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Research article

Spatiotemporal patterns of honeybee health within different regions of Spain (2012-2020)

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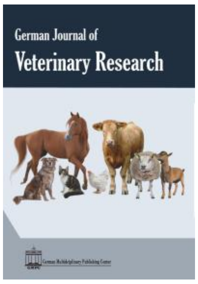

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Abstract

Globally recognized for their indispensable role in pollination, honeybees (*Apis mellifera*) not only enhance agricultural yields but also play a critical role in maintaining ecological balance. In Spain, the main producer of honey in Europe, with over 36,475 beekeepers and more than three million colonies, the dynamic landscape of apiculture reflects a complex interaction of traditional practices and modern challenges. This study analyzes data from the Spanish National Surveillance Program of Honeybee Colonies Losses between 2012 and 2020, focusing on the spatial-temporal dynamics of several pathogens, pesticides, and management practices that could affect the directional distribution and rates of mortality and strength of apiaries using geographical and scan statistics tools. Spatial analysis indicated annual variations in directional trends in apiaries' strength and mortality. Apiary strength shows regional fluctuations, with significantly lower values in autumn in the southwestern regions between 2017 and 2020 and in spring 2018 in the northwestern. The study also noted a substantial influence of Varroa destructor, *Nosema* spp., and pesticide exposure on apiary health, with seasonal variations. Clusters of high *Nosema* spp. spores load and Varroa infestations, previously detected in central-western and northeastern Spain, respectively, could be related to the cluster of high winter mortality observed in 2020, which included these regions. Our analysis not only reveals critical points of honeybee health but also contributes to a deeper understanding of geographical and seasonal factors that can affect their survival. The insights aim to support the development of sustainable beekeeping practices and robust agricultural policies.

Keywords: Honeybees, Spatial epidemiology, Surveillance, Varroa, Nosema, Pesticide exposure, Apiary health

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Introduction

The honeybee, *Apis mellifera*, has played a significant role in human society, not only as a source of honey and pollen but also in pollination, which is essential for both agricultural economies and ecosystem preservation (Alonso-Prados et al., 2020).

Spain, with 36,475 beekeepers and 3,097.647 colonies registered by the Ministry of Agriculture, Fisheries and Food (MAPA) of Spain, is a leading European honey producer. Beekeeping practices vary regionally, with 18% of beekeepers classified as professional, managing 80% of the colonies, mostly transhumant located in central and southern regions (MAPA, 2022). Over the last two decades, the increasing weakening and mortality of honeybees have been analyzed in multiple countries, particularly in Europe and North America (Meana et al., 2017; van Engelsdorp et al., 2009). Studies point to a multifactorial nature of bee colony mortality, emphasizing the role of infectious agents, pesticide co-exposure, and nutritional and environmental factors (Pérez-Cobo et al., 2022; Jacques et al., 2016).

To address these challenges, MAPA launched the national surveillance program of honeybee colonies losses in the last 12 years, in collaboration with the European Union reference laboratory (EURL) for bee health and the first pan-European active surveillance program on honeybee colony mortality (EPILOBEE) (Commission Implementing Decision, 2012). The national protocols were based on the guidelines designed by EURL for bee health and were implemented in each of the 17 participating member states to provide comparable data. The active surveillance involved inspections during autumn (A), spring (SP), and summer (SU) from 2012 to 2014. Colony losses (overwintering and seasonal colony mortality rate, measured respectively in SP and SU visits), strength (rated on an ascending scale from 1 to 5 across all periods- A, SP and SU), and the clinical prevalence of major diseases (such as varroosis and *Varroa destructor* infestation rates in autumn, American foulbrood (AFB), European foulbrood (EFB), nosemosis, deformed wing virus (DWV), acute bee paralysis virus (ABPV) and chronic bee paralysis virus (CBP) were estimated (Chauzat et al., 2014).

Given the importance of beekeeping in Spain, MAPA extended the baseline of the EPILOBEE program to include systematic surveillance of *Nosema* spp. spores loads each autumn, *Varroa destructor* infestation rates in spring (since 2018), and clinical intoxications up to the present. Additionally, systematic surveillance of residues of plant protection products (PPPs) and veterinary medicine products (VMPs) was conducted in bee bread honeycomb (BBHC) during the autumn of 2012 and spring of 2013 and 2016, analyzing 306 active substances (Perez-Cobo et al., 2022).

The analysis of spatial associations conducted in this study is crucial for understanding the complex interactions that impact the health of honeybees. Our goal is to explore the relationships between apiary strength/mortality rates and various risk factors, such as parasite or fungal loads (*Varroa destructor*, *Nosema* spp.), intoxication risks, and varroosis treatments. These insights could help improve beekeeping practices, enhance surveillance effectiveness, and support bee health initiatives. We analyze data from Spain's national surveillance to provide frequency estimates, mortality and strength rates, and their spatiotemporal distribution in apiaries and to identify potential risk factors that could influence them from 2012 to 2020.

Material and methods

Study area and national surveillance program

A spatiotemporal epidemiological analysis of honeybee colony strength and mortality rates was conducted across Spain using national data from MAPA's National Surveillance Program of Honeybee Colony Losses from autumn 2012 to spring 2020. A total of 1,143 apiaries were evaluated three times per year: during autumn, spring, and summer.

Sampling strategy

The sampling strategy followed the guidelines of the EURL for bee health (Chauzat et al., 2014) and was uniformly implemented across each autonomous community. Apiaries were selected based on the census of beehives registered in the general register of livestock holdings of Spain (REGA), proportionally distributed by the number of beehives in each region, and considering an expected prevalence of 15% with 5% absolute precision. If a randomly selected beekeeper declined to participate, another was randomly selected. Up to 13 colonies per apiary were investigated, assuming a 20% infection prevalence for pathogens and parasites. Although not all regions were able to participate in all campaigns, a total of 27,533 colony inspections and 45,502 laboratory analyses were conducted from 2012 to 2020 (MAPA, 2020).

Data collection and analysis recorded in the national database for each apiary

Epidemiological survey. Beekeeper details, apiary location (latitude and longitude coordinates, environment), management practices, treatments implemented for *Varroa destructor* control, health events, mortality and strength rates, clinical disease observations, and samples collected (systematic and clinical).

Winter mortality rate and autumn/spring strength. Winter mortality rates below 10% and colony strength with values above 2, ascending rated on a 1-5 scale, were considered normal.

Systematic analysis Nosema spp. spores load and the rest of the clinically suspected diseases. Conducted by the central veterinary laboratory of Algete. A load of ≥1 million spores per bee was considered the threshold to cause damage (Rennich et al., 2012).

Varroa destructor infestation rates. The official laboratories of each autonomous community performed systematic analyses. Although there is no harmonized criterion, a rate of >5% was considered harmful to the colonies' survival (Calatayud et al., 2018).

Treatment evaluation. Inspectors assessed the correct application of varroosis treatments based on VMP use, dosage, and treatment duration.

Residues analysis. PPPs and VMPs in BBHC were analyzed by the EURL for pesticide residues in fruit & vegetables and the Agri-food Arbitration Laboratory of Aravaca (MAPA). A risk evaluation of accumulated intoxication by contact was performed using the methodology described by Sanchez Bayo et al. (2014). Apiaries were classified into two categories: 1=high moderate risk of intoxication and 2= low risk of intoxication.

Exploration of the spatial directional trend of rates of apiary strength and mortality patterns

To determine whether the distribution of the strength and mortality rates of apiaries exhibits areas of concentration of higher values and their directional trends, the mean center (MC) and standard deviational ellipse (SDE) (ArcGIS PRO 3.2, ESRI Inc) tools were used on an annual and seasonal basis (autumn, spring, and summer), weighted by both strength and mortality rates. The SDE generates elliptical polygons containing 63% of the data points, calculated by measuring the standard deviation of the X and Y coordinates of each point from the MC of all points. This method allows us to visualize the central tendency, dispersion, and directional patterns of strength and mortality rates across different regions. The attributed values for each ellipse include the X and Y coordinates of the MC, the lengths of the long and short axes, and the orientation of the ellipse, providing valuable insights into potential hotspots and directional trends in colony health (Wang et al., 2015).

Spatiotemporal cluster analysis

Winter mortality rates, apiary strength in the autumn and spring months, *Nosema* spp. and *Varroa destructor* infestation rates, the risk of intoxication, and the application of treatments were considered the most representative indicators of apiary health for identifying spatiotemporal clusters. The scan statistics Bernoulli model for case-control data was applied using SaTScan™ software (v.9.6; www.satscan.org). For each analysis, mortality rates >10%, apiary strength ≤2, *Varroa* infestation rates >5%, *Nosema* spp. spores load ≥1,000.000 spores per bee, risk of moderate to acute intoxication, and incorrect treatment application were considered cases, while the remainder were considered controls (Table 1). The scan statistic calculates the expected number of cases within the scanning window using a Bernoulli distribution. 999 Monte Carlo replications were performed to determine the statistical significance of the likelihood ratio statistics for the identified clusters (Kulldorff, 1997).

Table 1: Variables for spatiotemporal analysis: winter mortality and seasonal strength in honeybee colonies.

Variable	Case (1)	Control (0)
Winter mortality	≥10% (high mortality)	<10% (normal mortality)
Autumn strength Spring strength	Strength ≤2 (low strength)	Strength >2 (normal strength)
Nosema spp. spores loads	≥1,000.000 spores per bee	<1,000.000 spores per bee
Varroa destructor infestation rates	>5%	<5%
Treatment correctly applied for varroosis control	No	Yes
Risk of intoxication	Risk of moderate to acute intoxication	Low risk of intoxication

Results

Exploration of the spatial directional trend of rates of apiary strength and mortality patterns

The spatial analysis of apiary strength and mortality rates from 2012 to 2020 revealed clear regional patterns that fluctuated annually, potentially influenced by sampling variability (Figures 1 and 2). Focusing on winter mortality, by spring 2013, 2014, and 2020, in which almost all regions participated, its distribution displayed no discernible pattern, except for 2014, showing a north-to-southeast orientation (Figure 3). From spring 2015 to spring 2017 the patterns observed differ from those described above, showing a northern pattern from west to east, due to several regions in the southern half of Spain were not able to participate (Figure 4). Nevertheless, a notable shift occurred in the spring of 2018 to 2019, where a pronounced change of pattern showed a shift to a north-south orientation in western Spain (Figure 3, Figure 4).

Spatiotemporal analysis

The spatial scan statistic identified significant clusters with high or low relative risk for each variable studied, highlighting the spatial and temporal dynamics of apiary health indicators.

Winter mortality rates. Two significant clusters were identified. The first, observed during the first half of 2020, covered the central, western, and northwestern regions of Spain with a radius of 324 km, showing high mortality rates exceeding 10%. The second cluster, smaller in size (139 km radius), indicated lower mortality in the southwestern and persisted from April 2016 to May 2020 (Table 2, Supplementary A).

Apiary strength. A persistent cluster with no risk of low strength was identified in the northwest from the autumn of 2013 to 2017. This trend changed completely in this area in the first half of 2018, when a cluster of lower strength (below 2) was identified, extending over the northern half of Spain (Table 2, Supplementary B-C).

Nosema spp. spore load. A cluster with a high spore load was mainly detected in the central-western region between the autumn of 2014 and the spring of 2019. Unlike the previous, a cluster with values lower than expected was identified in the eastern region of Spain between the autumn of 2012 and 2016 (Table 2, Supplementary D).

Varroa destructor infestation rates. Two clusters of high infestation rates were detected, one in the northeastern region, covering the Ebro River Valley and adjacent areas, from autumn 2013 to 2017 and the other in the south from spring 2019 to 2020. Lower infestation rates than expected were observed in a cluster in the central-western area of Spain from spring 2015 to 2019. (Table 2, Supplementary E).

Application of treatments for V. destructor control. Treatment application in autumn was also evaluated. A cluster with excellent application was identified from autumn 2014 to 2017 in the centralwestern region. Conversely, an extensive cluster of incorrect application cases was identified in the eastern half of Spain from autumn 2012 to 2014 (Table 2, Supplementary F).

Risk of intoxication: A large cluster (343.38 km radius), indicating moderate to high risk of poisoning, was detected in eastern Spain in the autumn of 2012 (Table 2, Supplementary G).

The statistical analysis using scan statistics Bernoulli model confirmed the high statistical significance (*p*-value <0.01) of these clusters compared to other areas.

Directional distribution of mortality:
Standard Deviational Ellipses (SDE) & Mean Center (2012-2020)

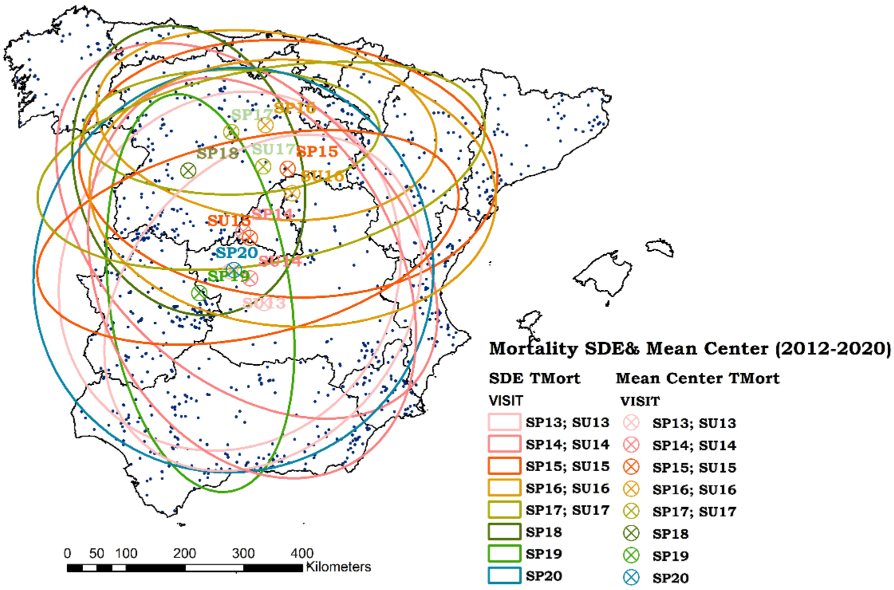

Figure 1: Directional distribution & Mean center of winter (SP) and spring (SU) mortality (2012-2020).

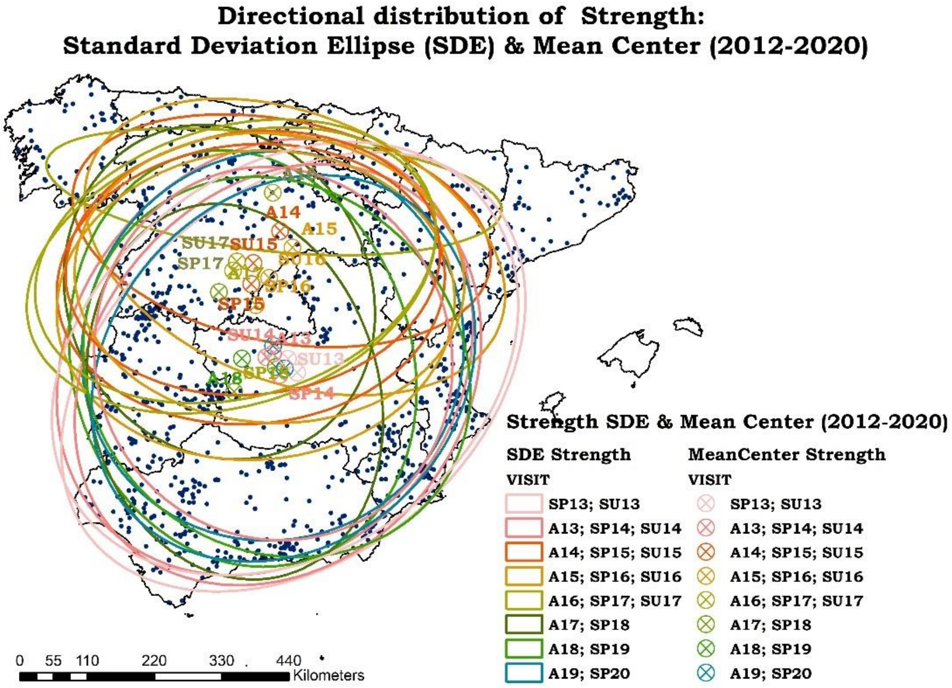

Figure 2: Directional distribution of strength of autumn (A), spring (SP), and summer (SU) (2012-2020).

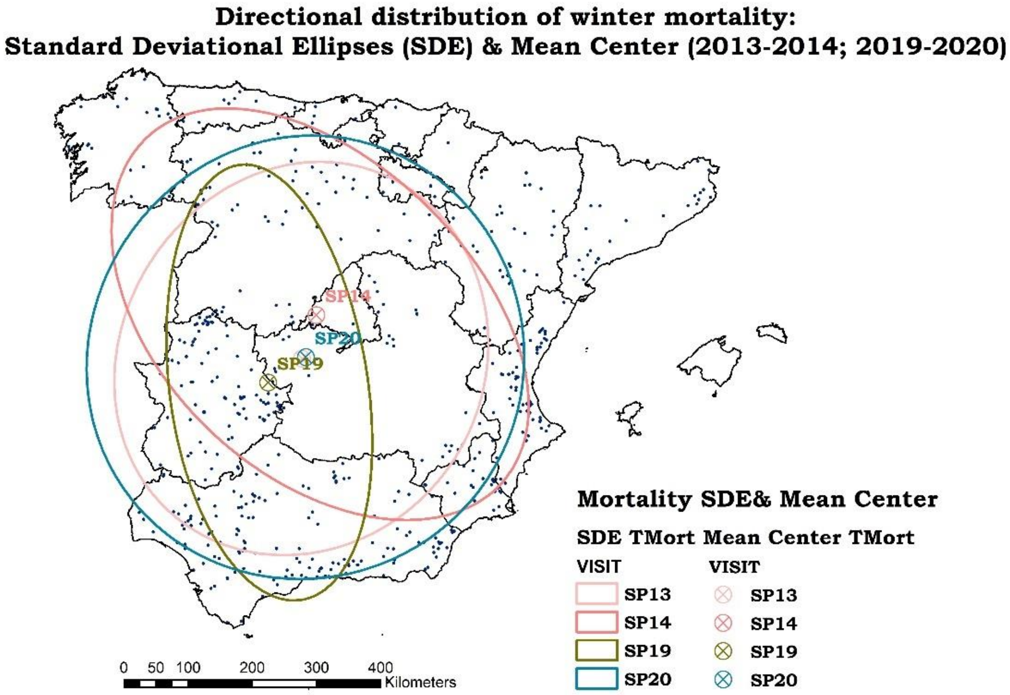

Figure 3: Standard deviation ellipses (SDEs) and mean center (MC) of apiary winter mortality (SP) occurring between the springs of 2013-14 and 2019-2020 reflect a pronounced change of pattern, showing a shift to a North-South orientation in the western region of Spain in SP19. Each ellipse is drawn from data collected during the annual spring visits (SP).

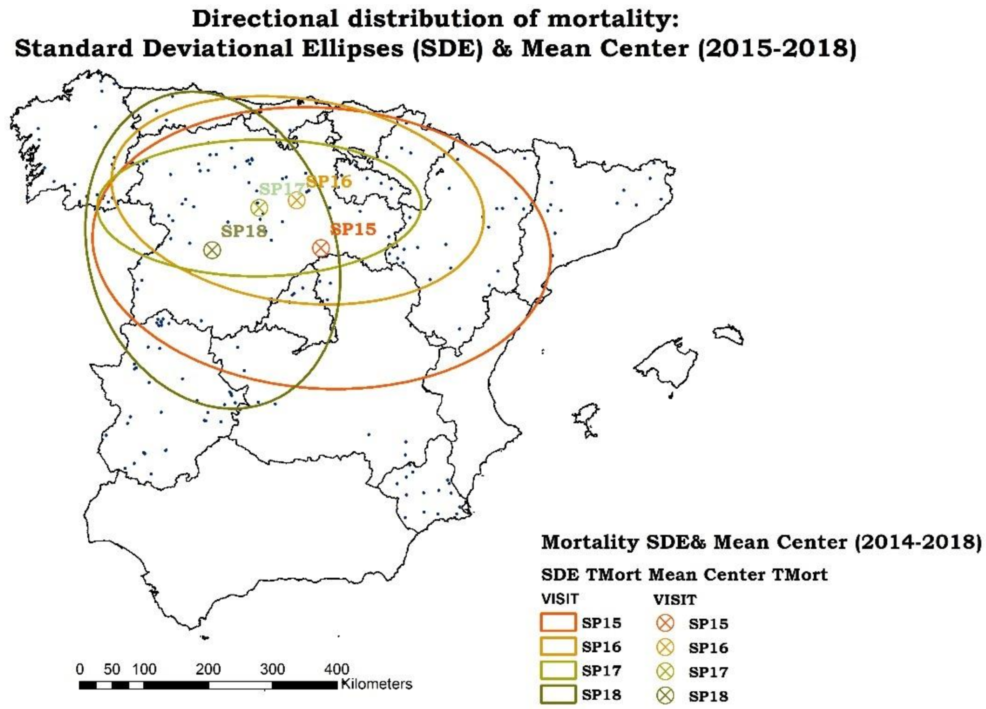

Figure 4: Standard deviational ellipses (SDE) and mean center (MC) represent the directionality of winter mortality in honeybee colonies across Spain from spring 2015 to spring 2018. A change of pattern, with North-South orientation was observed in the western of Spain in SP18. Several regions were not able to participate. Each ellipse is drawn from data collected during the annual spring visits (SP).

Discussion

Some honeybee health epidemiological studies have been carried out over the last 15 years to explain the colony loss phenomenon in Spain, yet in limited areas and apiaries (Meana et al., 2017). This study is extended over a wide geographical area with a larger number of apiaries and hives sampled for laboratory analysis over eight years, employing harmonized procedures. Nevertheless, despite this intensive sampling, data are not completely representative, especially for four campaigns (from autumn 2014 to spring 2018) since several southeastern regions were unable to participate.

Although spatiotemporal analysis is a tool often used to study the epidemiology of many animal diseases, it has not yet been widely implemented as a tool in honeybee health, which is reflected in the few studies in which it has been adopted these tools to examine very specific aspects such as winter mortality in Belgium (Roelandt et al., 2016) or *Varroa* distribution in Ontario-Canada (Sobkowich et al., 2022). This research delves into the assessment of the spatial distributions of these and other colony health indicators over a longer period, allowing for the study of trends.

In the spring of 2018, a major change in the spatial distribution of winter mortality rates was detected with the SDE, indicating the epidemiological center of major mortality in northwestern Spain. This is consistent with the winter mortality results recorded by the MAPA in that spring, in which the national mortality rate was 13.5% in contrast to the values above 20% recorded in the area covered by the SDE (MAPA, 2018). In the spring of 2020, a significant cluster of mortality was detected in central-north-western in eight years of study. The SDE for this spring did not detect any discernible pattern, indicating that the rate of winter mortality described for this year in Spain, 19.2%, was affecting equally the entire country (MAPA, 2020). The different patterns described from autumn 2015 to spring 2018 are influenced by the reduction of the area that could be sampled, highlighting that sampling, as already mentioned by (Lee et al., 2015), is one of the greatest challenges in the implementation of honeybee health monitoring programs.

SaTScan™ control-case study is more likely to identify clusters in sparsely populated areas, allowing detect hotspots along space-time. The clusters found underscore significant high mortality and low strength at different areas and times during the study.

Table 2: Spatiotemporal clusters of apiary health indicators across Spain. The table summarizes significant spatiotemporal clusters of apiary health indicators identified through saTScan™ analysis, displaying both high and low-value clusters across various regions in Spain. High/low-value clusters are marked by higher/lower observed-to-expected and relative risk

Variable	**Cluster**	**Location**	**Radius (km)**	**Start date**	**End date**	**Days**	**% Cases**	**Relative risk**	**Observed / Expected**	***p*-value**
Winter mortality (SP)	1	Central-north-western	324.58	01/03/2020	31/07/2020	152	88.6	2.92	2.71	3.5E-6
	2	Southeastern	139.81	01/04/2016	31/05/2020	1521	0.0	0.00	0.00	0.018
Autumn strength (A)	1	Southwestern	171.90	01/11/2017	29/02/2020	850	46.6	4.22	3.2	2.2E-6
	2	Northwestern	280.27	01/10/2013	31/10/2017	1491	0	0.00	0.00	3.9E-5
	3	Central-western	61.32	01/01/2014	31/10/2014	303	68.4	5.21	4.71	8.2E-3
Spring strength (SP)	1	Central north-western-	277.50	01/03/2018	31/07/2018	152	42.9	4.29	3.72	3.2E-4
Nosema spp. spore load /bee (A)	1	Central-western	162.50	01/11/2014	28/02/2019	1580	67.4	3.04	2.47	3.4E-10
	2	Central-eastern	216.59	01/12/2012	31/12/2016	1490	3.1	0.11	0.11	0.026
Varroa destructor infestation rates (A; SP)	1	Central-western	151.93	01/02/2012	30/06/2019	2706	3.1	0.17	0.2	5.4E-5
	2	Northeastern	267.01	01/10/2013	30/11/2017	1521	37.8	2.74	2.41	1.8E-3
	3	Southwestern	166.31	01/05/2019	31/07/2020	457	48.9	3.36	3.12	1.9E-3
Application of treatments for *V.destructor* control in autumn (A)	1	Central-western	148.93	1/11/2014	30/11/2017	1125	0.9	0.023	0.021	3.3E-16
	2	Central-eastern	345.38	1/10/2012	31/12/2014	821	63.4	1.63	1.88	4.7E-7
Risk of intoxication (A12-SP13-SU16)	1	Central-eastern	343.38	1/10/2012	31/12/2012	91	83.6	1.86	2.2	3.4E-7

The significant low-strength cluster registered in the central northwestern in the spring of 2018 could anticipate a meaningful increase in winter mortality in the central northwestern in the spring of 2020. The differences in mortality and strength values between apiaries may also reflect their potential association, also described by other authors**,** with parasitic load or management (Jacques et al., 2016; Meana et al., 2017). In this regard, the persistent cluster from the autumn of 2014 to the spring of 2019 showed moderate-high rates of *Nosema* spp. parasitism in central western of Spain could be related to the cluster of high winter mortality in the central-western peninsula later in 2020. Similarly, the significant low-strength clusters recorded in the central-northwestern in the spring of 2018 and in southwestern from autumn 2017 to spring 2020 could be partially explained by the clusters of high rates of *Varroa destructor* in the northeast, Ebro valley and adjacent areas between autumn 2013 and 2017 and in the southwest between spring 2019 and 2020. In addition, the Ebro Valley and adjacent areas were included in a cluster of wrong applications of varroosis control treatments recorded from 2012 to 2014.

The study also clearly reflects that in regions with significantly better management of varroosis control, such as that detected in central-western Extremadura, a significantly minor rate of *Varroa destructor* occurs.

The high-risk cluster for poisoning detected in central-eastern Spain could be connected to environmental factors, such as the concentration of almost 70% of national fruits and vegetables production, being Spain, according to MAPA, the first producer in the EU with more than 25% of the production.

Conclusions

This study has provided valuable spatial and temporal insights into various factors affecting mortality and strength of apiaries. The application of GIS and scan statistics tools has been instrumental in identifying areas and periods of significant bee health concerns, such as hotspots of parasite loads, incorrect management practices, and risks associated with active substance residues. These findings could facilitate targeted and effective intervention strategies to improve bee health in the affected regions. In future work, it will be essential to integrate additional environmental factors, such as climate and land use, into the analysis to refine further our understanding of the spatio-temporal distribution of colony losses. Moreover, specific statistical models should be applied to investigate the interplay between these factors and honeybee health in greater detail.

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Authors contribution. All authors contributed equally to the work.

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