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REVIEW

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Temporal and spatial distributions of animal and human rabies cases during 2012 and 2018, in Tunisia

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Abstract

Rabies is one of the most important zoonosis in Tunisia. In the last 6 years, a dramatic increase in the number of cases in animals had raised concern about the transmission dynamics of rabies and the effectiveness of established control measures. For a better understanding of the epidemiological features of rabies in Tunisia, data on animal and human rabies cases and post-exposure prophylaxis (PEP) protocol, for the period from 2012 to 2018, were analysed to describe the spatial and the temporal distributions of the disease and to guide targeted rabies control measures. Thus, the analysed data have shown that 2,642 animal and 25 human rabies cases were recorded between 2012 and 2018; only few case numbers were reported in wildlife. Time-related distribution showed that the incidence of dog rabies increases over the period of study, from 24,8/100,000 dog population in 2012 to 35,2/100,000 dog population in 2018. Dog seems to be the main reservoir of rabies as it accounted for more than 59% of the animal confirmed cases and the source for more than 80% of the human confirmed cases. Geographical distribution of animal rabies cases revealed the presence of clusters in the North and the Center of Tunisia; only a little number of animal cases were reported in the South. In contrast, the overall human rabies incidence was 0.03 per 100,000 population, during the period of the study. We have found that the incidence of human rabies increases by 13.6% from 2012 to 2015 and drops steadily by 23.41% over the second period (2015 to 2018). A high number of PEP was recorded during the 7-year period, with an average of 360 PEP per 100,000 inhabitants per year. Spatial analysis revealed the presence of clusters of PEP in the Northern and Central governorates. The present study pointed out the need to review the control strategy of rabies in Tunisia and conduct further studies on dog population to provide the basis for a new and efficacious policy of interventions and control program for rabies.

KEYWORDS

clusters, dog, epidemiology, hotspot, human, rabies, Tunisia, vaccination

1 | INTRODUCTION

Rabies is a viral disease caused by a lyssavirus virus of the Rhabdoviridae family. Rabies virus (RABV) accounts for the majority of rabies cases, globally. Rabies is an encephalomyelitis, which is always fatal whenever clinical signs appear. In 99% of the cases, domestic dogs are responsible of the rabies virus transmission (Hanlon et al.). However, domestic and wild animals may also be the victims of the disease. Industrialized countries have eliminated canine rabies since the first half of the twentieth century (Macpherson et al., 2013). This success was mostly attributable to the application of mass vaccination of dogs against rabies, leash laws and elimination of stray dogs. In contrast, rabies remains a very serious public health concern, in most of the developing countries, where the urban and suburban rabies is more and dogs (especially stray dogs) appear to be the main reservoir for the disease transmission. Rabies is present in all continents except Antarctica, with more than 95% of human deaths occurring in Asian and African regions (Taylor & Nel, 2015; World Health Organisation, 2019).

In the 1980's, the continuing social and economic burden of the disease in developing countries have led the WHO to initiate various programs aimed at the controlling of canine rabies by mass vaccination (Macpherson et al., 2000). These programs have resulted in the control of enzootic dog rabies in many urban areas, but have rarely been effective in eliminating the disease in the majority of these countries (Chadli, 1988; Mitmoonpitak et al., 1998). Several reasons explain the difficulties in conducting effective canine rabies control and elimination programs. These are attributable to the availability of financial and human resources, the accessibility to dog population and their high turnover rates, and the quality and the immune potency of used vaccines (vaccine brand and proper use after the respect of the cold chain requirements) (Touihri et al., 2011).

In Tunisia, rabies is endemic and cases in humans and animal are being recorded annually. A national control program against rabies was initiated in 1982, and was essentially based on annual vaccination of owned dogs, management of free-rooming dogs and post-exposure prophylaxis (PEP) (Seghaier et al., 1999). From 1982 to 1987, a considerable decrease in the incidence of rabies in dogs (33 cases/year), other species and humans was observed. Unfortunately, efficient outcomes have not been maintained (Seghaier et al., 1999) and only ring vaccination was conducted during the years 1988, 1989 and 1990. Consequently, a rapid increase in the incidence of rabies cases was noted (354 cases in 1991). Since 1992, annual vaccination campaigns were scheduled and fluctuations of the rabies incidence were recorded, with episodes of recrudescence and fall of incidence in the following years. Phylogenetic studies realized between 1992 and 2003 to explain the rabies epidemiological evolution during this period have proven the existence of two rabies virus variants circulating within the country (Amouri et al., 2011). The first variant (NCS), closely related to isolates from Algeria and Morocco, is circulating in the Northeast, Center, East and North of the country. The second variant (NW), affiliated with the Ethiopian and Sudanese strains, is circulating only in the North-western regions (Amouri et al., 2011). From 1992 to 2011, the incidence of rabies has stabilized with an average of 180 cases per year.

After a period of stability, rabies has occurred with higher incidence, in the Northwest and Northeast regions of the country, in 2012. In fact, a significant upsurge of rabies cases was noted in many governorates and the epidemiological situation of rabies became alarming (Kalthoum et al., 2015) since the Tunisian revolution of 2011. A drastic decrease in the number of submitted samples was observed (282 samples analysed in 2011 compared to 423 in 2010 and 415 in 2009) (http://www.rage.tn/Fr/situation-en-tunisie_11_269). This decrease coincided with a period of intense destabilization in Tunisia. Instability and insecurity resulting from the manifestations had indirect consequences on the disease surveillance (Ripani et al., 2017). Veterinarians were unable to collect samples and a notable variation in reporting cases was observed.

The economic situation and the socio-cultural behaviours towards animals in most developing countries, which are different from those in developed ones, may have contributed in the spread of animal rabies reservoirs (Hamta et al., 2019). In fact, such behaviours may explain the abundance of stray dogs, which are barely covered by parenteral vaccination and most of the time incriminated in rabies transmission (Belo et al., 2017). The lack of achieving a rabies-free status has big economic consequences, related to very costly mass vaccination campaigns of dogs that has to be continued along with increased and expensive post-exposure prophylaxis that has to be continued, most likely for better health services and a socio-cultural evolution (Youssef et al., 1998).

Due to the limited result of the parenteral vaccination program, oral vaccination has been recommended to increase the overall vaccination coverage of the entire dog population. Preliminary studies on oral vaccination were conducted in Tunisia, and have shown the efficacy of such approach; but the safety of the dog owners was questioned and not resolved (Youssef et al., 1998).

Studies on the evolution of rabies in space and time in Tunisia are almost inexistent; only few reports have documented the numbers of suspected samples and positives cases, leading to an epidemiological situation of rabies remaining poorly described.

For this, our aim was to answer questions related to the resurgence of animal rabies and provide relevant conclusion for an efficient strategy of the disease control in order to provide useful information to eliminate and eradicate rabies in humans by 2030 as recommended by the World Health Organization (WHO) (2018).

Descriptive studies and geographic mapping of field data (collected during the years 2012–2018), were realized, allowing analyses of the spatial and temporal evolution of animal rabies and the Post-Exposure Prophylaxis program, as well as evaluation of the impact of parenteral vaccination.

2 | MATERIALS AND METHODS

2.1 | Study area

Tunisia is located in the North of the African continent and covers an area of 165,000 km^2. It is subdivided into 24 governorates (administrative units) that are further divided into 264 delegations. Tunisia is bordered by the Mediterranean Sea in the North and the East, Algeria (965 km) in the West and Libya (459 km) in the South (Figure 1). Tunisian population was estimated at 11 million inhabitants in 2014.

2.2 | Animal rabies surveillance and control in Tunisia

Rabies is notifiable disease and case reporting is mandatory in Tunisia. Surveillance of rabies in animals is based on a passive surveillance; all suspected cases of any animal species showing clinical symptoms (bite, hypersalivation, etc.) or behaviour change (paralysis involving the limbs and a similar difficulty in swallowing) have to be reported to the regional veterinary services. Collected samples are then submitted to the laboratory of "Institut Pasteur de Tunis" (IPT). The reference technique used for rabies diagnosis is the Fluorescent Antibody Test (FAT), which detects the presence of rabies antigens in brain tissue of tested animals. Virus isolation and real-time RT-PCR technique using the SYBR-Green fluorescent intercalant were used for rabies diagnosis (Pasteur_Tunis,).

For suspected and vaccinated dogs, they are quarantined; whenever they show rabies clinical signs, they are euthanized and sampled for confirmation in the laboratory. If the result is positive, all person who were in contact with the confirmed case should be treated. An investigation in the outbreak and its circumstances is then conducted by the regional veterinary services.

The rabies control program in Tunisia established in 1982 was based on vaccination of dogs. Vaccination campaigns are conducted at fixed vaccination posts in urban and sub-urban areas and house-to-house campaigns are organized in rural areas. From 1992 to 2012, annual vaccination campaign was undertaken from May to July. Then, since 2012, annual vaccination campaign was rescheduled during March, April and May, as this period coincided with the school holidays and children are most likely to handle dogs and bring them for vaccination. However, from 2014, vaccination campaign was

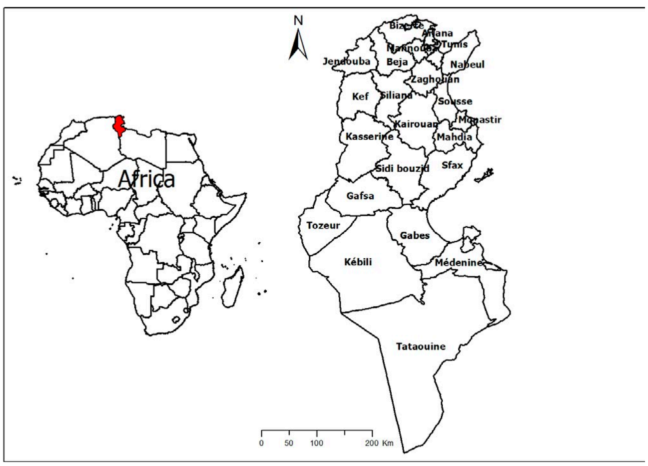


FIGURE 1 Study area

delayed during the first quarter of the year (from January to March) to avoid the confirmed impact of temperature and hot season on the quality of the vaccine (Pasteur_Tunis). Inactivated vaccine is used to immunize dog against rabies. Vaccination is free and obligatory and all dogs over 3 months of age must be vaccinated. In addition to vaccination campaign, other measures are implemented to prevent secondary outbreaks such as ring vaccination (owned dogs in the outbreak must be vaccinated), euthanizing infected animals as defined in the national legal basis and strays dogs. In parallel, PEP has been established in order to prevent the disease in humans (Ripani et al., 2017).

2.3 | Data collection

Data on animal rabies cases (dogs and other animal species) as well as vaccinated dog were collected from the annual reports of the National laboratory of rabies diagnostic at IPT (http://www.pasteur.tn/index.php?option=com_content&view=article&id=310&Itemid=639) and the General Direction of veterinary services (DGSV) (ref) for the period from January 2012 to December 2018. Statistics on "Post Exposure prophylaxis" (PEP) cases were provided by the Basic Health and Care Management department (Direction des Soins et de Santé de Base (DSSB)) of the Ministry of the Public Health.

Collected data were entered into a Microsoft Excel spreadsheet, and information on the animal species and the governorate from which the samples were received, and the date of sample acceptance by the laboratory were logged.

2.4 | Descriptive analysis

Data analyses of Human and dog rabies and PEP cases were used to assess the temporal evolution of rabies in the Tunisian context. The incidence of human rabies and PEP was calculated using the number of positive rabies cases or PEP acts divided by the recorded population during the same year, and adjusted to 100,000 inhabitants; The National Institute of Statistics (INS) providing the size of the human population in each governorate (http://www.ins.tn/). The incidence of dog rabies was determined by dividing the number of confirmed dog rabies cases by the dog population during the same year, adjusted to 100,000 dogs.

The vaccination coverage was calculated according to the following formulae:

$$\text{Vaccination coverage} = \text{Total number of vaccinated dog} / \text{Dog population during the considered year}$$

Seasonal distribution of dog rabies was assessed by aggregating the positive cases into the four seasons of the year.

Mapping and spatial analysis of dog and human rabies cases reported in the different governorates were performed using

TABLE 1 Results of the laboratory diagnosis of animal and human rabies in cattle in Tunisia between 2012 and 2018

Species	2012	2013	2014	2015	2016	2017	2018	Total
Dog	167	198	280	252	230	218	236	1581
Cattle	51	94	88	79	76	77	73	538
Cat	23	17	17	22	14	34	12	139
Horse	22	22	50	25	25	22	37	203
Goat	9	10	7	4	3	10	3	46
Sheep	9	16	32	21	17	11	19	125
Wildlife	1	2	1	2	1	1	0	8
Camel	0	1	1	0	0	0	0	2
Human	3	5	3	6	4	1	3	25

software R version 3.5 (R-Development-Core-Team, 2017). For descriptive comparisons between their frequencies (positive and negative cases, time interval in months and years, governorates and season period), the Kruskal–Wallis and the Chi-square tests were used; *p*-values lower than .05 was considered statistically significant at 95% confidence level. The correlation test was used to assess the relationships between the different parameters. The trend analysis of human rabies incidence was performed using Joint point Version 4.7.0.0 (Statistical Research and Applications Branch, National Cancer Institute) (Dragomirescu et al., 2018).

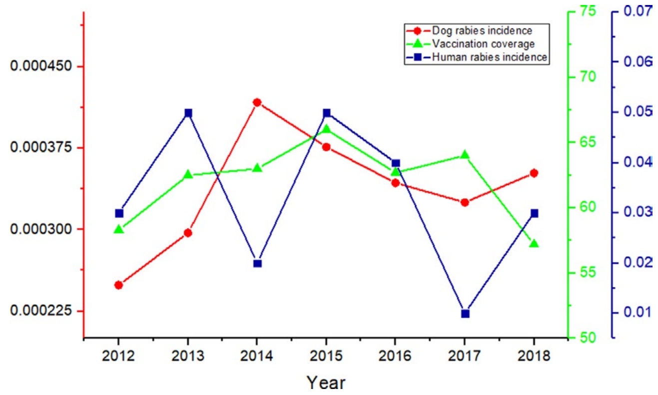


FIGURE 2 Human and dog rabies Incidence and the vaccination coverage in Tunisia between 2012 and 2018

2.5 | Spatial analysis

To investigate the spatial patterns of animal rabies cases along with PEP, the Moran's global index and the spatial autocorrelation by Cluster and Outlier Analysis Anselin were used at the governorate level (Aubry & Piégay, 2001; Le Gallo, 2002). These tools detect statistically significant clusters of high values (hotspots) and low values (cold spots). As a result, governorates were classified into five clusters:

- High-High: Governorates with high number of rabies or PEP cases surrounded by zones of the same category (high number of rabid animals or PEP);
- Low-Low: Governorates with low number of rabies or PEP cases surrounded by zones with low number of rabid animals or PEP;
- Low-High: Governorates with low number of animal rabies or PEP cases surrounded by zones with high number of rabid animals or PEP;
- High-Low: Governorates with high number of animal rabies or PEP cases surrounded by zones with low number of rabid animals or PEP;
- Non-significant: number of rabid animals or PEP cases randomly distributed (Oliveau, 2017).

The spatial analysis was performed using the package "spdep" of R software.

3 | RESULTS

3.1 | Descriptive statistical analysis of animal and human rabies in Tunisia

The results showed that between 2012 and 2018, 2,642 (37,9%) were confirmed positives among 6,966 samples of domestic and wild animals received and analysed by the National laboratory of rabies diagnostic at IPT, with an average of 377 animal cases per year. It appeared that dog infection accounts for 59.8% (1581/2642) of all detected rabies cases (226 dog rabies cases/ year). Cattles seemed to be the most affected species with 20.3% (538/2642) of total rabies cases, followed by horses (7.8% (203/2642)), sheep (5.2% (139/2642)) and goats (1.7% (46/2642)). However, only 0.3% (10/2642) of the confirmed cases were registered in camels and wildlife. During the same period, 25 human rabies cases have been confirmed with an average of three human cases/year (Table 1).

It is shown in the Figure 2 that, even though the vaccination coverage, which is under the recommended value (70%) of WHO (Coleman & Dye, 1996; World Health Organization, 2005), was relatively stable (Kruskal–Wallis chi-squared = 6, *p*-value = .4232), the incidence of dog rabies increased from 24,8 per 100,000 dog population in 2012 to 35,2 per 100,000 dog population in 2018. Besides, the incidence of human rabies was fluctuant throughout the studied period. Statistical differences of human rabies incidence with time were better illustrated in Figure 3; thus, an increase by 13.6% per year was shown from 2012 to 2015, followed by a decrease by 23.41% per year from 2015 to 2018. The results would suggest the absence of linear correlation between the incidences of both dog and human rabies ($r = -.07$).

3.2 | Rabies in dogs

Monthly distribution of dog rabies cases is presented in Figure 4, which shows that dog rabies cases are detected during all months of the study period, with an average of 18 cases registered per month. Although the dog rabies cases peaked during April and May, no statistically significant trend was observed (Kruskal–Wallis chi-squared = 18.823, $p = .06$) (Figure 4).

For rabies seasonality, dog rabies cases were aggregated by seasons and an average of 56 dog rabies cases was recorded per season. The seasonal plot showed that dog rabies cases are similar throughout the seasons and there is no evidence of seasonality effect on rabies cases in dogs, during the 7-year data period (Kruskal–Wallis chi-squared = 6.3583, $p = .09$) (Figure 5).

3.3 | Geographical distribution of animal rabies in Tunisia, during 2012–2018

The results of the spatial distribution of confirmed rabies cases in dog and others species in all governorates are shown in Figure 6. Such distribution differed significantly depending on the years of observation (Kruskal–Wallis chi-squared = 107.34, $p < .0001$). Among the 24 studied governorates, 23 registered animal rabies cases and only the governorate of Kébili (Southwest of Tunisia) did not record any animal rabies cases, during 2012–2018.

Our analysis also revealed that animal cases of rabies are intermittently present in five governorates of the South (Tozeur, Kébili, Gabes, Medenine and Tataouine) and low number (0 to 6 cases). However, the disease appeared to be confined to the North and the Center of the country. Thus, occurring rabies cases in Northern governorates (Tunis, Ariana, Ben Arous, Manouba, Nabeul, Zaghouan, Bizerte, Béja, Siliana, Kef and Jendouba) accounted for 64% (1693/2642) of all animal cases. Overall, the highest number of rabies cases was seen in the governorates of Jendouba and Nabeul, with 83 and 80 animal rabies cases, respectively. In the Center, the number of reported rabid animals represented 33.5% of all rabies cases. The highest number of rabies cases in the Center was shown in the governorates of Kairouan (49 cases) and Mahdia (45 cases), in 2015, and Sidi Bouzid (46 cases), in 2018 (Figure 6).

The hotspot analyses point out that the governorates identified as hotspots (High–High) (red colour, Figure 7) show significant clusters of a high number of occurring rabies cases. In fact, four significant clusters of animal rabies cases were identified during the first 4 years (2012–2015) of the study. These High–High clusters were located either in the Northern governorates (Jendouba, Béja, Kef and Bizerte), for the period from 2012 –2014, or the Central region (Sfax), during 2015; no significant clusters were found after 2015. The governorate of Nabeul have recorded outliers High–Low for rabies cases for two successive years (2015–2016). However, there was a persistent cold spot (Low–Low) in the governorate of Kébili (South of Tunisia) during a period of 5 years (Figure 7).

3.4 | PEP in Tunisia during 2012–2018

There are 306,418 PEP acts (average 406 per 100,000 inhabitants per year), reported during 2012 and 2018. Overall, the incidence of PEP showed a steady trend over time (Kruskal–Wallis chi-squared = 6, $p = .42$) with few fluctuations. The lowest incidence of PEP (363 cases

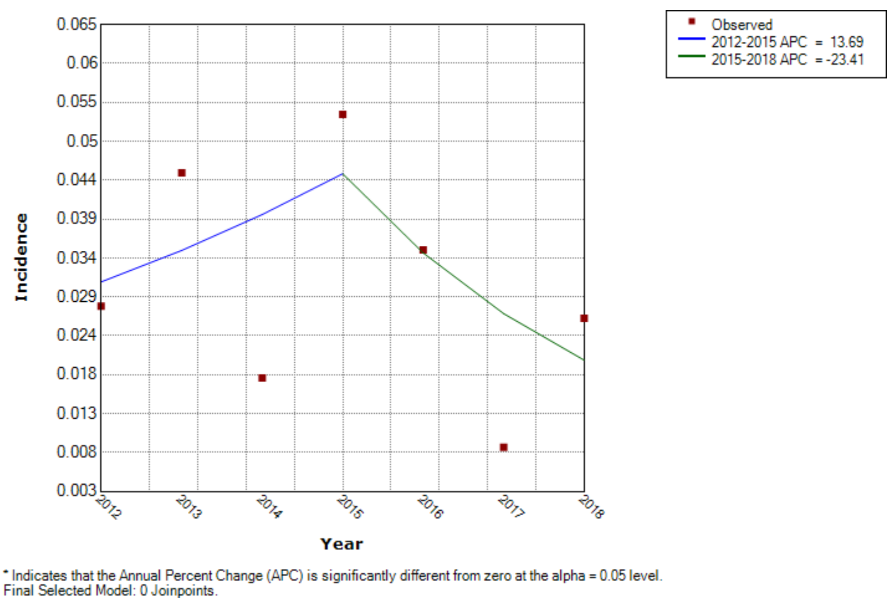


FIGURE 3 Join point analysis of human rabies incidence rates in Tunisia between 2012 and 2018

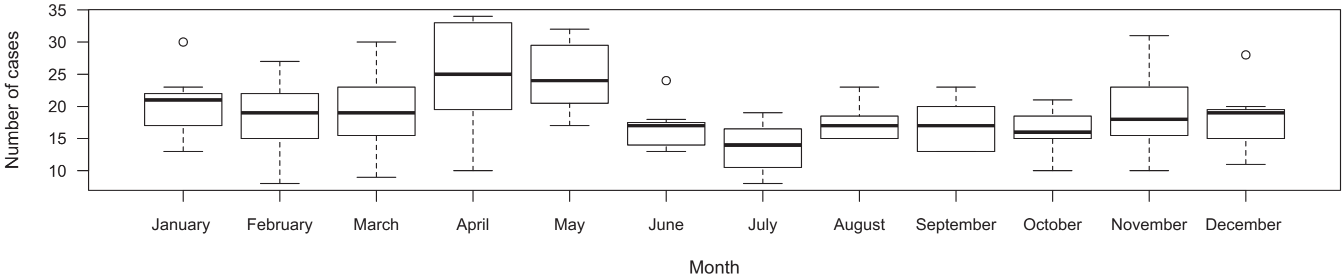
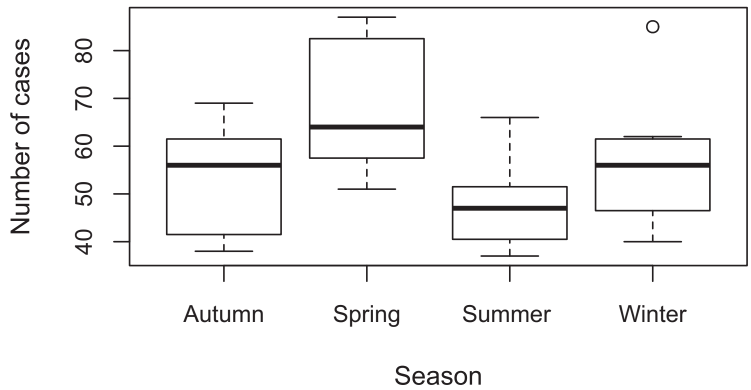


FIGURE 4 Monthly distribution of dog rabies cases in Tunisia, 2012 and 2018

FIGURE 5 Seasonal distribution of dog rabies cases in Tunisia, 2012–2018



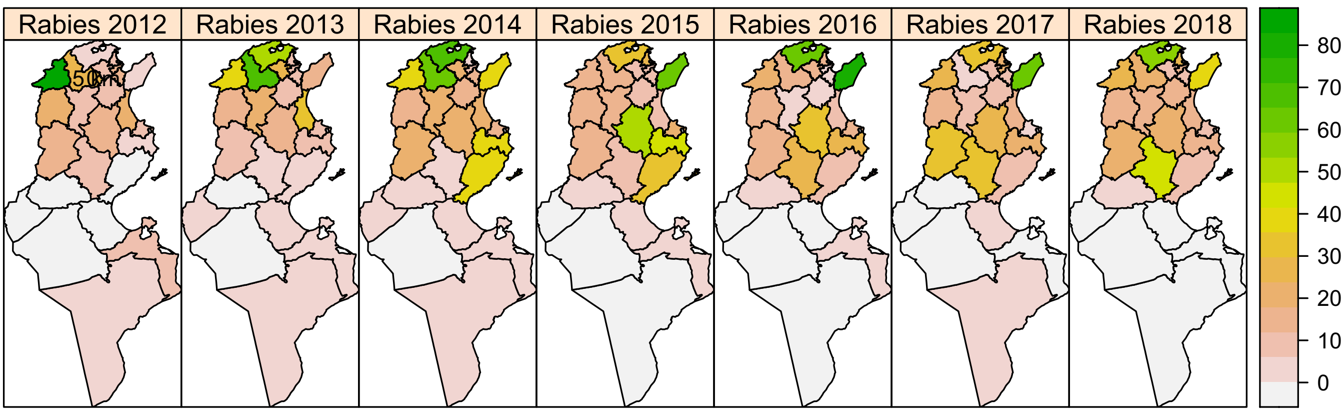


FIGURE 6 Geographical distribution of animal rabies cases in Tunisia, 2012–2018

per 100,000 inhabitants) was observed in 2012 and the highest one (438 cases per 100,000 inhabitants) reported in 2015. Geographical mapping revealed that such incidences are revealed mainly prevalent in the governorates of the Northwest and the Center. For this, Governorate of Zaghouan (North) showed the highest incidence (2,113 PEP per 100,000 inhabitants) in 2015, followed by the governorates of Jendouba, with 1,084 PEP per 100,000 inhabitants recorded in 2012, and Siliana (984 PEP per 100,000 inhabitants) and Kef (868 PEP per 100,000 inhabitants) during the study period. Whereas, the lowest incidence of PEP per 100,000 inhabitants was reported in certain governorates of the North (Tunis, Manouba and Ben Arous) and South (Kébili, Tataouine and Gabes), where the incidences of PEP were less than 120 PEP per 100,000 inhabitants (Figure 8).

The hotspot analyses revealed the presence of six clusters, of which four identified in the governorates of Jendouba, Kef, Siliana and Kairouan. With the exception of 2017, high–high clusters were detected in the North (Jendouba, Siliana and Kef) during the period 2012–2018 and the Center (Kairouan) in 2015. Persistent clusters of PEP were also reported in Jendouba and Kef. However, the Southern governorates (Kébili, Gabes, Tataouine and Medenine) remained as PEP cold spots, during 2012, 2013, 2014, 2016, 2017 and 2018 (Figure 9).

4 | DISCUSSION

The aim of the present study was to evaluate the temporal and the spatial trends of animal and human rabies cases as well as PEP acts, during the period 2012 and 2018 in Tunisia. In fact, our country has experienced a serious re-emergence of animal rabies during the

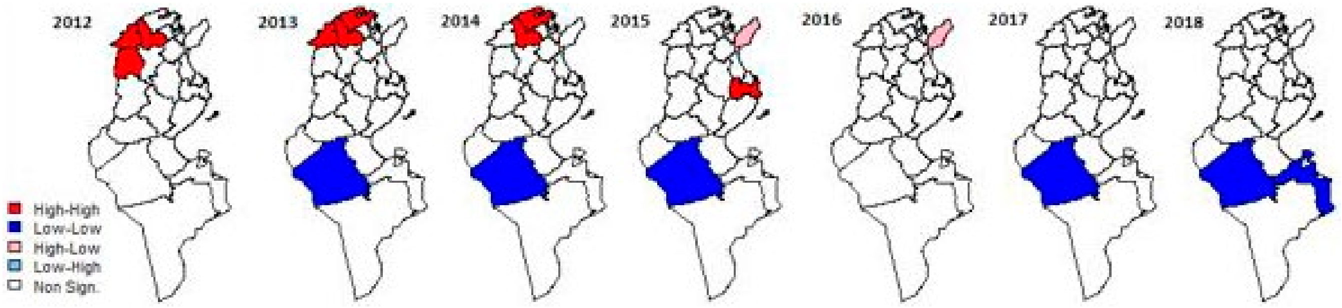


FIGURE 7 Geographical location of clusters of rabies cases in Tunisia, 2012–2018

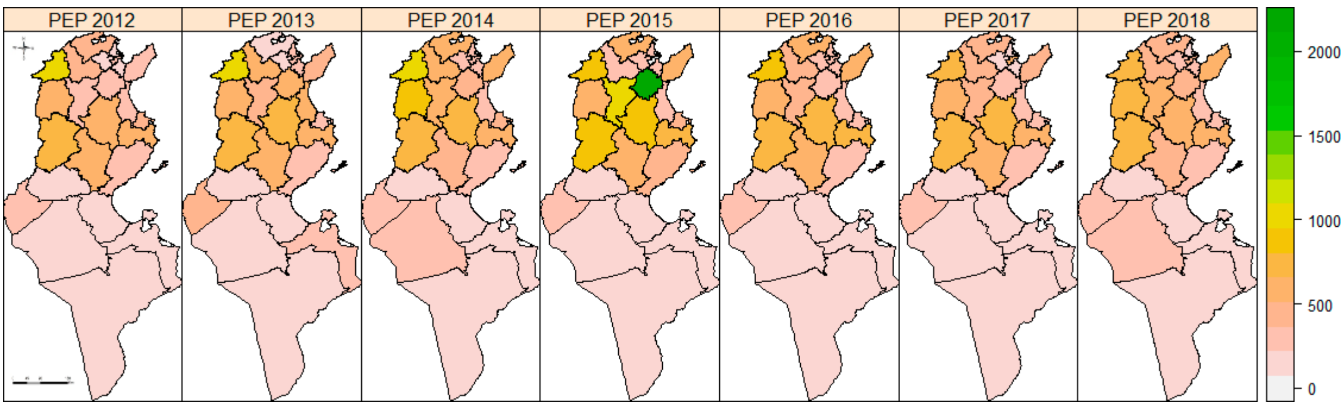


FIGURE 8 Geographical distribution of PEP in Tunisia, 2012–2018

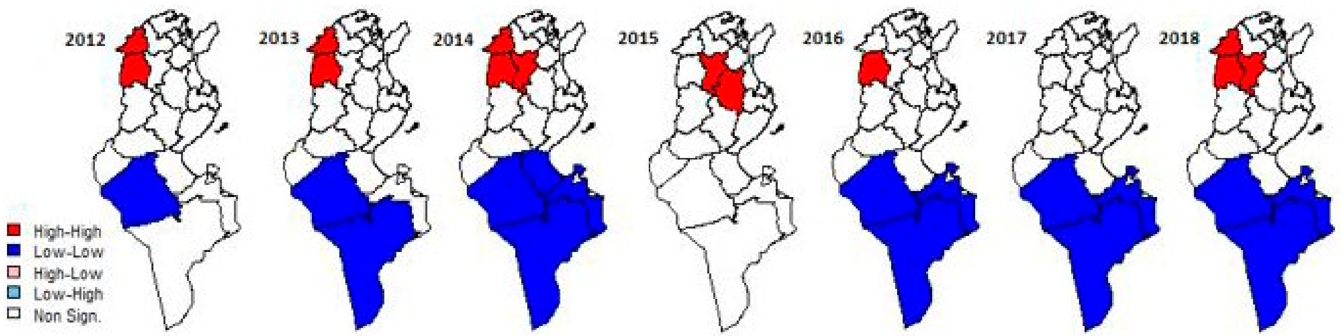


FIGURE 9 Geographical location of clusters of PEP in Tunisia, 2012–2018

last 7 years and rabies situation has changed markedly since 2012. Overall, a high number of animal rabies cases of animal rabies was reported, with an average of 377 confirmed cases per year. When comparing it with the number reported before 2012 (average 148 animal rabies cases per year), we notice that there was a significant increase in animal rabies cases; positive cases have then doubled during that period. This situation is probably related to the indirect consequences of the instability and insecurity after the revolution on the disease surveillance and to the substantial decrease in the number of vaccinated dogs from 421,531, in 2010, to 382,318 vaccinated dogs, in 2011 (Kalthoum et al., 2015). Consequently, a reduction by 9.3% in the number of vaccinated dogs was sufficient to induce a significant increase in the number of animal rabies cases as well as its incidence. Besides, the increase in the number of cases could be attributed to the improvement of the animal rabies monitoring in Tunisia. On the other hand, the number of samples submitted to the rabies diagnostic laboratory has doubled, going from an average of 452 samples/year, before 2012, to an average of 995 samples/year after 2012. It was demonstrated in a study on bovine rabies in Brazil that the increase by one unit in the number of samples submitted raises incidence by 0.57 (Rodenbusch et al., 2016).

As described, animal rabies is enzootic in Tunisia (Ripani et al., 2017). Such epidemiological situation of the disease is comparable with that of many other countries in North Africa (Morocco, Algeria, Libya, etc.) and the Middle East where the disease is endemic, too; both urban and sylvatic rabies cycles being also highlighted. Indeed, a high number of animal cases was recorded in these countries. In Algeria, 950 animal rabies cases were declared annually by the veterinary services, whereas an average of 386 cases was reported per year, in Morocco (McDowell & Rafati, 2014). In the

Middle East, rabies is endemic in Palestine, Iraq, Yemen and Omen (Bannazadeh Baghi et al., 2018).

As expected, dogs were found to be the most frequently reported rabid animals in our study, with an average of 226 cases per year, as 59.8% of confirmed cases were recorded in dogs and 40.2% in others species (bovine, ovine, caprine, equine, etc.). It is obvious that domestic dog plays the role of both a reservoir and a vector of the disease in Tunisia. This is quite different from some other countries (Europe, Mexico and North America) where wildlife animals play an important role in maintaining the viral infection, and low incidences (0.1%-5%) were reported in dogs (Wandeler et al., 1988). However, our observation is similar to that found in Algeria, Zambia and Ethiopia (Aleme, 2015; Kardjadj, 2016; Munang'andu et al., 2010) but not in Morocco, where cattle were the most infected species (Amraoui, 2016; Bakkali, 1985). Compatible with other studies, the analysis of livestock rabies data revealed that cattle were the most affected species (Baccar, 2018; Saadi).

It appears that rabies is being rarely detected in wildlife in Tunisia. This should be related to the fact that rabies cases in wildlife are more likely underreported due to the inaccessibility to such population. Currently, there is no national or local active surveillance program of rabies in wildlife and we lack relevant information on rabies cases in wildlife.

Previous phylogenetic studies in Tunisia have shown that the strains of rabies virus isolated from herbivores and wildlife animals are associated with domestic dogs' strains (Frontiers; FAO; Amouri et al., 2011). Further investigations are needed to understand the possible roles of wildlife in the transmission and persistence of rabies.

The incidence of dog rabies ranged between 24.8 and 35,2 per 100,000 dogs and its increase has been highlighted during the period of the study. The upsurge of rabies in dogs may be due to the increase in household landfills that contributed to the proliferation of stray dogs and the increase in the rabies risk through the dog's fighting and contact (Aleme, 2015; Al-Shamahy et al., 2013). Raymond et al. (2015) reported that stray dogs are often attracted by decomposed cooking waste or outdated food (Raymond et al., 2015). Besides, stray dog proliferation may have influenced the vaccination coverage and limit the result of the control program. Considering the lack of knowledge on stray dog population, studies using capture mark and recapture methods are needed as recommended by WHO to provide consistent estimates of such population and transmission dynamics of rabies.

Nevertheless, we consider that the incidence of dog rabies, in Tunisia, is low as compared with other countries where high annual incidences were observed in dogs such as Kenya (860/100,000 dogs), Ethiopia (412.83/100,000 dogs) and Chad (140/100,000 dogs) (Jemberu et al., 2013).

Despite the high incidence of dog rabies, a low incidence of human rabies is being recorded (0.03 cases per 100,000 inhabitants) in Tunisia. This result is different from that reported in other countries where rabies is endemic. Indeed, human rabies incidence is estimated at 0.06–0.08 cases per 100,000 inhabitants and 0.3 cases per 100,000 inhabitants, in Côte d'Ivoire and Chongqing (China), respectively (Qi et al., 2018; Tiembré et al., 2018). The low incidence of human rabies in Tunisia is one of the consequences of the implementation of a program for rabies control and the PEP actions. Thus, 306,418 PEP (an average of 360 per 100,000 inhabitants) were reported, between 2012 and 2018, in Tunisia. According to the study of Gautret et al. (2011), it was the highest and very close to that observed in Vietnam (375 PEP per 100,000 inhabitants) (Tran et al., 2019). The high number of PEP in Tunisia highlighted the efficiency of the awareness plan, the health education effort and the opportunity to reach rapidly the medical care after being bitten. A recent economic study on rabies has estimated at 4,911,382.78 $ for PEP acts from the total costs of rabies control and prevention which represents 85% (Aicha, 2019).

Our analysis showed no significant variation in the distribution of rabies cases according to months and seasons. This finding is in agreement with the result of a study conducted in the governorate of Manouba (Kalthoum et al., 2017) and the work of Lee et al. (2018) in Vietnam. It is important to note that the study samples are collected from animals with troubled behaviour, which may lead to an underestimation of the real number of dog rabies cases especially for asymptomatic ones, due to the lack of awareness among farmers and dogs' owners as well as the Government effort to raise awareness. Besides, cross-sectional serological studies on culling dogs can provide crucial information on seasonal risk differences.

In contrast, the seasonality of rabies was demonstrated in several countries as in Peru (Lima district) (Malaga et al., 1979), Bhutan (South Asia) (Dhand & Ward, 2011) and even in neighbouring countries (Morocco and Algeria) (El Harrak, 2011; Khayli et al., 2019). Thus, the high number of dog rabies observed in a given season was explained by increased fighting between dogs during the breeding season, amplifying thus the risk of transmission of the disease (Yadav, 2012). In others studies, the authors suggested that the change in the age structure of canine population (non-immune puppies) at a given time of the year could also explain the seasonal peaks of rabies (Douangngeun et al., 2017).

The results of the present study showed that animal rabies cases are commonly distributed throughout the country. In fact, animal rabies were reported in 23 of 24 governorates, with a predominance in the Northern part where a persistent disease transmission and the highest number of cases rabies were recorded (more than 64% of the total number of cases). The consistent increase on animal rabies cases seen in the North of Tunisia is likely to be linked to the rural areas characterized by high density of herbivore (cattle, sheep and goat) and dog population (25 dogs/ km^2 in the governorate of Nabeul, Ariana, Tunis and Ben Arous) (Kalthoum, 2014). It was shown that dog rabies become endemic in areas where the dog density exceeds 4 dogs//km^2 (Cleaveland & Dye, 1995). The governorate of Kébili in the South did not record any cases of rabies during the study period and very low number of animal rabies (less than 6 cases) were always reported in the Southern governorates. Similar results were reported in Algeria and Morocco, where the Northern areas were shown to be the most affected areas by the disease (Darkaoui et al., 2017; Yahiaoui et al., 2018). Variation in the number of animal rabies cases in Tunisia is depending on multiple factors such as insufficient level of immunity in dogs' population due to a high dog turnover (Touihri et al., 2011), no restriction of dog movement and poor management of disease outbreaks (absence of ring vaccination to prevent secondary cases and no culling of infected animals).

The spatial analyses have confirmed the results above and showed that statistically significant hotspots in the North, in 2012, 2013, 2014 and 2015 and persistent cold spots in the South (Kébili and Medenine). Geographical differences between governorates, in relation with the level of dog vaccination coverage, might have an effect on the clusters identified in this study. Unfortunately, data on dog vaccination are not available. Such information is important as it would provide information on whether the animal rabies clusters last for years or not, which may provide new guidelines for policy planning and decision-making for better rabies control.

The presence of host spots in the Northern governorates indicates that the risk of transmission of rabies to human is high. This result was confirmed by the identification of high clusters of PEP in these regions (Jendouba, Kef, Siliana and Kairouan), suggesting that the risk of rabies may be limited to specific areas. Indeed, the Northern governorates might be then considered as high-risk areas for human rabies exposure. The rural population in these areas ought to be more exposed to dog bites (lack of awareness and education along with frequent contact with dogs). These results are similar to those from studies conducted in Ethiopia and Asian countries (China and India) (Song et al., 2009; Sudarshan et al., 2007; Yibrah & Damtie, 2015). However, Southern governorates (Kebili, Gabes, Tataouine and Medenine) have recorded lower incidence rates of PEP and were identified as cold spots, between 2012 and 2018. Such low incidence was associated with the low density of canine population (<4 dog/km^2), the low number of animal rabies cases and of PEP.

Finally, the results would infer that the program control of rabies should be revised to adapt a strategy taking into account new findings. To fill the gap and provide a baseline data on rabies, further studies on stray and owned dogs should be conducted to update data on dog population size as well as vaccination coverage. To explore the role of wildlife in rabies transmission and persistence, longitudinal surveys may be implemented along with studies that take into consideration the environmental variables.

5 | CONCLUSION

This is the first study that explores the spatial distribution of animal rabies and PEP acts with a focus on clusters that explain the resurgence of rabies and its persistence, in Tunisia. The results showed that animal rabies remain a major problem in Tunisia and efforts to control it are insufficient. The findings highlighted the need on focusing on alternative means to reduce and hopefully eradicate rabies, as recommended by the WHO, like labelling vaccinated dogs with collars, applying new measures to control stray dogs, updating data on dog population and practicing ring vaccination within a distance of 3 km during outbreaks.

CONFLICT OF INTEREST

The authors certify no conflicts of interest.

AUTHOR CONTRIBUTION

Sana kalthoum: Conceptualization; Formal analysis; Methodology; Software; Writing-original draft; Writing-review & editing. **Kaouther Guesmi:** Methodology. **Raja Gharbi:** Methodology. **Mohamed Naceur Baccar:** Validation; Writing-review & editing. **Chedia Seghaier:** Validation; Writing-review & editing. **Malek Zrelli:** Validation; Writing-review & editing. **Chokri Bahloul:** Validation; Writing-review & editing.

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