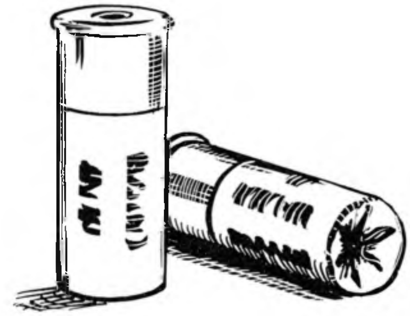
Methods of investigating the presence of ingested lead shot in waterfowl gizzards: an improved visual technique



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Waterfowl ingest spent lead gunshot whilst feeding in shot-over wetlands. The evaluation of the degree of exposure to shot has been based on examining the gizzards of birds, killed by hunters, for the presence of ingested shot. Several techniques have been employed previously for this purpose (including simple visual examination and x-ray) with varying degrees of success. In this paper we report on an improved visual examination technique and discuss the relative merits of different approaches.

For more than a century, poisoning of waterfowl through the ingestion of spent lead gunshot has been recognised as a mortality factor (Grinnell 1894). Waterfowl ingest shot along with grit and food. Ingested shot is often retained in the gizzard along with grit, and is rapidly mechanically eroded and dissolved by the stomach acids. The toxic salts formed are absorbed into the blood and poisoning occurs. Although the degree of poisoning depends upon a wide range of biological and ecological factors, estimates of annual waterfowl mortality from lead poisoning in the USA, before the introduction of non-toxic shot, were of the order of several million birds (USFWS 1986). Concern about this problem resulted in the introduction of the first bans on the use of lead shot in certain areas of the USA in the 1970s. By the 1992 hunting season, the use of lead shot for hunting waterfowl was banned throughout the USA, and replaced by non-toxic steel shot.

Increased awareness of lead poisoning of waterfowl in Europe in recent years has resulted in the introduction of statutory or voluntary bans on its use for hunting waterfowl. Denmark, the Netherlands and Norway have imposed statutory restrictions, and a number of other countries, including the UK, are evaluating the situation and the most appropriate implementation methods.

In other countries, particularly in southern Europe, research programmes have been initiated recently to investigate the severity and distribution of the problem. The most frequently used method of evaluating waterfowl exposure to lead shot is through the analysis of gizzards of birds shot by hunters for the presence of ingested shot. Several techniques have previously been employed for this purpose: x-ray (or fluoroscopy) of intact gizzards; x-ray of gizzard contents; simple visual examination of gizzard contents. Of these techniques, x-ray of gizzard contents has proved the most reliable, with the other two methods underestimating the number of gizzards containing shot (Montalbano & Hines 1978). However, the use of x-rays is not always possible for financial and logistical reasons (e.g. in field situations). In this paper we report on an improved visual examination technique. This method is compared with those previously used and the relative merits of different approaches are discussed.

Methods

Ninety-eight gizzards were collected from waterfowl shot in the Evros Delta, Greece, during the winters of 1989 and 1990 (see Pain & Handrinos 1990). Gizzards were cut in half and the contents washed into a dish with a water stream. Plant material and other food items were removed by flotation, and carefully inspected for any grit and shot, which was retained in the dish. The characteristics of ingested grit were recorded. The remaining gizzard contents were examined in the three following ways: 1) the contents of each gizzard were placed into a plastic petri dish (@ 7 cm diameter) and x-ray photographs were taken using a Ray-omix at 40 Kv for 0.15 seconds; 2) gizzard contents were examined visually with no optical aids (i.e. simple visual examination) by two observers and the presence of shot or lead fragments recorded - one of the observers (A) had previous experience of identifying shot, the other (B) did not; 3) gizzard contents were thinly spread out onto a multi-compartmental rectangular plastic tray, with a surface area of 11 cm^2; sections of the tray were scanned systematically under a binocular microscope (x4-x16 magnification) by both observers independently and randomly. The presence of shot was noted, and verified where possible by scratching the surface with a scalpel (lead is soft and easily cut revealing a shiny grey/silver surface.

Results

Number of correctly identified samples

Using the x-ray method, 20% of samples were found to contain shot. This was assumed to represent the actual number of samples with shot, as x-rays have previously been established as the most accurate technique (Montalbano & Hines 1978). When samples were examined using a microscope, 100% and 90% of the samples containing shot (as established by x-ray) were correctly identified by observers A and B respectively (Table 1). However, several false positive results were recorded (Table 2). Observer B reported 3 of 4 false positive results as small lead fragments rather than discrete shot.

With simple visual examination, both observers correctly identified 80% of samples containing shot (Table 1). Four false negative results were recorded by both observers, and two of these were from similar samples. All but one of the false negatives recorded resulted from misidentification of discrete shot, rather than small lead fragments.

Neither observer reported false positive or negative results for gizzard samples from Gadwall *Anas strepera* or Wigeon *A. penelope*.

Number of correctly identified shot

In total, 45 discrete shot and nine small fragments were found in all samples using x-ray. Significantly fewer shot were recorded by both observers using simple visual examination (paired T-test, 97 df $T = 2.18$, $P = 0.032$; $T = 2.15$; $P = 0.034$). There was no significant difference between the number of shot recorded using x-ray or microscope techniques, but both observers recorded significantly more shot by using the microscope than with sim-

Table 1. The incidence of shot in waterfowl gizzard samples using different identification techniques.

	n	x-ray	Number of samples recorded to contain shot: microscope examination A	microscope examination B	simple visual A	simple visual B	Average grit size[1]
Pochard *Aythya ferina*	15	8	8	7	7	6	3
Pintail *Anas acuta*	8	3	3	3	2	3	
Mallard *A. platyrhynchos*	15	5	5	5	5	4	2.8
Shoveler *A. clypeata*	9	0	0	1	0	0	2.6
Teal *A. crecca*	33	3	4	5	1	2	0.5-2
Gadwall *A. strepera*	15	1	1	1	1	1	<1
Wigeon *A. penelope*	3	0	0	0	0	0	<1
Total	98	20	21	22	16	16	
Total no. of shot or fragments in all samples		54	47	47	35	35	

A = experienced observer, B = non-experienced.
[1] = From Pain 1990 and Pain & Handrinos 1990.

Table 2 - Lead shot in gizzard contents : number of false positive or false negative results (relative to x-ray results) with simple visual and microscope examination methods.

	With microscope		Simple visual examination	
Observer	A	B	A	B
False positive	1	4	0	0
False negative	0	2	4	4

From a total sample size of 98, of which 20 were found to contain shot when x-rayed.
A = experienced observer, B = non-experienced observer.

ple visual examination (paired T-test, 97 df T = 2.41, $P = 0.018$; T = 2.77, $P = 0.007$). Many of the shot missed using simple visual examination were small fragments.

Discussion

Of the methods previously used to investigate the presence of lead in waterfowl gizzards, the x-ray or fluoroscopy of whole gizzards is the simplest and most rapid, given ready access to the necessary equipment. However, shot are easily missed using this technique, particularly when gizzard contents are tightly packed together, such as often occurs in the impacted intestines of lead-poisoned birds. Montalbano & Hines (1978) found that 28% of gizzards containing shot were missed using this technique, probably due to masking of small eroded shot by grit.

The x-ray of gizzard contents has generally been accepted as the most reliable indicator of the presence of shot (Montalbano & Hines 1978, Anderson & Havera 1985, Anderson *et al.* 1987), although misinterpretation of weak x-ray signals may result in small errors (<10%, Montalbano & Hines 1978).

One problem associated with all radiological techniques is that the source of shot cannot be determined from x-rays, i.e. shot which has been ingested or entered ballistically (been shot in). However, when gizzards are opened, manual examination of the gizzard lining for the presence of shot entry holes, and a rapid scan of gizzard contents enables this potential source of error to be removed. Shot that have entered ballistically are dark grey, of normal size and angular with flattened uneroded surfaces. Ingested shot are eroded and pitted, often undersized, and are often flattened or irregular in shape.

The accuracy of simple visual examination of gizzard contents varies considerably between studies, probably in relation to sites, grit sizes ingested, feeding habits of different species, and observer experience. In other studies, 46-92% of gizzards containing shot have been identified using this technique (Longcore *et al.* 1982, Anderson & Havera 1985). The percentage success rate in the present study (80%) is similar to that reported by Montalbano & Hines (1978). The main problem associated with simple visual examination of samples appears to be an inability to distinguish between seeds, certain types and similar sizes of grit and eroded shot. The results of the present study suggest that simple visual techniques are probably sufficient for identifying shot when the gizzard contents are primarily sand (<1 mm in diameter), such as is found in the gizzards of Wigeon and Gadwall (although sample sizes were small in this study).

The use of a microscope and systematic searching of a sample tray is a considerable improvement upon simple visual searches, as far fewer shot are missed. This was true both of the number of samples found to contain shot, and the total number of shot or lead fragments found (65% without a microscope, 80-85% with - excluding false +ve or -ve results). However, several false positive result were reported (as small lead fragments) by the inexperienced observer using the microscope technique. This error could be reduced by observer training and experience, or by selectively taking x-rays of samples with only small fragments recorded.

The authors would like to thank the Tour du Valat Foundation for funding partially this study.

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