Environmental Pollution **82** (1993) 201–205

ORGANOCHLORINE AND HEAVY METAL CONTAMINATION IN NON-VIABLE EGGS AND ITS RELATION TO BREEDING SUCCESS IN A SPANISH POPULATION OF LESSER KESTRELS (*Falco naumanni*)

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(Received 15 May 1992; accepted 19 August 1992)

Abstract

*Residues of organochlorines, polychlorinated biphenyls (PCBs), and heavy metals (mercury, cadmium, lead, copper and zinc) were measured in unhatched eggs of Lesser Kestrels (*Falco naumanni*) collected in southern Spain in 1988–1991. Although contaminants were detected in all eggs, the amounts were generally below levels known to have negative effects on reproduction. This is consistent with the relatively high hatching rate (about 80%) in the studied population. The nestling mortality was severe, however, apparently due to starvation. It cannot be discounted that pesticides had an indirect effect on the kestrel's breeding success by reducing the populations of prey.*

INTRODUCTION

The Lesser Kestrel (*Falco naumanni*) is a small colonial falcon which has experienced a dramatic reduction in numbers in the last three decades. Once considered one of the most common raptor species in Europe (Bijleveld, 1974), it is now seriously endangered (Cramp & Simmons, 1980; Biber, 1990). It has been suggested that agricultural pesticides are the cause of this population decline (Garzón, 1977; Cramp & Simmons, 1980). Bijlsma *et al.* (1988) reported that the low hatching rate in the Lesser Kestrel colonies that they studied might be related to pesticides. However, to the authors' knowledge, no study on organochlorine and/or heavy metal residue levels in the species has been published.

This paper reports on organochlorine and heavy metal contents in deserted or addled Lesser Kestrel eggs collected in the low Guadalquivir river valley. This is an intensively cultivated area (Fernández Alés *et al.*, 1992) where the use of agricultural chemicals is widespread. Additionally, some selected breeding parameters in the colonies where the eggs were collected have been monitored. In the presence of heavy poisoning a low hatching rate due to infertility or eggshell breakage would be expected (Newton, 1979).

Environ. Pollut. 0269-7491/93/$06.00

STUDY AREA AND METHODS

Breeding success

The study was carried out in 12 Lesser Kestrel colonies in Sevilla province (southern Spain) and covered four breeding seasons (1988–1991). The study area, of about 10 000 km^2, is quite homogenous (Fernández Alés *et al.*, 1992). It is a mixed farmland area—the lower part of the Guadalquivir valley—intensively cultivated with cereals, sunflowers, olive trees and irrigated fields. The climate is typically Mediterranean, with a temperate rainy winter, which allows the wintering of some Lesser Kestrels (Negro *et al.*, 1991), and a hot, rainless summer (Font, 1983). The main prey of the Lesser Kestrels in the area are beetles in winter and grasshoppers in summer (Franco & Andrada, 1977). The kestrels hunt them mainly in grassy field margins and in cereal crops (Negro, 1991).

Three colonies (those located in the villages of Arahal, Mairena del Alcor and Morón) were surveyed every year, four colonies (Clavinque, Santo Domingo, Caracol and Lebrija) were visited during two breeding seasons, and five additional colonies (Carmona, Marchenilla, Castellar, Manzanilla and Sevilla) were visited only during one breeding season. All the nests in the colonies were located and were visited at least twice to determine brood size and to ring individually the young and some adults. In order to avoid disturbance during incubation, the authors visited only a selected number of nests to determine clutch size. In estimating clutch size, clutches of one egg, probably abandoned before clutch completion and which never hatched, were excluded. Eggs that failed to hatch remained in the nest, so eggs disappearing near hatch were assumed

to have produced chicks which died and went undetected (the same assumption was made for Common Kestrels (*Falco tinnunculus*) in Cavé (1968) and Village (1986). The colonies were searched for dead nestlings after ringing, so the estimation of the number of fledglings must be near the true figure.

The eggs for contaminant analyses (41 for heavy metals and 32 for organochlorines) were collected in July, at the end of the reproductive season, when it was apparent that those eggs were not going to hatch. Collecting eggs at random during the incubation period was deliberately avoided because the authors did not want to reduce the breeding success of the birds, which are strictly protected in Spain.

To find whether the levels of each particular contaminant affected hatching success, those clutches in which at least one egg had been analysed were considered. For each variable, two groups of clutches—below and above the median value—were compared. When more than one egg was analyzerd per clutch, the mean value for the clutch was used. In the case of DDE, two critical values were considered: 5 ppm, a value for which slight eggshell thinning has been noted in several raptors (Kiff *et al.*, 1979; Coon, 1983; Wiemeyer *et al.*, 1984), and 10 ppm, which caused 30% thinning in eggshells of American Kestrels (*Falco sparverius*) (Peakall *et al.*, 1973). The levels of the rest of the organochlorine compounds resulted to be very low (see Table 1) and no critical value has been considered.

Analytical methods

The conventional analytical procedures for the determination of organochlorine contaminants involve solvent extraction and clean-up of the extract with liquid partitioning and adsorption chromatography. The analysis of samples was carried out essentially as previously described (Tessari & Savage, 1980). Thus, the samples (5 g) were dispersed in anhydrous sodium sulphate and the mixture extracted with hexane–acetone. Organochlorine compounds were isolated by a liquid–liquid partitioning process that consisted of dissolving the extract in 15 ml of hexane and extracting four times with 30 ml of acetonitrile saturated with hexane. Four phases were further combined. Aqueous dilution of the acetonitrile extract served to back partition the organochlorine compounds into hexane. The organic phase was then purified chromatographically on a Florisil column; clean-up was achieved using 25% diethyl ether in hexane as eluate. The eluate was evaporated to dryness, diluted accordingly with hexane, and an aliquot analysed by gas chromatography.

Gas chromatography analysis was done on a Hewlett-Packard 5890 equipped with Ni 63 Electron Capture Detector. A 30-m-long capillary column covered with RSL-200 was used. Chromatographic conditions were as follows: detector 280°C; injector 300°C; temperature programme, isothermal phases at 180°C (1 min) and 250°C (30 min), with intermediate temperature increase rate of 2°C min^{-1}. Quantification was done comparing the peak areas in the sample with those in corresponding standards. Polychlorinated biphenyls (PCBs) were quantified by summation of peak areas from seven major peaks in Aroclor 1260. Recoveries of organochlorine compounds ranged from 89 to 101%. The detection limit was 1 $\mu g\ kg^{-1}$. Levels were expressed in $\mu g\ g^{-1}$, wet weight.

For heavy metals (cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn)) 0·5 g of sample was accurately weighed into a Teflon cup and 4 ml of 8 M nitric acid was added. The cup was covered and put on a hot plate at 90°C for 2 h. The final solution was filtered through Whatman paper. The filtering was carried out to a volume of 10 ml with distilled water and used for Cd, Pb, Cu and Zn determination by atomic absorption

Table 1. Organochlorine and heavy metal residue levels (ppm, wet weight) in Lesser Kestrel eggs collected in southern Spain, 1988–1991

	Number of eggs	Mean	Geometric mean	SD	Range	CV
αHCH	32	0·000 7	0·000 6	0·001	ND–0·007	142·9
βHCH	18	0·111	0·033	0·244	ND–0·942	201·8
γHCH	32	0·005	0·002	0·008	ND–0·034	160·0
Dieldrin	14	0·002	0·001	0·008	ND–0·034	390·0
Heptachlor epoxide	32	0·055	0·013	0·198	ND–1·137	360·0
DDE	32	2·088	0·658	3·904	0·046–19·773	187·0
TDE	14	0·007	0·002	0·016	ND–0·061	228·6
DBF	32	0·007	0·002	0·012	ND–0·058	171·4
DDT	32	0·014	0·001	0·062	ND–0·351	442·9
ΣDDT[a]	32	2·109	0·684	3·904	0·066–19·783	185·1
ΣIO[b]	32	2·514	0·757	4·756	0·069–19·896	189·2
PCBs	32	0·231	0·148	0·297	0·019–1·510	128·6
Hg	41	0·379	0·275	0·717	0·110–4·830	189·2
Cd	41	0·710	0·673	0·236	0·380–1·120	33·2
Pb	41	0·892	0·103	0·931	ND–2·78	104·4
Cu	41	3·073	1·859	2·478	0·18–7·13	80·6
Zn	41	12·319	11·602	4·067	4·17–21·81	33·0

[a] ΣDDT = DDE + DBF + DDT (excluding TDE).
[b] Summation of insecticidal organochlorines (excluding βHCH, Dieldrin and TDE).

Table 2. Breeding parameters of Lesser Kestrels in southern Spain (mean ± SD), 1988–1991[a]

Parameters	1988	1989	1990	1991	All years	KW[b]
Clutch size	3·94 ± 0·62 (19)	4·08 ± 0·89 (87)	4·11 ± 0·76 (26)	4·2 ± 0·81 (70)	4·11 ± 0·82 (202)	$P = 0·43$ NS
Egg mortality	0·84 ± 0·80 (13)	0·68 ± 0·60 (16)	0·61 ± 0·65 (13)	0·80 ± 0·15 (51)	0·76 ± 1·24 (93)	$P = 0·30$ NS
Nestling mortality	0·85 ± 1·15 (42)	1·69 ± 0·78 (26)	1·33 ± 1·17 (13)	0·57 ± 0·75 (14)	1·17 ± 1·11 (133)	$P = 0·003$ **
Fledged young	2·16 ± 1·37 (112)	1·53 ± 1·24 (126)	1·80 ± 1·17 (125)	2·31 ± 1·51 (141)	1·95 ± 1·37 (504)	$P = 0·000$ ***

[a]The numbers in parentheses are the numbers of pairs.
[b]KW—Kruskal–Wallis test (for differences between individual years); NS—not significant; *$P < 0·05$; **$P < 0·001$; ***$P < 0·001$.

(Haseltine *et al.*, 1981). Hg was measured by flameless atomic absorption using a mercury hydride system. The lower limit of reportable residues was 0·02 $\mu g\ g^{-1}$. Recoveries of heavy metals ranged from 87 to 96%. Levels were expressed in $\mu g\ g^{-1}$ wet weight.

RESULTS

All the eggs analysed contained detectable residues of organochlorine compounds (Table 1). In general, these levels were very low (< 1 ppm in 37·5% of the samples), although five eggs (15·6%) contained levels above 5 ppm. In one egg, the level was 19·8 ppm (19·7 ppm of DDE). Hexachlorocyclohexanes were detected in 81·2% of the eggs, although at low levels. Heptaclor epoxide and DDE appeared in all the analysed eggs. DDE appeared in the highest concentrations. PCBs were also present in all eggs, but apart from one sample (1·5 ppm), always below 1 ppm. To calculate the summation of insecticidal organochlorines (ΣIO), βHCH, dieldrin and TDE, were excluded as these compounds were analysed in a limited number of eggs (see Table 1).

Heavy metals appeared in all the samples analysed (Table 1). Zinc showed the highest values, followed by Cu, Cd, Pb and Hg. The levels of Hg were low, even though one sample had 4 ppm. Pb was detected in 29 out of 41 eggs (70·7%). In eight eggs (19·5%) Pb concentration was above 2 ppm. Cd was detected in all the samples, but only seven (17·1%) eggs had more than 1 ppm.

Breeding parameters

Clutches of two to five eggs were recorded, with a frequency distribution of eight clutches of two (4%), 34 clutches of three (16·8%), 86 clutches of four (42·6%) and 74 clutches of five (36·6%). The mean clutch size was 4·11 eggs (SD = 0·82, n = 202). There were no significant differences in clutch size from year to year (Table 2). The percentage of unhatched eggs, about 20% including deserted clutches, was nearly constant between years. Eggshell breakage as an obvious reason for hatching failure was never observed. The authors did not observe either significant differences in hatching rates between clutches above the median and clutches below the median values of the different heavy metals (Table 3). In the case of DDE, the only organochlorine residue at significant levels, no differences in the hatchability of clutches below and above 5 ppm were observed. Additionally, one egg had more than 10 ppm of DDE, and belonged to a clutch of two non-viable eggs. Nesting mortality varied significantly between years (Kruskal–Wallis test, $P < 0·01$) and ranged from 0·57 nestlings per laying pair in 1991 to 1·69 in 1989 (Table 2). Accordingly, differences in mean breeding success between years were significant (Kruskal–Wallis test, $P < 0·01$), and ranged from 1·53 to 2·31 fledglings per laying pair. Considering all monitored breeding attempts (n = 504), breeding success was 1·95 fledglings per laying pair (SD = 1·37).

Of 139 nestlings found dead, 125 (89·9%) apparently have died from starvation. These losses were attributed to starvation because the young which were found freshly dead were underweight for their age, they did not show external infections or parasites, and they were usually the youngest in their broods. Predation by Barn Owls (*Tyto alba*) accounted for six nestlings (4·3%), one nestling (0·7%) suffered cannibalism by an adult Lesser Kestrel and seven (5%) showed infections and traumatisms.

Table 3. Comparison (χ^2 test) of hatching rates between clutches below and above the median value of DDE and heavy metals

Residues	DDE		PCB		Mercury		Lead		Cadmium		Copper		Zinc	
	Without	With 5 ppm	Without	With 0·13 ppm	Without	With 0·26 ppm	Without	With 0·5 ppm	Without	With 0·59 ppm	Without	With 2·13 ppm	Without	With 12·2 ppm
Eggs hatched (%)	36·8	43·8	44·1	32·4	42·9	33·3	38·5	40·5	42·3	36·7	31·4	40·3	37·1	38·5
Number of eggs	38	16	34	37	49	33	52	30	26	60	35	62	35	52
Probability	0·865 NS[a]		0·442 NS		0·386 NS		0·922 NS		0·801 NS		0·514 NS		0·920 NS	

[a] NS—not significant ($P > 0·05$).

DISCUSSION

The levels of contaminants detected may be an overestimation of the true figure in the Spanish population, as non-viable eggs were analysed. In any case, the detected levels can be considered relatively low. Considering that the area of study is heavily treated with agricultural chemicals, even lower levels of contamination would be expected in other parts of Spain.

In most eggs, organochlorine residues were below levels known to have adverse effects on survival or reproduction in birds (Stickel *et al.*, 1966; Hernández *et al.*, 1986; González & Hiraldo, 1988; Stendell *et al.*, 1988). Nonetheless, as in any other bird population (Newton, 1979), a few individuals contained very much higher residues than the rest. The study area is quite homogenous (see Introduction) in land-use and possibly in intensity of chemical treatments. The variability detected could therefore be related to differences in the African wintering area. In Alaskan Peregrines (*Falco peregrinus*), for example, it was demonstrated that high pesticide levels were due to ingestion of contaminated prey in Central America during the winter (Fyfe *et al.*, 1976). Furthermore, the Lesser Kestrels studied here were partially migratory (Negro *et al.*, 1991). About 20% of the adults stay at the colonies year round, while the rest of the adults and all the young migrate to Africa. These migrants could be inhabiting areas with different pollutant levels.

The ranges in the levels of heavy metals were narrower than in the organochlorines, except the Hg, in which, however, all samples except one were below 0·5 ppm. Pheasants' eggs containing 0·5–1·5 ppm of Hg had significantly lower hatchability than controls (Fimreite, 1971), but, even taking into account possible interspecific differences (Fimreite *et al.*, 1971), it is unlikely that the Lesser Kestrel eggs were adversely affected by their Hg content. For the rest of the heavy metals, no information relating residue levels and effects on the eggs has been found. Th heavy metals analysed appeared at similar levels to those reported in other predatory birds in Spain (Hernández *et al.*, 1986, 1988; González & Hiraldo, 1988). Nonetheless, the levels of Pb were, as expected, lower than those found in carrion-eating raptors, which sometimes swallow lead-shot (González & Hiraldo, 1988).

The hatching rate of the studied population (about 80%, considering addled eggs and deserted clutches) can be considered high and is even higher than that of other species of similar size which are not considered to be seriously affected by pollutants at present (Sparrowhawk (*Accipiter nisus*) 60%, Newton, 1986; European Kestrel, 64%, Village, 1990). In fact, the hatching rate of a Lesser Kestrel population in Austria in the prepesticides era (66%, Bernhauer in Cramp & Simmons, 1980) was lower than in this study. Additionally, 62% of the eggs laid hatched in the Lesser Kestrel colonies studied by Bijlsma *et al.* (1988). These authors argued that this low hatching success was due to pesticide contamination, although they did not give data on residues in egg contents. These results could be human-induced, however, because the investigators visited the colonies repeatedly during the incubation period and handled most of the eggs to take measurements. It is known that frequent visits to bird colonies can depress breeding success (Tremblay & Ellison, 1979).

In conclusion, the hatching rate of the population studied here was not severely affected by contamination. It cannot be discounted, however, that the pollutants had an indirect negative effect on breeding success. Nestling mortality (35%) was very high in comparison to other raptors (see the review of Newton, 1979) and seemed to be due to starvation. This could be caused, among other possible reasons, by a sudden reduction of prey after chemical treatments. The dead prey (mainly insects) would not be consumed by the kestrels, so that bioaccumulation of pesticides possibly would not be a serious problem for the adult Lesser Kestrels. On the other hand, the pesticides could also indirectly affect the kestrels by maintaining prey populations at low levels throughout the breeding season.

ACKNOWLEDGEMENTS

M. de la Riva helped to collect the eggs. A. Krupa revised the English. Two anonymous referees made valuable suggestions. The study was supported by the CICYT, project no. PB 90-1021. One of the authors (J.J.N.) was in receipt of an FPI grant from the Ministry of Education.

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