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WILD RODENTS OF PONTINE ISLANDS **AS** BIOINDICATORS OF ENVIRONMENTAL QUALITY

I RODITORI SELVATICI DELLE ISOLE PONTINE **COME** BIOINDICATORI DELLA QUALITA' AMBIENTALE

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ABSTRACT

An investigation was performed in Pontine islands (Ponza, Palmarola, Zannone) within the researches performed in the Latium region aimed at locating risk and control areas using wild Rodents as bioindicators. Serological analyses and mutagenctic tests were applied on collected animals (*Mus domesticus*, *Rattus norvegicus*, *Rattus rattus*). Serological examinations show: positive sera (17.6%) against *Leptospira icterohaemorrhagiae* only in mice and rats from Ponza; positive sera versus ***Rickettsia mooseri*** (7%), *Toxoplasma gondii* (7%) and *Leishmania donovani* (12.5%); no antibodies against Hantaviruscs wcre detected. Results obtained by the micronucleiis test show that rodent populations from Pontine Islands may be considered as **control** for ihe environmental inutagencsis studies.

Key words: Rodents, Insulariiy, Bioindicators, Zoonoses, Environmental Mutagencsis.

RIASSUNTO

Il lavoro **fa** parte di **un** progetto di ricerca che ha lo scopo di individuare aree esposte a rischio e aree di controllo attraverso l'applicazione di **un** sistema integrato di indagini su Roditori selvatici utilizzati come bioindicatori. Test sierologici e di mutagenesi sono stati effettuati **su** Murini infestanti, raccolti nelle isole Pontine (Ponza, Palmarola, Zannone) individuate come presumibili aree di controllo. **Il** test di agglutinazione microscopica (MAT) ha evidenziato sieropositività nel **17%** dei Murini raccolti a Ponza. Il test di immunofluorescenza indiretta per la ricerca delle agglutinine IgG per Haninan virus, risultato negativo in tutti gli animali esaminati, è indicativo dell'assenza del virus nel territorio. Il test di fissazione del complemento per la ricerca di anticorpi contro le Rickettsie ha mostrato una positività solo per *Rickettsia mooseri* in 2 *R. norvegicus* e 1 *R. rattus* di Ponza e 2 *R. rattus* di Palmarola. I risultati ottenuti con il test dei micronuclei evidenziano che nelle tre specie esaminate la frequenza media di eritrociti policromatici micronucleati (MPCEs) si attesta tra i valori più bassi registrati in altre popolazioni delle stesse specie raccolte nel Lazio. **i** risultati ottenuti offrono **un** primo contributo alla conoscenza dei Murini delle Isole Pontine e puntualizzano la necessità di condurre monitoraggi periodici per il controllo delle popolazioni infestanti e delle zoonosi da essi trasmesse.

Parole chiave: Roditori, Insularità, Bioindicatori, Zoonosi, Mutagenesi ambientale.

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INTRODUCTION

This paper is part of a research program aimed to identify risk and control areas, employing an integrated system of analyses on natural populations of small mammals used as bioindicators (Ieradi et al., 1984; Cristaldi et al., 1985; Cristaldi et al., 1990). Some wild rodent pest species (*Mus domesticus*, *Rattus norvegicus*, *Rattus rattus*) are being used for their widespread distribution in Mediterranean areas (Amori et al., 1984), their relatively small home range and the abundant biological data available on laboratory strains. As far as Environmental Impact Assessment (EIA) is concerned, pest rodents can be considered at the same time as indicators of environmental modifications and risk factors, for zoonoses they can transmit to humans and to other animals and damage they can cause to property (Ieradi, 1990).

This type of research includes:

- individuation of murine species and analysis of their distribution on the area to be examined;
- application of serological and bacteriological analyses on murine species in order to determine their role as vectors of zoonoses;
- use of mutagenetic tests (micronucleus test and sperm abnormality assay) in order to determine the effects on resident populations of chemical and/or radioactive pollutants present in the environment.

Pontine Islands (Ponza, Palmarola and Zannone), not being polluted by chemical contamination from industrial and agricultural plants, were chosen as control areas.

In this paper the preliminary results of serological analyses and micronucleus test are reported; serological investigation were carried out in order to verify the extent of the infections (leptospirosis, haemorrhagic fever with renal syndrome, leishmaniosis, rickettsiosis, toxoplasmosis) in wild murine species living in Pontine Islands and the micronucleus test in order to control genetic damage. The micronucleus test is a simple and rapid cytogenetic test *in vivo* used for evaluating the clastogenic potential of exposure to chemicals and radioactive substances (Schmid, 1976; Jenssen & Ramel, 1976; Carriott & Grahn, 1982).

The combination of those two methods (test of micronuclei and serological assays) was also performed to verify the possibility of a correlation between the frequency of micronuclei and the presence of infections in the examined samples, as was recently demonstrated by Ormiston et al. (1989) for babesiosis.

MATERIALS AND METHODS

The survey was carried out on Ponza, Palmarola and Zannone Islands from May 31st to June 20th 1988. These islands, the most important of the NW group of the Pontino Archipelago, are between 40" 52' and 40" 58' parallels and 0' 23' and 0° 37' meridians; Ponza is the only inhabited island. The Archipelago is of volcanic origin and it is formed by eruptive rocks (Carrnassi et al., 1983). The

Tab. 1 – List of the wild Rodents collected in Pontinc Islands.
Lista dei Roditori selvatici mccolii nelle isole Pontine.

SAMPLES	TRAPPING SITE	SEX	AGE (MONTHS)	WEIGHT (GRAMS)	MPCES/1000 PCES
PONZA *Mus domesticus*					
PNZ 21	Calettella	M	≥11	16	0
PNZ 23	Capo Bianco	M	3+5	14	0
PNZ 24	Capo Bianco	F	< 4	12	0
PNZ 25	Capo Bianco	F	≤11	12	0
PNZ 26	Monte 3 Venti	M	≥ 1	10	0
PNZ 21	Giancos	F	≥ 3	16	0
PNZ 28	Gianws	F	– 3	16	0
PNZ 29	Gianws	M	– 3	12	3
PNZ 30	Giancos	F	2 2	6	0
PNZ 32	Capo Bianco	hi	< 3	8	1
PNZ 35	Monte Guardia	M	< 4	14	0
PNZ 36	Capo Bianco	M	> 5	18	1
PNZ 37	Giancos	M	> 2	8	2
PNZ 38	Monte Guardia	M	– 3	14	1
PNZ 39	Monte Guardia	F	> 5	20	1
PNZ 41	Giancos	F	s 5	14	2
PNZ 42	Giancos	F	> 3	16	4
PNZ 43	Giancos	F	≤ 5	16	1
PNZ 44	Monte Guardia	F	– 3	12	1
PNZ 45	Monte Guardia	F	2 3	18	2
PNZ 46	Porto	F	7+8	18	0
PNZ 49	Porto	F	≤ 5	18	1
PNZ 50	Giancos	F	18+24	20	0
PNZ 52	Giancos	M	– 3	12	0
PNZ 53	Giancos	M	≤ 2	10	1
PNZ 59	Porto	M	– 3	12	0
PONZA *Rattus norvegicus*					
PNZ 22	Capo Bianco	M	1 2	20	1
PNZ 31	Capo Bianco	M	< 2	20	2
PNZ 34	Capo Bianco	F	< 2	20	1
PNZ 47	Capo Bianco	F	>12	356	0
PNZ 48	Le Forna	F	>12	302	1
PNZ 54	Porto	F	2 2	30	1
PNZ 55	Porto	M	> 2	34	0
PNZ 56	Porto	M	> 2	36	0
PNZ 57	Porto	F	> 2	32	0
PNZ 58	Porto	M	> 2	34	0
PONZA, PALMAROLA E ZANNONE *Rattus rattus*					
PNZ 33	Linguana	F	> 12	168	0
PNZ 51	Linguana	F	<12	158	0
PLM 2	Marina S. Silverio	M	> 2	52	0
PLM 4	Marina S. Silverio	M	2+5	56	2
PLM 5	Marina S. Silverio	F	2 3	54	0
PLM 6	Marina S. Silverio	M	< 2	38	1
PLM 7	Marina S. Silverio	M	> 5	244	0
PLM 8	Marina S. Silverio	F	> 5	188	1
PLM 9	Marina S. Silverio	F	< 5	134	1
ZNN 1	Casa di Caccia	F	> 5	164	0
ZNN 2	Casa di Caccia	M	7 5	136	0

phytocoenoses are typically mediterranean; climax vegetation is mainly constituted by *Quercetum ilicis gallo-provincialis* Br. Bl., replaced by *Erica arborea* maquis in the northern slopes (Nimis, 1986)

Palmarola lies on the eastern edge arid is inhabited just by a few people from spring to autumn; houses are built on the only beach (Marina di San Silverio). Zannone lies on the northern edge of the Archipelago and is completely covered by mediterranean wood. This island, inhabited only by the guardian and his family, is part of National Park of Circeo since 1979 (Ortese, 1952).

A total of 51 murine rodents was collected: 28 *Mus domesticus*, 10 *Rattus norvegicus*, 13 *Rattus rattus*. On the island of Ponza collecting stations were located in the following areas: Porto, Le Forna, Giancos, Monte Guardia, Capo Bianco, Monte dei Tre Venti, Linguana.

In the collecting station "Porto" traps were posed into the garden of the Cathedral, in the village round the Belvedere, on fields and vegetable-gardens and in a cove (Calettella), located between the harbour of Ponza and Punta della Madonna, characterised by sand and mediterranean wild plants.

The station named "Le Forna" is located under a cane field and near a waste water dranage; the "Giancos" station is situated near the houses on the cultivated fields and non; "Monte Guardia" is the highest peak of the island (m. 283); trapping station is near a small Chapel on abandoned fields; "Capo Bianco" station is located in a mediterranean wood area near the sludges of an out of use incinerator; "Monte dei Tre Venti" station is between mediterranean wood anti field-pastures; "Linguana" station is located in an almost wild mediterranean wood. In Palmarola traps were set just behind the only beach in the NW of the island. In Zannone traps were located under and around the house of the guardian of the Park ("Casa di Caccia").

Collected animals were transferred to an especially equipped laboratory on Ponza and sacrificed with ethyl ether, weighed and numbered.

The age of Rodents was determined using the method of tooth wear (Karnoukova, 1971; Keller, 1974); the outlines of the teeth of the lower and upper jaw of the left side were drawn with **a** tube mounted on a binocular stereoscopical microscope at the following magnifications: **x** 12 fur rats and **x** 25 for mice; moreover head-body lenght, weight and general aspect of the skull were considered.

Blood (by a cardiac puncture) and bone marrow (from femurs) samples were immediately taken for serological assays and for the micronucleus test, respectively.

SEROLOGY: sera were assayed to detect antibodies against Ieptospires by the microscopic agglutination test (Faine, 1952). Living strains belonging to the following serotypes: *icterohaenzorrhagiae, copenagheni, bratislava, canicola, grippotyphosa, saxkoebing, hardjo, ballum, tarassovi, pomona*, were used as antigens.

A modification of indirect immunofluorescence test (Lee et al., 1978) was employed for detecting IgG antibodies against Hantaviruses (HTN viruses). Sera from Rodents initially were diluted 1:32 in phosphate buffer at pH 7.4. Vero-E-6 cells were infected with the following hantaviruses: Hantaan prototypes (strain

76/118), Seoul **(SEO)** and the Puumala serotype (PUU). Rodent serum samples were considered positive only at dilutions of 1:32 or greater.

The micro-Complement Fixation test was employed to detect antibodies against *Rickettsia conori, R. mooseri* and *Coxiella bumeti;* soluble antigens of ***R. mooseri*** and ***R. conori*** were supplied by the **WHO** Reference Center for Rickettsiosis of Bratislava (Czechoslovakia) **arid** C. *burneti* antigen was commercially available; minimum diluition titre was 1:8, maximum 1:256. The indirect haemoagglutination reaction and the direct agglutination test were used for the detection of antibodies against *Leishmania donovani* and for the detection of antibodies against *Toxoplasma gondii,* respectively.

TEST OF MICRONUCLEI (Matter and Schmid, 1971): both femurs were dissected and their marrow cells were flushed out with fetal bovine serum, the celi suspension was centrifugated at 800 rpm for 5 min and the cell pellet was resuspended and smeared on clean glass slide. Smears, fixed with methanol, were stained with May-Grunwald Giemsa (Cole et ai., 1979). Counting of micronuclei is carried out determining the frequency of micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes (Mc Gregor et al., 1987).

RESULTS AND DISCUSSION

SEROLOGICAL ASSAYS (tab. 2): serological esamination by the microagglutination test (MAT) revealed a positive reaction in 6 (17.6%) out off 34 rodents examined (14 *Mus domesticus,* **9** *Rattus norvegicus* and **11** *Rattus rattus):* agglutinins against *Leptospira icterohaemorrugiae* were found in two *R. norvegicus* and in four *Mus* captured at Ponza, at a titre variable from **1/100** to 1/1600.

Antibodies against Hantaviruses were not detected in 32 rodents examined.

The micro-Complement Fixation test showed a positive reaction only against *Rickettsia mooseri*, the pathogenic agent of murine typhus, on five (17.8%) out of the 28 examined animals (titres from **1/8** to 1/16): two *R. norvegicus* and one *R. rattus* captured at Ponza and two *R. rattus* captured at Palmarola.

Two sera from animals captured at Ponza, out of the 28 examined (7%), shown antibodies against *Toxoplasma gondii*: one *M. domesticus* with a high antibody titre (1/4096) and one *R. norvegicus* with the minimum assayed titre (1/64).

Antibodies against *Leishmania donovani,* were found only in three *Mus domesticus* of Ponza (12.5%), out of 24 examined samples at the minimum assayed titre (1/64).

Detection of antibodies against ***L.*** *icterohaemorrhagiae* in *R. norvegicus* and ***Mus*** *domesticus* collected at Ponza indicates that leptospiral infection is present on that island. Comparing these results with those from murine populations of Rome (Amaddeo et al., 1989; Ieradi et **al.,** 1989; Amaddeo et al., 1991) we can see that the percentage of seropositive murines for *Leptospira icterohaenzorrhagiae* from Ponza is smaller (17.6%) than from wild Rodents collected at Rome **(50.4%).** Moreover, no agglutinins against ***L.*** *ballum* were found in the examined serum samples, whereas they were present in *M. domesticus* and *Rattus rattus* captured in

Tab. 2 – Serological analyses for detecting antibodies against *Leptospira icterohaemorrhagiae* (tab. 2.1), *Rickettsia mooseri* (tab. 2.2), *Leishmania donovani* (tab. 2.3), *Toxoplasma gondii* (tab. 2.4).
Analisi sierologiche per la rilevazione di anticorpi contro Leptospira icterohaemorrhagiae *(fah.2.1),* Rickettsia mooseri *(tab. 2.2),* Leishmania donovani *(tab. 2.3),* Toxoplasma gondii *(tub. 2.4).*

SPECIES	NO. SERA	No. POS.		TITRES			
Tab. 2.1							
Mus domesticus	14	4		1/100	1/200	1/1200	1/1400
Rattus norvegicus	9	2		1/1200	1/1600		
Rattus rattus	11	0					
TOTAL	34	6	17.6%				
tab. 2.2							
Miu domesticus	9	0					
Rattus·norvegicus	9	2		1/8	U16		
Rattus rattus	10	3		1/8	1/16	1/16	
TOTAL	28	5	17.86%				
tab. 2.3							
Mus domesticus	7	3		1/64	1/64	1/64	
Rattus norvegicus	7	0					
Rattus rattus	8	0					
TOTAL	22	3	13.63%				
tab. 2.4							
Mus domesticus	9	1		1/4096			
Rattus norvegicus	9	1		1/64			
Rattus rattus	10	0					
TOTAL	28	2	7.1%				

Rome, even though at low percentage (6.6% and 7.7% respectively). It must be underlined that it was shown in various animal species that *L. ballum* is incapable of inducing high values of antibody titres (Hataway et al., 1982). No antibodies were found on the islands of Palmarola and Zannone against *Leptospira* sp. in any of the black rats captured. It can therefore be hypothesized that environmental conditions of the island, with no fresh water supply and almost with no human settlements, were not suitable for the survival of those microorganisms.

The negative antibodies response against the different serotypes of the Hantaviruses indicates the probable absence of HTN virus in the murine populations of Pontine islands. Comparing this result with that from murine populations sampled in Rome (Nuti et **al.,** 1990), where a positive response was recorded in *R. norvegicus* (52%), *Mus domesticus* (19%) and *R. rattus* (17%), we can see a similar situation to that observed by Lee et **al.** (1978) on Jeju island

lab. 3 – Bone marrow micronucleus test.
Test dei micronuclei nel midollo rosso delle ossa.

SPECIES	No. OF ANIMALS	MIN	$\bar{x}$ (SE)	MAX
Mus domesticus	26	0	0.8 (0.29)	4
Rattus norvegicus	10	0	0.6 (0.22)	2
Rattus rattus	7	0	0.7 (0.28)	2

(Korea). On that island, located in front of a continental area, endemically subject to haemorrhagic fever, Hantaviruses were not present in the captured rodents.

Results obtained with the micro-Complement Fixation test for detecting antibodies against *Rickettsia mooseri* would indicate the circulation of this microorganism (Lillini et ai., 1991), but as murine typhus practically disappeared from official reports (Vitale et al., 1988), further investigations would be necessary.

TEST OF MICRONUCLEI (tables 1 and *3):* the micronucleus test carried out on 47 animals (26 *Mus domesticus*, 10 *Rattus norvegicus*, 11 *R. rattus)* shows that the mean frequency of MPCEs/1000 PCEs (tab. 3) is included among the lowest values found in other natural populations of Latium (range: *Mus domesticus* 0.6±0.3 - 2.5±0.3; *Rattus norvegicus* 0.4±0.2 - 4.5111.5). Moreover, such value is significantly lower than that recorded in laboratory breeds used as control (1/1000) and shows absence of a genetic damage. Consequently, the values recorded in such populations can be considered **as** control values for studies on environmental mutagenesis of wild Miirines.

Furthermore in animals examined no significant correlation was found between the frequency of micronuclei and zoonoses.

Studies on *Mus domesticus* on island environments (Amori et al., 1986) report a normal karyotype (2n=40) for Ponza island; studies on chromosome evolution and applied aspects of territory management on Eolian islands (Cristaldi et al., 1987) indicate a strong interaction between karyotype evolution and passive transportation of rodents through trading. This causes a parallel spreading of zoonoses transmissible by rodents. The results of this research suggest some considerations on the Pontino Archipelago. On Mediterranean islands, where natural or seminatural conditions still exist (e.g. Palmarola and Zamione) the typical distribution of *R. rattus* was observed (Amori et al., 1984). On islands like Ponza, where human presence is considerable because of trades and settlements, the situation of murine populations is more complex: *R. rattus* seems to be confined to areas where the Mediterranean maquis is quite intact (Linguana); the presence of *Mus* is generalized even on uninhabited areas, whereas *R. norvegicus*, favoured by continuous water supplies to the island, can be mainly found on environments as dumps, drains, etc.

In conclusion the results from this study confirm wild Rodents as bioindicators also in uncontaminated areas, which could be used as control sites (Tommasi et al.,

1990). Furthermore for monitoring environmental quality, it is necessary to repeat the investigation in different periods of the year. In fact, the spreading of human activities, associated with an increase of tourism, particularly during the warm season, has as a consequence an inadequate management of solid and liquid wastes that could cause, particularly on Ponza island, an increase of the "murine risk".

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