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FIRST EPIZOOTIC OF RABBIT HEMORRHAGIC DISEASE IN FREE LIVING POPULATIONS OF *ORYCTOLAGUS CUNICULUS* AT DOÑANA NATIONAL PARK, SPAIN

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ABSTRACT: The first known epizootic of rabbit hemorrhagic disease (RHD) occurred in two free-living wild rabbit (*Oryctolagus cuniculus*) populations at Doñana National Park, Spain. Rabbit population density was not correlated to RHD mortality. Only adult animals were affected; males and females had similar mortality rates.

Key words: Epizootic, mortality, rabbit hemorrhagic disease, wild rabbit, population density, radiotracking, *Oryctolagus cuniculus*.

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is a viral disease, first described in the People's Republic of China (Liu et al., 1984). The causative agent of the disease may be a calicivirus (Ohlinger and Thiel, 1991). The disease is characterized by hemorrhages located mainly in the lung, and results in degenerative and necrotic lesions in the liver. Since 1988 RHD has been reported in many European countries. In early summer 1988, Argüello et al. (1988) described the RHD from the Iberian Peninsula. The disease quickly spread across Spain. Previously unexposed populations of European rabbit (*Oryctolagus cuniculus*) had a high morbidity and mortality in the first contact with the virus (Xu, 1991).

European wild rabbits are native to the southern Iberian Peninsula. There are few references on RHD epizootiology in wild rabbit populations (Lohlinger and Eskens, 1991; Maess et al., 1989). Our objective was to describe the effects of the first epizootic of RHD on a free-living wild rabbit population in Doñana National Park, in southwest Spain.

MATERIALS AND METHODS

Doñana National Park (37°10'N, 6°23'W) occupies approximately 550 km^2 on the northern bank of the Guadalquivir River mouth. The climate is Mediterranean, with hot dry summers (August mean temperature is 25 C) and mild rainy winters (January mean temperature is 11 C, with approximately 500 mm annual rainfall).

Three main biotopes (marshes, scrub, and dunes) are present (Valverde, 1958). Two areas were selected for study: El Acebuche, an area of low rabbit density situated in the scrubland, and La Vera, an area of high rabbit density in the scrub-marsh ecotone.

From October 1988 to October 1992, we monitored the rabbit population at La Vera through capture-recapture, radiotracking, and direct observations from a tower with the use of a telescope. Rabbits in warrens were captured by ferreting, while animals remaining in the scrub were captured with gill nets (Shepherd and Williams, 1976).

Auto censuses (13 km long) were conducted three times each month at dusk on consecutive days, using the line-transect technique, and used with the program TRANSECT described by Burnham et al. (1980); we measured the sighting distances at the perpendicular distance from the line of transect to the rabbit and used these data to estimate rabbit population density.

Rabbit pellet counts (Taylor and Williams, 1956) were conducted monthly in fifty 3.14 m^2 plots in both study areas; these occurred from September 1988 to October 1992 at El Acebuche and from September 1991 to October 1992 at La Vera. In both areas data from the first month were omitted because they contained pellets >1 mo old. A *t*-test (Sokal and Rohlf, 1969) was used to compare the rabbit abundance obtained through pellet counts between La Vera and El Acebuche for the time period in which data of both sites were available.

Rabbit populations were compared before and after the onset of RHD in both areas with an analysis of variance (ANOVA) (SAS Institute, 1990). For La Vera, we used the line transect data; for El Acebuche, we used the rabbit pellet abundance data. To compare the population tendencies between both sites, a Spearman correlation (Sokal and Rohlf, 1969) was performed between the same time periods.

Fifty-five rabbits (17 adult males, 16 adult females, and 22 young rabbits) were fitted at La Vera with radiocollars with activity posture sensing (Biotrack, Wareham, England). From October 1989 to December 1990, radiocollared animals were checked daily at early morning and at night to determine their positions and activities.

Mortality rates were obtained by using the Micromort program (Heisey and Fuller, 1985). This estimation was based on calculating daily mortality rates occurring in the interval covered by radiotracking. Differential mortality between males and females due to RHD from 14 April to 30 May (first and last radiotagged rabbit found dead from RHD), was compared with the following test (Heisey and Fuller, 1985):

$$Z = \frac{M_\delta - M_\varphi}{V_\delta + V_\varphi + 2(Cov_{\delta\varphi})^{1/2}}$$

M = mean mortality; V = variance; Cov = covariance.

As part of this study, 10 ha of the La Vera site were searched daily. All ill or dead rabbits found after October 1988 were collected at Doñana National Park.

The site of each dead animal found was located on a map. Gross lesions and cause of mortality were noted for each animal. A direct hemagglutination test (HA) was used to detect RHD antigens in liver, spleen, and lung tissues of the first 55 freshly dead rabbits found in the study area (all adults), as described by Pu et al. (1985). The test, carried out in micromethod, was performed by the Regional and National Health and Animal Breeding labs (Laboratorio de Sanidad y Producción Animal, Consejería de Agricultura y Pesca, Córdoba; and Departamento de Sanidad Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid). Median titer values were calculated by the techniques of Argüello et al. (1988).

RESULTS

Deaths of wild rabbits due to RHD were reported on 14 January 12 km north of La Vera, outside of the Park. On 30 March, 16 dead animals due RHD were found 5 km north of La Vera, in the Park. We detected RHD for the first time in El Acebuche on 12 March 1990. Dates on which the first (21 April 1990) and last (23 May 1990) radiotagged animals were found dead from RHD were considered the respective first and last dates of the epizootic wave through La Vera. Forty-five animals (radio-tagged or unmarked) were found dead in the intensively surveyed 10-ha area in La Vera. The last unmarked animal found in these daily surveys was on 30 April; this was 23 days before the last radiotagged animal was found dead). About 300 dead rabbits were collected by the game officers in an undetermined area all over Doñana.

The most common gross lesions found at necropsy were poor blood coagulation, petechial hemorrhages in almost all organs, catarrhal gastritis and enteritis, hyperemia of the tracheal mucosa, and lung congestion and edema with multifocal hemorrhages. Direct HA tests in all liver, spleen and lung tissues were positive; the HA titers for these three organs were 2^{15}, 2^{13}, and 2^{14}, respectively. There was little variation in titers among each tissue.

We recognized RHD as a cause of death only in adult individuals. Neither juvenile nor subadult (<4 mo, ≤780 g) rabbits were found affected by the disease. Mean rabbit mortality rate caused by RHD was estimated as 55 ± 27% at 95% confidence limits. Mortality of adult males (51%) was not different ($Z = 1.152$; $P = 0.125$) than females (60%). After 30 April 1990, no RHD-dead rabbits were found during the daily surveys in Doñana. No other species were found dead due to any disease during the RHD epizootic; hares (*Lepus capensis*) were common at Doñana National Park.

The population indices had similar patterns at both areas, with an increase in rabbit abundance each year after rainfall in spring and early summer and a decrease during winter; this occurred in spite of the disease. El Acebuche, a low rabbit density area, had a similar decrease in rabbit abundance as that observed in La Vera, a high density area. A high correlation ($r_s = 0.8332$, $P < 0.001$) existed between rabbit abundance in both populations.

Mean (±SE) pellet counts were 13.82 ± 4.96 for El Acebuche and 28.87 ± 9.45 for La Vera; the counts were significantly different ($t = 2.75$, $P < 0.002$). Following occurrence of RHD, the mean pellet counts

and density decreased significantly (ANOVA $F_{1,41} = 21.24$; $P < 0.0001$ for El Acebuche, and $F_{1,36} = 14.31$; $P = 0.0006$ for La Vera).

DISCUSSION

The period of time over which carcasses were found at La Vera (32 days) was very similar to the duration of epizootics reported in domestic rabbits (42 days, Rossell et al., 1989). Xu et al. (1985) and Xu (1991) also reported that susceptibility to RHD was clearly influenced by age; only adult animals have been found dead.

The lesions observed in dead rabbits were similar to those reported by Marcato et al. (1991). Although Liu et al. (1984) did not find differential susceptibility between males and females, Rossell et al. (1989) reported higher mortalities in breeding domestic rabbit females (90%) than in males (70%). We found a higher mortality among wild females than males, but the differences were not significant. Wild female rabbits use warrens more intensively than males and thus may have increased contact with other individuals or carcasses. Also, the breeding period occurs during spring; pregnancy and lactation could induce an immunosuppression of does.

The delay between the appearance of RHD in El Acebuche and La Vera may have been because human movements were heavily restricted in Doñana to prevent artificial spread of the epizootic. These recommendations were better followed at the Biological Reserve (a restricted access area which includes La Vera) than in the outer parts of the National Park, where El Acebuche is located. El Acebuche is the public information point of the Park, and has a high daily visitation.

Weather and predator abundance may have affected the absence of new reports of RHD mortality at Doñana after the epizootic. High temperatures in the study areas in late spring and summer may have been unfavorable to disease maintenance or transmission; the disease is more common in times of colder temperatures (Liu et al., 1984). Stress may predispose a host to RHD (Rossell et al., 1989), but no abnormal temperatures or precipitation were noted at the time of the epizootic.

Probably due to a great reduction in rabbit density, predators were concentrating in areas like La Vera where rabbits were still abundant in the Park (P. Ferreras, pers. comm.), and so, a high predator density may make difficult the finding of sporadic deaths by researchers before the scavengers do. We believe that radiotracking was a good method to study the spread of wildlife diseases, because it allowed us to find dead rabbits only a few hours after their death. Also, since 30 April 1990, no rabbit mortality has been detected except among those rabbits which have been radiomarked.

Factors associated with incidence and maintenance of the disease in free living populations, such as possible vectors, reservoir species and asymptomatic carriers, are areas for future research at Doñana.

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