Veterinary Parasitology 199 (2014) 283–288

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short Communication

Blood-feeding patterns of horse flies in the French Pyrenees

F. Baldacchino[a,*], L. Gardès[b], E. De Stordeur[a], P. Jay-Robert[a], C. Garros[b]

[a] UMR 5175, Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), Université Paul-Valéry (UM3), 34199 Montpellier Cedex 5, France
[b] UMR 1309 CMAEE, CIRAD, INRA, 34398 Montpellier, France

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 10 October 2013
Accepted 13 October 2013

Keywords:
Tabanidae
Blood meal
Cytochrome *b*
Livestock
Wild ungulates
Besnoitiosis

ABSTRACT

Horse flies can mechanically transmit *Besnoitia besnoiti*, the agent of bovine besnoitiosis. Although previously limited to enzootic areas, especially the French Pyrenees Mountains, bovine besnoitiosis is now considered a re-emerging disease in western Europe. To improve understanding of the role of horse flies as mechanical vectors, this study investigated their blood-feeding ecology in the eastern French Pyrenees, in two high-altitude summer pastures whose main domestic ungulates were cattle, and in a wildlife park with native fauna. Species-specific PCR assays were conducted to identify the sources of blood meals: wild boar, horse, cattle (or bison), sheep (or mouflon), goat, red deer, roe deer and izard (or Pyrenean chamois). In La Mouline pasture, tabanids ($N = 20$) fed on red deer (70%) and cattle (30%). In Mantet pasture, tabanids ($N = 24$) fed on cattle (52%), red deer (20%), wild boar (16%), horse (8%) and sheep (4%). In the wildlife park, *Tabanus bromius* ($N = 32$), the most abundant species collected, fed on red deer (85%), bison (9%) and wild boar (6%). Despite relatively high densities in both the pastures and in the wildlife park, small wild ungulates (izard, mouflon and roe deer) were not detected as a source of blood meals. Only two mixed blood meals were identified in two specimens of *T. bromius*: cattle/horse for the specimen collected in the pastures, and bison/wild boar for the specimen collected in the wildlife park. Our findings showed that tabanids display a level of opportunistic feeding behaviour, in addition to a preference for red deer, the latter being particularly true for *Philipomyia aprica*, the most abundant species collected in the pastures.

1. Introduction

Recent epidemiological data confirm an increased number of cases and a geographic expansion of bovine besnoitiosis in cattle herds in Europe (Alvarez-García et al., 2013). Previously, this disease was only encountered in enzootic areas, especially the French Pyrenees Mountains (Jacquiet et al., 2010). Arthropods such as horse flies may play a role in the mechanical transmission of *B. besnoiti* from cattle with chronic or asymptomatic infections. The mechanical transmission of *B. besnoiti* between cattle has been demonstrated in experiments with African tabanids (Bigalke, 1968). The persistent feeding behaviour of horse flies is favourable to mechanical transmission, although they most often continue to bite the same host following an interrupted meal (Desquesnes et al., 2009). Mechanical transmission principally occurs within a group of animals: for example, in a herd or at gathering points. Pyrenean summer pastures could be considered areas with a high risk of transmission, as cattle there are highly exposed to biting flies, and animals with chronic or asymptomatic infections can mix with healthy livestock in the same herd. Moreover, cattle graze near wild ruminants in mountain pastures; the role of wild ungulates as a wild reservoir of *B. besnoiti* remains unknown. In a recent serosurvey of *Besnoitia* sp. infection in wild ruminants in Spain, sera samples from roe deer and red deer were seropositive, but

* Corresponding author at: Route de Mende, 34199, Montpellier cedex 5, France. Tel.: +33 4 67 14 23 21; fax: +33 4 67 14 24 59.
E-mail address: frederic.baldacchino@cefe.cnrs.fr (F. Baldacchino).

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.10.009

Table 1
Estimated relative densities of animals in each valley during the summer of 2011.

	Cattle	Sheep	Goats	Red deer	Roe deer	Izards	Mouflons	Horses	Wild boar
La Mouline	Low	Very low	Absent	Low	Low	Low	Very low	Very low	Low
Mantet	High	Low[a]	Low	Very low	Very high	Very high	Very low	Very low	High

Absent ($N = 0$), very low ($N = 0.1–1$), low ($N = 1.1–5$), high ($N = 5.1–10$) and very high ($N > 10$).
N is the number of individuals per km^2.
[a] A flock of sheep was only present in Mantet between mid- and end-June.

only one sample from each species was clearly identified as *B. besnoiti* (Gutiérrez-Expósitoa et al., 2013).

Understanding the biting intensity and blood-feeding behaviour of tabanids is crucial to assessing how problematic they are and their potential role as mechanical vectors (Magnarelli and Anderson, 1980). Although blood-meal analysis has been widely applied to haematophagous dipteran, especially mosquitoes, tabanids have received little attention in this area. Studies of tabanid feeding patterns have been conducted in North America (Magnarelli and Anderson, 1980; Wilson and Richards, 1969), Africa (Gouteux et al., 1989) and Australia (Muzari et al., 2010a), but previously none had been carried out in Europe. Moreover, all of these studies were based on immunological methods, which mainly detect species-specific targets, but might not distinguish between blood meals obtained from closely related host species (Mukabana et al., 2002). In this study, we considered that tabanid blood-meal sources might be identified with greater specificity and sensitivity using host species-specific PCR-based assays (Garros et al., 2011).

Using a molecular-based approach, this study investigated the blood-feeding patterns of horse flies in the French eastern Pyrenees. Tabanids were collected in summer pastures where mainly cattle are present, and in a wildlife park sheltering species representative of wild Pyrenean fauna. Our objectives were to estimate the proportion of feedings on domestic and wild ungulates, as well as the prevalence of mixed blood meals. To this end, PCR-based assays were optimized for tabanids, and their sensitivity to blood-meal digestion was determined. The implications for horse fly populations and the mechanical transmission of bovine besnoitiosis are discussed.

2. Materials and methods

2.1. Study areas and tabanid collection

Tabanids were captured in the French eastern Pyrenees, in high-altitude summer pastures in the valleys of La Mouline (2°14′ E 42°37′ N) and Mantet (2°18′ E 42°28′ N), and in the Les Angles Wildlife Park (2°04′E 42°34′N). The estimated relative densities of hosts in each valley are noted in Table 1. These estimates are based on sightings by park rangers and on harvest report from hunters.

The wildlife park is home to Pyrenean fauna still present in the natural environment (red deer [$N = 33$], European roe deer [$N = 6$], mouflon [$N = 44$], izard [$N = 25$], Spanish ibex [$N = 16$], brown bear [$N = 2$], wolf [$N = 11$] and marmot [$N = 5$]) or no longer present in the wild in this region for many centuries (European bison [$N = 5$], reindeer [$N = 10$] and fallow deer [$N = 36$]). This study site was chosen as all potential tabanid hosts are available in a small area (0.37 km^2) and their exact densities are known, which is not the case in mountain pastures.

The sampling design in the summer pastures was the same as in the study by Baldacchino et al. (2013). In the wildlife park, six Nzi (Mihok, 2002) traps baited with octenol were set between or within the animal enclosures. The octenol was dispensed in polyethylene sachets as in Torr et al. (1997). Collections were made between 9 July and 1 August 2012. One trap was moved on 23 July because no flies were collected at the initial position. The tabanids were identified using Chvala's key (1972) and kept in 95% ethanol before molecular analysis.

2.2. Blood-meal digestion and species-specific PCR assays

To determine PCR sensitivity in relation to blood-meal digestion, *T. bromius* females were used, as this species is very common in Europe. Wild females were collected in the field, kept in screen cages under laboratory conditions (22–26 °C, 40–60% relative humidity) and supplied *ad libitum* with water and sucrose solution. 24 *T. bromius* were fed to repletion with citrated bovine blood provided with a Hemotek 5W1 Membrane Feeding System (Hemotek Ltd., Accrington, England). A Parafilm® membrane was stretched over the aperture of the meal reservoir of the feeder, and a small hole punctured in the middle of the membrane to allow a drop of blood to escape at the surface. Feeders were placed under the cages between 11:00 and 18:00. Flies ($n = 6$) were killed in groups by freezing at 24, 48, 72 and 144 h after feeding and stored in 95% ethanol at −20 °C before analysis. The blood-meal size of fed females was assessed after dissection by weighing the gut. Unfed female tabanids were used as a control ($n = 6$).

Host identification analyses were made on specimens collected in the summer pastures and the wildlife park. Individuals were dissected to examine signs of engorgement. The midguts that had visible traces of blood were isolated in 95% ethanol and stored at −20 °C. DNA was extracted using a commercial kit (NucleoSpin Tissue, Magerey-Nagel, Düren, Germany) on each individual midgut ground in 200 μL of PBS buffer.

Genomic DNA extracts from tabanid midguts were amplified by polymerase chain reaction (PCR) on a PTC-100 thermal cycler (MJ Research, Alameda, CA, USA) using specific primers.

Host primers based on cytochrome *b* (cyt *b*) were selected from Garros et al. (2011) for identifying wild boar, horse, cattle (or bison), sheep (or mouflon), goat, red deer, roe deer and izard (same primers as chamois).