First isolation of *Parafilaria bovicola* from clinically affected cattle in Belgium

B. Losson, C. Saegerman

The observation of the first two outbreaks of bovine parafilariosis in Belgium led to a preliminary epidemiological investigation conducted through a telephone survey among veterinarians in a limited area around the outbreaks. Typical clinical signs consisting of bleeding spots and areas of oedema were recorded, and the aetiology was confirmed through the observation of typical embryonated eggs of *Parafilaria bovicola* in the exudate. The localisation of the lesions on the withers, neck, back and, to a smaller extent, the rump of the animals, and their first appearance in early spring, were additional useful epidemiological observations. The clinical herd prevalence in the area was 14·1 per cent (95 per cent confidence interval 11·3 to 17·2 per cent), suggesting that bovine parafilariosis is established in the area.

BOVINE parafilariosis is a vectorborne parasitic disease caused by development of the nematode *Parafilaria bovicola* in the subcutaneous and intermuscular connective tissues of cattle and buffaloes. The disease is characterised by the seasonal occurrence of bleeding spots on the surface of the skin, surrounded by yellowish subcutaneous oedematous eosinophilic areas. The life cycle of the parasite is indirect and involves species of muscid flies as intermediate hosts. In Europe, *Musca autumnalis*, the face fly, is considered to be the main vector. *P bovicola* is found in Africa, Asia and southern Europe (Alzieu and others 1999, Taylor and others 2007), and in the 1970s it was introduced into Sweden, where it is now well established and is responsible for substantial economic losses in beef production (Gibbons and others 2000) due to the trimming of carcases and downgrading of hides (Taylor and others 2007).

Veterinary Record (2009) **164,** 623-626

B. Losson, DVM, PhD, DipEVPC, Laboratory of Parasitology and Parasitic Diseases,
C. Saegerman, DVM, PhD, DipECVPH, DipAppAnimEpid, Research Unit of Epidemiology and Risk Analysis, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Boulevard de Colonster 20, B43, 4000 Liège, Belgium

E-mail for correspondence: blosson@ulg.ac.be

This paper describes the first two outbreaks of bovine parafilariosis observed in Belgian cattle and the results of preliminary epidemiological observations conducted in a limited area around the outbreaks.

Materials and methods

The two outbreaks of parafilariosis were identified on farms in Oreye (farm 1) and Faimes (farm 2), both close to Liège in the east of Belgium.

The animals were examined on the farms and samples of serohaemorrhagic exudate were collected from them into sterile saline. At the laboratory these samples were centrifuged for five minutes at 157 *g* and the sediments were transferred on to glass slides. A coverslip was applied and each sample was observed under the microscope at x 400 and x 1000 to identify any parasites.

Epidemiological inquiry

Involvement of veterinary practitioners An epidemiological investigation was carried out by means of a questionnaire sent to 43 veterinary practitioners active in large animal practice around the outbreaks, in an area covering 1050 km^2 and containing 562 herds holding 60,451 cattle.

Questionnaire

The questionnaire was prepared in accordance with the available scientific information and pre-tested through the collaboration of five veterinarians working at the Faculty of Veterinary Medicine, University of Liège. It focused on the following topics: where the cases occurred, the percentage of herds and cattle affected, the month in which they occurred and the climatic conditions, the breed and the age of the cattle affected (less than six months, six to 11 months, 12 to 24 months and over 24 months), the distribution of the skin lesions, the history of the cases and the application of any treatments, and the effects on milk production and body condition.

Statistical analyses

The prevalence of parafilariosis in the different veterinary practices and the herd prevalences with 95 per cent confidence intervals (CI) were estimated according to an exact binomial distribution.

Each veterinarian classified the three most frequent sites of the skin lesions in rank order, and the first, second and third most common sites were scored 3, 2 and 1, respectively. The sum of the points allocated to each site by all the vets who responded was calculated.

Results

Case reports

On April 18, 2008, the Laboratory of Parasitology and Parasitic Diseases at the University of Liège was contacted by two large animal practitioners who reported the presence of bleeding spots on the neck and withers of one animal on one farm (farm 1) and on several animals on another (farm 2). These signs had not previously been seen by these veterinarians. Both farms were visited on April 22, 2008.

On farm 1, one adult Belgian white and blue bull aged five years and weighing approximately 1000 kg was affected. Its neck, shoul-

FIG 1: Breeding bull on farm 1, showing bleeding spots on its withers

FIG 2: Embryonated thin-shelled egg of *Parafilaria bovicola* isolated from serohaemorrhagic exudate from skin lesions on the bull on farm 1. Bar=20 µm

ders, withers and to a smaller extent thoracic areas showed inflammation and oedema. The areas affected were 1 to 5 cm in diameter, and painful on palpation. In some areas there was a small central hole in the skin from which there was a serohaemorrhagic exudate, which streaked and matted the surrounding hair. Only one active bleeding spot was observed (Fig 1), but there were many older ones visible. Apart from this skin condition the animal was apparently normal. Its owner indicated that the same clinical signs had been observed on the same animal in the spring of 2007, but no treatment had been given.

A presumptive diagnosis of parafilariosis was made, and a sample of the serohaemorrhagic exudate was collected in sterile saline for investigation.

On farm 2, the farmer indicated that the same clinical signs had been observed in a fairly large proportion of his 400 Belgian white and blue cattle. Most of the animals had been turned out, but five adult cows were available for examination. Similar observations were made, consisting of recent or old bleeding spots, mainly on the neck, withers and shoulders. The general condition of the animals was good. Similar observations had been made by the owner in 2007 but the animals were left untreated because the cause of the skin condition was unknown and it appeared to have no economic impact. A sample of serohaemorrhagic exudate was collected in sterile saline.

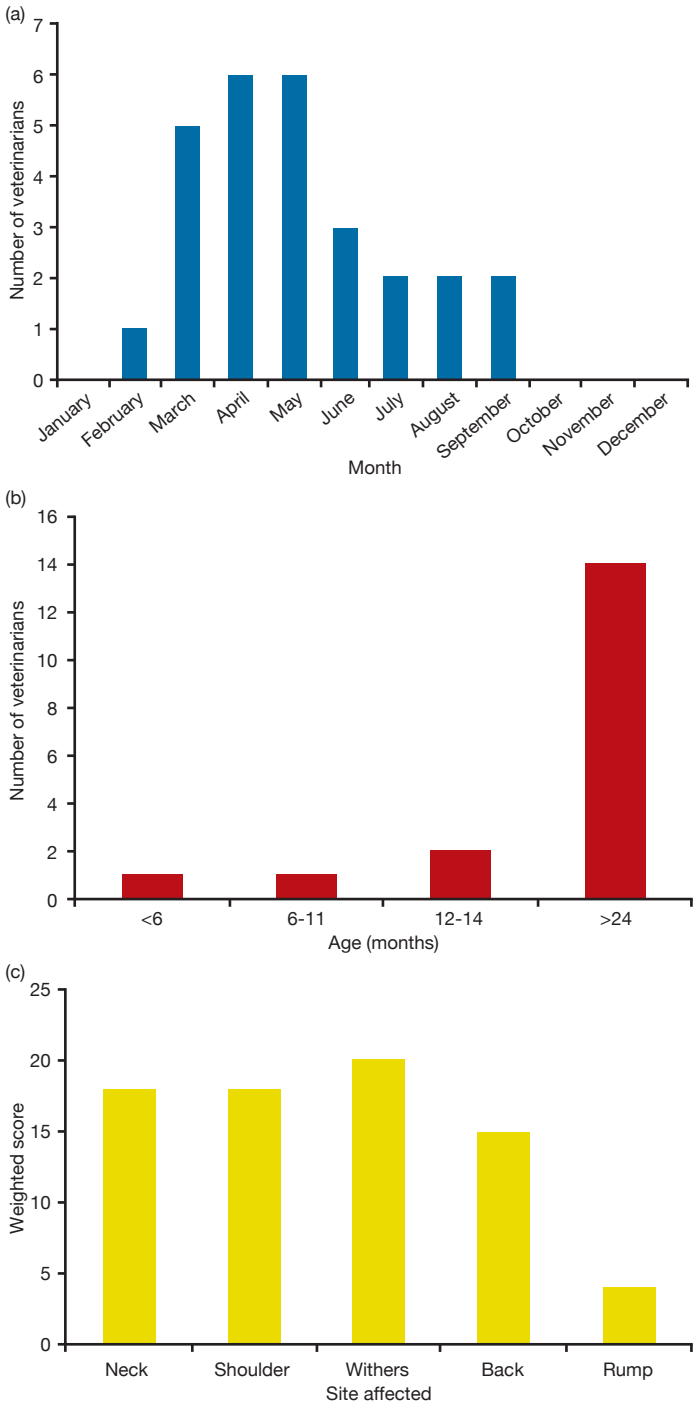

FIG 3: Numbers of veterinary practitioners reporting bleeding spots on the skin of cattle (a) in each month; (b) in animals of different ages. (c) Weighted scores of the skin lesions at different sites on the animals' skin

The differential diagnosis comprised hypodermosis, contusions during handling and transport, and bites inflicted by tabanids.

In each sample, small numbers of the small thin-shelled and embryonated eggs of *P bovicola* were observed (Fig 2), indicating that *P bovicola* was responsible for the condition.

On farm 1, the bull was given a single injection of 0·2 mg/kg doramectin (Dectomax, Pfizer) on April 22, and when the animal was examined 22 days later, all the bleeding spots had disappeared and the skin had regained its normal appearance.

Epidemiological investigation

The 43 veterinary practitioners active in large animal practice locally were contacted. Sixteen of them (37 per cent, 95 per cent CI 23 to 53

per cent) had observed clinical signs of parafilariosis in 79 of the 562 herds managed by the 43 veterinarians giving a herd prevalence of 14·1 per cent (95 per cent CI 11·3 to 17·2 per cent). The within-herd prevalence was estimated to be 1 per cent, 3 per cent, 5 per cent, 10 per cent and 30 per cent by seven, two, two, one and one of the 16 practitioners, respectively; the three other veterinarians had observed the disease but were unable to estimate the within-herd prevalence.

Herds with Belgian white and blue animals were apparently more commonly affected. More cases were observed in the spring of 2008 (Fig 3a) but they were not associated with particular climatic conditions. Eleven of the veterinarians indicated that they had observed the same clinical signs in 2006 and/or 2007, and three of them had observed the condition on the same animals in two consecutive years. A decrease in milk yield or a loss of body condition was reported in approximately 12·5 per cent of cases. More than two-thirds (68·9 per cent) of the veterinary practitioners had observed the same clinical pattern sporadically in the past without discovering its cause, and approximately 20 per cent of the animals had shown similar clinical signs two years consecutively.

The skin lesions particularly affected animals more than two years old (Fig 3b), and most of them were observed on the withers, neck and back (Fig 3c). Most of the animals with parafilariosis were left untreated, but a few were treated with a macrocyclic lactone including injectable doramectin (Dectomax; Pfizer), injectable ivermectin (Ivomec; Merial and different generic products) according to the recommendations of the manufacturers. The results of all the treatments were considered to be very good whatever the drug used, and the animals recovered completely.

Discussion

These two outbreaks of parafilariosis in cattle are the first to be recorded in Belgium. Typical clinical signs consisting of bleeding spots and areas of oedema were observed, and the aetiology was confirmed through the observation of typical embryonated eggs in the exudate from the skin lesions. The localisation of the lesions on the withers, neck, back and, to a smaller extent, the rump of the animals and their appearance in early spring were additional useful epidemiological observations.

The simultaneous outbreaks on two farms approximately 10 km apart prompted a preliminary epidemiological survey in the local veterinary district, which covers 1050 km^2 and contains approximately 60,500 cattle. The 43 veterinarians active in large animal practice and resident in the district were contacted and 16 of them indicated that they had already observed the clinical signs in 2008, or sporadically during previous years. One had observed the same condition 10 years ago, but most of them had observed bleeding spots during the previous two to three years. The absence of any major clinical impact, the easy confusion with accidental wounds or the bites of tabanids or other biting flies, and the lack of information and awareness among the veterinary profession probably account for this late identification of the disease.

In Sweden (Lundquist 1983), South Africa (Nevill 1984) and France (Alzieu and others 1999), ovipositional bleeding is strongly seasonal. In Europe, blood spots first appear in late winter (March), reach a peak in April to May and then decline. In the present study, all but one of the infected animals were Belgian white and blue breed. However, the area investigated is well known for its large beef cattle population, and no breed predisposition to bovine parafilariosis has been reported.

In South Africa, Nevill (1984) reported a relationship between the prevalence of blood spots and the age and sex of the animals. Bulls, heifers and steers less than two years of age bled the most and adult cows bled the least. In the present study, many of the veterinarians had observed active bleeding spots in breeding bulls, and the skin lesions particularly affected animals over two years of age, including cows, in contrast with the observations of Nevill (1984), and Alzieu and others (1999). Young double-muscled Belgian white and blue cattle are treated routinely with endectocides to control endoparasites and ectoparasites, particularly psoroptic mange (Losson and others 1999). Several endectocides are considered to be highly effective against *P bovicola* (at least against the adult stage), and this could explain this unexpected observation. However, other factors, such as the identity of the vector in tropical Africa and in northern Europe, whether or not the vector has a winter diapause, and the dates of turnout and housing in cold temperate areas such as Belgium and Sweden, could play a role.

This preliminary epidemiological survey indicates that bovine parafilariosis is established in this area of Belgium. The disease is often introduced by the importation of breeding bulls (Webster and Wilkins 1970). In the Netherlands, van Wuijckhuise and others (2007) reported bleeding spots in a bull imported from France. The bull was culled to prevent the risk of the disease being spread by the widely distributed fly vector *M autumnalis*.

Nationwide studies are needed to set up an early detection system for bovine parafilariosis, and to assess the distribution of the parasite, its economic impact and the threat it represents for neighbouring countries, and the population dynamics of its specific vector, *M autumnalis*.

Acknowledgements

The authors thank the veterinary practitioners for their contribution to this study. They also thank M. Horemans for access to the cattle population data, and M. Lekimme and L. Hartert for their scientific and technical assistance. The study was financed by the University of Liège.

References

ALZIEU, J. P., DUCOS DE LAHITTE, J., BOURDENX, L. & SCHELCHER, F. (1999) Parafilariose bovine: attitude diagnostique et thérapeutique. *Bulletin des GTV* **2,** 109-115

GIBBONS, L. M., ZAKRISON, G. & UGGLA A. (2000) Redescription of *Parafilaria bovicola* Tubangui, 1934 (Nematoda: Filarioidea) from Swedish cattle. *Acta Veterinaria Scandinavica* **41,** 85-91

LOSSON, B., LONNEUX, J. F. & LEKIMME, M. (1999) The pathology of *Psoroptes ovis* infestation in cattle with a special emphasis on breed difference. *Veterinary Parasitology* **83,** 219-229

LUNDQUIST, H. (1983) *Parafilaria bovicola* (Tubangui 1934) established in Swedish cattle. *Nordic Veterinary Medicine* **35,** 57-68

NEVILL, E. M. (1984) Seasonal abundance and distribution of *Parafilaria bovicola* ovipositional blood spots on cattle in South Africa. *Onderstepoort Journal of Veterinary Research* **51,** 107-114

TAYLOR, M. A., COOP, R. L. & WALL, R. L. (2007) Parasites of cattle. In Veterinary Parasitology. 3rd edn. Eds M. A. Taylor, R. L. Coop, R. L. Wall. Blackwell Publishing. pp 129-130

VAN WUIJCKHUISE, L., PEUTZ, J., ROUMEN, M., BORGSTEEDE, F. & KOCK, P. (2007) Parafilariasis: a new parasitic disease of cattle in the Netherlands. *Tijdshrift voor Diergeneeskunde* **21,** 820-824

WEBSTER, W. A. & WILKINS, D. B. (1970) The recovery of *Parafilaria bovicola* Tubangui, 1934 from an imported Charolais bull. *Canadian Veterinary Journal* **11,** 13-14