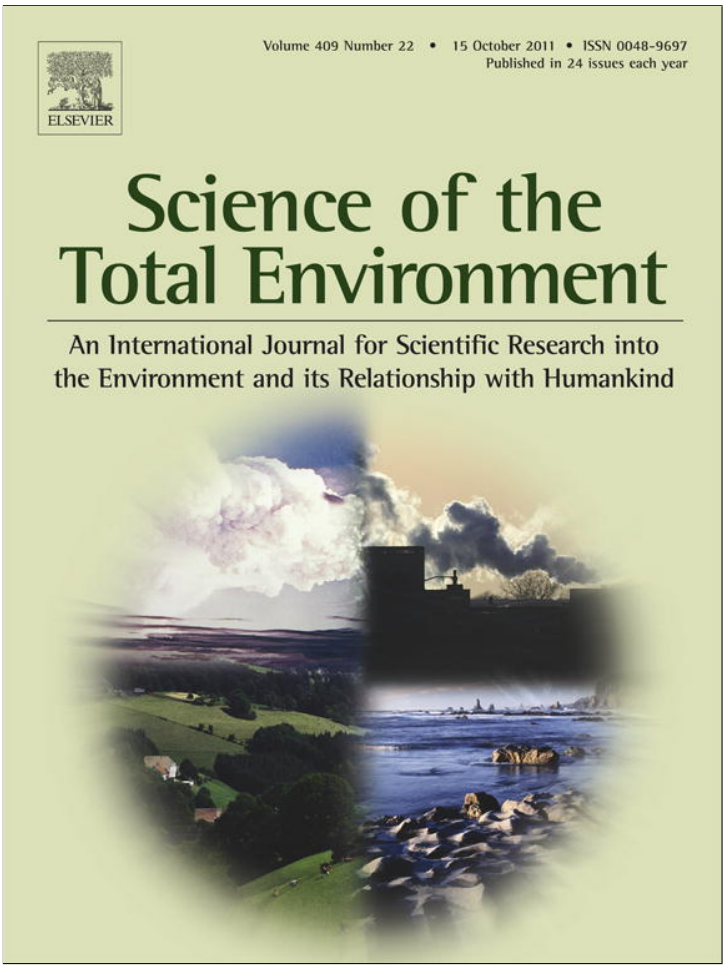

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Side effects of rodent control on non-target species: Rodenticides increase parasite and pathogen burden in great bustards

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ABSTRACT

For many years anticoagulant rodenticides have been used in vole control campaigns, in spite of the proven risk of secondary poisoning of non-target predators and scavengers. In this paper we analyse for the first time great bustard exposure and intoxication by anticoagulant rodenticides in Spain, based on residues found in the livers of 71 bustard carcasses collected during 1991–2010. Ten individuals contained chlorophacinone and one flocoumafen. Chlorophacinone level was significantly correlated with the pathogen and parasite burden of intoxicated birds. Moreover, through the last 12 years the annual number of great bustards that present chlorophacinone in liver collected in our study areas was correlated with vole peaks at a nearby area, suggesting that the ingestion of rodenticide was proportional to the amounts spread in the fields. We conclude that rodenticide consumption is a regular event among great bustards when baited cereal is spread on fields, and that this may cause chronic weakening of intoxicated individuals, possibly affecting their survival. Future rodent control actions should consider these negative side effects on non target granivorous steppe and farmland species, particularly when they are globally threatened.

1. Introduction

Anticoagulant rodenticides are used worldwide as the main tool for rodent control during plagues (Anderson and Kluge, 1986; Erickson and Urban, 2002; Olea et al., 2009). Chlorophacinone is a first-generation indandione anticoagulant rodenticide synthesised from the coumarin-based compound warfarin. First-generation rodenticides are predecessors of second-generation anticoagulant rodenticides (SGARs), such as difenacoum, bromadiolone, brodifacoum and flocoumafen, which are much more acutely toxic. The use of first-generation rodenticide baits is less strictly regulated in Spain than that of SGARs, and is permitted in extensive control campaigns due to their bioaccumulative dosage effect; SGARs have a lethal effect with a single ingested dose (Buckle et al., 1994).

Anticoagulant rodenticides have been reported as a significant risk for non-target species that may consume the toxic bait (primary poisoning) or by secondary poisoning when predators eat contaminated prey (Berny et al., 1997; Shore et al., 2003; Brakes and Smith, 2005; Sage et al., 2008; Sarabia et al., 2008; Walker et al., 2008; Olea et al., 2009; Winters et al., 2010). Chlorophacinone, despite being a first-generation rodenticide, has been shown to have secondary toxicity among raptors, owls, mustelids, and foxes (Mendenhall and Pank, 1980; Albert et al., 2010; Fournier-Chambrillon et al., 2004).

In Spain, authorised or illegal rodent control campaigns using anticoagulant rodenticides have been carried out to reduce potential crop damages, particularly during vole plagues. These actions have recently increased and extended to larger regions due to the pressure exerted by farmers on authorities. Common vole (*Microtus arvalis*) populations are widely distributed in agricultural landscapes of the region Castilla y León in the northern half of Spain (González-Esteban and Villate, 2002). They have been regularly reported as plagues, and disseminating cereal treated with chlorophacinone has been the usual way to control them (Olea et al., 2009). Spanish agricultural landscapes hold significant population of farmland and steppe birds, which at present are the most threatened bird group in Europe, with 83% of the species subject to unfavourable status (BirdLife International, 2004). Among these are great bustards (*Otis tarda*), little bustards (*Tetrax tetrax*), black-bellied sandgrouse (*Pterocles orientalis*) and Montagu's harriers (*Circus pygargus*), which may consume baited cereal grain or poisoned rodents. Rodent control campaigns are thus potentially dangerous for these non-target species. According to Olea et al. (2009), after the last outbreak of common voles declared by the regional government of Castilla y León in 2007, some campaigns of vole poisoning with chlorophacinone were carried out. Since then, poisoned individuals of some bird and mammal species (*Buteo buteo*, *Melanocorypha calandra*, *Columba livia*, *Lepus granatensis*) have been reported (Olea et al., 2009). So

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far, poisoned great bustards have not been reported in Spain. This problem is particularly relevant for long-lived species, in which adult mortality is a major factor affecting population viability, and may cause local extinctions of small populations. In these species, establishing the exact causes of mortality is fundamental to improve their conservation. However, most frequently only the proximate cause of death is determined (i.e. power line or car collisions, shot), while possible ultimate causes remain undetected.

The great bustard *O. tarda* L. is a large steppe bird inhabiting cereal farmland of the Iberian Peninsula. The species has suffered marked declines during the last decades, and today is considered Globally Threatened and qualifies as Vulnerable in the Red List of Threatened Species (IUCN, 2010). Spain holds the largest population, with 30,000–35,000 individuals (>60% of the world population, Palacín and Alonso, 2008; Alonso and Palacín, 2010). The future of this species is endangered in some Spanish regions as a consequence of habitat degradation caused by agriculture intensification and urban or infrastructure development, as well as to non-natural mortality due mainly to collisions with power lines (Palacín et al., 2004).

In this paper we investigate the impact of using the indandione chlorophacinone baits for rodent control in Spain. We examine rodenticide consumption as a possible cause of poisoning and subsequent chronic weakening or death of great bustards. We also discuss the possibility that rodenticide ingestion may affect the sensorial capacity of great bustards, and increase the probability of collision with power lines, which currently represents the main adult mortality cause in this species (authors unpubl. data), as well as its relation with the parasite and pathogen burden.

2. Material and methods

During a comprehensive study on great bustard health status, 71 dead individuals were fully necropsied. Twenty three were marked birds found during a long term radio-tracking study, 9 died some time after arrival to bird rehabilitation centres, and the rest were found dead in the field. Fifty birds were collected under power lines and had clear symptoms of having died through collision with the cables. Most carcasses collected in the wild were partially decomposed or predated when found.

All bustards were collected between 1991 and 2010 in various Spanish regions: 67.1% in Madrid, 21.4% in Castilla-la Mancha, 5.7% in Castilla y León, 2.9% in Andalucía, 1.4% in Extremadura, and 1.4% in Navarra (Fig. 1). We distinguished juvenile birds (individuals under 1 year) from adults (over 1 year). Bustards were conserved thawed at −20 °C until complete necropsy was performed. The proximate cause of death was tentatively determined, but macroscopic lesions sometimes indicated a different underlying cause. To determine whether dead bustards had accidentally ingested poisoned rodents or treated grain we analysed bustard livers, looking for the indandione anticoagulant chlorophacinone. This anticoagulant is the most commonly used to control vole plagues in the study areas (it is provided directly and freely by the Castilla y León government to farmers), either with or without authorization. Rodenticide mixed with cereal grain is spread as bait on the fields using sowing machines. The maximum authorised dose is 20 kg seeds/ha (with 0.0075% chlorophacinone), but higher dosages are probably being used as a rule in most regions. Rodent species present in the study areas include the Mediterranean pine vole (*Microtus duodecimostatus*), wood mouse (*Apodemus sylvaticus*), house mouse (*Mus musculus*), western Mediterranean mouse (*Mus spretus*) and brown rat (*Rattus norvegicus*) (Palomo et al., 2007), all of them being considered by farmers as plague when numbers are relatively high. The same baits used in the authorised vole eradication campaigns in the region Castilla y León were probably also used by farmers in the neighbour regions Madrid and Castilla-La Mancha (see Fig. 1), either for rodent eradication of locally declared plagues or as preventive campaigns.

In the first instance we decide to analyse the bustard rodenticide exposure by a multi-residue analysis of eight anticoagulant rodenticides in bustard liver by using liquid chromatography combined with heated electrospray ionisation tandem mass spectrometry following Vandenbroucke et al., 2008. Due to the lack of positive results and the vole campaigns using chlorophacinone baits, we decided to measure also the chlorophacinone levels in bustard livers.

Chlorophacinone was analysed through high performance liquid chromatography (HPLC) (Fauconnet et al., 1997), modified according to Sarabia et al. (2008). Briefly, chlorophacinone was extracted with dichloromethane from 1 g of liver. The extract was dried and resuspended with 2.5 ml of a 20:80 v/v mixture of methanol and 65 mM phosphate buffer (pH 7.6). Chlorophacinone was extracted

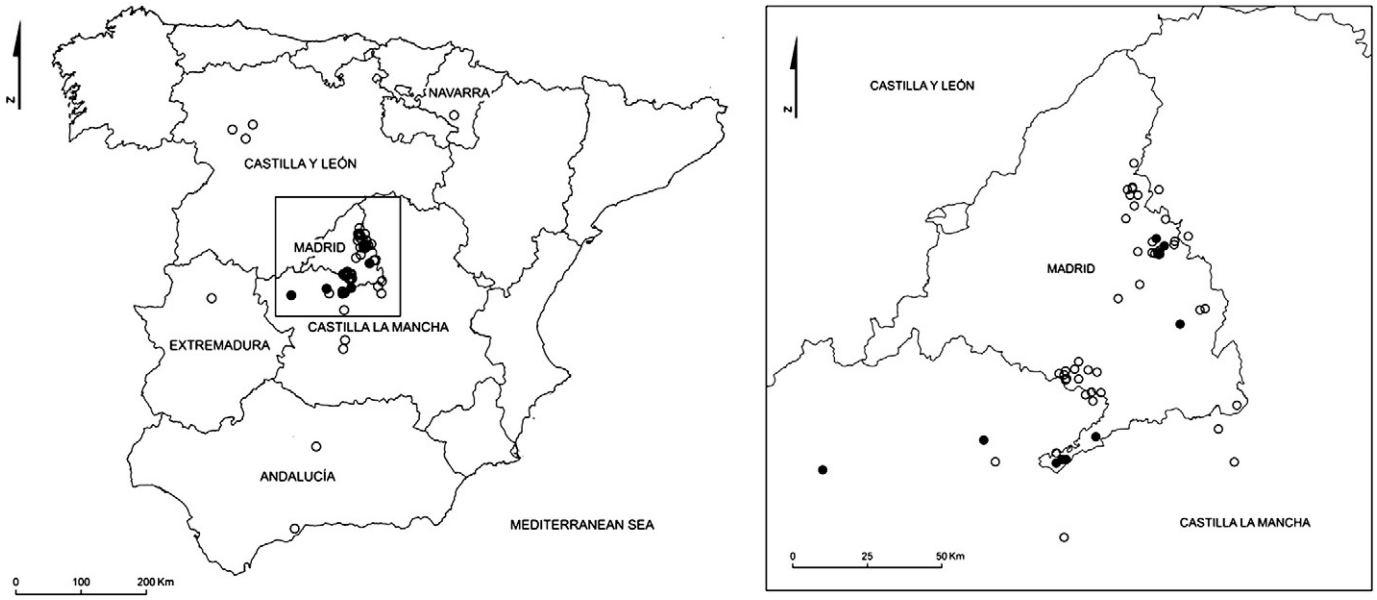

Fig. 1. Map of Spain showing the distribution of great bustards with (black dots) and without chlorophacinone residues (open dots). Borders between administrative regions are shown, and the names of the regions where bustard carcasses were collected are given.

with an Upti-Clean solid phase extraction column. The recovery obtained using this method was 61% for liver samples spiked with chlorophacinone standard.

The HPLC system consisted of an Agilent 1100 series high performance liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, an on-line degasser, an autosampler, an automatic injector, a thermostated column compartment and diode array detector. ChemStation (Agilent) software package was used to control the instrument, data acquisition and data analysis. The HPLC column was a Zorbax Eclipse XDB-C8. The flow rate used was 1 ml/min of a 55:45 v/v mixture of 65 mM phosphate buffer and acetonitrile (pH7.6). Diode array detector was monitored at 281 nm. Calibration was performed with liver spiked with 0.0125–10 μg/g of chlorophacinone. Linear correlation coefficient was 0.998. Variation coefficient of calibration slopes was 10.3%. Chlorophacinone limit of quantification was 0.080 μg/g of liver (wet weight).

In addition, flocoumafen determination was performed in a bustard suffering severe bleeding. The technique used was firstly described by Jin et al. (2007). Briefly, flocoumafen was extracted from the liver by using a solid–liquid method using ethyl acetate and warfarin as internal standard. Detection was performed on a mass spectrometer by negative electrospray ionisation in multiple reactions monitoring mode. Calibration curves were linear from 0.1 to 100 ng/g, with a detection limit of 0.05 ng/g. Relative standard deviations were less than 8.0% intraday and less than 10.8% interday. Minimal recovery was more than 83.7%.

Pathogens were surveyed by PCR as in Vogel et al. (2011), and Blanco and Lemus (2010). Parasites were surveyed from the cloaca by direct faecal smear following, or by direct intestine observation (Greiner and Ritchie, 1994; Clyde and Patton, 2001; Forbes and Fox, 2005). Once the pathogen and parasite results were obtained, we determine the pathogen and parasite burdens. These were defined as the number of pathogen or parasite species found on each bustard.

Spearman's rank correlation tests were used to examine relationships between chlorophacinone level and pathogen or parasitic burden. The relationship between cause of death (trauma/no trauma) and presence/absence of chlorophacinone was determined through Fisher's exact probability test (Siegel and Castellan, 1988). We classified the level of liver chlorophacinone into three categories: *absence* (chlorophacinone level = 0 ng/g – below detection limit – wet weight), *low* (<1000 ng/g wet weight) and *high* (>1000 ng/g wet weight), in order to analyse possible effects of the rodenticide on the number of parasites and/or parasite burden. Categories were intended to determine the possible total single rodenticide bait ingestion, the possible successive ingestions (chlorophacinone, as a first generation rodenticide is a bioaccumulative rodenticide — Nahas, 1987; USEPA, 1998; Erickson and Urban, 2002) and the kinetic behaviour of this rodenticide, i.e. excreted very fast during the first week and very slow during the next months (up to 6 months, Lechevin and Vigie, 1992). Mann–Whitney test was used to examine relationships between chlorophacinone level and parasitic and pathogen burden. All statistical analyses were performed using IBM SPSS Statistics Version 19.

Finally, we predicted that high vole abundance would determine a generalised use of rodenticides, and consequently cause higher impact on great bustards. To test this prediction, we compared annual common vole abundance in a nearby area – just 70 km northwest from our main study site in northeastern Madrid – for which data were available (Fargallo et al., 2009; J. A. Fargallo pers. com.) with annual number of chlorophacinone exposed found bustards using non-parametric Spearman correlation. There was a certain delay in vole plagues raising social alarm, as revealed by the frequency of news about this issue in different media. Therefore, we excluded the first vole abundance peak (years 1997–98) because in these years farmers were not yet alarmed by the plague and the use of rodenticides was not extended.

3. Results

The most common mortality cause identified was trauma (70.4% of the 71 great bustards, 42 of them due to collision with power lines) (Table 1). Other causes were predation, disease (hyperparasitization, aspergillosis, starvation), hunting or undetermined. Chlorophacinone residues were found in ten individuals (14.1% of the sample) that we suspect had ingested treated grain or poisoned voles, although the latter were not found in the stomachs. All poisoned individuals were from central Spain (Fig. 1). Liver chlorophacinone concentrations ranged from 82 to 3800 ng/g wet weight (w.w.) (see Table 1). In most cases (83.3%), the levels recorded were above those associated with chlorophacinone poisoning (≥200 ng/g w.w.), as is reflected by the macroscopic associated lesions (see above). The other anticoagulant residue, flocoumafen, was found at a dosage of 52.8 ng/g, which is compatible with a lethal dose.

The most common macroscopic lesions observed in great bustards suffering chronic rodenticide toxicosis ($n = 10$) were generalised haemorrhages (66.7%) with hepatomegaly (100%) (including areas of focal necrosis −83.3%-). Haemorrhages were located mainly in the thoracic area (66.7%), but also in the cervical area (33.3%), *pectoralis sternobrachialis* (50%) and *pectoralis thoracobrachialis* (33.3%) muscles, with "haemothorax" (that may also be due to collisions), and pelvic limb external haemorrhages (100%). Because bustards also present pathological signs suggesting collision, only the lesions that presumably were due to chlorophacinone intoxication were taken into account.

There was no significant association between presence of chlorophacinone and death by trauma (Fisher's test, $p = 0.38$) (Table 2). Chlorophacinone level was significantly correlated with the number of pathogens (pathogen burden; $r_S = 0.78$, $p = 0.000$) and parasites (parasite burden, $r_S = 0.47$, $p = 0.000$) (Fig. 2). Pathogen burden differed significantly among *absence*, *low* and *high* levels (post-hoc test: *absence-low* levels $U = 21.5$, $p = 0.000$; *absence-high* levels $U = 0.000$, $p = 0.000$; *low-high* $U = 6.00$, $p = 0.01$), while for parasite burden this difference was only significant between the *absence* and *low-high* (post hoc test: *absence-low* $U = 54.0$, $p = 0.003$; *absence-high* $U = 6.00$, $p = 0.001$), but not between *low* and *high* (post hoc test: $U = 4.00$, $p = 0.074$) (see Fig. 2).

Finally, yearly abundances of common voles at an area close to our main study site in northeastern Madrid were significantly correlated with the frequency of chlorophacinone exposed bustards in our sample (one-tailed $r_S = 0.553$, $p = 0.031$; Fig. 3).

4. Discussion

Our study reports on the first documented great bustard exposed to rodenticides in Spain. The results demonstrated an increase in parasite and pathogen burden of intoxicated individuals, and raise concern about possible effects on the health or even survival of the birds. High parasite loads have been shown to affect reproductive rates and survival of several bird species (Moller, 1990; Lehmann, 1993; Brown et al., 1995). Since the great bustard is a globally threatened species, and steppe birds are one of the most endangered bird groups worldwide (BirdLife, 2004), these results are particularly relevant, and should be taken into account by conservation authorities. The only published record about intoxicated great bustards reported high mortality rates after rodent control in the Caspian region during the 1960s, and suggested this might have contributed to local great bustard population decreases (Belik, 1997).

Great bustards are omnivorous, and both treated seeds and poisoned rodents may potentially form part of their diet. Rodent consumption has not been observed in Iberian great bustards, but has been reported for central European populations (Glutz et al., 1973; Cramp and Simmons, 1980). Spanish great bustards were thus probably intoxicated through consumption of treated cereal baits,

Table 1
Details about parasites and pathogens in chlorophacinone exposed and flocoumafen intoxicated bustards, and comparison with non-intoxicated individuals.

Cause of death	N	Sex	Age	Year of death	Pathogens found	Parasites found	Liver chlorophacinone level (ng/g wet weight)	Total pathogen burden	Total parasite burden
Power line collision	1	Female	Juvenile	2007	*PAST*, *MYCOP*	*HAEM*, *LEUC*, *OTID*	239	2	3
	1	Female	Adult	2007	*ASP*, *CHLAM*.	*LEUC*, *PLAS*, *OTID*	395	3	3
	1	Male	Adult	2003	*MYCOP*, *CHLAM*, *GUM*, *PARA*	*LEUC*, *OTID*	448	4	2
	1	Male	Juvenile	2007	*CHLAM*, *POX*, *WEST*	*HAEM*, *LEUC*, *PLAS*, *OTID*, *IDIO*	126	3	5
	1	Male	Adult	2007	*ECOLI*, *PAST*, *CHLAM*, *GUM*, *PARA*, *POX*, *WEST*.	*HAEM*, *LEUC*, *OTID*, *CAPI*	2948	7	4
	1	Male	Adult	2010	*ASP*, *PAST*, *PSEU*, *CHLAM*, *GUM*, *PARA*, *POX*, *WEST*	*HAEM*, *LEUC*, *OTID*, *IDIO*	2295	8	4
	1	Female	Juvenile	2010	*ASP*, *ECOLI*, *WEST*	*OTID*, *IDIO*	449	3	2
	1	Male	Adult	2008	*ECOLI*, *PAST*, *CHLAM*, *GUM*, *PARA*, *POX*, *WEST*	*HAEM*, *LEUC*, *PLAS*, *OTID*	2376	7	4
Undetermined	1	Female	Adult	1999	*ASP*, *ECOLI*, *CHLAM*, *POX*, *WEST*	*HAEM*, *PLAS*, *OTID*	88	5	3
Poisoning	1	Female	Adult	2004	*SALM*,*ECOLI*, *CHLAM*, *GUM*, *PARA*, *POX*, *WEST*	*HAEM*, *PLAS*, *OTID*, *CAPI*	1196	7	4
Other collisioned bustards	42						–	2.53 ± 1.31	1.84 ± 0.76
Non-collisioned bustards	19						–	1.95 ± 1.27	1.52 ± 1.02

Fungi: *ASP*: *Aspergillus fumigatus*.
Bacteria: *ECOLI*: *Escherichia coli* enterotoxigenic strain, *SALM*: *Salmonella sp*, *PAST*: *Pasterella multocida*, *PSEU*: *Pseudomonas aeruginosa*, *CHLAM*: *Chlamydophila psittaci*, *MYCOP*: *Mycoplasma sp*.
Viruses: *GUM*: *Gumboro disease virus*, *PARA*: *Paramyxovirus*, *POX*: *poxvirus*, *WEST*: *West Nile virus*.
Haemoparasites: *HAEM*: *Haemoproteus* spp *(telfordi*, *tendeiroi)*, *LEUC*: *Leucocytozoon* spp (*Otidis*), *PLAS*: *Plasmodium* spp.
Helminths: OTID: *Otiditaenia conoides*, IDIO: Idiogenes CAPI: *Capillaria* sp.

since cereal seeds are common in their diet (Lane et al., 1999, unpublished data). We do not discard occasional ingestion of poisoned rodents, which might have shown increased susceptibility to predation due to their altered behaviour (Cox and Smith, 1992).

The chlorophacinone is bioaccumulative, being excreted at a very slow rate from the liver (Lechevin and Vigie, 1992). Our results show that the pathogen and parasite burdens increased in intoxicated individuals, probably affecting the health status. In particular, the parasite *Otiditania conoides* appeared in huge quantities in great bustard faeces and intestines (Illescas-Gómez and Gómez, 1980, 1981; García-Montijano et al., 2002). The interaction and intensification of the toxic effects of chlorophacinone with pathogens have been recently described in Vidal et al. (2009), who found that toxic concentrations were lower in voles showing *Francisella tularensis*. Lower doses may thus provoke high effects in bustards. The pathogen burden of bustards increased with the mere presence of chlorophacinone, independently of the dosage. However, the increase was more marked above chlorophacinone levels of 500 ng/g. The influence of chlorophacinone on parasites is also clear, but compared to pathogens, the rodenticide level needs to be higher to cause a significant increase in parasite burden.

The link between presence of chlorophacinone and cause of death is unclear, but some researchers point out that although rodenticide concentration was not enough to kill specimens, it was enough to decrease their sensorial capacity and make them prone to suffer accidents (Albert et al., 2010). In our case, the relationship between presence of chlorophacinone and a traumatic death did not reach statistical significance. However, our limited sample still suggests that chlorophacinone exposed bustards might be slightly more susceptible to accidents (mostly collision with power lines). Accidents caused four times more deaths than other causes among exposed birds, compared to only 2.2 times more in non-exposed birds. Although the sample size of exposed birds is small, results support this evidence.

Table 2
Numbers of great bustards for which a death cause was determined, in relation to presence or absence of chlorophacinone residues in their carcasses. † Fisher's exact probability test.

	Cause of death		Total	p †
	Trauma	Others		
Absence of chlorophacinone	42	19	61	0.380
Presence of chlorophacinone	8	2	10	
Total	50	21	71	

The persistence of chlorophacinone in large birds is unknown. The only published results are from poultry species (Riedel et al., 1990), and their kinetic behaviour differs from that of bustards (Bailey et al., 1998a,b). We have not found poisoned voles or treated grain in the gizzards of chlorophacinone exposed bustards, so the rodenticide ingestion time remains unknown. However, according to the concentrations obtained, there are both, *low* levels (<1000 ng/g wet weight) and *high* levels (>1000 ng/g wet weight), which suggests that chlorophacinone has different liver clearance, showing a fast period and a slow one (Lechevin and Vigie, 1992). However it is not possible to be sure that the concentration differences measured in the liver are not a consequence of difference of ingested quantity.

As for flocoumafen, this rodenticide is nowadays one of the most commonly used rodenticides in agricultural areas (it is freely X to farmers by the local government), together with the so-called second generation rodenticides, such as difenacoum, bromadiolone, or brodifacoum. They were introduced between 1975 and 1985 to replace warfarin, to which rats had become resistant (Cowan et al., 1995). The second generation anticoagulant rodenticides are approximately 100–1000 times more toxic than warfarin and other first-generation compounds, a single feeding bout of bait being sufficient to kill a rodent (Nahas, 1987; USEPA, 1998). They also have long biological half-lives in tissues such as the liver (Eason et al., 1996; Huckle et al., 1989; Parmar et al., 1987). Both attributes enhance their potential to cause secondary poisoning in predators. Rodents usually die several days after consuming bait containing second-generation compounds, and thus can be captured and eaten by a predator during this time. Scavengers may also feed on carcasses of poisoned animals. Because of their foraging habits, all predators could potentially be exposed to second-generation rodenticides by eating contaminated prey, and could also die after a single ingestion. This is the most challenging and serious disadvantage of the second-generation rodenticides compared to the first-generation ones. Massive poisoning with first-generation rodenticides is still relatively harmless when compared with the large scale use of the more persistent second-generation rodenticides, which imply a higher risk of secondary

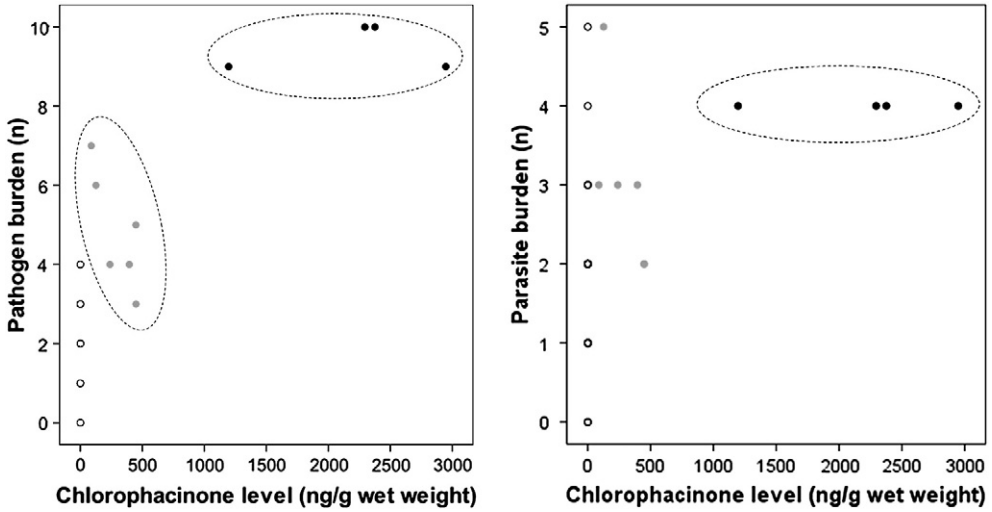

Fig. 2. Relationship between pathogen burden, parasite burden and chlorophacinone level in rodenticide intoxicated bustards. Black dots correspond to chlorophacinone high level (>1000 ng/g wet weight), grey dots to chlorophacinone low level (<1000 ng/g wet weight) and open dots to non-intoxicated bustards. Intoxicated bustards with high level (continuous line ellipse) had a significantly higher pathogen burden than intoxicated bustards with low level (dotted line ellipse) and non-intoxicated bustards. There was no significant difference in the parasitic burden between bustards with high and low level of chlorophacinone.

poisoning for scavengers and predators (Berny et al., 1997; Walker et al., 2008; Sage et al., 2008).

There was a clear correlation between the curve reflecting presence of chlorophacinone in dead bustards through the last 10 years and vole peaks at an area of Castilla y León where rodenticide use was allowed, just 70 km northwest from our main study site in northeastern Madrid (Fargallo et al., 2009). Because we know, through our long-term radio-tracking project, that none of the chlorophacinone exposed individuals radio-tagged in Madrid (30%) visited the treated areas of Castilla y León, we can be reasonably sure that these birds ingested the rodenticide in Madrid.

This suggests that it was the social alarm caused by vole plagues reported from the neighbour region Castilla y León that prompted farmers from Madrid, and probably also Castilla La Mancha, to spread chlorophacinone baits on their fields as a preventive measure. Common voles are much scarcer in Madrid and Castilla La Mancha where most of our bustards were found, but plagues of this or similar rodent species might have also occurred in these two regions. The amount of rodenticide spread in Madrid and Castilla La Mancha was probably either proportional to the incidence of the plague reported for Castilla y León, or to real demographic cycles of local rodent species similar to those of common voles. This was most probably the cause of the correlation found, which suggests that ingestion of rodenticide by the bustards was proportional to the amounts spread in the fields.

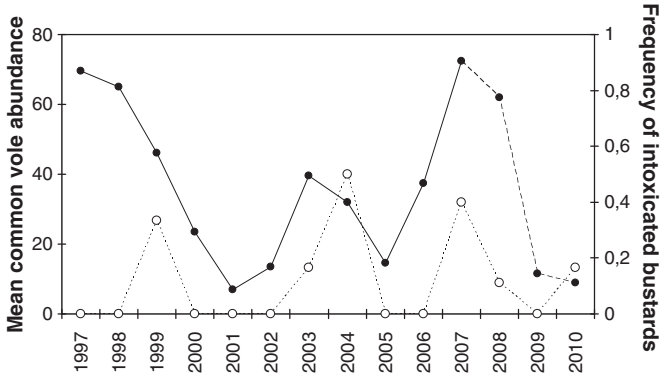

Fig. 3. Annual variation in abundance of common vole (*Microtus arvalis*) in Campo Azálvaro (Castilla y León region) (black dots; from Fargallo et al., 2009; data for 2008–2010, J. A.Fargallo, pers. com), and frequency of chlorophacinone exposed bustards in our sample (open dots).

The benefits of extensive rodenticide application have been seriously debated because there is always a risk for non-target species (Berny et al., 1997; Shore et al., 2003; Fournier-Chambrillon et al., 2004; Olea et al., 2009; Vidal et al., 2009). Our results raise high concern about the negative side effects of rodenticide use for granivorous species. This extends the impact of rodenticides reported previously for predators and scavengers, and suggests that poisoned baits of cereal seeds should only be used when they can be conveniently monitored in order to avoid negative side effects on granivorous non-target species. Moreover, an official authorisation should be required to use these products in plague areas, and their use out of these areas should be banned and punished. In sum, authorities should cautiously balance all positive and negative consequences of rodenticide use when these actions may affect any carnivorous, scavenger and also granivorous non-target species, particularly if these are threatened.

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