

Research of skin *microfilariae* on 160 horses from Poland, France and Spain

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SUMMARY

Cutaneous onchocercosis can affect horses. Adult forms and larvae of *Onchocerca* are responsible for the troubles. Only a few epidemiological studies have been carried out in France on this topic. This preliminary study is aimed at establishing a method of detection of cutaneous larvae so as to perform later an epidemiological study in our country.

One hundred and sixty skin biopsies were realised on horses from Poland, France and Spain in order to detect onchocerca larvae. These samples were cut into little slides, immersed in physiological solution, that was centrifuged and the residue analysed with a microscope. Six foreign horses were found positive (5/95 Polish and 1/18 Spanish). None of the forty seven horses from France was detected positive. These results are similar to the ones established by Collobert, who found a prevalence of 1% on 368 horse samples in 1995. This investigation allowed us to try a simply, reliable and not expensive detecting method, that should be used more frequently in usual practice by veterinarians.

Keywords : *Onchocerca cervicalis* - *Microfilariae* - Horse - Research - Identification - Skin.

RÉSUMÉ

Recherche de microfilaries cutanées sur 160 chevaux provenant de Pologne, de France et d'Espagne. Par M. T FRANCK, J. COLOMBET, C. HUGNET, J. DUCOS DE LAHITTE, L.M. DESMAIZIÈRES, M. DELVERDIER et M. FRANC.

L'onchocercose cutanée est une maladie pouvant affecter les équidés. Elle est consécutive à l'infestation par des vers du genre *Onchocerca*, et son épidémiologie est peu documentée en France.

Cette étude préliminaire a pour objectif de mettre au point la méthode de recherche des microfilaries cutanées en vue de réaliser une étude de prévalence dans notre pays.

Des biopsies cutanées ont été réalisées sur 160 chevaux provenant de Pologne, de France et d'Espagne pour dépister les porteurs de microfilaries. Ces prélèvements ont été fragmentés, mis en suspension dans du soluté physiologique de chlorure de sodium. Le culot de centrifugation a été examiné au microscope. Six chevaux étaient porteurs de microfilaries (5/95 chevaux polonais et 1/18 chevaux espagnols). Sur les 47 chevaux français examinés, aucun n'a été trouvé porteur de parasites. Cela est en conformité avec les résultats obtenus par Collobert en 1995 qui avait trouvé une prévalence de 1% sur 368 échantillons. Cette enquête a permis d'étudier une méthode de dépistage simple, fiable et peu onéreuse. Cette méthode de diagnostic devrait être utilisée plus souvent dans la pratique courante.

Mots-clés : *Onchocerca cervicalis* - Microfilaries - Cheval - Recherche - Identification - Peau.

Three species of *Onchocerca* can infect horses: *O. cervicalis*; *O. gutturosa* and *O. reticulata* [20]. The adult forms of the first species is located in the funicular portion of the *ligamentum nuchae*, the second ones in the lamellar portion of this ligament and the third ones infest the connective tissue of the flexors tendons or the suspensory ligament of the fetlock [17]. These worms are very long and thin: up to 60 cm long and about 0.5 mm large. Females lay microfilariae which migrate to the dermis, causing cutaneous lesions called cutaneous onchocercosis. These larvae are transmitted by intermediate hosts belonging to *Simulium* spp or *Culicoides* spp [5].

Focal annular areas of alopecia, scaling or crusting are observed on the face and the neck, or more frequently on the ventral medianline. Pruritus can be observed too. Exceptionally, microfilariae can invade the eyes, and generate ocular signs [9, 13].

Most of infected horses do not develop clinical signs: it is supposed that cutaneous onchocercosis represents a hypersensitivity reaction to microfilarial antigens.

The cutaneous onchocercosis treatment uses endectocids, that only kill microfilariae and not adult forms [11]. Both ivermectin (0.2 mg/kg) or moxidectin (0.4 mg/kg) can be used [12].

It appears that cutaneous onchocercosis is common on horses in the United States of America. In fact, many surveys carried out there showed that about 20 to 100 % of horses are *Onchocerca microfilariae* carriers. These results are also found in Australia [3, 4, 8, 10, 11, 14, 16, 18, 21]. In France few epidemiological studies have been performed : in 1954 Moignoux established that almost 6% of horses living in Camargue had *Onchocerca microfilariae* [15]. Forty years later, in 1995, Collobert found that just one per cent of 368 French horses examined were positive [2].

There are many methods to detect onchocerca worms, adult forms and larvae. Looking for adult forms is a long and difficult technique that is only realised on corpses, so we decided to look over microfilariae carriers. The main objective of the present investigation was to propose a method of detection and coloration of the microfilariae in samples of horse's skin.

Material and methods

SAMPLES COLLECTION

Skin biopsies were made on anaesthetised living horses; skin samples were also taken on dead horses during necropsies made at Toulouse's National Veterinary School, and on dead horses from France, Poland and Spain at Narbonne's slaughterhouse. All these samples were collected in order to detect *Onchocerca* microfilariae.

PROCEDURES FOR DETECTING MICROFILARIAE

Microfilariae have an elective location, but usually they are also found in the dermis of the ventral midline [7, 19]. That's the reason why almost the skin samples were taken there, near the *umbilicus*, with a biopsy-punch for living horses, or with scissors for dead ones. Parts of scrotum were also collected during castration. The samples were dilacerated into little parts and immersed in physiological solution for 24 hours to allow the liberation of larvae from dermis. The suspension was then centrifuged at 3000 revolution per

minute during 5 minutes. The bottom of the solution was examined with a microscope [6]: microfilariae are 200-240µm in length by 4-5µm in diameter, unsheathed with a short tail. They are translucent and twist on themselves (Figure 1).

PROCEDURES FOR COLOURING MICROFILARIAE

Slides were made from the positive samples. They were first dehydrated in pure acetone at 4°C during one minute, in order to clear them before staining with Giemsa or with the histochemical stain used on dog blood in order to differentiate *Dirofilaria immitis*, *Dipetalonema reconditum* and other microfilariae. The acid phosphatase activity was demonstrated by the naphthol AS-TR phosphate method. [1].

Results

Six samples on the 160 observed contained microfilariae.

Giemsa- stained microfilariae take a purple color. All the larvae look like *Onchocerca cervicalis*: they are 212-214µm in length by 3.6-4.6µm in diameter, they have a cephalic part,



FIGURE 1. — *Onchocerca cervicalis* microfilariae : direct examination.



FIGURE 2. — *Onchocerca cervicalis* microfilariae Giemsa stained.

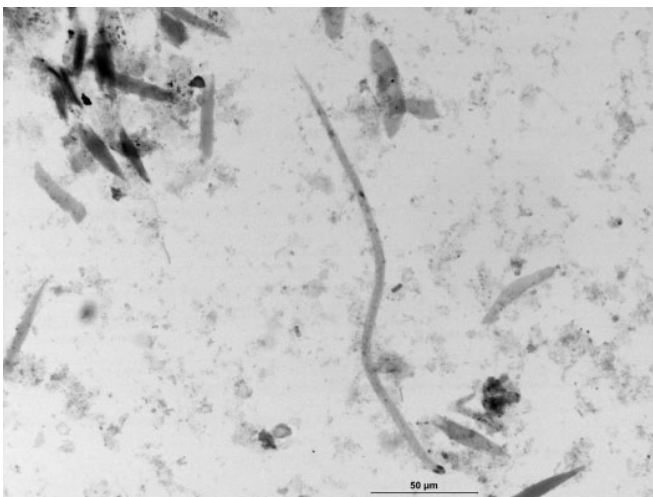


FIGURE 3. — *Onchocerca cervicalis* microfilariae with evidence of enzyme activity (red spots).



FIGURE 4. — Geographic repartition of the positive horses.

large and rounded and a posterior one, thin but short. (Figure 2). Those of *Onchocerca reticulata* measure 330-370µm and possess a long whiplash-like tail.

After histochemical staining, all the positive samples exhibit the same type of microfilariae. They are coloured light green, with two red spots of phosphatase activity : one near the cephalic part, and one at the middle part of the worm (Figure 3).

All the positive horses were more than two years old, and they all have been slaughtered at Narbonne. Among the six positive horses, two were female, three were castrated male, and one was a stallion.

None of the forty seven French horses was positive. Five of the ninety five Polish ones, and one of the eighteen Spanish ones were positive (Figure 4).

Discussion

The detecting method used is efficient, cheap, rapid and simple. It can be performed with little material, which is usually used by veterinarians.

This method can be used to diagnose onchocercosis on living animals.

Onchocercosis is known in Poland and Spain, and it must exist in France. In fact, there are many vector insects in our country. As we could not examine enough French samples, we can not conclude that Onchocercosis has disappeared. Moreover, as Collobert had shown in 1995 [2], this disease seems to be rare. We can assume that using ivermectin or moxidectin once or twice a year reduces the number of positive animals. Currently both ivermectin or moxidectin can be used in France (oral route is agreed only). This method of detection of cutaneous larvae will be used to perform an epidemiological study with the practitioners during the castration period.

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