




Monitoring of emerging myxoma virus epidemics in Iberian hares (*Lepus granatensis*) in Spain, 2018–2020

Ignacio García-Bocanegra¹  | Leonor Camacho-Sillero² | Javier Caballero-Gómez^{1,3}  |
 Montserrat Agüero⁴ | Félix Gómez-Guillamón² | Juan Manuel Ruiz-Casas⁵ |
 José Manuel Díaz-Cao¹  | Elena García⁶ | María José Ruano⁴ | Rafael de la Haza⁶

¹Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Córdoba, Córdoba, Spain

²Programa Vigilancia Epidemiológica Fauna Silvestre (PVE), Consejería Agricultura, Ganadería, Pesca y Desarrollo Sostenible, Junta de Andalucía, Málaga, Spain

³Unidad de Enfermedades Infecciosas, Grupo de Virología Clínica y Zoonosis, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Hospital Reina Sofía, Universidad de Córdoba (UCO), Córdoba, Spain

⁴Laboratorio Central de Veterinaria (LCV), Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain

⁵Consejería de Agricultura, Agua y Desarrollo Rural, Junta de Comunidades de Castilla-La Mancha, Toledo, Spain

⁶Área de Epidemiología, Subdirección General de Sanidad e Higiene Animal y Trazabilidad, Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain

Correspondence

Ignacio García-Bocanegra, Department of Animal Health, University of Córdoba, Campus Universitario Rabanales, 14071 Córdoba, Spain.
 Email: nacho.garcia@uco.es

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Abstract

Myxomatosis is an infectious disease caused by the myxoma virus (MYXV), which has very high mortality rates in European wild rabbits (*Oryctolagus cuniculus*). While sporadic cases of myxomatosis have also been reported in some hare species, these lagomorphs are considered to have a low susceptibility to MYXV infection. In the present study, we describe the spatiotemporal evolution and main epidemiological findings of novel hare MYXV (ha-MYXV or MYXV-Tol) epidemics in Iberian hares (*Lepus granatensis*) in Spain. In the period 2018–2020, a total of 487 hares from 372 affected areas were confirmed to be MYXV-infected by PCR. ha-MYXV outbreaks were detected in most of the Spanish regions where the Iberian hare is present. The spatial distribution was not homogeneous, with most outbreaks concentrated in the southern and central parts of Spain. Consecutive outbreaks reported in the last two years suggest endemic circulation in Spain of this emerging virus. A retrospective study carried out just after the first epidemic period (2018–2019) revealed that the virus could have been circulating since June 2018. The number of outbreaks started to rise in July, peaked during the first half of August and October and then decreased sharply until January 2019. The apparent mean mortality rate was 55.4% (median: 70%). The results indicated high susceptibility of the Iberian hare to ha-MYXV infection, but apparent resistance in the sympatric hare species present in Spain and less infectivity in European rabbits. The novel ha-MYXV has had significant consequences on the health status of Iberian hare populations in Spain, which is of animal health and conservation concern. The present study contributes to a better understanding of ha-MYXV emergence and will provide valuable information for the development of control strategies. Further research is warranted to assess the impact of this emerging virus on wild lagomorph populations and to elucidate its ecological implications for Iberian Mediterranean ecosystems.

KEYWORDS

epidemic, ha-MYXV, Iberian hare, myxomatosis, Spain

1 | INTRODUCTION

The Iberian hare (*Lepus granatensis*) is an endemic species in the Iberian Peninsula and one of the most representative wild lagomorphs in terms of abundance and hunting interest. This species plays a key role in the ecology of Iberian Mediterranean ecosystems, being the staple prey of a large number of predators, including endangered species such as the Iberian lynx (*Lynx pardinus*), Iberian wolf (*Canis lupus signatus*) and the Spanish imperial eagle (*Aquila adalberti*) (Purroy, 2011). The Iberian hare is also among the main small game species, with about 930,000 animals harvested annually in Spain (MAPA, 2020). Although the information about the population densities of Iberian hares in Spain is limited, in some regions their densities have remained stable at local sites, whereas in others, there has been a decreasing trend in population size in recent decades (Ballesteros, Benito, & González-Quirós, 1996; Carro & Soriguer, 2017). Conservation of the Iberian hare is threatened by different natural and anthropogenic factors, including predators and hunting pressure, fragmentation and loss of habitat, use of herbicides and pesticides, weather conditions, roadkill and disease (Duarte, 2000; García-Bocanegra et al., 2019; Sánchez-García et al., 2012). This species has been shown to be susceptible to different infectious and parasitic diseases (Fernández-Aguilar et al., 2013; Ruiz-Fons, Ferroglio, & Gortázar, 2013; Sánchez-García et al., 2012). On the other hand, several pathogens that affect other hare species (Wibbelt & Frölich, 2005) have not been detected in the Iberian hare, although the available information about its health status is still very scarce.

Myxomatosis is an infectious disease caused by the myxoma virus (MYXV; family *Poxviridae*; Genus *Leporipoxvirus*), which is mainly transmitted by biting arthropods or direct contact with infected animals (Kerr, 2012). MYXV is considered a classic example of host–pathogen coevolution following a species jump (Alves et al., 2019; Kerr et al., 2015). MYXV infection induces benign cutaneous fibromas in its natural host, the South American forest rabbit (*Sylvilagus brasiliensis*), while it causes severe and often fatal disease in European rabbits (*Oryctolagus cuniculus*) (Bertagnoli & Marchandeau, 2015). After MYXV was illegally introduced into France in 1952, the virus spread rapidly throughout Europe causing mortality rates of up to 90% in wild rabbit populations (Fenner & Ratcliffe, 1965). Myxomatosis is currently endemic in most European countries, including Spain, with annual epizootic cycles causing high mortality in susceptible domestic and wild rabbit populations (Villafuerte et al., 2017). Even though hare species are considered to be mostly resistant to MYXV infection, sporadic cases of myxomatosis have been reported in the European brown hare (*Lepus europaeus*) and mountain hare (*Lepus timidus*) (reviewed in Kerr et al., 2015). Between mid-July and the end of October 2018, high mortalities associated with a novel recombinant MYXV strain (ha-MYXV or MYXV-Tol) were detected in Iberian hare populations in Spain and Portugal (García-Bocanegra et al., 2019; OIE, 2018). Molecular studies revealed that an insertion or recombination event with respect to the MYXV Lausanne reference strain may have been involved in the

cross-species jump and increased virulence in its new host (Águeda-Pinto et al., 2019; Dalton et al., 2019). The main aim of the present study was to describe the spatiotemporal evolution and main epidemiological findings of the ha-MYXV epidemics in Iberian hares in Spain in the period 2018–2020.

2 | MATERIALS AND METHODS

After the first outbreaks of the novel ha-MYXV isolate in an Iberian hare population in Andalusia (southern Spain) were notified on 10 July 2018 (García-Bocanegra et al., 2019), a national passive surveillance program, coordinated by the Spanish Ministry of Agriculture, Fisheries and Food, was launched across Spain. Between July 2018 and April 2020, a total of 372 hunting estates and protected areas that reported cases compatible with myxomatosis in Iberian hares in this country were investigated/surveyed. The study period was divided into two consecutive epidemic periods: P1, between July 2018 and April 2019, and P2, between May 2019 and April 2020.

Whenever possible, between one and 16 (mean = 3) full carcasses or eyelid samples were collected from clinically affected Iberian hares and wild rabbits found dead in each investigated area. Only samples from animals with the presence of lesions compatible with myxomatosis were collected. Clinically affected hunted hares were also included, although most of the samples (92%) were from animals that were found dead. During the study period, a total of 1,404 and 47 samples of Iberian hares and wild rabbits, respectively, were obtained by the authors, generally in collaboration with the gamekeepers, and sent to the Animal Health laboratory (University of Cordoba, Spain) (93 fresh full carcasses of Iberian hares) for postmortem examination and sampling of carcasses, or directly to the Central Veterinary Laboratory in Algete (Spanish National Reference Animal Health Laboratory) for molecular analysis. Total DNA was extracted with the MagAttract® 96 Cador® Pathogen Kit (QIAGEN, Germany) following the manufacturer's instructions. For the detection of DNA of both the classical MYXV strains and the novel ha-MYXV isolate, a conserved region of the M071L or M005L/R gene was amplified by PCR or real-time PCR, respectively, as previously described (Cavadini, Botti, Barbieri, Lavazza, & Capucci, 2010; Dalton et al., 2019; Duarte et al., 2014, 2015). Using TaKaRa LA Taq DNA polymerase (TaKaRa, Japan), a specific ha-MYXV PCR was carried out with forward and reverse primers M009L-F (5'-CGCAGGTCCACGTATAAACC-3') and M009L-R (5'-CGAACGTATCATTAGACAATG-3') (Dalton et al., 2019). Data on location and date of sampling were also gathered from each surveyed area.

Additional epidemiological information was gathered in P1 through on-site interviews with gamekeepers at the investigated areas, using a standardized questionnaire. In most cases, this information was also verified by the authors during the visit. For each investigated area, the following data were recorded: location data, date of onset and end of clinically affected animals, clinical signs observed, clinical cases of myxomatosis in sympatric wild rabbits

during or before the outbreak, estimated number of affected or dead Iberian hares, estimated number of clinically affected animals, estimated mortality rate, hare densities before the outbreak and the presence of other affected hare species.

In the present study, an outbreak was defined as an investigated area with at least one Iberian hare infected by ha-MYXV and confirmed by PCR. Investigated areas where Iberian hare mortality compatible with myxomatosis was observed but could not be confirmed by laboratory analysis due to the absence of samples were considered suspected areas.

3 | RESULTS

Between July 2018 and April 2020, a total of 487 Iberian hares from 372 investigated areas were shown to be positive for ha-MYXV infection by PCR. The classical MYXV strain infection was not detected in the Iberian hares analysed. In the 372 ha-MYXV-confirmed areas, 210 outbreaks were detected in P1, 162 in P2, and ha-MYXV outbreaks were detected in 16 of these positive areas in both periods. ha-MYXV outbreaks were confirmed in 141 localities in 11 of 17 Spanish regions. A total of 78 and 63 ha-MYXV-positive localities were detected in P1 and P2, respectively, and in 35 of these positive localities, ha-MYXV outbreaks were successively reported in both study periods (Figure 1). The spatial distribution of ha-MYXV was not homogeneous across Spain; the highest numbers of outbreaks were reported in southern and central regions.

During P1, epidemiological information was obtained by questionnaire in 312 investigated areas: 176 were confirmed as

ha-MYXV-positive areas and the remaining 136 were considered as suspected areas. The first clinically affected Iberian hares were observed on 20 June 2018 on a hunting estate in the province of Cuenca (central Spain). The number of outbreaks started to rise from July, peaked during the first half of August and October and then decreased sharply until January 2019. The last sick animals were observed in early March 2019 on a hunting estate located in Valladolid province (northwest Spain) (Figure 2). In most of the surveyed areas (74.7%), the first clinically affected hares were observed between mid-July and mid-October 2018. In 58.9% of investigated areas in P1, the maximum number of cases was reported between early September and mid-October of the same year (Figure 3). In the surveyed areas, the mean interval between the first and maximum number of cases was 31.3 days and ranged between 0 and 73 days. The mean duration of outbreaks in P1 was 115 days (ranging between 3 and 406 days). A decreasing trend in mean duration of the outbreaks was observed, falling from 167 days in July 2018 to 30 days in January 2019 (Figure 4).

The frequency of clinical signs associated with ha-MYXV infection in Iberian hares in Spain during P1 is shown in Figure 5. The clinical signs most commonly observed by the interviewed gamekeepers were conjunctivitis (63.4%), myxomas (33.5%), blepharitis (32.9%) and anogenital swelling (20.2%). Cachexia, epistaxis, prostration, dyspnoea, convulsions, paralysis, diarrhoea, sudden death and opisthotonos were also observed. The number of animals with clinical signs compatible with myxomatosis was similar between males and females in most of the surveyed areas (77.6%). In 53.8% of surveyed areas, adult animals were found to be more frequently affected than juveniles, in 36.5% of affected areas, the distribution across age classes was similar, whereas

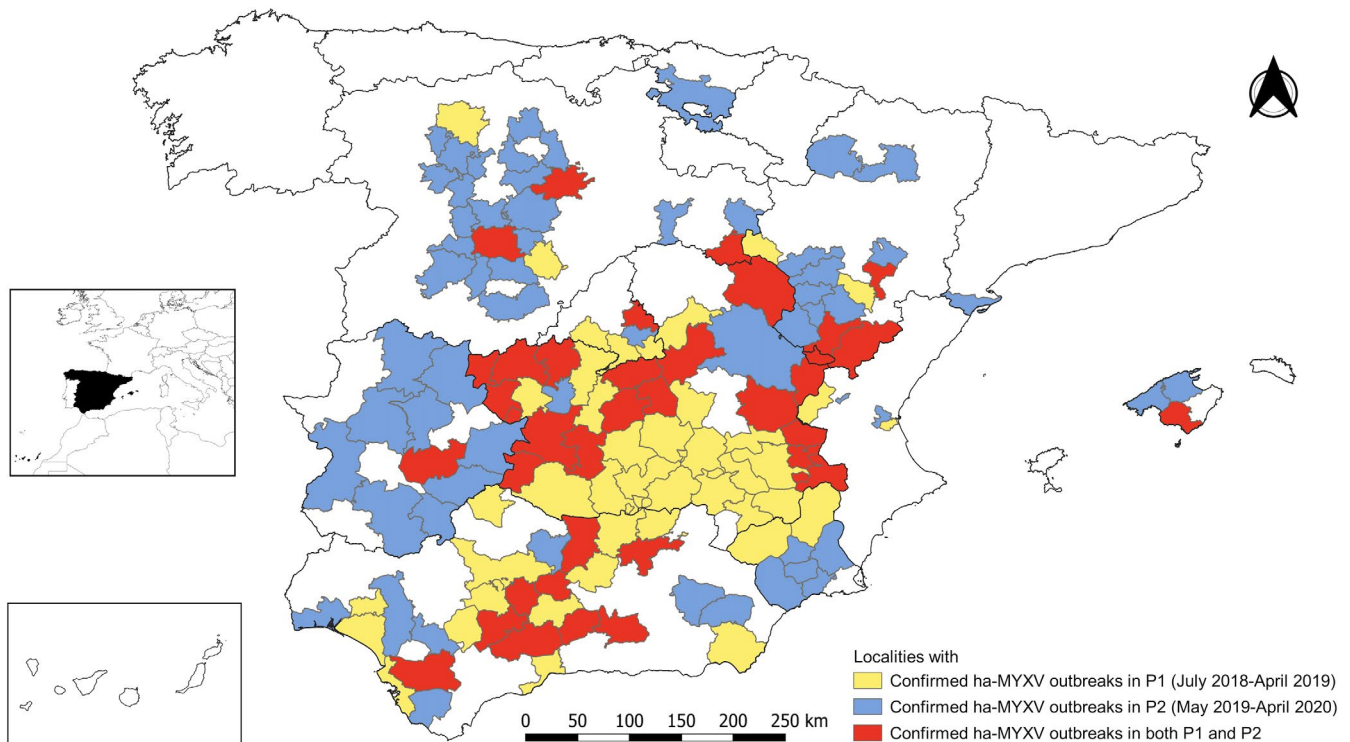


FIGURE 1 Spatiotemporal distribution of ha-MYXV outbreaks at regional level in Spain during the period 2018–2020

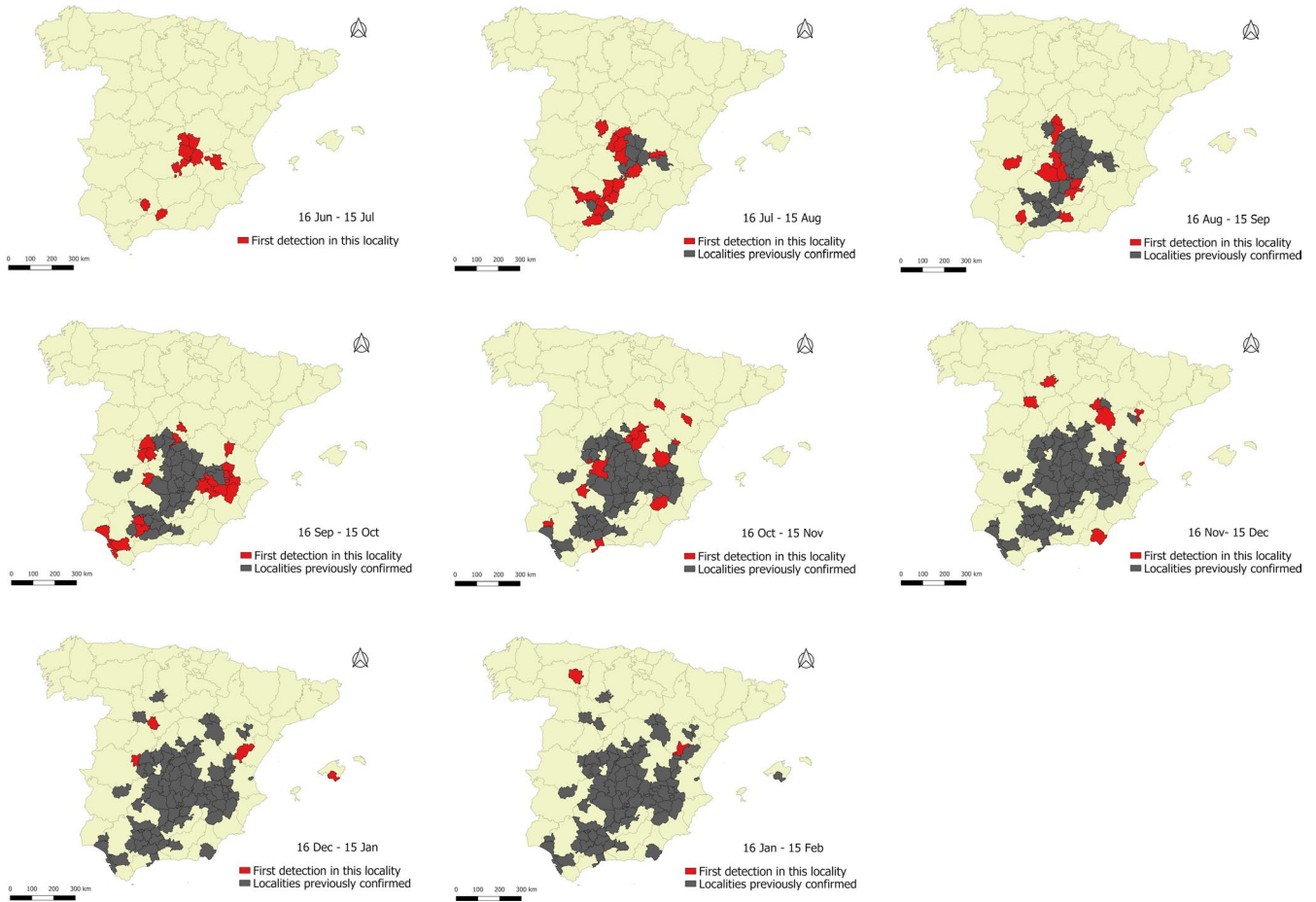


FIGURE 2 Spatiotemporal evolution of ha-MYXV outbreaks at regional level in Iberian hares in Spain during P1, 2018-2019

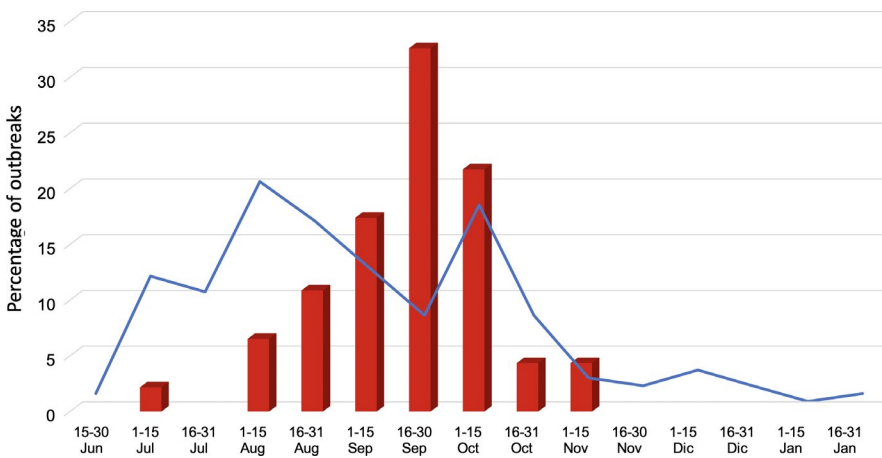


FIGURE 3 Temporal evolution (by fortnight) of the first (line) and maximum number (bars) of ha-MYXV outbreaks in Iberian hares in Spain during P1, 2018-2019

in the remaining 9.6% of areas, the gamekeepers interviewed indicated that juvenile Iberian hares were the most frequently affected animals.

During P1 and P2, neither clinical cases nor mortality compatible with myxomatosis were reported in the other two hare species present in Spain: the European brown hare and the Broom hare (*L. castroviejoii*). Even though most of the surveyed areas (75.7%) had myxomatosis outbreaks in wild rabbits in the two years preceding P1, cases of disease were only observed in this species in 27% of

areas investigated in this first epidemic period. A total of 47 clinically affected wild rabbits (seven sampled in P1 and 40 in P2) from localities with ha-MYXV cases in Iberian hares were also analysed by PCR to detect MYXV DNA. Classical MYXV strain infection was detected in 20 rabbits, whereas ha-MYXV DNA was confirmed in two animals. ha-MYXV-infected rabbits were sampled in August 2018 and November 2019 in the provinces of Toledo and Cuenca (both central Spain), respectively.

FIGURE 4 Mean duration (in days) of ha-MYXV outbreaks according to month of onset of the first myxomatosis case in Iberian hares in Spain during P1, 2018–2019

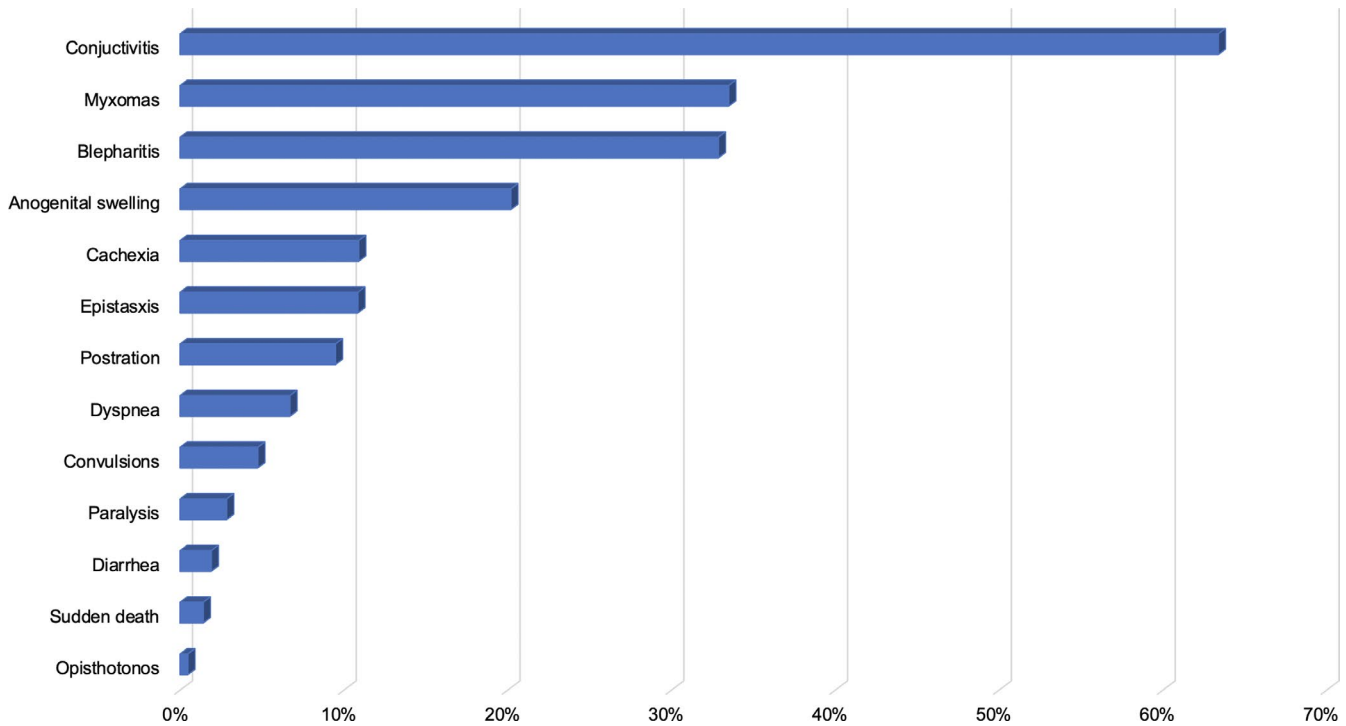
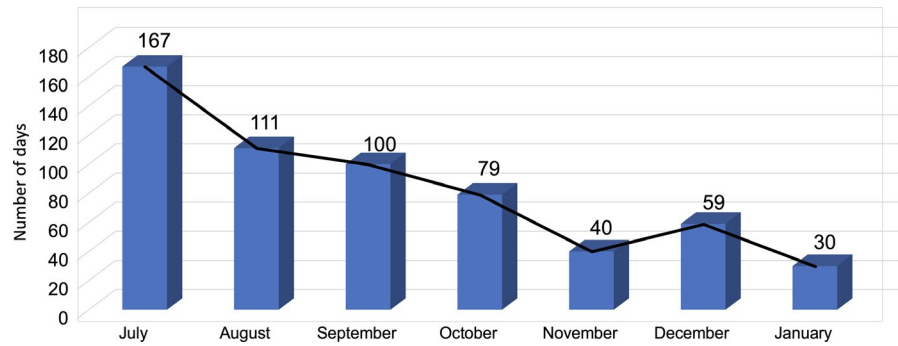


FIGURE 5 Percentage of clinical signs associated with ha-MYXV outbreaks in Iberian hares in Spain during P1, 2018–2019

During P1, a total of 10,297 Iberian hares (mean: 40.4 hares/affected area) were estimated to have been found dead in the field by interviewed gamekeepers. The estimated number of sick live hares was 900 (mean: 3.5 hares/affected area). The estimated mean mortality rate was 55.4% (ranging between 0% and 100%). Apparent mortality rates were above 70% in 38% of the surveyed areas, between 15% and 70% in 38% of areas, and below 15% in the remaining 24% of areas. The spatial distribution of cases within the affected areas was homogeneous in most of the areas surveyed (83.5%).

4 | DISCUSSION

In the present large-scale study, we describe the evolution and main findings of the first myxomatosis epidemic causing high mortality in hares worldwide. Between July 2018 and April 2020, a total of 372 ha-MYXV outbreaks were confirmed in Iberian hares, providing evidence of the cross-species transmission event by MYXV

(Águeda-Pinto et al., 2019; Dalton et al., 2019). In spite of the fact that the number of cases may be underestimated as a result of the difficulties of finding dead hares in the field, the detection of ha-MYXV-infected hares in most of the Spanish regions where this species is present further highlights the widespread dispersal of this novel virus in Spain. The occurrence of outbreaks was not homogeneous, since most were concentrated in southern and central parts of Spain. Differences in hare population densities, climatic and environmental conditions or surveillance efforts are possible factors accounting for the geographical variations observed (Villafuerte et al., 2017).

The high number of outbreaks reported in P1 and P2 as well as the cases detected in both periods consecutively in 35 localities (Figure 1) suggests endemic circulation of ha-MYXV in Iberian hare populations in Spain in the last two years. This finding is consistent with the endemic occurrence of myxomatosis in wild rabbit populations in recent decades (Calvete, Estrada, Villafuerte, Osácar, & Lucientes, 2002; Villafuerte et al., 2017).

While the first confirmed outbreak was notified in July 2018 on a hunting estate in Andalusia (southern Spain) (García-Bocanegra et al., 2019), the information obtained from the areas investigated in the present study suggests that the virus could have been circulating in other Spanish regions at least one month before that, since mortality compatible with myxomatosis was observed in June 2018 in two affected areas in the provinces of Cuenca and Toledo (both central Spain).

Peak incidence was observed in summer and autumn, which is consistent with the temporal distribution of myxomatosis in wild rabbits (Calvete et al., 2002; Ferreira et al., 2009; Villafuerte et al., 2017). This temporal evolution as well as a decreasing trend in mean outbreak duration during P1 may be related to the greater abundance of competent vectors during the summer season. It has been shown that *Xenopsylla cunicularis*, a potential myxomatosis vector, is the most abundant flea species in wild rabbits in Spain, with the highest abundance index detected during the summer months (Osácar et al., 2001). Nevertheless, the high spatiotemporal dissemination of the ha-MYXV outbreaks suggests that other competent vectors may also be responsible for long-distance spread to isolated populations. In this context, the role of *Culicidae* in MYXV transmission has previously been documented (Fenner & Ratcliffe, 1965; Merchant et al., 2003; Ross & Tittensor, 1986), since some species are able to travel long distances and keep the MYXV active for long periods (Fouchet, Guitton, Marchandeu, & Pontier, 2008). High mosquito density has been shown to be a risk factor for MYXV exposure in wild rabbits in southern Spain (García-Bocanegra et al., 2010). Interestingly, ha-MYXV outbreaks were reported in the Balearic Islands in both P1 and P2. Taking into account the distance between that region and mainland Spain (more than 200 km), it seems unlikely that ha-MYXV was introduced by infected mosquitoes carried on the wind. Transportation of infected vectors by ship, aircraft or fomites, and restocking with infected hares from mainland Spain for hunting purposes are possible hypotheses for ha-MYXV introduction into these islands. In any case, since the reason for the widespread distribution of this virus remains uncertain, so that entomological surveillance programs and molecular analyses of potential competent vector species of ha-MYXV are needed to elucidate sources of transmission in the Iberian hare populations.

Clinical signs observed in the affected hares were similar to those found previously in this species during the first ha-MYXV outbreaks in Spain and Portugal (Águeda-Pinto et al., 2019; Carvalho et al., 2020; García-Bocanegra et al., 2019) and also to classic rabbit myxomatosis (Calvete et al., 2002; Rosell et al., 2019), but contrast with those reported in the European hare, in which subclinical or mild myxomatosis has been described (Barlow et al., 2014; Collins, 1955). The detection of clinical ha-MYXV infection exclusively in Iberian hares indicates apparent resistance among other hare species present in mainland Spain, which is consistent with previous reports (Barlow et al., 2014; Kerr et al., 2015). It should be noted that in some affected areas in northern Spain, the Iberian hare and European hare are sympatric species (Gortázar et al., 2007). The hypothesis about differences in ha-MYXV susceptibility between lagomorphs

is also supported by the limited number of myxomatosis cases observed in wild rabbits in the surveyed areas. Consistent with our results, ha-MYXV infections have been detected in Iberian hares but not wild rabbits also in Portugal (Carvalho et al., 2020; OIE, 2019). Nevertheless, the susceptibility of the wild rabbit to this novel virus cannot be totally ruled out, since ha-MYXV DNA was confirmed in two animals sampled during the study period. Furthermore, an ha-MYXV outbreak causing high mortality was also confirmed on a domestic rabbit farm in Murcia province (southeastern Spain) in October 2019 (MAPA, unpublished data). These findings raise questions on ha-MYXV cross-transmission between Iberian hares and European rabbits. Additional experimental and phylogenetic studies would provide valuable information about the origin and evolution of this emerging virus, as well as elucidate the direction of interspecies transmission (rabbit-hare vs. hare-rabbit).

Gamekeeper estimates of mean apparent mortality in P1 (55.4%) were very similar to the 56.7% obtained by García-Bocanegra et al. (2019) during the first outbreaks. Mortality of more than 70% was detected in 38% of the investigated areas, which is consistent with the high mortality rates observed after the introduction of MYXV in wild rabbits in Europe in the early 1950s (Fenner & Ratcliffe, 1965). The larger number of hares found dead compared to the number of sick animals observed in P1 could be associated with acute or hyperacute forms of myxomatosis in Iberian hare populations, as has been suggested previously (Carvalho et al., 2020). ha-MYXV infections were observed in individuals of different sexes and age classes. Similar ha-MYXV exposure levels between sexes in most of the affected areas have also been previously reported during the epizootics of myxomatosis in wild rabbits in Spain (Calvete et al., 2002; García-Bocanegra et al., 2010). In 53.8% of the surveyed areas, interviewed gamekeepers pointed out that more adults than juvenile hares were affected, which contrasts with the higher resistance to MYXV infection reported in adult European rabbits (Calvete et al., 2002; Fenner & Ross, 1994; Villafuerte et al., 2017). This finding could be explained, at least in part, by the absence of immunity to ha-MYXV infection in Iberian hare populations, as well as the fact that it is more difficult to find juveniles in the field than adult hares.

In conclusion, our results provide evidence of the rapid and widespread distribution of ha-MYXV in Iberian hare populations in Spain, which is of both animal health and conservation concern. The high number of outbreaks detected consecutively in P1 and P2 indicates active endemic circulation of this novel virus in this country in the last two years. The limited number of myxomatosis cases among sympatric wild rabbits, as well as the absence of outbreaks in other hare species, suggests differences in susceptibility to ha-MYXV between lagomorph species. The results obtained contribute to a better understanding of ha-MYXV emergence and provide valuable information for the development of control strategies. Risk-based surveillance programs, captivity breeding, controlled sanitary restocking, specific vaccination programs against ha-MYXV, reduced hunting pressure and elimination of hares found dead in the field, are possible measures that could help limit the circulation of ha-MYXV in Iberian hare populations. Further studies are warranted to assess

the impact of this emerging virus on the health status of wild lagomorph species and to elucidate its ecological implications for Iberian Mediterranean ecosystems.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Ethical statement is not applicable as samples were collected from dead animals or from animals legally hunted by authorized hunters with the correct permits and license and with the permission of landowners. This study did not involve purposeful killing of animals.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the authors upon reasonable request.

ORCID

Ignacio García-Bocanegra  <https://orcid.org/0000-0003-3388-2604>

Javier Caballero-Gómez  <https://orcid.org/0000-0002-6241-3439>

José Manuel Díaz-Cao  <https://orcid.org/0000-0002-8119-7057>

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