Incidence of histomonosis in turkeys in France since the bans of dimetridazole and nifursol

M-P. CALLAIT-CARDINAL, S. LEROUX, E. VENEREAU, C. M. CHAUVE, G. LE POTTIER, L. ZENNER

Between April 2003 and March 2005, 113 outbreaks of histomonosis were recorded in standard turkey farms in France, and 15 cases were recorded in turkey breeding centres. Most of the cases were in north-west France, the principal farming area for turkeys. The majority of the cases occurred during the hottest months, from April to September. Large numbers of cases occurred among birds from four to eight weeks of age, but there were some cases in three-week-old birds and some in birds up to 17 weeks of age. In most of the standard turkey flocks the mortality was less than 10 per cent, but it was above 30 per cent in nearly 20 per cent of the outbreaks. In the breeding flocks, the average mortality was 60·2 per cent. The size of the flocks, the sex of the birds and the age at which the first clinical signs appeared did not seem to influence the mortality.

Veterinary Record (2007) **161,** 581-585

M-P. Callait-Cardinal, DVM, PhD, DipEVPC,
E. Venereau, DVM,
C. M. Chauve, DVM, PhD, DipEVPC,
L. Zenner, DVM, PhD, DipEVPC,
Unité Mixte de Recherche ENVL/INRA, 958 Protozoaires Entéricoles des Volailles, École Nationale Vétérinaire de Lyon, 69280 Marcy l'Etoile, France
S. Leroux, MSc,
G. Le Pottier, MSc,
Comité Interprofessionnel de la Dinde Française, 11 rue de Plaisance, BP24, 35310 Mordelles, France

Correspondence to Dr Zenner

IN 2004, 625,000 tonnes of turkey meat were produced in France, constituting 32 per cent of poultry production and 10 per cent of total meat production. Of the 25 European Union countries, France is the largest producer of turkeys, with 41 per cent of total production in Europe, followed by Germany (370,000 tonnes), Italy, the UK and Poland (OFIVAL 2004, Magdelaine and Braine 2005). One of the diseases affecting turkeys is histomonosis, a parasite-borne disease that causes severe typhlohepatitis (McDougald 1998, Zenner and others 2002). It is also known as infectious enterohepatitis or blackhead disease. The disease is characterised by necrotic areas in the liver and caeca. The life cycle of the parasite *Histomonas meleagridis* is ill defined, and the roles of both the caecal nematode *Heterakis gallinarum* and the earthworm in the life cycle need to be clarified. In particular, the route of *Histomonas* infection in the absence of the *H gallinarum* vector needs to be confirmed (Hu and others 2004).

Among the criteria that characterise the various types of turkey farms, the presence or absence of a range is related to the likelihood of the birds coming into contact with the parasite and its vector. 'Traditional' farms, using 'lightweight' turkeys, producing quality and/or origin-controlled birds, must meet precise specifications, which in particular require that the birds spend several weeks outdoors. This is not the case for standard turkey farms using 'heavyweight' birds, which are housed throughout their life; these birds make up the vast majority of farmed birds. Moreover, until the Council of Europe banned the practice, factory-farmed turkeys systematically received a dietary supplement of dimetridazole (200 ppm), authorised until May 15, 2002, or nifursol (50 to 75 ppm), authorised until March 31, 2003, as prophylaxis against histomonosis (Callait and others 2002). The ban on these means of control is causing serious problems, and histomonosis is becoming a threat to turkey producers and to an industry that was previously relatively untouched by this disease, with no cases having occurred in standard production for decades.

To assess the consequences of the withdrawal of these products on turkey production in France, a procedure to investigate cases of histomonosis on farms was set up immediately after the withdrawal of nifursol and continued for two years, from April 2003 to March 2005. Several epidemiological features of the disease were defined during these investigations, and this paper describes the results.

MATERIALS AND METHODS

Data collection

The Interprofessional Committee for the French Turkey (Le Comité Interprofessionnel de la Dinde Française [CIDEF]) is an organisation that coordinates the production of turkeys in France and receives information on all the flocks on factory farms. Its members represent approximately 80 per cent of all turkey farmers, mostly with factory farms producing broiler birds and breeding centres. Since 2003, additional data have been required from any farmer with a clinical outbreak of histomonosis. A clinical outbreak was defined by the observation in most of the dead birds of serohaemorrhagic or caseous lesions of the caeca, with the production of yellow exudates, and target-like circular depressions on the liver, as described by BonDurant and Wakenell (1994). The outbreaks included farms where the diagnosis was established either by veterinarians or by specially trained technicians. For each case a questionnaire was completed, which provided details of the geographical location, the number of flocks and buildings affected, the number of birds affected in each flock, the age of the birds when the first clinical signs appeared, the month when they first appeared, the mortality in the affected flocks and the sex of the affected birds. It was also possible to add comments likely to be of interest to the researchers. The information in the questionnaires was collected at CIDEF and analysed at the INRA/ENVL laboratory in Lyon.

Analysis

The information collected was analysed by examining two periods of 12 months, the first between April 2003 and March 2004, and the second between April 2004 and March 2005.

The data were separated into two groups, providing first, a general and environmental study of the disease (geographical distribution of cases, mortality and seasonal variations) and secondly, a study of the susceptibility of the birds to the infection (variations with age, sex, the size of the groups and the type of production). Each affected flock constituted a statistical unit. A flock may consist of several thousand turkeys and a farm can have several flocks. For each variable, the analysis looked at two factors: the distribution of the number of cases and a comparison of the average mortality in each flock.

The following variables, each with two to nine categories, were subjected to statistical analysis: the year (year 1 and year 2), the region (grouped according to administrative areas and geographical proximity: Bretagne and Basse Normandie; Pays de Loire; Nord/Pas de Calais and Picardie; Centre and Bourgogne; Rhône-Alpes; Aquitaine and Poitou/Charentes; Languedoc/Roussillon and Midi Pyrénées), the period (April to September and October to March), the age of the birds in weeks when the first clinical signs appeared (from three to 11 weeks or more), the size of the flocks (limits were set

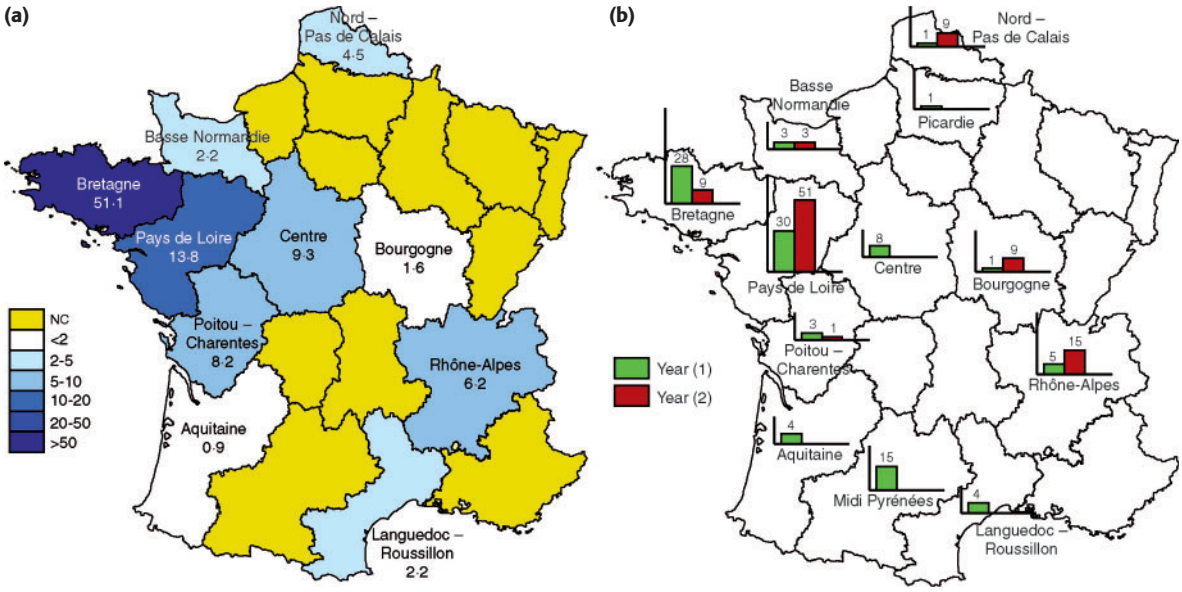

FIG 1: (a) Geographical distribution (percentage) of standard turkey production (average proportions of birds reared in different regions of France [source CIDEF]); (b) distribution (percentage) of cases of histomonosis in different regions from April 2003 to March 2004 (year 1) and from April 2004 to March 2005 (year 2). NC Not counted

to obtain subsamples of a homogeneous size, coherent with observations in the field: fewer than 4000 birds per flock, 4000 to 7000, 7000 to 9000, and more than 9000), sex (flocks composed of males, flocks composed of females, and mixed flocks) and the type of farm (broiler turkeys or breeding birds).

The statistical tests and graphical analyses were carried out by using R software (Ihaka and Gentleman 1996), with a significance threshold set at 5 per cent. The chi-squared test was used to compare the number of cases per region as a function of the number of birds affected, taking into account the geographical distribution of the farms. Comparisons of the average mortalities observed within the flocks for the different variables were made by using the non-parametric Wilcoxon or Kruskal-Wallis rank sum tests as a function of the number of terms, because the data were not normally distributed, even after logarithmic transformation. For the same reason, Wilcoxon's rank sum test was used to compare the average number of cases during the periods April to September and October to March, and the average age of the turkeys when the first clinical signs appeared in the two years, and to compare the average mortality as a function of the type of production unit.

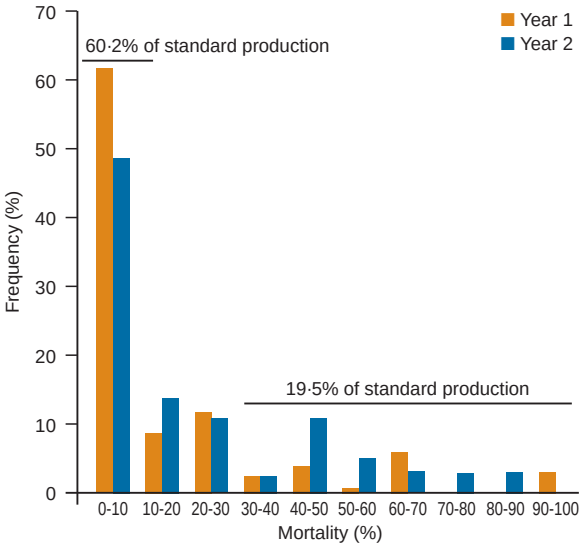

FIG 2: Distribution of mortalities in 113 standard turkey flocks with histomonosis in France from April 2003 to March 2004 (year 1) and from April 2004 to March 2005 (year 2)

RESULTS

One hundred and thirteen cases of histomonosis were reported between April 2003 and March 2005 in factory-type production units, of which 80 cases (70·8 per cent) occurred in the first year and 33 cases (29·2 per cent) occurred in the second. The disease occurred mainly in the Pays de Loire and Bretagne regions (Fig 1), although other regions were affected. The geographical distribution of the cases was significantly different from what would have been expected from the production figures for each region ($P<0·001$), mainly because the number of cases in the Pays de Loire and Bretagne regions was proportionately much lower than the production figures for these regions.

The mortality within each flock varied from virtually 0 to 100 per cent (Fig 2), with an average of 17·5 per cent during the first year and 20·3 per cent in the second; the difference between the two years was not significant. The mortality was less than 10 per cent in 60·2 per cent of the flocks, but mortalities above 30 per cent occurred in 19·5 per cent of the outbreaks.

During the first year the largest numbers of cases occurred in August and October, with 20 and 13 cases, respectively (Fig 3a). During the second year there were fewer cases overall, but there were three peaks, in May, September and December, with five, six and five cases, respectively. Regrouping by season showed that in both years the average monthly frequency of cases of histomonosis was higher in the period from April to September (10·7 per cent per month) than in the period from October to March (5·9 per cent per month) (Fig 4); the difference was not quite significant ($P=0·07$). In the first year the average monthly mortality was generally stable, but in the second year it was notably higher in the flocks affected between May and August (Fig 3b). However, in neither year was it significantly different in the periods from April to September (19·0 per cent) and from October to March (17·1 per cent).

Factors affecting the disease susceptibility of the birds

Cases were reported in birds aged from three to 17 weeks. In the first year, the first clinical signs appeared significantly later, in birds aged on average 7·5 weeks, compared with 6·4

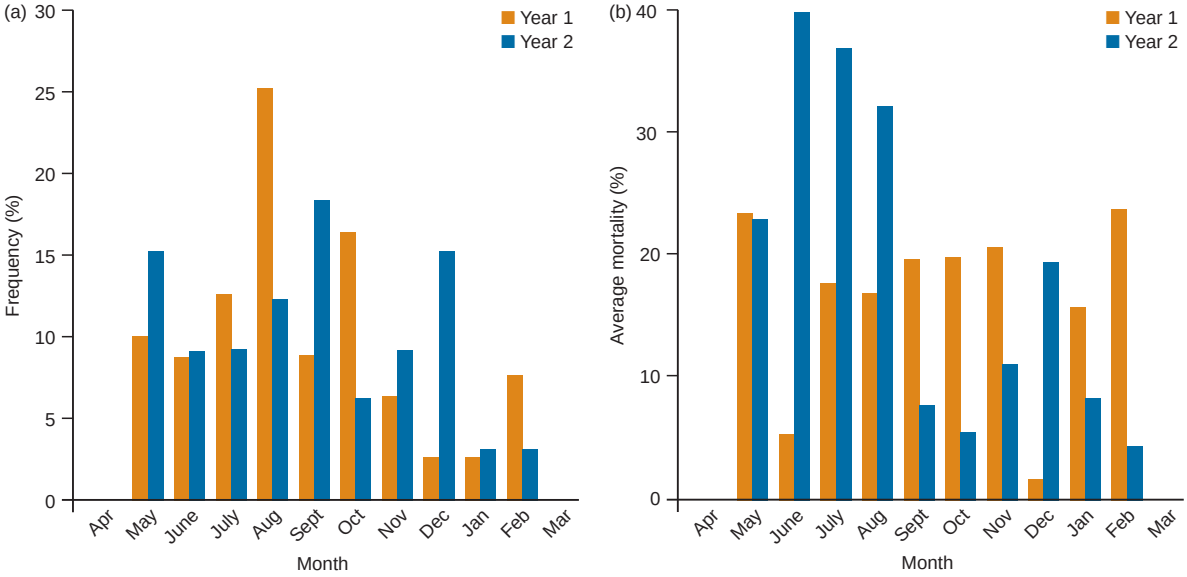

FIG 3: (a) Monthly distribution of cases and (b) average mortalities in outbreaks of histomonosis in France from April 2003 to March 2004 (year 1) and from April 2004 to March 2005 (year 2)

weeks in the second year (P=0·01); in both years the largest number of cases was in birds between four and eight weeks of age (Fig 5a). The average mortalities as a function of the age of the birds were not significantly different during the two years (Fig 5b).

The average mortality among the groups composed entirely of females (22·2 per cent) was not significantly different from that among the groups composed entirely of males (24·2 per cent). However, the average mortality among the groups composed of both males and females (9·9 per cent) was significantly lower (P<0·001) (Fig 6).

The size of the affected groups varied from 1700 to 19,200 turkeys per flock, with an average of 7000. The mortality was not significantly affected by the size of the flock (Fig 7).

Flocks of birds kept for breeding were also affected by histomonosis, with 12 outbreaks reported in the first year and three in the second, all of them in the Bretagne and Pays de Loire regions. The mortalities observed during these outbreaks were generally high, on average 60·2 per cent; only one of them had a mortality less than 10 per cent, and in 12 mortality was more than 30 per cent. In five of the 15 outbreaks, 100 per cent of the birds died. The mortality was thus significantly higher among the breeding birds than among the broiler turkeys (P<0·001).

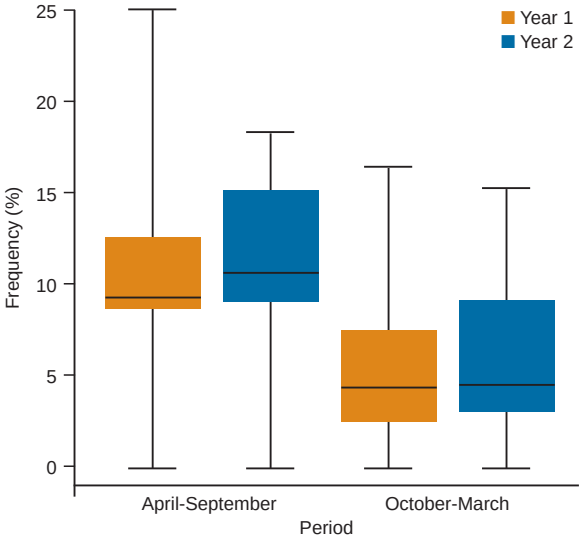

FIG 4: Average monthly frequency of cases during April to September and October to March in outbreaks of histomonosis in France from April 2003 to March 2004 (year 1) and from April 2004 to March 2005 (year 2)

DISCUSSION

Histomonosis as a disease affecting turkeys had been largely forgotten, owing to the systematic use of efficient prophylactic drugs on most farms (McDougald 2003). However, in March 2003, nifursol, the last of the authorised effective products, was banned in Europe and the question arose as to the possible re-emergence of the disease. To answer the question, a reporting system was set up in conjunction with the turkey farmers' professional organisation, to assess the initial consequences of the withdrawal of this treatment and to contribute new epidemiological evidence about the disease in turkeys.

The number of outbreaks reported during the two years of the study was almost certainly below the actual number. Owing to the high level of interdependence within the industry, it can be estimated that the reported figures represent approximately 80 per cent of the actual cases on farms producing standard and breeding birds. Outbreaks occurring on 'traditional' farms have not been included; these are less numerous and there is less contact and exchange between farms. The inclusion criteria for the study were also very strict, requiring the presence of both serohaemorrhagic or caseous caecal lesions and the characteristic yellow, target-like necrotic foci on the liver. Under these criteria some cases were probably not reported, but this was necessary to eliminate any doubtful cases of histomonosis.

A total of 113 cases were reported during the two years, 80 of them during the first year. In the previous years no cases had been reported on farms producing either standard or breeding turkeys, clearly showing that the outbreaks of the disease were associated with the withdrawal of nifursol. The first cases occurred in May 2003, a month during which no live turkeys had received nifursol. One possible explanation for the larger number of cases reported during the first year could be the fact that the summer of 2003 was particularly hot and dry, and this suggestion is supported by the observation of a clear seasonal effect. Bearing in mind that the number of turkeys established on farms is generally stable each month, the comparison of the number of cases reported per month during the periods April to September and October to March shows that in both years there were many more cases reported in the hotter months between April and September. Histomonosis therefore seems to occur more frequently in the hottest months, although no corresponding rise in mortality was observed. Heat and prolonged drought are therefore likely to be risk factors for triggering the disease on farms where the parasite is present.

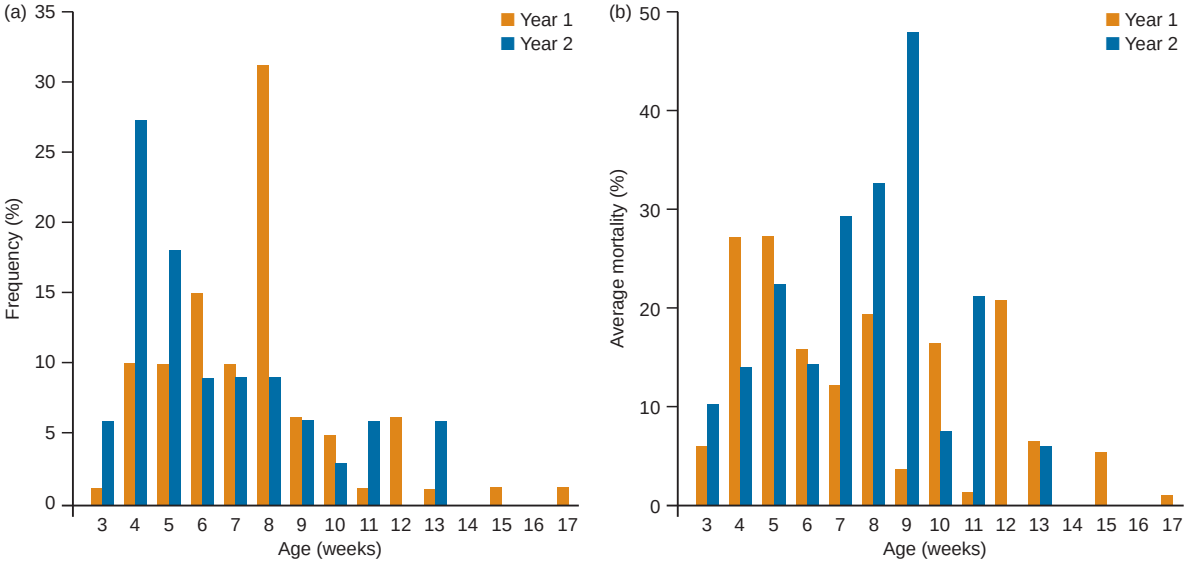

FIG 5: Relationships between the age of the birds when the first clinical signs appeared and (a) the frequency of cases, and (b) the average mortality, in outbreaks of histomonosis in France from April 2003 to March 2004 (year 1) and from April 2004 to March 2005 (year 2)

The geographical distribution of the cases was associated to some extent with the importance of the regions in terms of their turkey production, but not in absolute terms. The most affected regions were those with the largest numbers of farms. However, in the first and second years 30 per cent and 51 per cent of the cases, respectively, were reported in the Pays de Loire region, although this region accounts for only 13·8 per cent of total production; in the same periods the Bretagne region, which accounts for 51·1 per cent of turkey production, had only 28 per cent and 9 per cent, respectively of the reported cases. One possible explanation for these apparent anomalies may be the existence of parasitic strains of different virulence in these regions. This hypothesis needs to be investigated.

The average age of the birds when the first clinical signs appeared was different in the two years. In both years the largest number of cases appeared in birds aged between four and eight weeks, but they appeared significantly earlier during the second year. The first clinical signs may occur until the birds are 17 weeks of age, in contrast with generally accepted data (McDougald 2003, Zenner 2005); Norton and others (1999) have described a clinical episode on one farm in 15-week-old birds, with a high mortality.

The mortality among the flocks was very variable; it was less than 10 per cent in more than half of the cases, but more than 30 per cent in 20 per cent of them. These cases with high mortalities can result in farmers being in constant fear of an epidemic. These observations show that the mortality is the result of factors that will be important to identify, including environmental factors, genetic factors relating to the host and the parasite, the immunological status of the birds, and the management of the flocks.

Among these factors, the size of the flocks and the age at which the first clinical signs appeared did not influence the mortality. The average mortality among flocks of either male birds or female birds was similar at just over 20 per cent, but the average mortality among mixed-sex flocks was significantly lower at approximately 10 per cent. In these mixed flocks the highest mortality was in one or other of the sexes, although not necessarily always the same sex, without the other sex being particularly affected. However, in mixed-sex flocks the sexes are separated physically by bar-

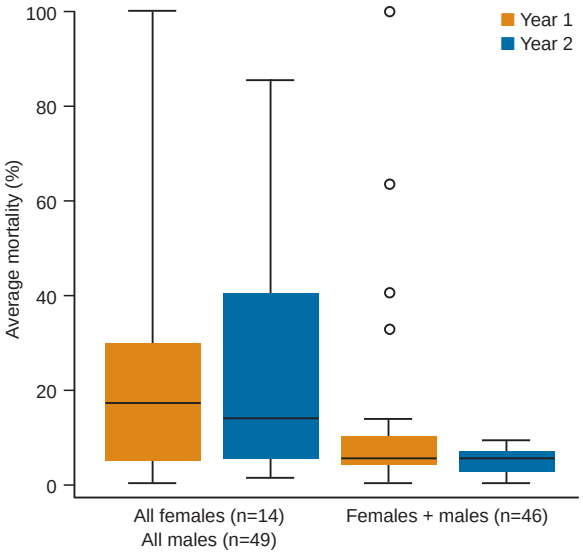

FIG 6: Average mortality in outbreaks of histomonosis in France from April 2003 to March 2004 (year 1) and from April 2004 to March 2005 (year 2) in flocks of all male or all female birds and flocks of both sexes

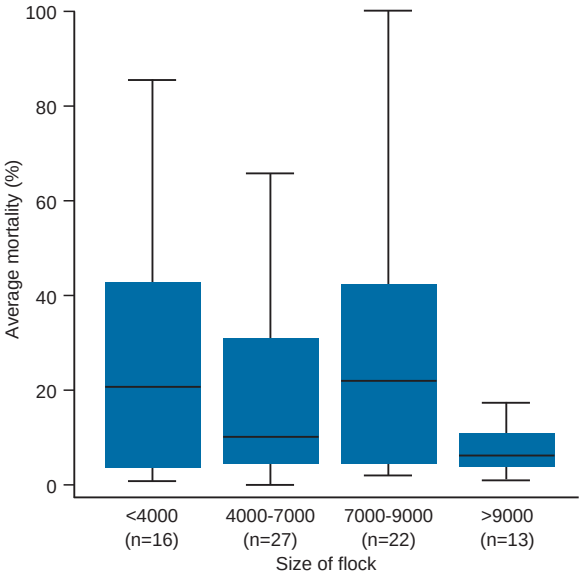

FIG 7: Relationships between the size of the flocks and the average mortalities in outbreaks of histomonosis in turkeys in France from April 2003 to March 2005

riers in the buildings when the birds are only a few weeks old. These barriers may prevent the direct transmission of the disease from bird to bird, as has been suggested in an experimental study of the rapid transmission of blackhead infection (McDougald and Fuller 2005). Another difference in the mortalities observed was that the mortality on the farms producing breeding birds was much higher than on the farms producing standard birds. The reason for this difference is uncertain, but may be associated with the fact that they are of different genetic strains and raised under more restricted conditions.

This work was the first epidemiological field study into clinical histomonosis covering more than 100 flocks. The results show that cases of the disease occurred shortly after the withdrawal of nifursol, which had been the only authorised drug for controlling the infection, and they explain the anxiety of France's turkey farmers. The authors consider that it would be interesting to study the situation of histomonosis in other European producers of turkeys, such as Germany, Italy or the UK.

References

BONDURANT, R. H. & WAKENELL, P. S. (1994) *Histomonas meleagridis* and relatives. In Parasitic Protozoa. Vol 9. 2nd edn. Ed J. Kreier. San Diego, Academic Press. pp 189-206

CALLAIT, M. P., GRANIER, C., CHAUVE, C. & ZENNER, L. (2002) In vitro activity of therapeutic drugs against *Histomonas meleagridis* (Smith 1895) . *Poultry Science* **81**, 1122-1127

HU, J., FULLER, L. & MCDOUGALD, L. R. (2004) Infection of turkeys with *Histomonas meleagridis* by the cloacal drop method. *Avian Diseases* **48,** 746-750

IHAKA, R & GENTLEMAN, R. (1996) R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics* **5,** 299-314

MAGDELAINE, P. & BRAINE, A. (2005) Le marché français et européen de la dinde. Proceedings of Journée Nationale des Professionnels de la Dinde. Pacé, France, June 2, 2005. pp 1-17

MCDOUGALD, L. R. (1998) Intestinal protozoa important to poultry. *Poultry Science* **77,** 1156-1158

MCDOUGALD, L. R. (2003) Other protozoan diseases of the intestinal tract. In Diseases of Poultry. 11th edn. Eds Y. H. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald. Ames, Iowa State University Press. pp 1001-1006

MCDOUGALD, L. R. & FULLER, L. (2005) Blackhead disease in turkeys: direct transmission of *Histomonas meleagridis* from bird to bird in a laboratory model. *Avian Diseases* **49,** 328-331

NORTON, R. A., CLARK, F. D. & BEASLEY, J. N. (1999) An outbreak of histomoniasis in turkeys infected with a moderate level of *Ascaridia dissimilis* but no *Heterakis gallinarum*. *Avian Diseases* **43,** 342-348

OFIVAL (2004) Le marché des produits avicoles. In Le Marché des Produits Carnés et Avicoles – France Union Européenne Monde. Paris, Ofival Edition. pp 289-379

ZENNER, L. (2005) Données actuelles sur l'infection à *Histomonas meleagridis* chez les volailles. *Bulletin de l'Académie Vétérinaire de France* **158,** 161-166

ZENNER, L., CHOSSAT, L. & CHAUVE, C. (2002) L'histomonose de la dinde, une maladie d'actualité. *Bulletin des Groupements Techniques Vétérinaires* **15,** 155-158