Diversity in the Genera *Avitellina* and *Thysaniezia* (Cestoda: Cyclophyllidea): Genetic Evidence

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ABSTRACT: The isoenzyme electrophoretic study of 2 species of cestodes, *Avitellina centripunctata* and *Thysaniezia ovilla*, sampled in African (Senegal) domesticated ruminants, revealed a complex of cryptic species. Four species of *Avitellina* were found in sheep and goats and 1 in cattle. Two species of *Thysaniezia*, 1 specific to cattle and the other to sheep, were also revealed. Despite a probable preponderant selfing mode of reproduction, the existence of the detected species was confirmed by high levels of genetic differentiation.

KEY WORDS: Cestoda, *Avitellina*, *Thysaniezia*, ruminants, isoenzyme electrophoresis, specificity.

*Avitellina centripunctata* (Rivolta, 1874) Gough, 1911, and *Thysaniezia ovilla* (Rivolta, 1878) Skrjabin, 1926, are 2 cestode species found in the small intestine of numerous herbivorous mammals (Schmidt, 1986). In domesticated ruminants, numerous other species of these two genera of Anoplocephalidae have been described by different authors, none of which is valid (Spasskii, 1951; Troncy et al., 1981). It is difficult to explain this lack of parasite diversity considering the heterogeneity of potential hosts and their diets.

In this article, we present a population genetic study, based on isoenzyme electrophoresis, of African (Senegal) *A. centripunctata* and *T. ovilla*, sampled in sheep, goats, and cattle. This enabled us to test the genetic homogeneity and the degree of specificity within the 2 cestode species. This study revealed a broader diversity of species and a narrower range of host specificity than suspected.

Material and Methods

**Sampling of the worms**

Two morphological species of parasite were studied, *Avitellina centripunctata* and *Thysaniezia ovilla* (Cyclophyllidea: Anoplocephalidae). Cestodes were taken from the small intestine of cattle (*n* = 30), sheep (*n* = 80), and goats (*n* = 38) from the Dakar (Senegal) slaughterhouse during the summer of 1992. For *A. centripunctata*, prevalences were 15, 8, and 7% in sheep, goats, and cattle, respectively. For *T. ovilla*, prevalences were 6 and 13% in sheep and cattle, respectively (goats not infected). The exact origin of each host is unknown; they may come from any region of the northern part of Senegal. Parasites were kept alive in physiological saline (0.9% w/v NaCl). After being identified under a dissecting stereoscope, the cestodes were stored in liquid nitrogen. It is known that such treatment prevents the contamination with host enzymatic material (e.g., Nadler, 1987; Johnson and Hoberg, 1989; Chilton et al., 1992). Parasites were then carried to Montpellier (France) on dry ice.

**Preparation of the worms**

In the laboratory, worms were thawed. One portion was fixed in alcoholic Bouin fixative, stained with aceto-carmine, mounted in Canada balsam (Martoj a and Martoja, 1967), and observed under a light microscope. This enabled a precise diagnosis of the cestodes, using the criteria described by Schmidt (1986). At this time, no morphological heterogeneity could be found within each of the 2 species. Another portion of each parasite, corresponding to a volume of 0.5 ml, was homogenized in Eppendorf tubes filled with an equal volume of distilled water, centrifuged at 12,000 rpm for 1 min, and the homogenates were used as the protein source.

**Electrophoresis**

Starch gel electrophoresis was performed as described by Renaud and Gabrion (1988). The enzyme systems studied and their corresponding Enzyme Commission numbers were as follows: glucose phosphate isomerase (GPI, EC 5.3.1.9), hexokinase (HK, EC 2.7.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), and

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Table 1. Genotypes observed for *Avitellina centripunctata*. The 4 genetic entities observed (A1, A2, A3, and A4) are shown separately. The cestodes came from 3 host species: sheep, goat, and cattle (represented by the third letter S, G, and C, respectively). Alleles were numbered according to their anodal mobility.

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<thead>
<tr>
<th></th>
<th>GPI</th>
<th>MDH</th>
<th>PEP-A</th>
<th>HK</th>
<th>NP</th>
<th>PGM</th>
<th>ME</th>
<th>N*</th>
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</table>

* Number of individuals.

Table 2. Genotypes obtained for *Thysaniezia ovilla* from sheep (TS) and cattle (TC). Alleles were numbered according to their anodal mobility.

<table>
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<th>MDH</th>
<th>PEP-A</th>
<th>NP</th>
<th>PGM</th>
<th>ME</th>
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<td>2/2</td>
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<td>16</td>
</tr>
</tbody>
</table>

* Number of individuals.

Results

Species diversity

The different genotypes obtained are presented in Tables 1 and 2 for *A. centripunctata* and *T. ovilla*, respectively. It can be noted that the 2 "species" actually correspond to 2 species complexes. *Avitellina centripunctata* is subdivided into 4 genetically distinct species. Out of the 7 loci studied, each species displayed a level of fixed allelic differences ranging from 71 to 100% (Table 1). Two species, discriminated with 7 (out of 8) diagnostic loci (87% of fixed differences), were observed for *T. ovilla* (Table 2).

Parasite specificity

In the *A. centripunctata* complex (Table 1), parasite specificity isolates the small ruminants (sheep and goats) from the large ruminants (cattle), with 1 species (A4) specific to cattle and the remaining 3 found in sheep and goats. For *T. ovilla*, the 2 species observed displayed a strict specificity (Table 2) for cattle and sheep.

Within-species diversity

Within-species genetic heterogeneity could only be found in *A. centripunctata* species infecting small ruminants (Table 1). This heterogeneity is represented by rare alleles in several of the loci studied. These loci are PEP-A and PGM for species A1; PEP-A, HK, and ME for species A2; and HK and ME for species A3 (Table 1). Within outcrossing species, homozygosity is highly unlikely for rare alleles (more likely to be found at a heterozygous stage) (Hartl and Clark, 1989). No heterozygote could be found, even for rare alleles. This strongly suggests that selfing may be the preponderant mode of reproduction for these cestodes.

Discussion

As demonstrated in similar studies, parasite species diversity is often much more complex...
than what morphological taxonomy has previously postulated. This is true for various kinds of parasitic organisms: cestodes (Reudin et al., 1985; Renaud and Gaborin, 1984, 1988; de Chambrier et al., 1992), trematodes (Versans et al., 1989), nematodes (Nascetti and Bullini, 1982; Andrews et al., 1989; Chilton et al., 1992), acanthocephalans (de Buron et al., 1986), and caligid copepods (Zeddman et al., 1988).

The level of biological diversity characterized within the cestodes studied appeared much higher than what has been reported (e.g., Euzéby, 1966; Soulsby, 1968; Troncy et al., 1981; Schmidt, 1986). Specificity was found to separate worms infesting small ruminants (sheep and goats) from those found in large ruminants (cattle). For certain kinds of organisms, in particular cestodes, selling may make species characterization more difficult (Lymbery, 1992). Here, the high levels of genetic differentiation strongly validate the 6 species characterized, even for those represented by few individuals.

Species that self are likely to display high heterozygote deficiencies and, thus, low levels of polymorphism (homozygosity lowers the effective population size, i.e., accelerates drift) (Li, 1976). Accordingly, no variation was found within the 2 cryptic species of Thyssaniezia and within 1 Avitellina species (cattle parasite). Some loci studied appeared polymorphic within 3 Avitellina species. No heterozygous individuals could be observed within these 3 species. Hosts probably came from a wide area. However, some migration must occur due to human activity (host migrations). Attributing the observed absence of heterozygotes to population structuring would require a total geographical isolation between the different units (no migration). High levels of selfing thus represents a suitable explanation.

In the small intestine of African domesticated ruminants, species of Avitellina and Thyssaniezia coexist with other cestodes: Stilesia globipunctata, Moniezia expansa, and M. benedeni (e.g., Euzéby, 1966), some of which are themselves species complexes (unpubl. data). The ecological factors allowing such a species diversity remain unknown. However, differences in intermediate hosts and in host grazing behaviors may explain heterogeneities in host infections. The goats, for example, were rarey infected, compared to sheep. Moreover, it is probable that all these coexisting species display different ecological and transmission strategies. This remains to be studied. It is probable, as well, that the effect of anthelmin tic treatments is different on these different parasite species. Consequently, the control of these diseases of veterinary importance may be more complicated than expected.

Literature Cited


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Meeting Schedule

HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1994

(Wednesday) 9 February 1994 Animal Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD

(Wednesday) 6 April 1994 Johns Hopkins University, Baltimore, MD

(Saturday) 7 May 1994 Joint Meeting with the New Jersey Society for Parasitology, at the New Bolton Center, University of Pennsylvania, Kennett Square, PA

October 1994 Site to be announced

November 1994 Site to be announced

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