considered as a rabies-free country, although there were outbreaks of the
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27 70 disease in neighbouring countries [8], which might have contributed to re-introduction of the disease into
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29 71 Greece. In November 2011, a new case of rabies was detected in FYROM, 300 m from the Greek borders, in a
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31 72 dead red fox. Then, in October 2012, an outbreak of rabies was recorded within the Greek territory. Since then,
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33 73 many cases of the disease have been diagnosed in animals, mainly in red foxes (*Vulpes vulpes*), in the country.
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35 74 Phylogenetic analysis of virus isolates showed that these belong to EE group [7].
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38 75 Red foxes are adaptable omnivorous carnivores distributed across all continents in the northern hemisphere.
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40 76 They have an important ecological and socio-economic role as a game species, as well as a key wildlife host of
41
42 77 rabies virus [9]. Despite absence of a population estimate in Greece, red foxes are considered to have
43
44 78 widespread distribution and high abundance in the country [10]. The adaptable and opportunistic nature of the
45
46 79 species has enabled these animals to inhabit suburban and urban areas of Greece. Their close proximity with
47
48 80 human populations and the resulting possible close contact of red foxes with ownerless free-roaming dogs or
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50 81 cats, for which there is limited management throughout Greece, support the hypothesis that red foxes were key
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52 82 contributors to rabies risk for public health.
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3 83 Objectives of this work were (i) geographic analysis of the 2012-2014 outbreak of animal rabies in Greece
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5 84 using GIS and (ii) comparative analysis of animal cases with data of potential human exposure to rabies together
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7 85 with environmental data, in order to provide information for risk assessment, effective monitoring and control.
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11 87 **METHODS**

14 88 15 16 89 **Study area**

18 90 Greece borders with Albania to the northwest, FYROM and Bulgaria to the north and Turkey to the northeast.
19
20 91 Total surface of the country is 131990 km². The study area includes the local administration units, where cases
21
22 92 of rabies have been diagnosed in animals, as well as administration units adjacent to those. Total surface of that
23
24 93 area is 65167 km².

25
26 94 Greece is characterized by (i) presence of large mountain areas (approx. 80% of total surface of the country),
27
28 95 (ii) extensive coastline (approx. 15000 km), (iii) many island complexes in the Aegean and the Ionian seas and
29
30 96 (iv) large climatic diversity (29 climatic zones according to the Thorn Waite classification). In general, climatic
31
32 97 conditions in the country are typically Mediterranean: summer is hot and dry and winter is usually mild. Rainfall
33
34 98 occurs mostly in autumn and winter.
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37 38 39 40 100 **Passive surveillance of the disease and cases of rabies in animals**

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42 101 An already enforced national program for passive surveillance of rabies, carried out according to Directive
43
44 102 99/2003/EC, which provided collection and examination of wild and domestic animals found dead or suspected
45
46 103 of having rabies, was enhanced in 16 prefectures along the northern and eastern land borders of Greece, after
47
48 104 November 2011, when a fox rabies case was officially reported by FYROM near the Northern Greek borders.
49
50 105 After laboratory confirmation of a rabid fox in October 2012 in Greece, collection of samples under the passive
51
52 106 surveillance program was extended in 2013 throughout the country, with the objective to achieve a more
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54 107 efficient surveillance of the disease and to obtain more accurate results on circulation of the virus in the country.
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3 108 Notification of suspect rabies cases according to the Greek legislation on rabies control makes mandatory by
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5 109 any person working with animals (farmers, private veterinarians, staff of local authorities responsible for control
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7 110 of ownerless free-roaming dogs and cats etc.) to immediately report any case of suspicion of rabies in animals.
8
9 111 In addition, a public awareness campaign, conducted by the Animal Health Directorate of the Ministry of Rural
10
11 112 Development and Food, targeted the general public, aimed to increase awareness and necessity for notification
12
13 113 of all suspect cases. Further, game wardens and forestry officers were involved in collection of dead wild
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15 114 animals or animals suspect for rabies and their delivery to the competent regional veterinary authorities for
16
17 115 subsequent appropriate submission to the national reference laboratory for animal rabies. The national rabies
18
19 116 control and eradication program in animals, as well as the passive surveillance program for the disease were
20
21 117 coordinated by the Animal Health Directorate of the Ministry of Rural Development and Food (i.e., the official
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23 118 veterinary service of the country).

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27 119 Details of animal rabies cases (October 2012-June 2014) and information regarding organisation and
28
29 120 implementation of the wildlife oral vaccination campaigns have been provided by the Animal Health
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31 121 Directorate. Laboratory diagnosis of rabies in animals is undertaken at the Athens Veterinary Centre of the
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33 122 Ministry of Rural Development and Food, where the Greek national reference laboratory for rabies in animals is
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35 123 located. Geographical coordinates of the locations, where cases of rabies were recorded, were obtained from
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37 124 official game wardens.
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42 126 **Cases of human potential exposure to rabies**

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44 127 Shortly after the identification of the first rabid animal, an algorithm including recommendations for
45
46 128 management of human cases was provided to physicians. Criteria included geographic location (high, medium,
47
48 129 low risk area), category of exposure, animal species involved, availability of the animal for evaluation in a
49
50 130 defined time margin, presentation of the animal during incident and provoked or justified attack.
51
52 131 Simultaneously, a notification system was established for data collection regarding the human cases potentially
53
54 132 exposed to rabies. Details of people who were potentially exposed to rabies in Greece from October 2012 to
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56 133 June 2014, have been provided by the Hellenic Centre for Disease Control & Prevention (HCDCP), a service of
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3 134 the Ministry of Health. Exposure might have arisen from contact with (i) animals (wild, ownerless free roaming
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5 135 or domestic) suspected to be rabies-infected or (ii) rabies vaccine baits.

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7 136 Relevant data that were collected, included type of exposure, classification of exposure, animal species
8
9 137 suspected to be rabies-infected involved (wildlife or domestic animal), location of the incident and type of anti-
10
11 138 rabies treatment. For classification of exposure, the guidelines of the World Health Organisation were used;
12
13 139 class III exposure involved mostly transdermal bites, single or multiple, class II exposure involved mostly minor
14
15 140 scratches or abrasions with no bleeding and class I exposure involved touching or licking on intact skin. In
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17 141 relation to location of the incident, risk assessment of rabies circulation in the geographical area was also taken
18
19 142 into account; in high risk areas (i.e., areas within a 50 km radius from the location where a case of rabies was
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21 143 diagnosed), treatment administration was always recommended; in medium risk areas (i.e., all other areas of
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23 144 administrative units in Northern Greece that were not classified as high risk), treatment administration was
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25 145 recommended only when, according to the description of the exposed person, the animal was considered suspect
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27 146 of rabies (non-provoked attack and/or signs [as described by the person affected] compatible with rabies);
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29 147 finally, in low risk areas (i.e., all areas that were not classified as high or medium risk), treatment administration
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31 148 was recommended only when, according to the description of the exposed person and with additional evaluation
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33 149 by a veterinarian the animal was suspect of rabies (non-provoked attack and/or signs compatible with rabies).
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40 151 **Oral anti-rabies vaccination areas**

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42 152 The area where oral anti-rabies vaccination programmes had been applied, was determined by the Animal
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44 153 Health Directorate, in collaboration with the Directorate of Technical Studies, Structures and Topography, also
45
46 154 a service of the Ministry of Rural Development and Food, in accordance with EU guidelines and
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48 155 recommendations. The selected areas included (i) a 50 km 'buffer zone' around the location of each laboratory
49
50 156 confirmed rabies case within the Greek territory, (ii) each local administrative unit where cases of animal rabies
51
52 157 had been diagnosed and (iii) all adjacent to above administrative units.

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55 158 According to the national Greek oral rabies vaccination (ORV) program for immunization of wildlife, which
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57 159 was issued before initiation of each ORV campaign and was approved by the relevant EU services, all urban and
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3 160 suburban areas had been excluded of target areas of vaccination. Vaccine-baits were aeri-ally distributed by
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5 161 fixed-wing aircrafts. The method did not allow operations in areas with high density inhabitation [11]. An
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7 162 additional parametre contributing to the choice of the vaccination area, was the potential of increased human
8
9 163 exposures to the vaccine used. The vaccine selected for the first oral vaccination campaign in Greece was the
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11 164 SAG2 vaccine (Rabigen® SAG2; Virbac, Carros, France), a live attenuated rabies virus (strain SAG2) vaccine.
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13 165 As the vaccine contained a live attenuated strain of the virus and there was lack of studies regarding safety of
14
15 166 the vaccine to humans after direct contact, exposure to the vaccine required adequate post-exposure rabies post-
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17 167 exposure prophylaxis[12]. In future vaccination campaigns, the manual distribution of vaccines within suburban
18
19 168 areas might be examined.
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25 170 **Environmental parametres**

26
27 171 Climatic variables were derived from the WorldClim version 1.4. software [13] (University of California,
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29 172 Berkeley, USA). Land uses and population density were derived from the Corine Land Cover 2000 database
30
31 173 (European Environment Agency, Copenhagen, Denmark). Boundaries of local administrative units were
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33 174 retrieved from the national open data catalogue (www.geodata.gov.gr; Institute for the Management of
34
35 175 Information Systems, Athens, Greece). Distance from permanent water sources and altitude values were
36
37 176 extracted from a digital elevation model (DEM). GIS layers were created to represent locations of towns and
38
39 177 villages, distance to the nearest village, distance from water presence, road networks, wildlife refuges and
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41 178 livestock farms using the ArcGIS 10.1 GIS software (ESRI, Redlands, USA). All data layers were converted to
42
43 179 a common projection, map extent and resolution, when used for the MaxEnt modelling. All other environmental
44
45 180 variables were featured data type (land uses, distance from farms etc.), which were converted to raster dataset
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47 181 with the same resolution and cell size using the conversion tool from the spatial analyst extension. All statistical
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49 182 analyses were performed with SPSS 19.0 (IBM Analytics, Armonk, USA).
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54 184 **Environmental niche model (ENM)**

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3 185 In the Maxent modelling, the pixels of the study area defined the area, where distribution of the Maxent
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5 186 probability had been defined. Pixels with occurrence records constituted the sample points and the features were
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7 187 environmental parametres (climatic factors, vegetation, topography etc.). Maxent method required presence-
8
9 188 only data, utilised both continuous and categorical data and included efficient deterministic algorithms and
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11 189 mathematical definitions [14]. Red fox rabies cases (n=40) and red fox negative samples of passive surveillance
12
13 190 randomly selected (n=180) were used as occurrence points for the ENM procedure. Maximum entropy
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15 191 modelling, using the MaxEnt software ver. 3.3.3, (Princeton University, Princeton, USA), was employed to
16
17 192 predict the appropriate ecological niches for red fox rabies cases [14]. Goodness of fit of the model predictions
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19 193 was evaluated by the mean area under the curve (AUC) of the receiver operating characteristic curve (ROC).
20
21 194 The Jackknife procedure was used to reduce number of environmental variables to only those that showed a
22
23 195 substantial influence on the model. According to Ceccarelli et al. [15], testing was repeated with the Jackknife
24
25 196 procedure until all remaining variables had a positive effect on the total gain.

26
27 197 Descriptive statistics were extracted with frequencies and medians of the distributions. In most cases, median
28
29 198 values were preferred instead of means, due to a non-normal distributions of the continuous variables [16].
30
31 199 Above data, along with coordinates of the places where rabies cases were detected in animals, were imported
32
33 200 into the GIS environment using Arc GIS 10.1 software (ESRI, Redlands, USA). During spatial analysis, lakes,
34
35 201 dams, rivers and urban areas were excluded (as vaccine-baits were unnecessary and should not have been
36
37 202 dropped near human activity areas or water supply networks respectively). Environmental criteria for
38
39 203 methodology of rabies vaccination (e.g., vegetation, distance from increased population density, water presence)
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41 204 were used to determine the area for implementation of the oral vaccination programme in each of the 24
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43 205 regional units in Greece.
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51 **RESULTS**

52 53 208 54 55 209 **Cases of rabies in animals and passive surveillance data**

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3 210 In total, 48 animal rabies cases were diagnosed in Greece during the period October 2012 to June 2014. Details
4
5 211 of all cases are presented in Table 1. Most cases (40/48, 83%) involved red foxes. Other animal species involved
6
7 212 were dogs (five cases), cattle (two cases) and a cat.

9
10 213 Further, samples examined for passive surveillance purposes during the reference period originated from 956
11
12 214 animals: 174 (18%) in 2012, 551 (58%) in 2013 and 231 (24%) in 2014. Of these, most samples (488/956
13
14 215 [51%], 103 in 2012, 289 in 2013 and 96 in 2014) originated from red foxes. Samples from other wild animal
15
16 216 species examined during passive surveillance originated from bat, beech marten, brown bear, European badger,
17
18 217 European brown hare, European wild boar, golden jackal, least weasel, mink, monkey, red squirrel, various
19
20 218 rodent species, roe deer, wildcat and wolf. Samples also originated from domestic animal species; cat, cattle,
21
22 219 dog, goat, horse, pig and sheep. In all cases, samples examined for passive surveillance purposes from wildlife
23
24 220 or domestic animals as above were found negative for rabies.

25
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27 221 Of the 74 local administrative units in Greece, cases of animal rabies had been diagnosed in eight (11%). Of
28
29 222 all cases, 80% were diagnosed in three administrative units in central northern Greece (Kilkis, Pella,
30
31 223 Thessaloniki). Kilkis was the administrative unit, where most cases had been diagnosed, 18/48 (38%) of all
32
33 224 cases. In total, 30 large towns and 2,893 smaller towns or villages were located within the 50 km buffer zones
34
35 225 around each of the 48 confirmed cases of rabies. Passive surveillance samples were collected in total from 43
36
37 226 (58%) local administrative units throughout Greece, from 22 in 2012, 38 in 2013 and 35 in 2014, from which
38
39 227 samples had been collected. Most samples, 428 of the 956 (45%) originated from administrative units where
40
41 228 cases of rabies had been diagnosed. Details are presented in Table 2 and Fig. 2.

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43
44 229 Of all cases, 75% were diagnosed in locations with an altitude of <343.5 m and within a distance of 1 km
45
46 230 from human settlements. Median (range) altitude of locations where cases occurred, was 228 m (10-1,076)
47
48 231 above sea level (Table 3, Fig. 3). Further, 50% of all cases were detected within a distance of 201 m from a
49
50 232 body of water, 75% of all cases were detected within 350 m of wildlife refuges and 75% of all cases within 351
51
52 233 m from a provincial road and 517 m from a forest road network (Table 3).

53
54
55 234 Samples for passive surveillance were collected from locations with mean altitude of 354 m (range 0-1,492
56
57 235 m). In particular, samples from red foxes were collected from locations with mean altitude of 391 m (0-1,476

236 m), within a distance of 1,612 m (100-12600) from a human settlement; samples from dogs were collected from
237 mean altitude of 248 m (0-1,123).

238 Most cases (52%) of rabies occurred in cultivated land. Cases were also recorded in natural areas (forests,
239 shrub land, agroforestry formations) (29% of all), as well as in human settlements or surrounding areas (19%).
240 Mean (range) distance of locations where a case occurred, from a livestock farm was 201 m (0-2101). Of all
241 cases, 73% occurred in areas with increased livestock density and grazing activities according to the kernel
242 density estimator.

244 **Predictive ENM for red fox rabid cases**

245 The environmental variable with highest gain when used in isolation was annual precipitation, which therefore
246 appeared to have the most useful information by itself. The environmental variable that decreased gain the most
247 when it was omitted, was distance from farms, which therefore appeared to have most information that was not
248 present in other variables. Regularized training gain was 2.047, training AUC was 0.943 and unregularized
249 training gain was 2.459. Maximum entropy analysis in the region of Kilkis, where most cases of the disease
250 were recorded, revealed that relative contribution of environmental variables included cultivated land in
251 combination with livestock activity. Regularised training gain was 1.536, training AUC was 0.921 and
252 unregularized training gain was 2.008.

254 **Exposure of humans to rabies**

255 The HCDCP recorded a total of 1,060 people, who were potentially exposed to rabies from October 2012 to
256 June 2014. Of these, 889 people were presented after dog bite. In most cases, exposure involved **ownerless free-**
257 **roaming** dogs (65%), whilst **ownerless free-roaming** cats were involved less often (8%); also, 11 (1%) people
258 had reportedly come into direct or indirect contact with vaccine baits. Details are in Table 4. Further, 721 people
259 (68% of all) had reported type III exposure, 310 (29%) type II and 19 (2%) type I exposure, whilst no
260 information regarding type of exposure was available in 10 (1%) people.

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3 261 Most cases of anti-rabies treatment were administered to people in areas, where rabies animal cases had also
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5 262 been reported. Anti-rabies treatment administered to potentially exposed people included: vaccine
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7 263 administration (578 people, 54.5%), vaccine and anti-serum administration (462 people, 43.5%) or rabies anti-
8
9 264 serum administration (20 people, 2%). In some cases, anti-serum administration was initiated while vaccine was
10
11 265 not readily available but soon after, the implicated animal was evaluated as free of clinical signs or tested
12
13 266 negative for rabies and prophylactic treatment was then discontinued without the addition of vaccine. In
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15 267 relation to year of administration, anti-rabies treatment was given to 22 (2%) people in 2012, to 574 (54%)
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17 268 people in 2013 and to 464 (44%) people in 2014.
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270 Oral anti-rabies vaccination program

271 In 2013 and 2014, oral anti-rabies vaccination programs were implemented in 24 local administrative units,
272 where animals had been considered to be at increased risk for infection and disease. Total surface of the area,
273 where the oral anti-rabies vaccination was performed, was approx. 60,000 km². Average density of distributed
274 baits, was 22 to 25 baits km⁻², depending on surface factors and year of the campaign.
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276 DISCUSSION

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278 Re-emergence of rabies in animals in Greece

279 Animal rabies has re-emerged in Greece in 2012. The outbreak of the disease has depended upon the situation in
280 neighboring countries, all of which had reported animal rabies cases in the previous to the outbreak years [6, 18,
281 19]. In fact, there is a geographical similarity, in terms of altitude and land use, of the area of Kilgis (a border
282 administrative unit of the country) with the respective unit Selemlis in FYROM, where a rabid red fox was
283 diagnosed in 2011. Results of relevant studies have confirmed phylogenetic similarity of the Greek rabies viral
284 strains with strains of the virus from rabies cases in FYROM, Bulgaria, Bosnia-Herzegovina, Montenegro and
285 Serbia [7]. These findings, allied to the location of the initial cases of the disease, i.e., close to the north
286 boundary of the country, support a hypothesis of southwards expansion of the disease from the Balkan

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3 287 countries. This hypothesis could be supported by the fact that mountains can form a substantial barrier to the
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5 288 spread of foxes into a territory, hence this could be a reason for the southwards spread of the disease, from
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7 289 FYROM into Greece, rather than into other areas of that country. However, identification of rabies at high
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9 290 altitudes, recently described in Bulgaria and Italy [5, 20] highlighted the permeability of natural barriers.
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11 291 Although red foxes, the most important reservoir for maintenance of rabies in wildlife in Central or South
12
13 292 Europe [2, 21-24] are well-suited to a wide range of landscape and can potentially spread rabies in every type of
14
15 293 environment, the majority of rabid red foxes (16/40) were recorded in the area of Kilkis in a mean altitude of
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17 294 210.1 m. Thus, it seems that landscape configuration characterized by gentle slopes influenced animal
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19 295 movement and rabies dissemination. Rabies dissemination has occurred through fox social structures and the
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21 296 wider dispersal of young foxes. This may be further affected by a complex interaction of various factors,
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23 297 including environmental parameters, e.g., landscape configuration (shape of available habitat, terrain roughness,
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25 298 disturbance etc.), which relate to dispersal of wildlife.

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27 299 Sporadically, rabies can spill over from wildlife to domestic animals, which may then attack people. In general,
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29 300 ownerless free-roaming dogs were responsible for the majority of cases of potential virus transmission to
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31 301 humans (65% of cases with treatment administration). In Greece large and unrecorded numbers of ownerless
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33 302 free-roaming dogs live in urban areas. Legal framework endorses reproductive only control schemes along with
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35 303 proper education of owners. Municipal authorities are responsible by law for management of urban free-
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37 304 roaming animals including the post-exposure procedure of search, capture and prompt evaluation of the
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39 305 involved animal. High number of incidents and lack of available resources pose significant limitations in
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41 306 performing the aforementioned tasks. Voluntary animal welfare societies collaborate with local authorities and
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43 307 contribute to reproductive control and animal welfare issues.

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45 308 Most cases of the disease have been recorded in areas of low altitude: agricultural land close to human
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47 309 settlements. The maximum entropy analysis revealed that distance from farms contributed with the highest
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49 310 proportion to define environmental niche profiles for rabid foxes. Notification of suspect rabies cases was,
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51 311 according to the national rabies control and eradication program, mandatory for all parties involved. Forestry
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53 312 officers and game wardens (who, due to the nature of their duties, mostly operate at high altitudes) were
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3 313 involved in collection of dead animals and delivery to regional veterinary officers. Further, cultivated land in
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5 314 combination with habitats offering cover (i.e., forests, shrub land, agroforestry formations) constituted 81% of
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7 315 the buffer zones surrounding the locations of rabies cases. Maximum entropy analysis conducted specifically in
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9 316 the area of Kilkis, where most cases of rabies in foxes were recorded, revealed that cultivated land in
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11 317 combination with livestock activity had the highest contribution in the environmental niche profiles for rabid
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13 318 foxes, a finding consistent with known fox ecology [17].
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320 **Potential for transmission of rabies to people**

20 321 Incidence of rabies in humans in Europe remains extremely small (less than five cases yearly). No human cases
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22 322 have been reported in Greece during the current outbreak in animals, as the result of applying strict preventive
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24 323 and effective control measures of rabies in domesticated animals, as well as in wild animal population.
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27 324 Management of human exposure cases during the outbreak had some difficulties, because of (i) the large
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29 325 numbers of exposed people to whom treatment should have been performed, and (ii) the frequent unavailability
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31 326 of the animal (often ownerless free-roaming dogs or cats) involved in the incident for detailed veterinary
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33 327 examination. These reasons have led to increased number of treatment cases of people during the rabies
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35 328 outbreak. Nevertheless, cases of potential exposure after biting by domestic carnivores (dogs, cats) have also
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37 329 been reported, which calls for implementation of compulsory vaccinations of these animals and of proper
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39 330 education of owners and attack victims for post-exposure procedures (i.e. exchange of contact details, veterinary
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41 331 evaluation).
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44 332 Certain criteria were set from Competent Health Authorities in form of algorithm to guide physicians to
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46 333 assess the need to administer post-exposure prophylactic anti-rabies treatment in potentially exposed humans.
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48 334 Due to the fact that a universal algorithm may not include every single parameter due to complexity reasons,
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50 335 physicians were encouraged to individualize, as possible, each case bearing in mind that irrational
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52 336 administration of treatments may result in unnecessary side effects and even exhaust available consumables.
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54 337 This could explain the fact that post-exposure prophylactic treatments were administered to humans even at
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56 338 areas far away and well-isolated from the locations of clinical cases of rabies.
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5 340 **Oral anti-rabies vaccination program**
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8 341 Oral vaccination of foxes is a proven, effective method for elimination of rabies in wildlife population. In
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10 342 Europe, 24 countries have already implemented oral vaccination of foxes [25]. Many countries (Bosnia &
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12 343 Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, FYROM, Germany, Kosovo, Montenegro, Poland,
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14 344 Romania, Serbia, Slovenia) have reported significant reduction of incidence risk or elimination of rabies after
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16 345 implementation of oral vaccination campaigns [19, 26-36]. Oral vaccination of foxes has been considered
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18 346 successful if the number of rabies cases is reduced by over 90% in a period up to 10 years after initiation,
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20 347 although, in many countries, this target has been achieved during a period of five years [25].
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23 348 Extent of the outbreak indicates the need for a prevalence estimate of the virus circulation in wild animals in
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25 349 northern Greece [8], for correct implementation of preventive measures to control further expansion of the
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27 350 disease. Moreover, increased passive surveillance throughout the country will be necessary to detect any
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29 351 possible new cases of the disease.
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34 353 **Concluding remarks**
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36 354 Application of oral vaccination program of wildlife and mandatory vaccination of domestic animals has
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38 355 contributed to limiting the disease, with no new cases since 2014. Implementation of oral vaccination
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40 356 campaigns of red foxes for a number of subsequent years will assure eradication of the disease among wildlife
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42 357 and will contribute to the rabies free status of the country in the future. Rabies reoccurrence in Greece
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44 358 emphasises the need for ongoing passive and active surveillance for important zoonotic diseases, even when
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46 359 these are believed to have been eradicated. Enhanced passive surveillance in the cross-border areas and in areas
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48 360 with intense human activity will permit early detection of new cases. Multidisciplinary co-operation of medical
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50 361 and veterinary authorities and institutes is necessary for collecting all data needed for taking appropriate disease
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52 362 control and elimination measures.
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57 364 **FINANCIAL SUPPORT**
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3 365 Part of the outcomes of this research that was conducted at the Faculty of Veterinary Medicine of the University
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5 366 of Thessaly, received partial funding from the European Union Seventh Framework Programme (2007-2013)
6
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9 368 costs related to the passive surveillance of the disease (samples analysis) as well as the costs for the
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11 369 implementation of the oral rabies vaccination campaigns were co-financed by the European Commission and the
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13 370 Greek state budget.
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17 18 372 **CONFLICT OF INTEREST**

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53 463 LEGENDS OF FIGURES

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58 465 **Fig. 1.** Animal rabies cases diagnosed in Greece during the period 1951 to 1989.
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3 466 **Fig. 2.** Map of geographical location of animal rabies cases diagnosed in Greece in conjunction with the negative animal
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5 467 samples of the passive surveillance and the rabies post-exposure prophylaxis in humans (Oct. 2012-Jun. 2014)

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7 468 **Fig. 3.** Map of geographical location of animal rabies cases diagnosed in Greece and FYROM, in relation to topographic
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9 469 configuration at landscape level.

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23 483 Table 1. Details, in chronological order, of confirmed cases of rabies in animals in Greece (October 2012-June
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25 484 2014).

Case ID	Animal species involved	Date of occurrence	Administrative unit	Location
1	Red fox	19/10/2012	Kozani	Siatista
2	Dog	19/11/2012	Kastoria	Ieropigi
3	Red fox	07/12/2012	Kilkis	Metaksoxori
4	Red fox	12/12/2012	Kilkis	Kato Potamia
5	Dog	20/12/2012	Pella	Aridaia
6	Red fox	24/12/2012	Pella	Aridaia
7	Red fox	31/12/2012	Kilkis	Metalliko
8	Red fox	31/12/2012	Kilkis	Stavrochori
9	Red fox	31/12/2012	Pella	Loutraki
10	Red fox	14/01/2013	Kilkis	Kilkis

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11	Red fox	18/01/2013	Kilkis	Antigonia
12	Red fox	31/01/2103	Kilkis	Koromilia
13	Red fox	20/02/2013	Kozani	Kteni
14	Cat	28/02/2013	Trikala	Agrielis
15	Red fox	28/02/2013	Kilkis	Koromilia
16	Red fox	11/03/2013	Kilkis	Vafeioxori
17	Red fox	13/03/2013	Kilkis	Xorigi
18	Red fox	22/03/2013	Trikala	Kastraki
19	Red fox	01/04/2013	Kilkis	Paroethio
20	Red fox	11/04/2013	Trikala	Platanos
21	Red fox	22/04/2013	Kilkis	Koromilia
22	Red fox	29/05/2013	Pella	Idraia
23	Red fox	06/06/2013	Thessaloniki	Sindos
24	Dog	18/06/2013	Serres	Emmanouil Pappas
25	Red fox	05/07/2013	Pella	Piperia
26	Red fox	24/07/2013	Kilkis	Lipsidrio
27	Red fox	07/08/2013	Kilkis	Kolchida
28	Red fox	28/08/2013	Pella	Mayrovouni
29	Red fox	04/10/2013	Thessaloniki	Nea Filadelphia
30	Red fox	10/10/2013	Thessaloniki	Drymos
31	Red fox	10/10/2013	Thessaloniki	Proxoma
32	Red fox	16/10/2013	Pella	Orma
33	Red fox	17/10/201	Kilkis	Pirgotos
34	Red fox	24/10/2013	Thessaloniki	Mellisochori
35	Red fox	01/11/2013	Pella	Orma
36	Cattle	20/11/2013	Kilkis	Miriophyto
37	Cattle	22/11/2013	Kilkis	Miriophyto
38	Red fox	19/12/2013	Pella	Neochori Almopias
39	Red fox	09/01/2014	Kilkis	Mayroneri
40	Red fox	15/01/2014	Thessaloniki	Modi
41	Red fox	15/01/2014	Thessaloniki	Euaggelismos Laggada
42	Red fox	15/01/2014	Thessaloniki	Neochorouda
43	Red fox	15/01/2014	Thessaloniki	Vasiloudi
44	Dog	16/01/2014	Thessaloniki	Oraiakastro
45	Dog	26/02/2014	Larissa	Elassona
46	Red fox	17/03/2014	Trikala	Agios Nikolaos
47	Red fox	31/03/2014	Trikala	Nea Pefki

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3 48 Red fox 12/05/2014 Pella Orma
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Table 2. Geographical locations of cases of rabies diagnosed in Greece and of animals from which samples were collected for surveillance purposes (October 2012-June 2014).

Administrative unit	Rabies cases (n)	Samples collected for surveillance purposes (n)
Kilkis	18 (38%)	64 (6.5%)
Thessaloniki	10 (21%)	102 (10.5%)
Pella	10 (21%)	40 (4%)
Trikala	5 (10%)	15 (1.5%)
Kozani	2 (4%)	81 (8.5%)
Kastoria	1 (2%)	43 (4.5%)
Larisa	1 (2%)	8 (1%)
Serres	1 (2%)	75 (8%)
Ioannina	0	65 (7%)
Drama	0	54 (5.5%)
Evros	0	54 (5.5%)
Florina	0	54 (5.5%)
Attiki	0	39 (4%)
Grevena	0	36 (4%)
Pieria	0	25 (2.5%)
Xanthi	0	24 (2.5%)

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3	Kavala	0	23 (2.5%)
4	Halkidiki	0	22 (2.5%)
5	Karditsa	0	20 (2%)
6	Rodopi	0	19 (2%)
7	Thesprotia	0	18 (2%)
8	Imathia	0	8 (1%)
9	Preveza	0	8 (1%)
10	Evia	0	7 (0.5%)
11	Arta	0	6 (0.5%)
12	Viotia	0	6 (0.5%)
13	Lefkada	0	5 (0.5%)
14	Aetoloakarnania	0	4 (0.5%)
15	Ilia	0	4 (0.5%)
16	Lakonia	0	4 (0.5%)
17	Magnisia	0	4 (0.5%)
18	Evritania	0	3 (0.5%)
19	Fokida	0	3 (0.5%)
20	Argolida	0	2
21	Fthiotida	0	2
22	Messinia	0	2
23	Ahaia	0	1
24	Arkadia	0	1
25	Hania	0	1
26	Iraklio	0	1
27	Korinthia	0	1
28	Dodecanisa	0	1
29	Rethymno	0	1
30	Total	48 (100%)	956 (100%)

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Table 3. Environmental parametres in locations, where cases of rabies in animals had been diagnosed in Greece (October 2012-June 2014).

Parametre	Median	Range
Annual rainfall (mm)	467.50	440.00-917.00
Temperature (°C)	14.05	9.50-15.80
Distance from water (m)	660.9	0.00-2898.2
Altitude (m)	227.50	10.00-1076.00
Distance from human settlement (m)	291.42	0.00-3759.00
Distance from wildlife refuge (m)	190.00	0.00-927.07
Distance from forest road (m)	284.61	0.00-1440.00
Distance from provincial road (m)	190.00	0.00-927.00
Distance from livestock farm (m)	201.25	0-2101.00
Livestock index	0.76	0.33-2.95

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16 529 Table 4. Details of reported source of exposure of humans, who received post-exposure prophylactic anti-rabies
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18 530 treatment.

Source of potential exposure	Details	Number of cases	Proportion (of all cases)
Animals		1,048	99%
Dog		889	84%
	ownerless free-roaming	690	65%
	domestic	135	
	farm	38	
	hunting	11	
	not reported	15	
Cat		95	10%
	ownerless free-roaming	81	8%
	domestic	14	
Red fox		26	2%
Other animal species		38	4%
Bat		16	
Stone marten		5	
Cattle		3	
Mouse / rat		3	
Wild boar		3	
Brown bear		2	
Horse		2	
Golden jackal		1	
Pig		1	
Weasel		1	
Wolf		1	
Vaccine bait		11	1%

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3		Direct contact	7	
4		Indirect contact		
5		through dog that consumed a bait	4	
6		Unknown	1	
7		Total	1,060	100%
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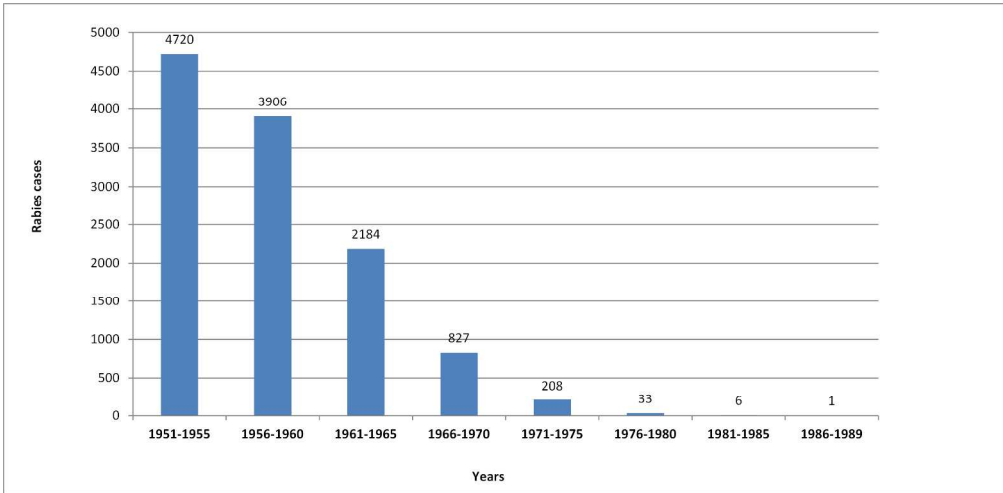
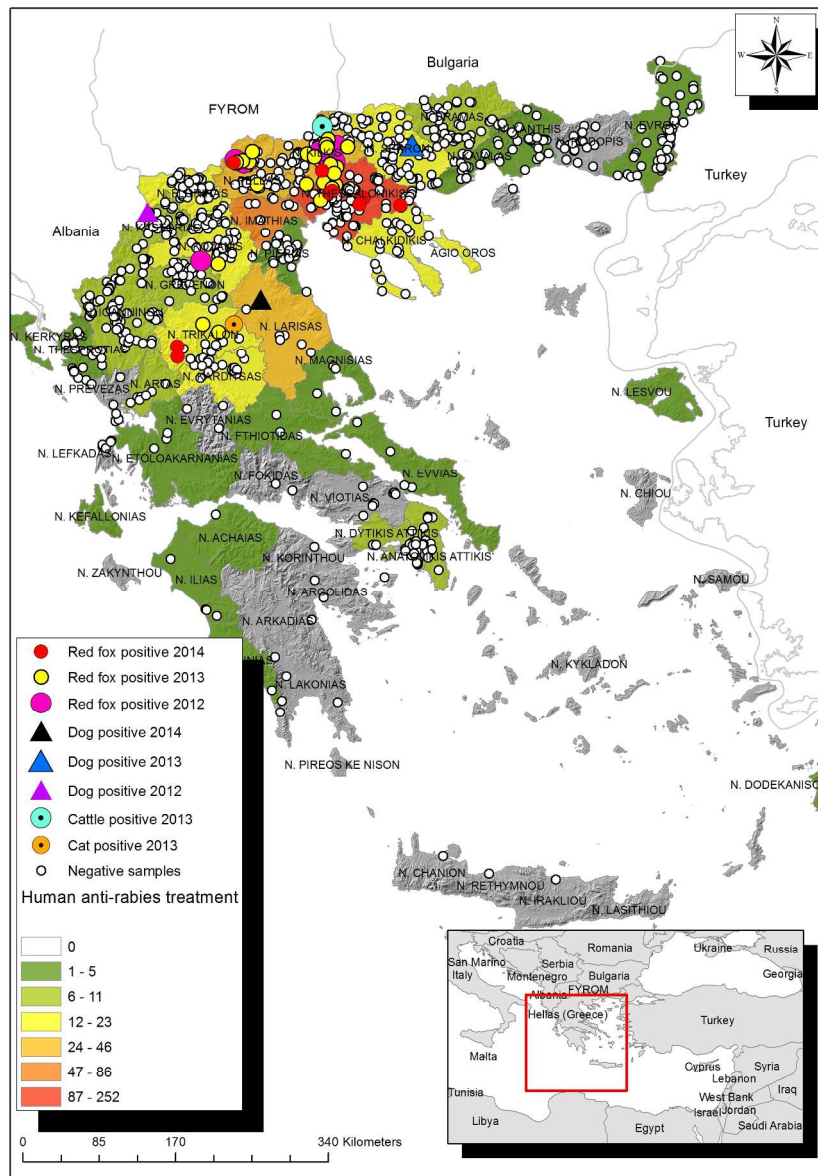


Figure 1: Cases of rabies diagnosed in animals in Greece during the period 1951 to 1989. 247x121mm (300 x 300 DPI)

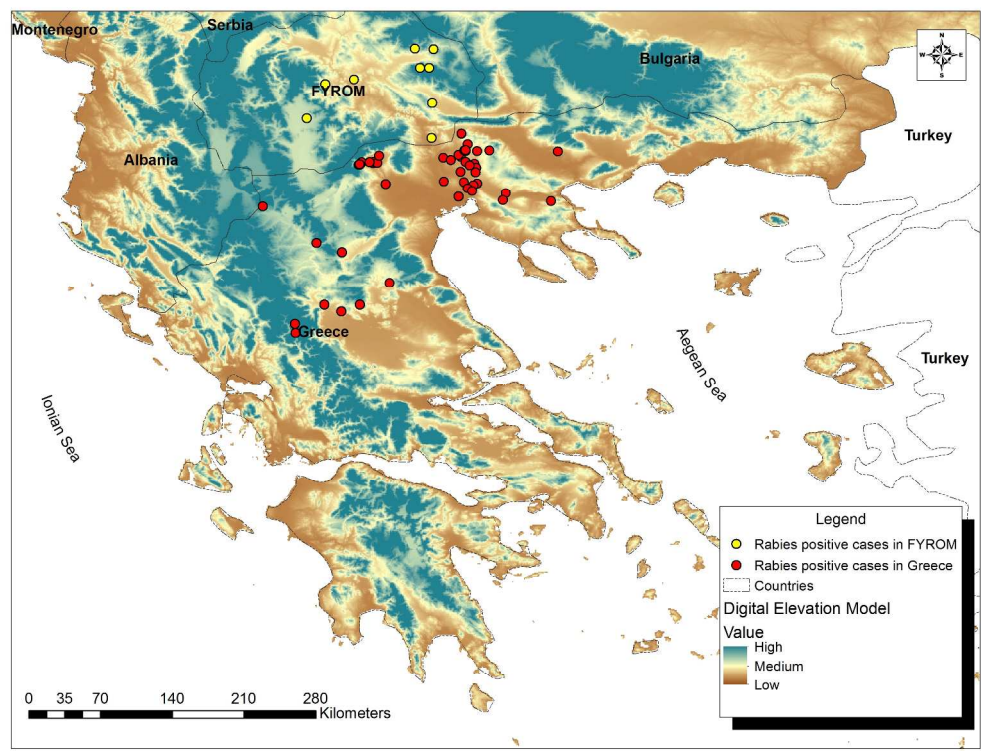
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210x296mm (300 x 300 DPI)

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279x215mm (300 x 300 DPI)

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