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Descriptive study of an avian pox outbreak in wild red-legged partridges (*Alectoris rufa*) in Spain

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SUMMARY

This study describes the dynamics and epidemiology of an outbreak of avian pox in free-living, red-legged partridges (*Alectoris rufa*) in southern Spain. Between March 2000 and January 2001, 115 free-living, red-legged partridges (70 juveniles, 45 adults) were captured and radio-tagged. This, together with the necropsy of 44 carcasses (10 juveniles, 34 adults) found in the study area, and the inspection of 108 shot birds (74 juveniles, 34 adults) after a hunting drive in October, permitted a close monitoring of the course of the disease. Forty-one per cent of radio-tagged juveniles but none of 45 radio-tagged adults showed pox-like lesions at the time of capture, recapture, or necropsy. At least 40% of the juveniles that survived into the hunting season, but only 2·9% of the adults inspected at the same time, showed lesions suggestive of infection with avian poxvirus. The survival of juveniles during the peak of the outbreak was much lower than that of the adults, but we found no significant differences between the survival probabilities of juvenile partridges with and without pox-like lesions. Nevertheless, some birds may have developed lesions after their capture. The occurrence and course of the disease in a managed area with intense predator control underlines the need for studies on the combined influence of diseases and predators on population dynamics. Also the need for early detection of diseases for the management of game species is emphasized.

INTRODUCTION

Avian pox is an infectious and contagious viral disease of worldwide distribution that has been reported in more than 60 species of free-living birds representing 20 families [1, 2], and especially in Galliformes [3–5]. In commercial poultry, fowlpox was a common and harmful disease, which is why routine vaccination programmes are used in many farms in Spain. Various authors attribute the decrease of the occurrence of avian pox in commercial poultry not only to the massive vaccination programmes but also to a marked increase in hygiene [6]. Nevertheless, the disease still persists in backyard chicken flocks and can spill over to wild birds. More recently, there has been concern that future changes in poultry production facilities, such as moves to free-range production, could again increase the risk of avian pox outbreaks (H. M. Hafez, personal communication). Despite the species specificity of avian poxviruses, infections in populations of wild birds could present a risk for poultry [7]. Even considering the decrease in problems caused in poultry production, avian pox is still a significant pathogen which can have serious effects on wild Galliformes [2, 8, 9].

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Although the virus is very stable in the environment, transmission in wild birds mostly depends on mosquitoes acting as mechanical vectors, or on the presence of small skin lesions that enable the virus to penetrate the skin. It has also been suggested that younger individuals are more vulnerable, and that birds that survive the infection develop immunity that lasts 12–18 months [8]. Thus, the transmission of this virus would be expected to be linked to factors which influence mosquito populations, such as temperature and rainfall, and to factors affecting the host's immunity, such as the proportion of naive individuals. The mortality rate may depend on the virulence of the strain, on the age-related susceptibility of the hosts, and on the occurrence of concurrent infections [5, 10].

There are few papers that describe avian pox outbreaks and their effect on free-living bird populations. The morbidity rates described in free-living Galliformes range from 2 to 54% [3–5, 11, 12], and the mortality rates described to date are low (0·6–1·2%) [3]. In many cases, higher mortality rates have been suspected, but a lack of adequate population data made accurate estimates impossible [5]. In experimental infections of 5- to 7-day-old domestic turkey poults with wild turkey (*Meleagris gallopavo*) poxvirus, mortalities ranged from 21 to 27% [5]. While some authors have found age-related differences in the susceptibility of Galliformes to avian pox [5, 13], others have suggested that infection rates are not related to the age or sex of the birds but rather to the sampling localities [3].

The red-legged partridge (*Alectoris rufa*) is important as a game species and as part of Mediterranean ecosystems in south-western Europe. Avian pox is common and widespread among free-living, red-legged partridges throughout the Spanish mainland (D. Fernández-de-Luco, personal communication, and authors' own data), but to date nothing is known about the relevance of the disease to the population dynamics of this species. This is the first detailed description of an outbreak of avian pox in wild partridges.

METHODS

Study area

The study was carried out in a 1000-ha private hunting estate in Medina Sidonia (Cádiz, southern Spain, 05° 58′ W, 36° 27′ N). Most of the range is devoted to intensive agriculture including large beetroot, wheat and sunflower fields. Only 5% of the surface is covered by a Termomediterranean scrub of *Pistacia lentiscus* and introduced *Opuntia ficus*. The altitude ranges from 44 to 148 m a.s.l. and the average annual rainfall for the period 1975–2000 was 674 $\pm$ 192 mm. The only permanent water is a shallow river that flows from West to East through the hunting area.

Game management practices include artificial watering and supplementary feeding with wheat from June to October, and intense predator persecution. As a consequence of this, Egyptian mongooses (*Herpestes ichneumon*) and weasels (*Mustela nivalis*) are the most common (but still scarce) carnivores in the study area, and red foxes (*Vulpes vulpes*) are extremely rare. Raptors observed during the fieldwork included most frequently Montagu's harriers (*Cyrcus pygargus*) and long-eared owls (*Asio otus*), but a number of other species were observed occasionally.

Sample size and tagging

From March 2000 to January 2001, 45 adult and 70 juvenile free-living, red-legged partridges were captured with large handheld nets and spotlights, and tagged with radio-transmitters. Additionally, 10 non-tagged juveniles and 34 adults were found dead and necropsied, and another 74 juveniles and 34 adults were inspected after a hunting drive in October. In order to obtain a random sample only captured and hunted partridges were included in the prevalence calculations [5].

Captured birds were immediately processed and released. Three or four people participated in the capture sessions, and the average process time per bird was about 15 min. Juveniles with less than 250 g weight were fitted with a 2-g tail-mounted radio-tag (Biotrack, Dorset, UK) and a wing-tag. Juveniles over 250 g and adults were fitted with 9-g necklace radio-tags (Biotrack) and ringed. Juveniles were not sexed during their capture. Family groups were captured together whenever this was possible. Juveniles captured in the same group were recorded as group mates.

Avian pox diagnosis and lesion scores

The diagnosis of avian pox was based upon histopathological features that included the presence of large intra-cytoplasmic inclusions and confirmed by

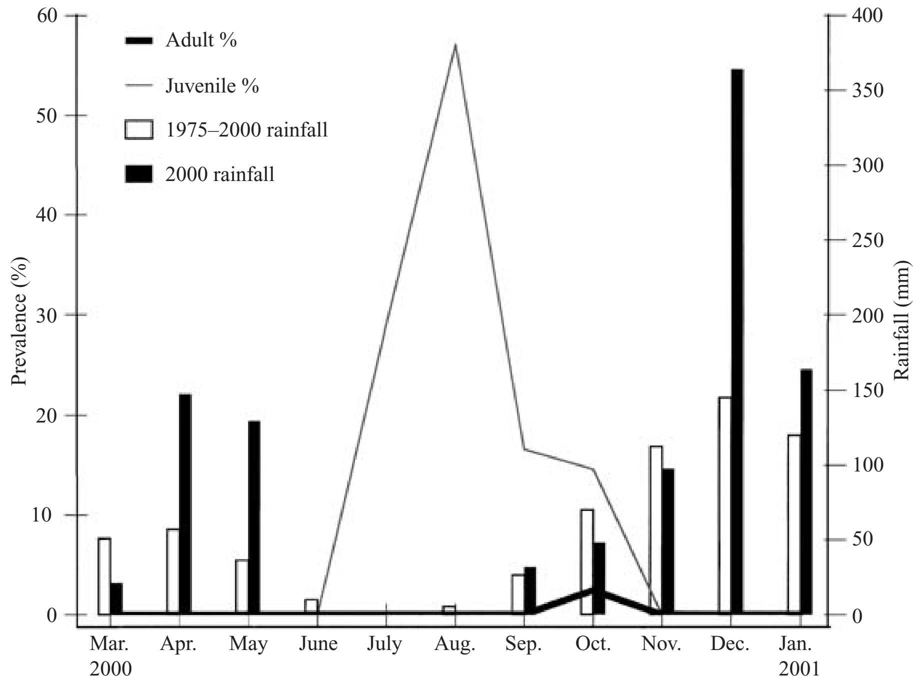

Fig. 1. Average monthly rainfall 1975–2001 (open bars), monthly rainfall in 2000 (solid bars), and percentage of red-legged partridges ($n=260$) showing pox-like lesions (lines), including all captures or recaptures ($n=152$), and the shot individuals ($n=108$) in Medina Sidonia (Cádiz, Spain).

ultrastructural examination and virus isolation [14]. The lesions ranged from single warts of less than 3 mm to severe proliferative skin lesions. The severity of the lesions, as defined in ref. [4], was graded for each captured or necropsied bird, from 1 (mild) to 3 (severe) and compared between months.

Survival analysis

Survival of radio-tagged partridges was estimated by aid of the Kaplan–Meier product limit estimate [15], and logrank tests were used to test against the null-hypothesis of lack of differences between data sets (adults *vs.* juveniles; juveniles with pox lesions *vs.* juveniles without visible lesions at capture time). Individuals shot at the beginning of the hunting season were treated as censored data. We used homogeneity tests and Kruskal–Wallis non-parametric analysis of variance where appropriate [16].

RESULTS

Timing of the outbreak

Total spring rainfall was 270 mm in 2000. This value is threefold the average (99 ± 60 mm) for this season recorded between 1975 and 2001. In fact, this was the highest spring rainfall recorded for the study area since 1975. Figure 1 shows the monthly rainfall and the percentage of birds showing lesions, including all captures or recaptures ($n=152$), and shot individuals ($n=108$). The first partridge juveniles with pox-like lesions were observed on 1 July, during the capture of birds for radio-tagging. The proportion of juveniles with lesions increased from July (30·3%) to August (60·6%, $\chi^2=4{\cdot}95$, 1 D.F., $P<0{\cdot}05$) and thereafter decreased until October. There was no difference in the average severity of the lesions described each month (Kruskal–Wallis, $\chi^2=6{\cdot}2$, 3 D.F., $P>0{\cdot}05$). After October, no bird with pox-like lesions was observed in the study area.

Morbidity data

Pox cases were aggregated in family groups more than would be expected at random ($\chi^2=26{\cdot}9$, 10 D.F., $P<0{\cdot}01$). Thus, group mates of pox-positive individuals had higher chances of becoming infected than group mates of healthy ones.

From June to September, gross lesions indicative of avian pox were observed on 29 out of 70 juvenile partridges (41·4%), either at their first capture, during subsequent recaptures, or at necropsy. The probability of detecting pox-compatible lesions was higher

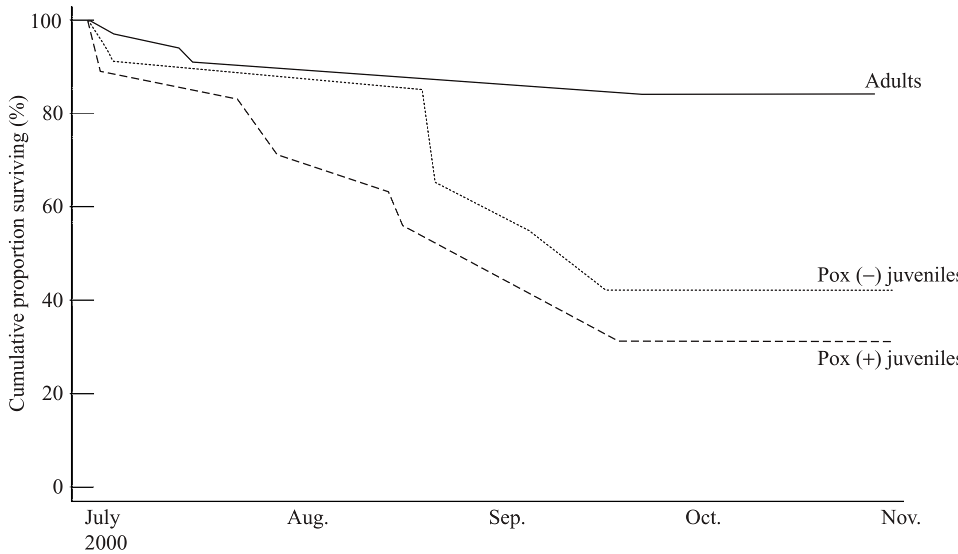

Fig. 2. Kaplan–Meier survival functions of radio-tagged adult and juvenile red-legged partridges in Medina Sidonia (Cádiz, Spain) from 1 July 2000 to 31 October 2000, during the peak of an avian pox outbreak.

($\chi^2 = 7{\cdot}9$, 1 d.f., $P < 0{\cdot}01$) in individuals that were inspected more than once between June and October (e.g. at capture and at recapture or necropsy, $n = 16$). This subsample had a prevalence of 75%. Thus, the prevalence for the total sample must be considered a minimum estimate since not all birds could be inspected at recapture or necropsied, and since early lesions may have been missed. In contrast, no radio-tagged adult inspected between March 2000 and January 2001 during capture or at necropsy showed similar pox-like lesions ($n = 45$ birds).

In the hunted sample (8 October 2000), 11 of 74 juveniles (14·9%) still had mild pox-like lesions. Nineteen additional juveniles (25·7%) showed scars that suggested healed pox lesions at this time [1]. Thus, at least 40% of those juveniles that survived into the hunting season showed evidence of contact with avian poxvirus. In contrast, only 1 out of 34 adults (2·9%) inspected at the same time showed scars. No sex-related differences in the prevalence were found in the subsample of shot juveniles (males 56%, females 75%, $\chi^2 = 1{\cdot}50$, 2 d.f., $P > 0{\cdot}05$).

From July to September 2000, eight juvenile non-tagged partridges were found dead in the study area and necropsied. Five of these (62·5%) had pox-compatible lesions. Such lesions were observed only in 1 of the 34 necropsies performed on adult partridges found dead in the study area between March 2000 and January 2001 (a non-tagged male found in October). In this case, the lesions were rated as severe and affected not only the feet and legs but also the eyelids and the ceres. An intense concomitant parasitosis (2×10^5 *Eimeria* sp. oocysts, 100 *Cheilospirura gruveli* and 200 *Trichostrongylus* sp. eggs/g of faeces) probably contributed to the death.

Partridge survival during the outbreak

Figure 2 shows the survival estimates for adult and juvenile red-legged partridges during the outbreak. Adults had a higher (WW = 7·75, test statistic = 3·38, $P < 0{\cdot}001$) survival probability (mean 83% ± 28) than juveniles radio-tracked during the same period (mean 38% ± 30). We found no differences between the survival of juveniles with (mean 31% ± 28) and without pox-compatible lesions (mean 42% ± 31, WW = −1·28, test statistic = −0·66, $P > 0{\cdot}05$). Nevertheless some of the birds classified as 'without lesions' may have acquired the disease after their capture and, if no recapture or necropsy was possible, some of those may have remained unnoticed. We did not find differences in the severity of disease (U test, $Z = 0{\cdot}27$, $P > 0{\cdot}05$) between tagged and non-tagged individuals during the inspection of the hunted birds.

DISCUSSION

To our knowledge, this is the first detailed epidemiological description of an outbreak of avian pox in free-living, red-legged partridges. Similar descriptions only exist from an outbreak among wild bobwhite quail (*Colinus virginianus*) in the Eastern United

States [3]. Radio-tracking and capturing permitted a very thorough observation of the course of the outbreak in juvenile birds [17]. Unfortunately, few adults could be examined during the peak of the outbreak, thus limiting the information on this age group. Further, due to the high number of losses, less data than expected could be collected from juveniles.

Numerous authors have suggested that the fitting of wild birds with radio-transmitters may negatively affect their survival, compared to non-tagged controls [18]. In our study, capture and manipulation of the partridges for radio-tagging could have caused stress-related immunosuppression and thus have increased the susceptibility of the birds to the disease. Nevertheless, we found no differences in the severity of the disease between tagged and non-tagged individuals during the inspection of the hunted birds.

Poxvirus infection rates may differ between sampling localities, with important differences even on adjoining properties [3]. There is no accurate data on other estates close to the study area, but cases reported by the gamekeepers were sporadic rather than massive. The peak of the outbreak coincides with presumably high mosquito densities due to the exceptionally high rainfall in spring, and with the chick season of the red-legged partridge in the area. Davidson et al. [3] reported birds with lesions from July to the end of March, which was probably related to the humid climate and well-known mosquito density in their study area.

Consistent with the literature (e.g. [5, 11, 13]) most of the birds affected in our study were juveniles. This contrasts with Pagés' statement that pox-incidence in farmed red-legged partridges is higher in adults [19]. The higher prevalence in juveniles may be related to their poorer immune competence, to the lack of acquired immunity, or to the fact that their skin is softer and less densely feathered, and thus more easily wounded or punctured by mosquitoes. Behavioural aspects could also influence the infection in juveniles, as those belonging to groups with affected individuals were more likely to develop lesions than those from groups without affected individuals. As juveniles mingle continuously during the day and huddle together during the night, transmission within family groups might be produced by mechanical contact. The probability of getting stung by the same mechanical vector also increases due to this behaviour (J. Lucientes, personal communication).

The source of the virus that caused the outbreak is still to be determined. It could (i) be enzootic, (ii) have been introduced by infected farm-bred partridges used for restocking on neighbouring estates and, (iii) result from a spill-over from infected domestic poultry. The absence of apparent disease in most adults might indicate it was endemic in the study area, being maintained as persistent infection in carriers [20]. Adult birds could have survived an infection and developed a specific immunity [8]. In order to get a clearer view of the disease dynamics molecular studies are under way to determine the origin of the poxvirus that was isolated from the observed lesions.

The low mortality found in this study is consistent with the findings of ref. [3] and the common understanding that avian pox is a mild self-limiting disease in most wild birds [1]. There was no significant relationship between survival and the presence or absence of pox-like lesions in juvenile partridges, but these results have to take into account the small sample size and the low power of the test. Moreover, early lesions may have been missed on some birds, and not all birds could be recaptured or examined after death.

On only a few occasions was the death of the affected birds related directly to pox, but more such cases may have gone unnoticed due to predation, and an adverse effect on body condition of the presence of pox lesions can be demonstrated in juvenile partridges [14]. This is consistent with the statements of other authors that indirect factors, such as increased susceptibility to predation due to depressed growth rates, debilitation and behavioural changes, may be even more important than direct mortality [5].

Predator control is very strong in the study area. This may on the one hand have reduced the removal of diseased partridges and thus have led to increased transmission. On the other hand it may have limited the effect of avian pox at the population level, since more sick birds may have been able to survive (and eventually heal) than if they had a 'normal' predation rate. This underlines the importance of studying the combined effect of predators and diseases in the regulation of natural populations [21].

In Spain, hunters commonly relate particularly wet springs with poor partridge years. The normal explanation is that rainy springs may reduce hatching of the eggs. Like Forrester [5], we speculate that avian pox also could also negatively affect game-bird populations in rainy years. In our opinion, the high pox prevalence reported here probably had an adverse effect at the population level, especially on the recruitment rate, but the lack of historical data on partridge density and productivity in the study area makes it impossible to test whether the observed mortality was additive or compensatory [22]. In any case, this outbreak may be due to very particular meteorological circumstances that do not occur every year.

The influence of diseases such as avian pox on partridge populations may require the management of hunting practices in order to maintain a stable and healthy core population. This might include regular censuses of the population, especially prior to the hunting season. Early detection of diseases, and sound data on population density, may then enable the adjustment of extraction quotas to reflect the actual situation.

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