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The causes of the mortality of eggs and nestlings of *Passer* spp.

J PINOWSKI, M BARKOWSKA, A H KRUSZEWICZ and
A G KRUSZEWICZ
Department of Vertebrate Ecology, Institute of Ecology PAS, 05-092 Lomianki, near Warsaw, Poland

Abstract. In the world-wide literature the role of predators and food shortage are considered as responsible for mortality of eggs and nestlings. In synantropic altricial hole-nestling birds such as sparrows *Passer domesticus* (L.) and *Passer montanus* (L.) the predation plays unimportant role and in spite of this, mortality of eggs and nestlings can exceeded 50%. The role of microorganisms, heavy metals, pesticides and food shortage were investigated as possible causes of embryo and nestling deaths. About 70% of eggs that not hatched were infested with such pathogens as *Escherichia coil, Staphylococcus epidermitis* and several, more rarely occurring others. Considerable percentage of nestlings died due to pathogenic impact of such factors as *Escherichia coli, Isospora lacazei, Candida* spp., heavy metals and pesticides. As one effect of such interaction, the level of sublethal doses of heavy metals and pesticides are much lower that reported in the literature.

Keywords. Causes of mortality; eggs; nestlings; *Passer* spp.

1. Introduction

Reproduction of the house sparrow [*Passer domesticus* (L.)] and tree sparrow [*Passer montanus* (L.)] was studied in different climatic zones of the world as a part of the International Biological Programme (1965-1974). This study showed that the number of young which were raised per female was determined to a large degree by mortality of embryos and nestlings (Dyer *et al* 1977). Predators and insufficient food supply could not be a sufficient explanation of this mortality, and we therefore turned our attention to diseases and toxic substances as factors determining the death of embryos and nestlings (Pinowski *et al* 1988; Kozlowski *et al* 1991a, b; Malyszko *et al* 1991; Pawiak *et al* 1991).

The goal of this paper is to present results on the influence and especially the interaction of microorganisms, heavy metals and pesticides on the development and mortality of house sparrow and tree sparrow eggs and nestlings. It is obvious that under natural conditions, these factors do not act independently.

2. Study area

The study was carried out in 1986-1989 in the villages and suburban zone of Warsaw and in three parks, Lazienki Park, Saxon Garden and the Zoological Garden, located in the center of Warsaw. A detailed description of the parks is given by Luniak (1981), Luniak *et al* (1986), and of villages and the suburban zone by Pinowski (1967, 1968) and Kot (1988).

3. Materials and methods

In each area, sparrows nested in Sokolowski type A nest boxes (Jablonski *et al* 1983). The nest boxes were checked every four days during laying and incubation and every day during the nestling period. Eggs that did not hatch were removed three days after the other eggs hatched and were examined under a dissecting microscope to see if they were fertilized. Also, the presence of pathogenic microorganisms (bacteria, fungi) was recorded along with the content of heavy metals. At daily intervals nestlings were weighted with a Pesola balance to the nearest 0·5 g. Nestlings were individually marked. Normally growing nestlings were etherized at an age of 13 days, prior to fledgling. Abnormally growing nestlings were etherized at an earlier age, when a decline in their body weight indicated that they might die soon. Normally growing nestlings were taken from the same nest boxes as abnormal nestlings as controls. Control nestlings were also collected from nest boxes with dead nestling. These categories of nestlings were examined individually for disease symptoms, load of pathogenic microorganism, content of heavy metals in their liver, protein and fat in their body, and pesticides in their fat. A total of 319 nestlings of house sparrow and 302 tree sparrow were analysed.

Specific bacteriological methodology and chemical analyses for heavy metals and pesticides are described in Karolewski *et al* (1991), Kozlowski *et al* (1991a, b), Malyszko *et al* (1991), Pawiak *et al* (1991) and Romanowski *et al* (1991).

4. Results

4.1 *Mortalily of eggs and nestlings*

Eggs mortality in both study areas was about 30% for house sparrow and about 25% for tree sparrow. Nestlings mortality ranged from 24 to 30% for both species (figures 1 and 2).

4.2 *Pathogenic agents of embryonic mortality*

From a total of 90 house sparrow eggs and 23 tree sparrow eggs, 67 and 74% respectively, were found to be infected with microorganisms. Rods of the Enterobacteriaceae family were prevalent in both house and tree sparrows broods and constituted 59% of the entire isolated microflora. Within the Enterobacteriaceae, *Escherichia coli* predominated in both sparrow species. The Micrococcaceae and the Streptococcaceae were also abundant. Strains isolated from eggs and dead embryos exhibit high proteolitic and lipolitic activities. Haemolytic activity was found for 32% strains of the isolated microorganism (Kozlowski *et al* 1991a; table 1).

Our research suggested that microorganisms in free-living birds were a possible important factor influencing eggs mortality in the wild.

4.3 *Pathogenic agents of nestlings mortality*

In suburban areas, the portion of house sparrow nestlings infected with pathogenic microorganisms in the group of dead or sick individuals was 64% which was

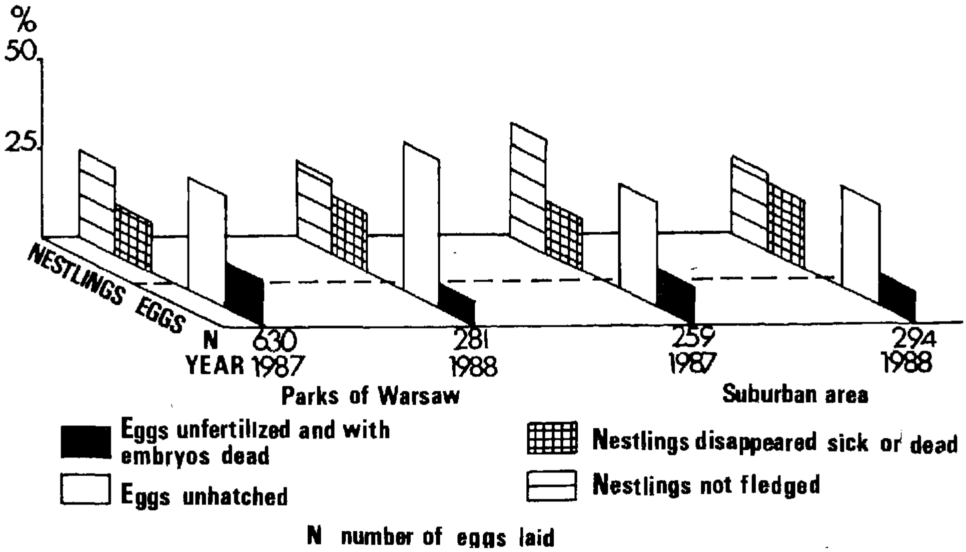

Figure 1. Mortality of embryos and nestlings of house sparrows (*P. domesticus*). (Reprinted with kind permission of the Deutsche Ornithologen Gesellschaft, from Pinowski *et al* 1988.)

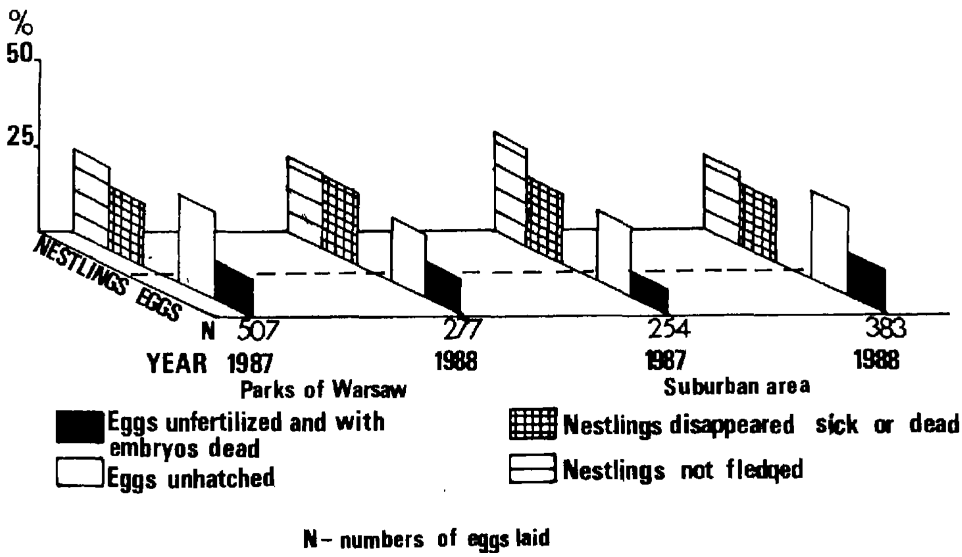

Figure 2. Mortality of embryos and nestlings of tree sparrows (*P. montanus*). (Reprinted with kind permission of the Deutsche Ornithologen Gesellschaft, from Pinowski *et al* 1988.)

significantly higher (t = 11·3; p < 0·0001) than in the group of nestlings without disease symptoms (normal growth rate, but carrying pathogens), where it was only 11% (figure 3). In suburban areas, similar data for tree sparrow are respectively 87% for nestlings infected with pathogenic microorganisms in the group of dead

Table 1. Species of microorganisms isolated from eggs and dead embryos of *P. domesticus* and *P. montanus* from Warsaw. Their enzymatic activity is also indicated (Kozlowski *et al* 1991a).

	Passer domesticus			*Passer montanus*		
Microorganism Family/Species	No. of strains	Activity proteolytic	lipolytic	No. of strains	Activity proteolytic	lipolytic
Escherichia coli	22	22	0	3	3	0
E. vulneris	2	2	0	1	1	1
E. fegusoni	1	1	0	0	0	0
Hafnia alvei	2	2	0	1	1	0
Kluyvera ascorbata	2	1	0	0	0	0
Citrobacter freundi	1	1	1	0	0	0
C. diversus	1	0	0	0	0	0
Enterobacteriaceae						
Edwardsiella ictaluri	2	1	1	1	0	0
Providencia rettgeri	1	1	0	1	1	0
P. stuarti	1	1	0	1	1	0
Proteus vulgaris	1	1	1	0	0	0
Enterobacter intermedius	1	1	0	0	0	0
E. aerogenes	1	1	1	0	0	0
Serratia plymuthica	1	1	0	1	1	0
S. fonticola	0	0	0	1	1	1
Bacillaceae						
Bacillus subtilis	1	1	0	0	0	0
Micrococcaceae						
Staphylococcus aureus	1	1	1	0	0	0
S. epidermidis	11	0	6	4	0	2
Streptococcaceae						
Streptococcus faecalis	6	4	1	2	2	1
S. thermophilus	2	2	0	1	1	0
Cryptococcaceae						
Candida albicans	1	1	0	0	0	0
Total	61	45 73·8%	12 19·6%	17	12 70·6%	5 29·4%

or sick individuals and 2·2% for nestlings without disease symptoms. Similar data for both species were obtained for nestlings from the parks of Warsaw (Pinowski *et al* 1988).

Among microorganisms which influence development and mortality of nestlings, significant roles were played by *E. coli* pathogenic strains, yeast-like fungi of the genus *Candida* and protozoans of the *Isospora* and *Entamoeba* groups. From house sparrow nestlings (n = 273), 63 strains of *E. coli* pathogenic to birds have been isolated. From tree sparrow nestlings (n = 207), 45 strains pathogenic to birds have been also isolated (Kozlowski *et al* 1991b) (table 2). Nestlings of house sparrows were more often parasitized by *E. coli* (pathogenic strains), yeast-like fungi, *Isospora* and *Entamoeba* spp. than were nestlings of tree sparrows. Presumably this was a consequence of closer contacts of house sparrows than tree sparrows with man and

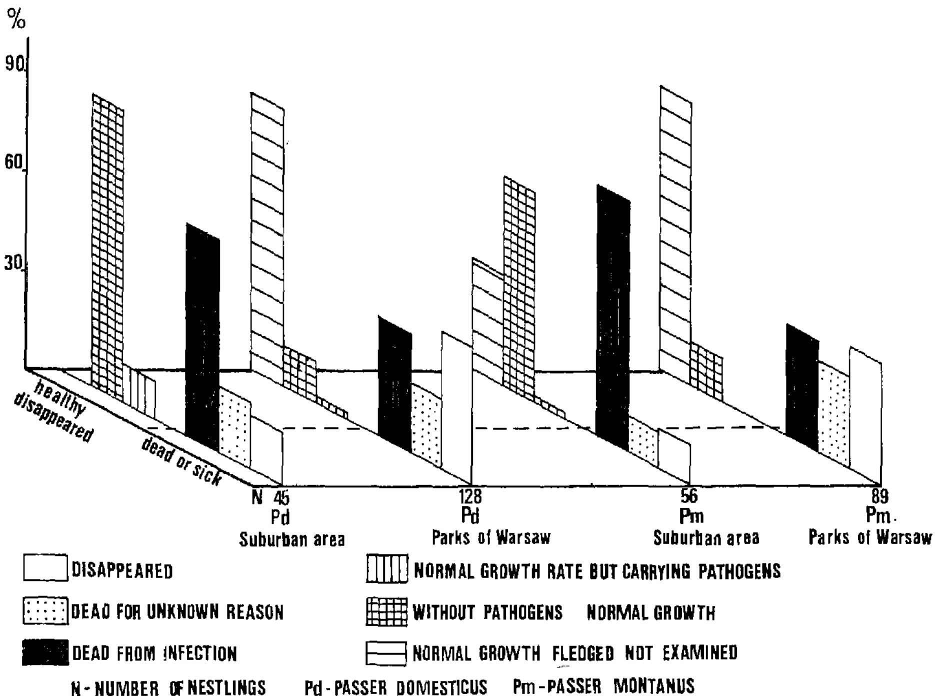

Figure 3. Diseases as causes of mortality in nestling *P. domesticus* and *P. montanus.* (Reprinted with kind permission of the Deutsche Otnithologen Gesellschaft, from Pinowski *et al* 1988.)

Table 2. The occurrence of *Coccidia, Entamoeba, E. coli* and yeast-like fungi in sparrow nestlings in Warsaw (Kozlowski *et al* 1991b).

	Passer domesticus			*Passer montanus*		
	Nestlings			Nestlings		
Microorganisms	Studied	Infected	%	Studied	Infected	%
Escherichia coli (pathogenic serotypes)	237	63	26·6	207	45	21·7
Yeast-like fungi	237	55	23·2	207	18	8·7
Coccidia	237	32	13·5	207	15	7·3
Entamoeba sp.	237	23	9·7	207	8	3·9

Statistic differences *P. domesticus* against *P. montanus:* for *E. coli*—χ^2 = 2·842; df = 2; *P* = 0·24; for yeast-like fungi-χ^2 = 34·182; df = 2; *P* < 0·001; for *Coccidia*—χ^2 = 9·221; df = 2; *P* < 0·009;for Entamoeba–χ^2 = 11·711 df = 2; *P* < 0·003.

domestic animals. All the pathogenic units mentioned above, except for *Isospora*, originate from man or domestic animals (Pawiak *et al* 1991).

4.4 *Influence of pesticides*

The suggestion that chlorinated hydrocarbons could account for the death of a certain number of nestlings is supported by the analysis of lipids extracted from the whole-body–the lipid basis, The highest insecticide (chlorinated hydrocarbons) levels for house sparrow and tree sparrow were 210 and 3050 ug.g^{-1} (lipid basis). These high values must have affected the health of nestlings (Karolewski *et al* 1991, Pinowski *et al* 1994a; figure 4),

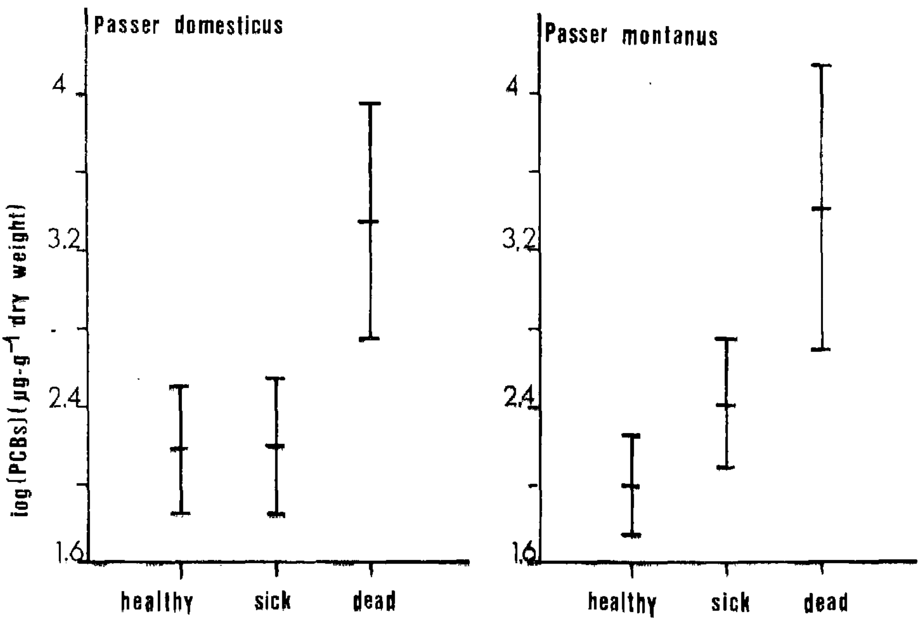

Figure 4. mean values of the logarithm of the concentration PCBs In the healthy, sick anti dead nestlings (Pinowskl *et al* 1994a). ANOVA: *P. domesticus*–F = 6·159; P < 0·002; *P. montanus*–F = 7·416; P <0·007,

4.5 *Influence of heavy metals*

A significantly larger proportion of dead birds was identified among house sparrows and tree sparrows nestlings in which the lead level was greater than 2 µg·g^{-1} of dry matter of the liver (pinowski *et al* 1994b). Significant relationships were not found between the concentration of cadmium in the liver and *the* percentage of bead, sick and healthy nestlings for both sparrow species (Pinowski *et al* 1994b),

4.6 *Interaction between lead, cadmium and bacteria*

The influence of interaction, between lead and bacteria from the Enterobacteriaceae group (primarily *E. coli*), on growth of weight of nestlings was identified, The mortality of the nestling was significantly higher, than would have been expected because of the additive effect of these several factors (Pinowski *et al* 1994c; figures 5 and 6).

Weight gains were observed to be negatively influenced by lead only for nestlings in which Enterobacteriaceae were identified in the intestines. This effect disappear

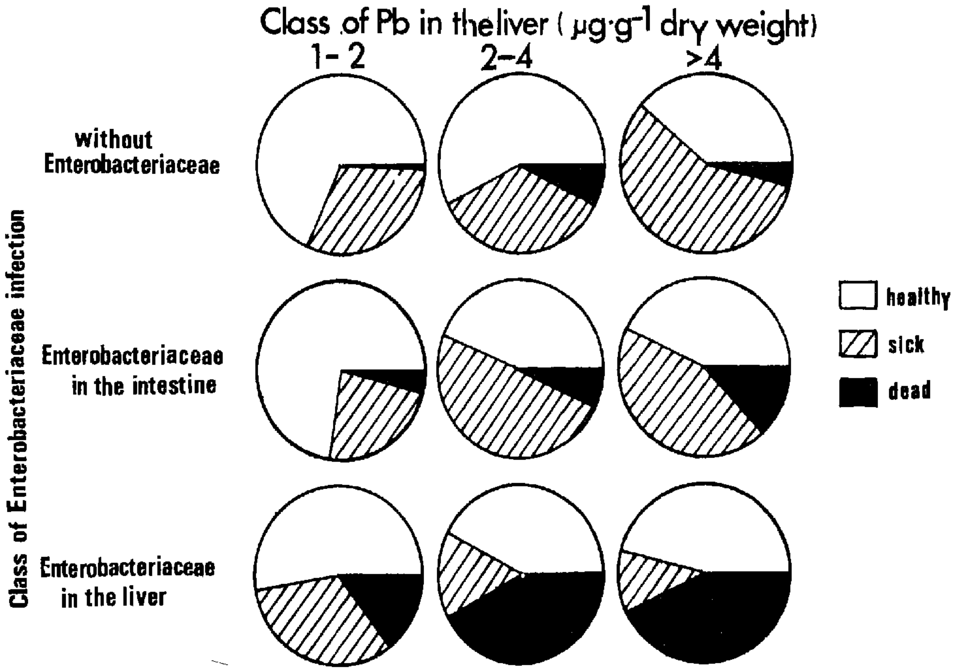

Figure 5. Healthy, sick and dead nestlings grouped according to varying concentration of lead (Pb) in the liver and level of Enterobacteriaceae infection of *P. domesticus* (Pinowski *et al* 1994c). χ^2 test for 3 category: Interaction-χ = 19·84; d.f. = 8; P < 0·01,

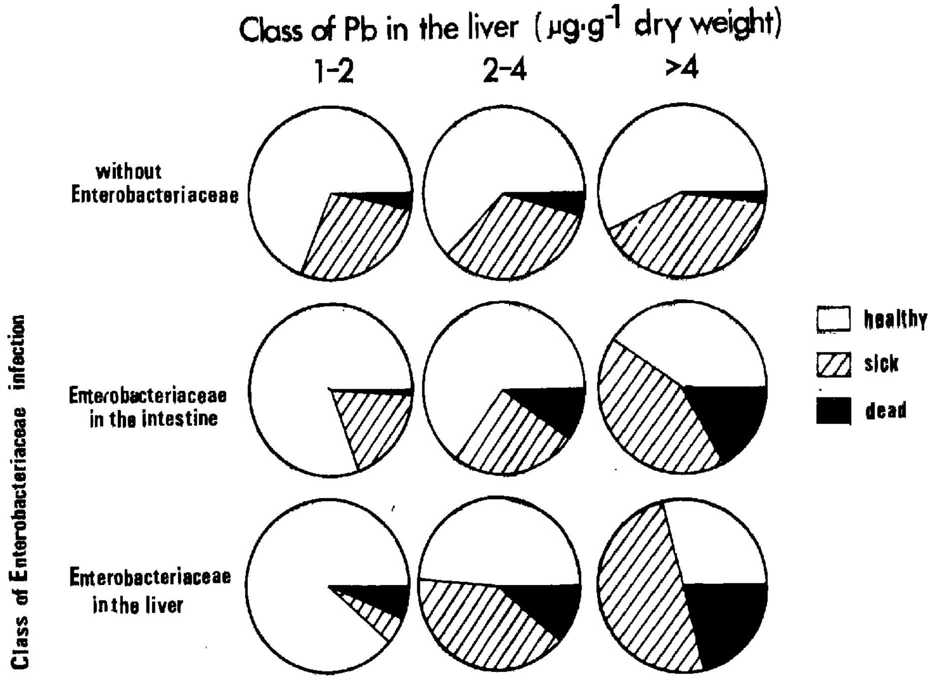

Figure 6. Healthy, sick and dead nestlings grouped according to varying concentration of lead (Pb) in the liver and level of Enterobacteriaceae infection of *P. montanus* (Pinowski *et al* 1994e). χ^2 test for 3 category: Interaction–χ = 17·810; d.f. = 8; P < 0·03,

when Enterobacteriaceae were found in the liver. At this time we can not explain this. A similar phenomena was observed in both sparrow species (figure 7).

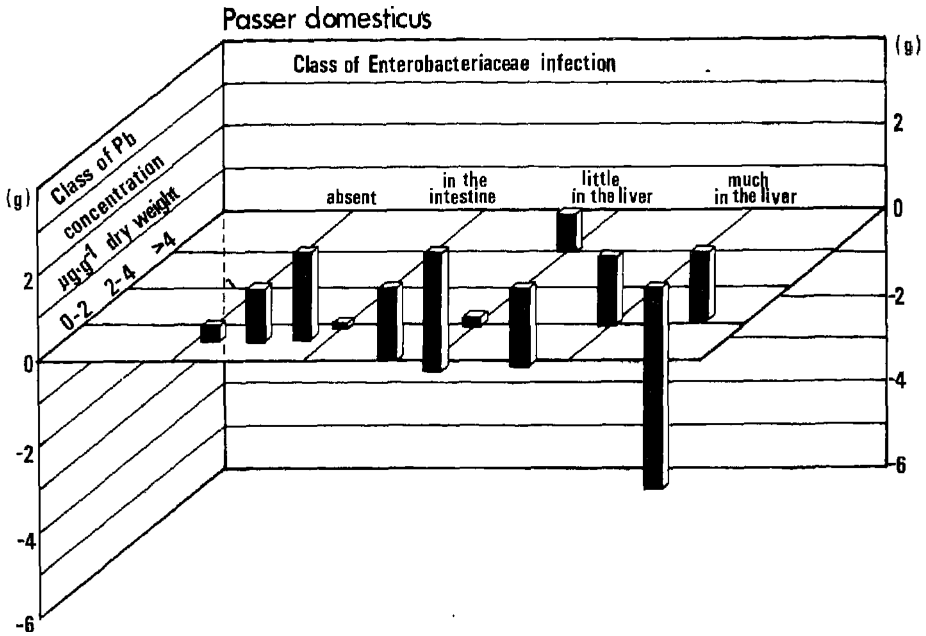

Figure 7. Mean deviations of the weight of nestlings on their final day of life from the value derived from the model curve in grouped with varying concentration of lead (Pb) in the liver and level of Enterobacteriaceae infection; *P. domesticus* nestlings which not dead. Two-way ANOVA:
Main effects–F = 4·195 $P < 0·001$; Effect of Enterobacteriaceac–F = 1·302, NS; Effect of Pb–F = 8·206; $P < 0·001$; Interaction–F = 1·803; NS.

The influence of cadmium on the development of nestlings was measured by the angle between the tangent to the Richards curve at the inflection point and time axis (see methods). The average values of this angle decreases as the concentration of cadmium in the liver increases by only in nestling in which Enterobacteriaceae were not identified in the liver. For birds in which Enterobacteriaceae were found in the liver these regularities were not observed (figure 8).

5. Discussion

There are numerous studies reviewing of the occurrence of microorganisms in poultry eggs or in other captive birds in aviary situations (Romanoff and Romanoff 1949, 1972; Harry 1957; Board 1966; Board and Fuller 1974; Bird 1981; Bruce and Drysdale 1983; Burley and Vadehra 1989). Little is known, however, about microorganisms harboured in eggs of free-living birds and their effect on embryo mortality (Sery and Strauss, 1960; Seviour *et al* 1972; Bernard 1989). Dead embryos and unfertilized eggs of house sparrow and tree sparrow nestlings in natural conditions in Warsaw and surrounding suburban areas contained microorganisms of

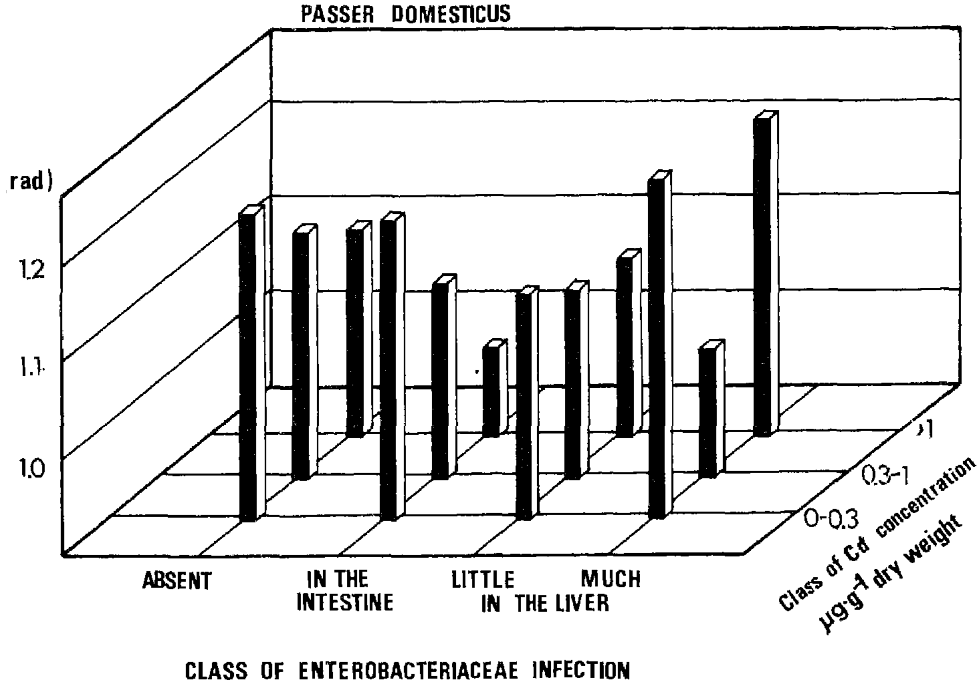

Figure 8 Mean angle between the tangent to the Richards' curve at its inflections point and time axis in groups of varying concentrations point and time axis in groups of varying concentrations of cadmium (Cd) in the liver and varying level of Enterobacteriaceae infection; *P. domesticus* nestlings which not dead.
Two-way ANOVA:
Main effect–F = 3·730; P< 0·003; Effect of Enterobacteriaceae–F = 0·471, NS; Effect of Cd–F = 8·288; P < 0·0003; Interaction–F = 2·613; P < 0·02.

the Enterobacteriaceae, Micrococcaceae, Streptococcaceae, Bacillaceae and Crypto-coccaceae. In dead embryos of both sparrow species *E. coli* predominated. Our research pointed to microorganisms in free-living birds as a possible important factor influencing embryos mortality in the wild (Kozlowski *et al* 1991a). The intestinal microorganisms and blood parasites of free-living adults are known for many species, however little is known about microorganisms of young birds especially about altricial nestlings and their influence on development and mortality (Box 1967; Kucera 1977).

According to our studies on nestlings of house sparrow and tree sparrow pathogenic strains of *E. coil*, yeast-like fungi as *Candida albicans* and C. *tropicalis, Isospora lacczzei* and other microorganisms can inhibit the development of nestlings and causes their death (Pinowski *et al* 1988; Kozlowski *et al* 1991a,b; Kruszewicz 1991; Malyszko *et al* 1991; Pawiak *et al* 1991).

In the literature there are only four papers analysing the effect of lead and cadmium on nestlings of altricial birds under natural conditions (Grue *et al* 1984, 1986; Nyholm 1989; Nyholm *et al* 1991). An experimental study on nestings of *Falco sparverius* has shown that lead concentrations in the liver greater than 2 $\mu g.g^{-1}$ (wet weight) may be associated with growth impairment, while greater than 5 $\mu g.g^{-1}$ (wet wt.) may be associated with impaired survival (Hoffman *et al* 1985).

The results of our study show that even relatively low lead (2 $\mu g.g^{-1}$) and cadmium (1 (dry wt.) concentrations in the liver can impair the development of altricial nestlings. Similar results we obtained for polychlorinated biphenyls.

6. Conclusions

(i) Microorganisms in free-living altricial birds are important factor influencing embryos and nestling mortality in the wild.
(ii) Relatively low lead and cadmium concentration in the liver can impair the development of altricial nestlings.

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