ENTERIC MICROFLORA IN ITALIAN CHIROPTERA

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Abstract - The authors present data obtained by bacteriological analyses of faeces collected, under sterility conditions, from 89 individual bats belonging to the following species: *Myotis daubentoni* (7), *Myotis capaccinii* (22), *Myotis myotis* (10), *Pipistrellus kuhli* (17), *Miniopterus schreibersi* (25), and *Tadarida teniotis* (8). The bats were collected from colonies in Lombardy, Emilia Romagna, Latium and Sicily. The analyses so far conducted have revealed the presence of 26 bacterial species, none of which is pathogenic to humans.

Riassunto - In questa breve nota vengono presentati i dati ottenuti dalle analisi batteriologiche condotte su feci, raccolte in sterilità, provenienti da 89 individui appartenenti alle seguenti specie di chirotteri: *Myotis daubentoni* (7), *Myotis capaccinii* (22), *Myotis myotis* (10), *Pipistrellus kuhli* (17), *Miniopterus schreibersi* (25), e *Tadarida teniotis* (8). I pipistrelli sono stati catturati in Lombardia, Emilia Romagna, Lazio e Sicilia. Le analisi finora effettuate hanno rivelato la presenza di ben 26 differenti specie di batteri, nessuna di esse può essere considerata patogena per l'uomo.

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1. Introduction

The Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" and Wilderness Studi ambientali have been collaborating since 1990 for the sanitary monitoring of bat colonies in the Italian territory. Their studies aim at a better knowledge and protection of these highly useful mammals. The few data available in the literature concerning the enteric microflora of bats refer almost exclusively to Neotropic and Malagasy species (Klite, 1965a, 1965b; Arata *et al.*, 1968; Mayoux *et al.*, 1970; Brygoo *et al.*, 1971; Cassel-Beraud & Richard, 1988). Data are particularly limited concerning Palearctic taxa. Pinus and Muller (1980) published a note on the enterobacteria present in the faeces of captive individuals of *Rhinolophus ferrumequinum*, *Myotis brandti*, *M. daubentoni*, *M. emarginatus*, *M. myotis* and *Nyctalus noctula*. Only recently Piraino *et al.* (1996) and Di Bella *et al.* (1997a; 1997c) have investigated the enteric microflora of bats captured in the wild. In the following list we report some ecological notes on the species which we have examined: *i*. Schreiber's Bat, *Miniopterus schreibersi* (Natterer in Kuhl, 1819). A typical troglophilous and migratory species. It forages between 5 and 20 meters from the ground, frequently far from its roosts, in open spaces or in proximity of crags. It is usually encountered in the plains and on the mountains at a low or mid altitude (we have found it in the Central Apennines up to 1050 m), in particular in gorges of karstic origin. Hunts also on meadows and pastures. Captures moths, diptera and coleoptera.

ii. Daubenton's Bat, *Myotis daubentoni* (Kuhl, 1819). An erratic species; hunts on the water, catching insects on the wing, along the riparial vegetation or on land among the plants, flying over the foliage, mainly near its roosts. The largest concentrations of bats of this species can be found in plain areas, in woods and parks near water. Its displacements for feeding have normally a 2-km radius, up to 10 kms in case of hunting on rivers and canals. Besides lakes, rivers and smaller waterbodies, it frequents also broadleaved and mixed woods, as well as human dwellings near its roosts. The diet of this bat includes mainly diptera (mostly Chironomids) and trichoptera (especially *Glossomatidae*) besides insects belonging to other groups; these are generally small sized insects, and feeding takes place always in flight.

iii. Long-fingered Bat, *Myotis capaccinii* (Bonaparte, 1837). This species probably moves not far away from its breeding colonies; it hunts preferably on water surfaces, apparently its preferred habitat. *M. capaccinii* is considered to be a typically troglophilous species in S. Europe, but it can frequent also buildings, provided they are placed in quiet areas, and near its feeding quarters. When these bats leave the roost, they disperse immediately over water stretches, some kilometers away. Their diet includes mainly diptera and trichoptera which are picked up from the water surface.

iv. Greater mouse-eared Bat, *Myotis myotis* (Borkhausen, 1797). A troglophilous species and occasional migrant. In the activity season it frequents mainly sparse woods and parks. Frequently it forages on the ground, capturing carabids, scarabs, grasshoppers, crickets and spiders. Its diet includes mainly non-flying arthropods; many investigations have found a clear predominance of Carabidae (Coleoptera); an important fraction is also represented by Orthoptera (mostly *Acrididae*), Diptera (*Tipulidae*) and *Arachnida*.

v. Kuhl's Pipistrelle, *Pipistrellus kuhli* (Kuhl, 1819). A sedentary species; it forages around the street lamps in towns, over water surfaces in gardens and in country environments. It is an essentially anthropophilous species, living at low and middle altitudes. In Italy it is the most common bat; it is less frequent in woodlands, but is normally mostly widespread in urban and semi-urban habitats, and in agricultural environments. Its reproductive colonies occupy the cracks of buildings (cornices, gutters, frames, tiles); isolated individuals have been found also in rock fissures and, occasionally, in hollow trees. It uses abandoned houses, barns, villas, historical buildings and condominiums. Its winter quarters are in buildings (probably the same ones as in summer) or natural cavities. It shares its sites with *P. pipistrellus* and *Hypsugo savii*. Hunts small flying insects, mostly lepidoptera, diptera and coleoptera.

vi. Free-tailed Bat, *Tadarida teniotis* (Rafinesque, 1814). A probably sedentary species; it can leave its roosts even for several kilometers. It can descend from passes or ascend from cliffs in order to reach its feeding grounds near lighted inhabited centres (even under street lamps, provided they are placed high up from the ground). Hunts also above waterbodies, describing large circles over the surface. Feeds on neuroptera and lepidoptera. Relatively common in mediterranean coastal areas, both along isolated sea cliffs and in large towns (Latina, Messina, Palermo, Reggio Calabria, Siracusa). It is also present in rocky areas on mountains, where it is apparently more rare. Recorded from the sea level up to an altitude of 2000 m. Its summer quarters are placed in rock cracks or in cliffs (also fissures in isolated large rocks), in buildings and in caves.

2. Materials and methods

We have examined faeces from 89 individuals belonging to two different families represented in Italy (Vespertilionidae and Molossidae). More precisely, the following numbers of bats were collected: 25 *Miniopterus schreibersi*, 7 *Myotis daubentoni*, 22 *Myotis capaccinii*, 10 *Myotis myotis*, 17 *Pipistrellus kuhli* and 8 *Tadarida teniotis*. Captures took place in the following localities:

i. Lombardy

1) Mixed colony with *Myotis daubentoni*, *M. nattereri* and *M. capaccinii* in a tower facing Lake Como near Lierna, Lecco. 2) Colony of *P. kuhli* in a building in Mandello sul Lario, Lecco.

ii. Emilia Romagna

1) Colony of *P. kuhli* in a house in the area of Boscone della Mesola, Ferrara.

iii. Latium

1) Mixed colony of *Rhinolophus ferrumequinum*, *Myotis myotis*, *M. capaccinii* and *Miniopterus schreibersi* in the Grotta degli Ausi, Colle Fornaro, Prossedi (Latina); 2) Colony of *Miniopterus schreibersi* in the Cisterne delle Terme di Nettuno, Ostia Antica, Roma.

iv. Sicily

1) Mixed colony of *Rhinolophus ferrumequinum*, *Myotis myotis*, *M. capaccinii* and *Miniopterus schreibersi* in the Grotta dei Puntali, Carini (Palermo); 2) Colony of *Tadarida teniotis* in a building of Palermo.

The sample collecting was made under sterile conditions, in order to avoid the contamination with environmental bacterial flora. The single bat individuals, after the capture, were placed for a short period inside sterile plastic containers and later released. Their faeces were removed with sterile buffers and placed in Amies-Stuart medium with particles of charcoal for transportation. Subsequently, in the laboratory, the buffers were sown using the methods already described by Piraino *et al*. (1996). In the same time an 3% Blood-Agar, for each sample, was incubated under anaerobiosis conditions, using a special container inside which the air was replaced by a mixture composed of N_2 (90,5%), CO_2 (5%) and H_2 (4,5%). For the identification of the isolated strains, we have used the systematic keys proposed by Krieg and Holt (1984) and Holt *et al*. (1994).

3. Results and discussion

From the 89 samples examined, we have isolated 26 bacterial species. In the faeces of *Miniopterus schreibersi* we found: *Hafnia alvei*, *Enterobacter cloacae*, *Escherichia blattae*, *Escherichia coli*, *Klebsiella oxytoca*, *Kluyvera ascorbata*, *Proteus mirabilis*, *Pseudomonas putida*, *Streptococcus faecalis* and *Citrobacter freundii*. In *Myotis daubentoni* we recorded the presence of *Citrobacter freundii*, *Enterobacter cloacae*, *E. agglomerans*, *Proteus vulgaris*, *Streptococcus faecalis*. From faeces of *Myotis capaccinii* we isolated the following species: *Hafnia alvei*, *Pseudomonas putida*, *P. cepacia*, *Acinetobacter calcoaceticus*, *Citrobacter freundii*, *Escherichia coli*, *E. adecarboxylata*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *E. sakazakii*, *E. agglomerans*, *E. aerogenes*, *E. intermedium*, *Proteus vulgaris*, *Morganella morganii* and *Streptococcus faecalis*. In the faeces of *Myotis myotis* we have recorded the presence of *Citrobacter freundii*, *Enterobacter agglomerans*, *E. cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Kluyvera cryocrescens*, *Proteus vulgaris* and *Streptococcus faecalis*. In *Pipistrellus kuhli* we detected the presence of the following species: *Escherichia adecarboxylata*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Enterobacter agglomerans*, *E. aerogenes*, *E. gergoviae*, *Proteus vulgaris* and *Streptococcus faecalis*.

Lastly, in the faeces of *Tadarida teniotis* we found: *Alcaligenes faecalis*, *A. denitrificans*, *Escherichia adecarboxylata*, *E. coli*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Enterobacter agglomerans*, *E. cloacae*, *E. sakazakii*, *E. taylorae*, *Proteus mirabilis*, *Morganella morganii*, *Yersinia frederiksenii*, *Streptococcus faecalis* and *S. faecium*.

In the grounds incubated under anaerobiosis we did not observe any growth. No one of the isolated strains showed any metabolic peculiarity. The main part of the isolated species belongs to the family Enterobacteriaceae; one species alone belongs to Pseudomonaceae, while another one to the Enterococci group D. These species dwell normally in the intestinal tract of many mammals and they have been already found in the faeces of some European bat taxa (Pinus & Muller, 1980; Piraino *et al.*, 1996; Di Bella *et al.*, 1997b). Among the Enterobacteriaceae, besides the species more commonly found, we have isolated 3 less frequent ones: *Kluyvera ascorbata*, *K. criocriescens* and *Escherichia blattae*. The presence of Kluyvera (*Kluyvera* sp.) was previously observed only in a Malagasy molossid *Chaerephon pumila* (= *Tadarida pumila*) (Cassel Beraud & Richard, 1988). The presence of *E. blattae*, on the other hand, is an unprecedented occurrence in the limited available literature.

No one of the isolated species belongs to the category of obliged pathogens, though *C. freundii* and *P. mirabilis* can be considered as primary agents of infection respectively to the cattle udder and to the urinary and gastroenteric apparatus of many mammalian species; a similar consideration applies to *E. coli*, which could nevertheless be included among the obliged pathogens due to its serotype as regards the other bacterial species which have been isolated, as *K. oxytoca*, *P. putida*, *S. faecalis*, and the same *C. freundii*, an opportunistic pathogenic type has been demonstrated.

The results of our investigations so far conducted in Italy do not show the diffusion of bacterial agents responsible for zoonosis through the faecal contamination by chiroptera. Therefore bats do not seem to constitute a danger to human health (Di Bella *et al.*, 1997b, 1997c). However, it is certainly useful to improve the knowledge on the enteric microflora of bats, in order to acquire further information on the digestive physiology and metabolism of chiroptera, as well as to monitor their possible role in the spreading of germs causing zoonosis.

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