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Evaluation of bloodsucking arthropod bite as possible risk co-factor in *Human herpesvirus*-8 transmission route

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Abstract. *Human herpesvirus*-8 non-sexual transmission occurs primarily from mother-to-child. The viral load in saliva is higher than in other human fluids. Moreover, there is evidence that bloodsucking arthropod bites induce an inflammatory/immune response that facilitates viral replication. We aim to explore possible risk factors in mother-to-child HHV-8 transmission associated with traditional methods which involve the use of saliva to relieve the irritation and skin reaction caused by arthropod bites. We administered questionnaires to 2244 children from several African countries and Italy. Descriptive statistics and logistic regression were used in the analysis of the answers to evaluate the relationships between the use of traditional methods and other risk factors. The use of traditional methods is high in Cameroon (63.0%) and Uganda (39.9%), intermediate in Senegal (26.7%) and Italy (21.7%), low in Madagascar (6.7%). Statistical analyses show significant direct relationships between the use of traditional methods, skin reactions to the bite and their duration in Cameroon, Uganda and Senegal. The use of saliva and herbs applied by the mothers on the child's skin, is a common habit in Africa. If this practice plays a role in the HHV-8 transmission, then, it could provide the basis for interventions capable of reducing the health impact of the infection in children in tropical areas.

Key words: HHV-8, saliva, mother-to-child transmission, promoter arthropods, questionnaires.

Human herpesvirus-8 (HHV-8) is a γ-herpesvirus considered to be the major determinant of Kaposi's sarcoma (KS) which is usually localised on limbs (Chang *et al.,* 1994). HHV-8 is also involved in the pathogenesis of primary effusion lymphoma (PEL), a rare form of non-Hodgkin lymphoma and of the benign lymphoproliferative disorder Multicentric Castelman disease (MCD) (Cesarman *et al.,* 1995; Soulier *et al.,* 1995). When Chang described HHV-8 for the first time, a new field of study and research regarding how this infection is transmitted and acquired opened up. This virus has emerged as an infectious agent of public health importance, mainly in sub-Saharan Africa where HHV-8 seroprevalence and risk for KS are high, but lower in Mediterranean countries, in United States, Northern Europe and Southern Asia (Dedicoat & Newton, 2003; Chatlynne & Ablashi, 1999). The route of HHV-8 transmission is uncertain. Several studies clearly show the association between sexual activity and HHV-8 infection (Kedes *et al.,*1996; Perna, 2000), and some studies have also suggested salivary transmission and a small but significant risk associated with drug injection (Corey *et al.,* 2002; Martin *et al.,* 1998). In United States and Europe, where the sexual behaviours associated with HHV-8 seropositivity include the number of sex partners, number of partners with KS and sex with an HIV infected partner (O'Brien *et al.,*1999; Tedeschi *et al.,* 2000), HHV-8 seroprevalence shows a range between 1-8% (Dedicoat & Newton, 2003; Chatlynne & Ablashi, 1999). In Southern Asia, although few studies had been performed, HHV-8 seroprevalence among adults shows ranges between 4.93% in Korea to 15.49% in Vietnam, and the infection is not associated with particular sexual behaviours (Chatlynne & Ablashi, 1999).

In Italy seroprevalence is reported to be 16.5% (Brown *et al.,* 2005) and 18.7% (Serraino *et al.,* 2001), while in sub-Saharan Africa it ranges from 40 to 70% in adults (Serraino *et al.,* 2001). Interestingly, among African children and Amerindian populations, HHV-8 seroprevalence ranges from 8% to 57% (Bourboulia *et al.,* 1998; Mbulaiteye *et al.,* 2003, de Souza *et al.*, 2007) according to the prevalence among adults in the different epidemiological contexts. This suggests that HHV-8 infection is not only transmitted sexually (Gessain *et al.,* 1999; Gessain, 2005; Plancoulaine & Gessain, 2005; Mbulaiteye *et al.,* 2005). Moreover, vertical transmission such as in utero, intrapartum and breast milk have been demonstrated to be minor contributors to the occurrence of HHV-8 in new born or very young children (Brayfield *et al.,* 2003; Mantina *et al.,* 2001; Brayfield *et al.,* 2004). Significant correlations have been reported with mothers', children's and siblings' HHV-8 serostatus (Mbulaiteye *et al.,* 2005). As saliva is the bodily fluid that most often harbours HHV-8 at greatest

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concentration, the exchange of saliva could be involved in HHV-8 mother-to-child horizontal transmission (Dedicoat & Newton, 2004; Corey *et al.,* 2002). The question is how children are exposed to maternal saliva? In Africa there are a number of common practices which involve maternal saliva (Wojcicki, 2003). We investigate the hypothesis that the use of saliva to soothe the itchy bites of bloodsucking arthropod vectors, or promoter arthropods, is involved in the transmission of HHV-8 (Coluzzi *et al.,* 2002). In particular, the arthropod does not inject the virus, but rather prepares the cellular and biochemical skin environment both injecting antihaemostatic molecules, and evoking a type 1 hypersensibility reaction at the bite site (Coluzzi *et al.,* 2002). Thus, the arthropod bite creates a point of entry for the virus which is introduced by mothers' (or caregivers') infected saliva applied to the child's bite site to relieve itching and reduce scratching (Coluzzi *et al.,* 2002). The local inflammatory reaction on the skin at the arthropod bite site follows the general trend of suppression of Th1 and induction of Th2 cytokines. Thus, as HHV-8 is avid in these cytokines, viral transmission could be facilitated (Ensoli *et al.,* 2001; Schoeler & Wickel, 2001; Asada, 2007).

Our aim is to study the following possible risk co-factors related to the role of promoter arthropods in HHV-8 transmission: (i) the use of traditional methods based on the application of saliva or premasticated herbs to children's skin to relieve itching on the bite site, (ii) the local inflammatory reaction related to child's response to the bite (skin itchiness, skin irritation, skin swelling, skin pain, skin mark, lasting reaction), and (iii) environmental factors which may be related to the presence of arthropods (stagnant water and domestic animals nearby domestic location). We carried out surveys using questionnaires in Italy, a Mediterranean basin country in which the HHV-8 seroprevalence is one of the highest in Europe and in four sub-Saharan African countries (Senegal, Cameroon, Uganda and Madagascar).

Materials and methods

Study area and subjects

The questionnaires were administered to 2244 children (age 5-15 years) interviewed at schools or health care centres. In Italy (Latium, Tuscany, Veneto regions) 1183 questionnaires were administered to 191 primary and 992 secondary school students in 2004. In Senegal (Thionck-Essyl: Casamance region; Horefondè: Matam region) to 104 students from primary school in 2007. In Cameroon (Djuttista, Nzong and Dschang: Western Cameroon) were administered to 200 primary school students in 2005. In Uganda (Kampala, Entebbe: Central Uganda; Rupa and Moroto: Northern Uganda) 533 questionnaires were administered in 2007 to 272 primary, 159 secondary school students, and 102 children from Namalu Health Centre (Karamoja region: North-Eastern Uganda). In Madagascar (Amboasary, Antsanomaro, Masihanaka, Fort-Dauphin: Southern Madagascar) questionnaires were administrated in 2007 to 224 children from local health care centres. Sample size was chosen in relation to the dimension of the local population.

Questionnaires

The experimental procedure included the distribution of anonymous questionnaires (Figure 1) which were completed with the help of trained local personnel. Formal permission was obtained from the local authorities (school and health care service directors). Signed informed consent was obtained from parents or caregivers of the children. The questionnaires were translated into the official language of each country (Italian, French and English).

Questionnaires were developed by one of the authors (Romano R.). The first section included sociodemographic questions about age, gender, home location (i.e. rural or urban area, access to water facilities and presence of stagnant or running water in the neighbourhood), presence of domestic animals (i.e. cows, sheep, goats, donkeys). The second section included questions about possible promoter arthropods (i.e. perception of insects in the environment, bite frequency, part of the body most bitten and when). The third section included questions on bitten skin reaction (i.e. itchiness, pain, irritation, swelling, skin mark, lasting of reaction). The last section concerned treatment received (i.e. traditional methods including saliva or premasticated herbs and pharmaceutical products).

Data analysis

SPSS, version 16, was used for statistical analyses. For each country descriptive statistics were calculated for each question related to the type of treatment received (saliva or premasticated herbs and pharmacological products) and risk co-factors. Yates-corrected χ^2 test, calculated Odds ratios (OR) with their corresponding 95% confidence intervals (CI) were used to investigate factors associated with traditional methods. Furthermore, binary logistic regression (BLR) was used to investigate the impact of different independent risk co-factors on the dependent dichotomous variable "use of traditional methods".

Risk factors investigated included symptoms related to the bite (itchiness, pain, irritation, swelling, skin mark and duration of reaction) and environmental factors (stagnant water and domestic/courtyard animals) which increase the presence of bloodsucking arthropods. These co-factors represent the most important ones related to the HHV-8 transmission route through promoter arthropods. We report only significant associations ($P<0.05$). Sam-

Questionnaire

Survey among primary and secondary school children.
Place of birth…… Nationality……

1.	Do you have brothers or sisters?	☐ yes	☐ no	
2.	Is your home located in:	☐ rural area distance > 3 km from the urban area		
		☐ urban area		
3.	Close to your home there are	☐ water stagnant (lakes, artificial canals, marsh)		
		☐ water no stagnant (rivers)		
		☐ none of the above		
4.	In which way does your household obtain water for domestic use?			
	(*a*) Piped (tap) waters inside the house			
	(*b*) Source or well (community or family) distance from house:		☐ > 200 m	☐ < 200 m
5.	Do you have any domestic animals?		☐ yes	☐ no
	If so, which?……			
6.	How many insects have you come across?	☐ few	☐ many	☐ none
7.	Have you ever been bitten by one?	☐ frequently	☐ rarely	☐ never
8.	If so, which kind of insect?	☐ mosquito	☐ wasp	☐ biting midge
		☐ other……...		
9.	If so, when?	☐ recently	☐ long time ago	☐ don't know/ remember
10.	On which part of your body?	☐ face	☐ thorax	
		☐ arm	☐ hand	
		☐ leg	☐ foot	
11.	Have you ever had fever after being bitten?	☐ yes	☐ no	
12.	Usually are you bitten during the:	☐ morning	☐ evening	☐ night
13.	Did you have a mark on your skin?	☐ yes	☐ no	
14.	What did you feel where the bite was?	☐ itchiness	☐ pain	☐ nothing
15.	What reaction did you have on your skin?	☐ irritation (redness)	☐ swelling	☐ nothing
16.	For how long did the bite bother you?	☐ < 3 days	☐ 3-7 days	☐ > 7 days
17.	What kind of treatment did you receive?			
	(*a*) Traditional methods using natural substances:		☐ herb	☐ saliva
	(*b*) Pharmaceutical products:		☐ cream	☐ lotion
	(*c*)		☐ nothing	☐ other
18.	If do you use herbs, which kind? ……...			
19.	Do you use them crushed?		☐ yes	☐ no
20.	If yes do you mix them with saliva after crushing?		☐ yes	☐ no

Figure 1. Questionnaire administered to children.

ple sizes differ slightly between tests because of missing values due to lack of answers.

Results

Table 1 shows responses to questions stratified by country. More than 90% of the interviewed children are bitten (data not shown).

The calculated frequencies with which saliva or premasticated herbs are used in each country were: Italy 17.6% (183/1042) and 4.2% (44/1042); Senegal 16.6% (15/90) and 9.3% (8/86); Cameroon 25.0% (50/200) and 45.5% (91/200); Uganda 21.9% (93/425) and 20.8% (88/424); Madagascar 4.2% (5/120) and 2.3% (3/120).

In Senegal, Cameroon and Uganda, there are statistically significant relationships between the use of traditional methods and different risk co-factors. In particular, in Senegal the use of traditional methods is directly associated with skin pain and skin mark (Table 2 and Table 3). In Cameroon, the use of traditional methods is directly associated with: skin itchiness, skin pain, duration of skin reaction and the presence of stagnant water near the home (Table 2 and Table 3). In Uganda, the use of traditional methods is directly associated with skin irritation, skin pain, skin mark, duration of skin reaction and presence of stagnant water near the home (Table 2 and Table 3). Data from Italy and Madagascar did not reveal significant relationships between use of traditional methods and these factors.

Table 1. Response to questions related to possible risk co-factors involved in HHV-8 transmission.

	Italy %	Senegal %	Cameroon %	Uganda %	Madagascar %
Use of traditional methods*	21.7 (226/1042)	26.7 (23/86)	63.0 (126/200)	39.9 (169/424)	6.7 (8/120)
Use of pharmacological products	81.6 (850/1042)	24.1 (21/87)	37.5 (75/200)	48.9 (209/427)	29.7 (35/118)
Arthropod bite on limbs	85.1 (888/1044)	66.0 (68/103)	70.0 (140/200)	75.4 (389/516)	68.9 (153/222)
Skin itchiness	85.1 (888/1044)	54.1 (53/98)	53.0 (106/200)	67.4 (355/527)	76.6 (164/214)
Skin irritation	67.7 (705/1042)	13.3 (13/98)	48 (96/200)	23.2 (122/525)	67.1 (143/213)
Skin swelling	57.4 (598/1042)	95.9 (93/97)	67.5 (135/200)	69.7 (366/525)	33.8 (72/213)
Skin pain	12.2 (127/1044)	46.2 (48/98)	55.0 (110/200)	28.8 (152/527)	11.7 (25/214)
Skin mark	45.8 (463/1010)	85.7 (84/98)	76.0 (152/200)	74.3 (379/510)	69.0 (145/210)
Lasting skin reaction > 3 days	25.8 (260/1009)	5.5 (5/90)	26.5 (49/185)	21.6 (109/505)	31.6 (48/152)
Presence of stagnant water nearby home location	30.8 (322/1044)	95.9 (94/98)	48.0 (96/200)	43.0 (228/530)	73.1 (158/216)
Presence of domestic animals	66.3 (682/1029)	95.0 (96/101)	93.4 (185/198)	69.2 (364/529)	66.1 (158/224)

* Use of traditional methods includes saliva or premasticated herbs.

Table 2. Comparison between use of traditional methods vs risk co-factors.

	Country	Sample size	Risk co-factors	% use traditional methods		OR (95% CI)	χ^2 (Yates-corrected)	*P*
				Co-factor present	Co-factor absent			
Use of traditional methods*	Senegal	88	Skin pain	45.3	7.1	10.00 (2.4547.36)	13.19	<0.001
		88	Skin mark	30.3	0	N/A	N/A	0.031**
	Cameroon	185	Lasting skin reaction > 3 days	78.3	52.7	3.25 (1.31-8.32)	6.98	0.008
		200	Presence of stagnant water nearby home location	66.7	50.9	1.92 (1.04-3.56)	4.45	0.035
	Uganda	421	Skin irritation	43.9	34.4	2.43 (1.52-3.90)	14.75	<0.001
		422	Skin pain	49.2	34.6	1.94 (1.26-2.99)	9.47	0.002
		410	Skin mark	44.4	29.5	1.91 (1.19-3.09)	7.36	0.007
		401	Lasting skin reaction > 3 days	56.0	35.2	2.34 (1.44-3.81)	12.62	<0.001
		421	Presence of stagnant water nearby home location	52.1	26.7	2.98 (1.94-4.58)	27.05	<0.001

* Use of traditional methods includes saliva or premasticated herbs; OR=Odds ratio ± 95% confidence interval.
** Fisher's exact test.

Table 3. Significant co-factors for the use of traditional methods: logistic regression.

	Country	Sample size	Risk co-factors	OR (95% CI)	*P*
Use of traditional methods*	Senegal	82	Skin pain	13.0 (3.3-50.7)	< 0.001
	Cameroon	184	Skin itchiness	4.1 (1.6-10.3)	0.003
			Skin pain	3.2 (1.3-7.9)	0.013
			Lasting skin reaction > 3 days	3.6 (1.5-8.6)	0.003
	Uganda	289	Skin irritation	1.9 (1.2-3.1)	< 0.001
			Lasting skin reaction > 3 days	2.1 (1.3-3.3)	0.010
			Presence of stagnant water nearby home location	2.4 (1.5-3.7)	0.004

* Use of traditional methods includes saliva or premasticated herbs.

Discussion

Our aim is to evaluate the use of traditional methods for the treatment of the local inflammatory reactions to arthropod bites and environmental factors which may be involved in HHV-8 transmission. The survey demonstrated that, in the countries studied, saliva or premasticated herbs are frequently applied by mothers (or caregivers) to the child's skin to relieve itching and scratching due to the arthropod bite. In Africa, the rate of use of traditional methods differs among countries: in Senegal, Cameroon and Uganda it is between 27% and 63%. Comparing HHV-8 seroprevalence in analysed countries and the frequency of traditional methods usage, we observe that the use of these methods is a very common habit (Table 1) both in Uganda and Cameroon, where HHV-8 seroprevalence rate is the highest in Africa (14-86% and 28-62% respectively) (Dedicoat & Newton, 2003). In Senegal, where HHV-8 seroprevalence rate is lower (14%) (Dedicoat & Newton, 2003), the use of traditional methods is also less widespread (Table 1). In Madagascar, where HHV-8 seroprevalence rates are not available, the use of traditional methods is less than 7%, this could be due to cultural differences. In fact, the island of Madagascar shows unique characteristics in terms of flora, fauna and human habits. As HHV-8 horizontal transmission occurs through saliva (Gessain, 2005; Plancoulaine & Gessain, 2005; Dedicoat *et al.,* 2004; Corey *et al.,* 2002; Wojcicki, 2003) these preliminary observations, about the different rate of traditional methods usage, could open new fields on how this infection is transmitted from mother to child. In Italy the majority of mothers (81.6%) apply pharmaceutical products to relieve the child's itching after the bite, reducing swelling and the skin mark, but also the use of traditional methods is common, about 20%. In Africa, the use of traditional methods is widespread: both saliva or premasticated herbs are applied on the child's skin. In particular, in Cameroon the rate of traditional methods usage is the highest (63.0%) among the countries studied.

In Senegal, Cameroon and Uganda a high proportion of children have a skin reaction after an arthropod bite and statistical analysis shows that it is directly associated with the use of traditional methods. Moreover, in Cameroon and in Uganda the use of traditional methods is high, and is associated with presence of stagnant water near the home which may be responsable for the presence of different species of arthropods. In effect, stagnant water can be considered as reservoir of arthropods: many are dependent on water for at least a part of their life cycle. Living near stagnant water, could increase the risk of being bitten by arthropods, indeed 90% of the African children report being bitten. We found this association only in Cameroon and Uganda, whereas in Italy, Senegal and Madagascar the direct relationship between use of traditional methods and stagnant water is not evident. As at the moment, entomological data relating on the studied areas are not available, and we can only infer that these differences among countries may be due to different resident species of arthropods. High exposure to arthropod bites causes increased local skin reaction and, consequently, a wider use of traditional methods by mothers to reduce children's discomfort. According to our hypothesis, promoter arthropods are a new category of vectors which do not transmit the virus directly, but prepare the skin environment for the infection. Through biting, the arthropods evoke an immediate/delayed hypersensitivity response which probably lasts more than three days (Table 2 and Table 3). This hypersensitivity reaction on the bite site causes the release of cytokines which creates a favourable skin environment for HHV-8 infection. The virus is probably introduced on the children's bite site through maternal saliva which is applied to reduce itching and scratching. This type of skin reaction is related to bloodsucking arthropods, particularly those associated with non-human hosts such as phlebotomous (*Phlebotomus* spp.), simuliums (*Simulium* spp.), biting midges (*Culicoides* spp. and *Leptoconops* spp.) and some species of mosquitoes (i.e. *Ochlerotatus, Coquillettidia* and *Aedes* spp.) (Coluzzi *et al.,* 2002). The trend shown by statistical analysis seems to be in agreement with our hypothesis: the skin inflammatory reaction, after the arthropod bite, determining itching and scratching, induces the mother to apply saliva or premasticated herbs to the bite site. Thus it seems that the following co-factors are involved in the HHV-8 infection route: skin reaction to the bite, the use of traditional methods and environmental factors such as stagnant water reservoirs. This hypothesis could represent a model of how HHV-8 infection passes horizontally from mother to child through saliva. From the literature it would appear that the saliva of seropositive subjects is a main reservoir of HHV-8; moreover, this study reveals that the use of saliva on the site of arthropod bite is very common. Thus, if this, non-sexual, horizontal transmission route were to be proven, it could provide the basis for interventions to reduce the impact of this paediatric viral infection in tropical areas.

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Malarial infections in sedentary and migratory passerine birds in Israel: description of new species

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Abstract. Our objective was to investigate the diversity of *Plasmodium* species in birds of the Rift Valley section in Israel. *Plasmodium merulae* Corradetti & Scanga, 1973 was previously reported in blackbirds (*Turdus merula* Linnaeus, 1758), that are resident. We also report and describe three other species and seven new species of *Plasmodium* from migratory birds in the north, and from Eilat at the southernmost tip of the Jordan Valley. New species are: *Plasmodium lusciniae* sp. n., *Plasmodium alloreticulatus* sp. n. and *Plasmodium paranuclearis* sp. n. from *Luscinia svecica* (Linnaeus, 1758), *Plasmodium phoenicuri* sp. n., *Plasmodium reticulatus* sp. n. and *Plasmodium synnuclearis* sp. n. from *Phoenicurus phoenicurus* (Linnaeus, 1758), and *Plasmodium bilobatus* sp. n. from *Acrocephalus schoenobaenus* (Linnaeus, 1758). The morphological affinities among the new described species and between *P. merulae* and *Plasmodium vaughani* Novy & MacNeal, 1904 are highlighted and discussed. The host birds belong to two families: Muscicapidae (*Turdus merula, Luscinia svecica and Phoenicurus phoenicurus*) and Sylviidae (*Acrocephalus schoenobaenus*). All the parasites species are affiliated to the so-called "*vaughani* complex" (Corradetti & Scanga 1973) which are small parasites that possess a characteristic refractile globule in their cytoplasm.

Key words: avian *Plasmodium*, migration, Kfar Rupin, Eilat, Jordan Valley.

Bird ringing at Kfar Rupin and at the International Birding and Research Center in Eilat (IBRCE), Israel, offered us an opportunity to investigate blood parasites of birds trapped routinely for ringing during the spring and autumn migrations en route between Eurasia and Africa. In previous communications, we described several new species of *Plasmodium* from raptors (Paperna *et al*., 2007), a Sunbird (Paperna & Yosef, 2010) and, in another communication (Paperna *et al.,* 2008), a series of very small *Plasmodium* species characterized by a fixed number (four) of merozoites. In this paper, we report *Plasmodium* infection from the resident blackbird, *Turdus merula* Linnaeus, 1758, and describe new species of *Plasmodium* from migratory birds: bluethroat, *Luscinia svecica* (Linnaeus, 1758), redstart, *Phoenicurus phoenicurus* (Linnaeus, 1758), both of which belong to the Muscicapidae Vigors, 1825. Furthermore, we present another related species from the sedge warbler, *Acrocephalus schoenobaenus* (Linnaeus, 1758), a bird belonging to the Sylviidae Leach, 1820.

Materials and methods

Blood samples were collected at the Kfar Rupin ringing station (32°27′N, 35°33′E) in 2002-2003 and at the Eilat station (29°33′N, 34°57′E) from 2004 to 2007.

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The data analyzed in this study were collected during the normal trapping work conducted at the respective ringing stations and no special efforts were made to trap any specific species. Hence, the data represent a random sample of passerines and we consider them to be representative of migration in Israel.

All trapped birds were ringed, sexed, aged and their biometric parameters measured. A blood sample was collected from the brachial vein of the trapped bird using a heparinized hematocrit capillary and two smears per bird were made onto clean glass slides and the bird was then released. Air-dried smears were flooded with absolute methanol and stained before drying for 1 h with 12% Giemsa (Merck), in pH 7.4 buffer phosphate. The entire stained smear was screened at ×1000, to include at least 30 000 erythrocytes.

Holotypes consisting of a characteristic schizont or gametocyte that were clearly delimited within a circle etched on the smear coverslip with a diamond, were also photographed. These types were deposited at the MNHN, in the collection of the Laboratoire de Parasitologie Comparée et Modèles Expérimentaux, under the numbers 61-66, drawer PXII, 440 LV.

Results

The common distinctive characters of all the new species described in this paper are the elongate shape of their gametocytes, the small size of their schizonts, the small number (maximum 8) of merozoites, and the presence in their cytoplasm of one or more refrac-

Table 1. Host list and diversity of *Plasmodium* infection. Age 0 = young of the year, age 1+ = older than a year, adult = older than a year and sexually mature, unk.= unkown.

Bird species	Ring number	Age	Sex	Locality	Date	*Plasmodium* species		
Acrocephalus schoenobaenus								
	S07832	age 1+	unk.	Eilat	13/03/2004	*P. ashfordi*	*P. bilobatus*	*P. polare* like
	S16156	adult	unk.	Eilat	11/09/2005	*P. bilobatus*	*P. hexamerium* like	
	S19977	adult	unk.	Eilat	07/09/2006	*P. bilobatus*		
Luscinia svecica								
	W84173	adult	♂	Jordan Valley	07/11/2002	*P. lusciniae*	*P. alloreticulatus*	
	S16380	age 0	unk.	Eilat	27/10/2005	*P. lusciniae*	*P. alloreticulatus*	
	S16441	adult	♂	Eilat	04/11/2005	*P. lusciniae*	*P. alloreticulatus*	
	S17142	age 0	♀	Eilat	10/11/2005	*P. lusciniae*	*P. alloreticulatus*	
	S20529	age 0	♂	Eilat	22/10/2006	*P.* sp. (only trophozoites stages)		
	S20559	adult	♀	Eilat	23/10/2006	*P. paranuclearis*		
Phoenicurus phoenicurus								
	S16299	age 0	♀	Eilat	18/10/2005	*P. phoenicuri*	*P. reticulatus*	*P. synnuclearis*
	S16471	age 0	♂	Eilat	05/11/2005	*P. phoenicuri*	*P. reticulatus*	*P. synnuclearis*
Turdus merula								
	C41082	age 0	♀	Jordan Valley	13/12/2002	*P. merulae*		
	C41093	age 1+	♀	Jordan Valley	13/12/2002	*P. merulae*		
	DD7797	age 0	♂	Jordan Valley	13/12/2002	*P. merulae*		

tile globules, the majority of which staining blue but some colorless. All of the parasites develop in erythrocytes causing no or rarely a slight, displacement of the nucleus. All differential diagnoses are made with the species possessing these characteristics.

Most of the studied hosts were infected with several species of *Plasmodium* (Table 1). In all the birds the parasitaemia levels were below 0.1%. Infection prevalence was higher in the three migrating birds species investigated from Eilat as compared to those from Kfar Rupin, and the bird species with the highest infection prevalence was the resident *Turdus merula* (Table 2). The host birds were divided into three age groups: age 0 = young of the year, age 1+ = older than a year, or adult = older than the year and sexually mature.

Table 2. Prevalence of *Plasmodium* infections [including *P.* (*Haemamoeba*) spp.]. In all birds level of parasitaemia was below 0.1%

Bird species	Locality	Number of bird examined	Positive infection
Acrocephalus schoenobaenus			
	Eilat	47	8 (17.0%)
	Jordan Valley	28	1 (3.6%)
Luscinia svecica			
	Eilat	87	18 (20.7%)
	Jordan Valley	56	6 (10.7%)
Phoenicurus phoenicurus			
	Eilat	78	9 (11.5%)
	Jordan Valley	3	0 (0.0%)
Turdus merula			
	Jordan Valley	7	3 (42.9%)

Plasmodium merulae Corradetti & Scanga, 1973 (Fig. 1A)

Material examined. Blood smear from *Turdus merula*, ring number C41093, adult, female, 127 g, Kfar Rupin, Jordan Valley, Israel, 13.12.2002; blood smear from *T. merula*, ring number C41082, age 0, female, 79.5 g, Kfar Rupin, Jordan Valley, Israel, 13.12.2002; blood smear from *T. merula*, ring number DD7797, age 0, male, 90.2 g, Kfar Rupin Jordan Valley, Israel, 13.12.2002.

Distribution. Macerata, Marche, Italy (type locality); new record for the Jordan Valley, Israel.

Hosts. Type host: *Turdus merula*.

Synonym. *Plasmodium vaughani merulae* Corradetti & Scanga, 1972.

The infection conforms with Corradetti & Scanga's (1972) description in *T. merula* caught in different regions of Italy: Lombardia, Emilia, Toscana, Lazio, Marche. The parasites are of small size, irregular shape, with an average 6 merozoites per schizont, and a refractile globule (Fig. $1A_{1-4}$). The merozoites remain in the host erythrocyte after segmentation (Fig. $1A_5$).

P. merulae with an identical description was equally found in all blackbird material from France (Landau & Chavatte, unpublished).

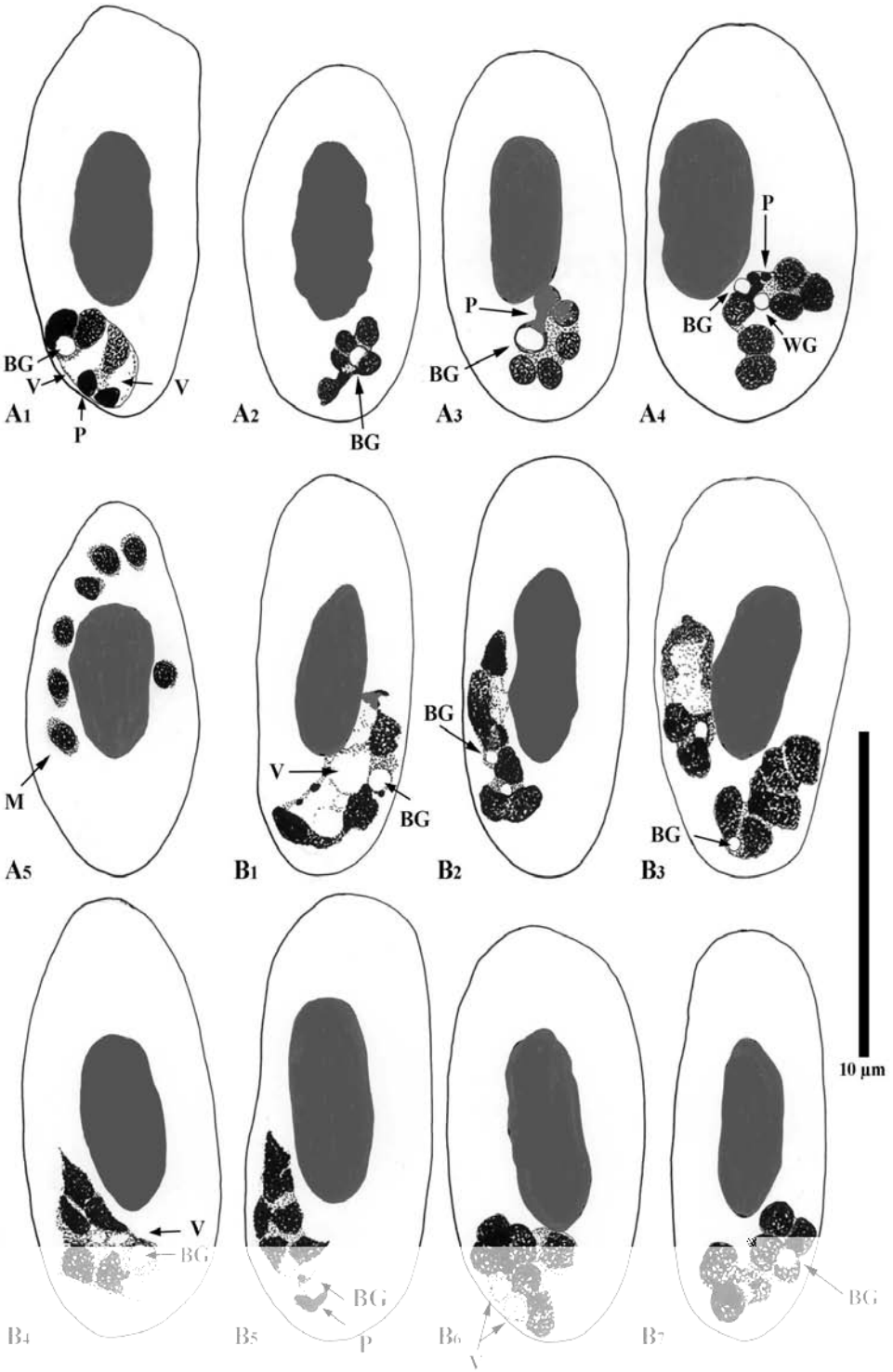


Fig. 1. A, *Plasmodium merulae* Corradetti & Scanga, 1973 from *Turdus merula* C41093; A_1, schizont with two nuclei, blue globule and vacuoles; A_2, five-nucleus schizont; A_3, four-nucleus schizont, the blue globule is linked to the pigment aggregate; A_4, six-nucleus schizont with blue and translucent globules linked with pigment granules; A_5, released merozoites in the erythrocyte cytoplasm; BG: blue globule, M: merozoite, P: pigment, V: vacuole, WG: white globule. B, *Plasmodium luscinae* Paperna & Landau sp. n. from *Luscinia svecica* W84173; B_1, young schizont with large vacuoles; B_2 & B_3, differentiating schizonts with residual cytoplasm; B_4-B_7, variations in the configuration of differentiated schizonts, all containing conspicuous blue globule; BG: blue globule, P: pigment, V: vacuole; Scale bar = 10 µm.

Plasmodium lusciniae Paperna & Landau sp. n. (Figs 1B, 2A)

Type material. Holotype: blood smear from *Luscinia svecica*, ring number W84173, adult, male, 15.5 g, Kfar Rupin, Jordan Valley, Israel, 07.11.2002 (MNHN, 446LV PIII-72). Paratypes: blood smear from *L. svecica*, ring number S16380, age 0, female, 12.2 g, Eilat, Israel, 27.10.2005; blood smear from *L. svecica*, ring number S16441, adult, male, 14.8 g, Eilat, Israel, 04.11.2005; blood smear from *L. svecica*, ring number S17142, age 0, female, 13.9 g, Eilat, Israel,10.11.2005.

Etymology. This species' name derivates from that of the host bird.

Distribution. Known only from Israel: Kfar Rupin (type locality) and Eilat.

Host. *Luscinia svecica* (type host).

The infection was mixed with two other species, *P. alloreticulatus* sp. n. and *P. paranuclearis* sp. n. (described below) (Table 1.).

Description. In the erythrocyte, the schizonts always maintain an apical or subapical position. Young schizonts contain large vacuoles (Fig. $1B_1$). In growing schizonts, the cytoplasm becomes residual (Fig. $1B_{2-4}$). Schizonts from the two-nuclei stage develop a conspicuous blue-staining refractile globule often accompanied by a second, blue or translucent, globule (Figs $1B_{3-7}$; 2A); a few vacuoles may be present as well (Fig. 2A). Both globules and vacuoles are usually allied with aggregates of pigment. The clustered schizonts are of irregular shape, either oblong (Figs $1B_5$; 2A), or rounded-ramified (Fig. $1B_6$). Schizonts divide to up to 5 or 6 nuclei.

Another species, whose schizonts have abundant cytoplasm, small nuclei, blue-staining globules and a progeny of 7-8 merozoites (Fig. $2B_{1,2}$), coexists with *P. lusciniae* in the type host W84173. The schizonts from this species differ, however, from the typical *P. lusciniae* in their abundance of cytoplasm and the conspicuously smaller and more numerous nuclei (7-8 instead of 5-6). However, since only a few schizonts were seen, we did not name this species.

Remarks. See remarks for *Plasmodium phoenicuri.*

Plasmodium phoenicuri Paperna & Landau sp. n. (Fig. 2C)

Type material. Holotype: blood smear from *Phoenicurus phoenicurus*, ring number S16299, age 0, female, 12.8 g, Eilat, Israel, 18.10.2005, (MNHN, 450LV, PI-218). Paratype: blood smear from *Phoenicurus phoenicurus* ring number S16471, age 0, male; 12.6 g, Eilat, Israel, 05.11.2005.

Etymology. This species' name derivates from that of the host bird.

Distribution. Known only from Eilat, Israel (type locality).

Host. *Phoenicurus phoenicurus* (type host).

Both *P. phoenicurus* hosts showed mixed infection with other species, *P. reticulates* sp. n. and *P. synnuclearis* sp. n. (described further on) (Table 1.).

Description. The schizonts have scant cytoplasm, and are clustered, rounded or slightly oblong (Fig. $2C_{1,2}$). Even when fully mature, they remain detached and smaller than the host-cell nucleus, which also never becomes displaced, maintaining a polar or subpolar position. Schizonts yield 5 or 6 merozoites (Fig. $2C_{3-5}$). A few schizonts with 7-8 nuclei are also found. All schizonts contain one or two conspicuous blue-staining refractile globules, which are exceptionally translucent (Fig. $2C_4$). The globule is often accompanied by a pigment aggregate, the latter only exceptionally refractile. The schizont also contains additional scattered pigment as well as translucent vacuoles.

Remarks. Both *Luscinia svecica* and *Phoenicurus phoenicurus* demonstrated mixed infection. Although trophozoites, early schizont stages and gametocytes, were observed, they could not therefore be ascribed confidently to one or other species in the mixed infection.

P. lusciniae and *P. phoenicuri* by their large nuclei and scant cytoplasm, are close to *P. merulae* Corradetti & Scanga, 1973. The schizonts in the three infections contain up to 6 merozoites and a conspicuous globule, sometimes linked with a refractile pigment granule. The globule stains blue as for that in *P. merulae*.

However, the oblong schizonts occur only among *P. lusciniae.* The mature schizonts of *P. phoenicuri* reach larger sizes than those of *P. merulae* or *P. lusciniae.*

In the infections of *L. svecica* and *P. phoenicurus* a few schizonts had more than the expected progeny of 6, as they developed 8 to 9 nuclei and their cytoplasm was more abundant, suggesting that they belong to a separate species.

Plasmodium reticulatus Paperna & Landau sp. n. (Figs 2D, 3A)

Type material. Holotype: blood smear from *Phoenicurus phoenicurus*, ring number S16299, age 0, female, 12.8 g, Eilat, Israel, 18.10.2005, (MNHN, 450LV, PXIII-77). Paratype: blood smear from *Phoenicurus phoenicurus* ring number S16471, age 0, male, 12.6 g, Eilat, Israel, 05.11.2005.

Etymology. The name reflects the main morphological trait: the reticulated cytoplasm.

Distribution. Known only from Eilat, Israel (type locality).

Host. *Phoenicurus phoenicurus* (type host).

Plasmodium infections in both host specimens were a mixture of several species: this species, *P. phoenicuri* sp. n. described above, and *P. synnuclearis* sp. n. described below (Table 1).

Description. Inside the erythrocytes, the parasites occupy a polar or subpolar position, rarely lateral. The on-growing schizonts show a reticulated shape and scant cytoplasm (Fig. $2D_{1,2}$). The cytoplasm contains one or two colourless or bluish globules, sometimes also a translucent vacuole and a con-

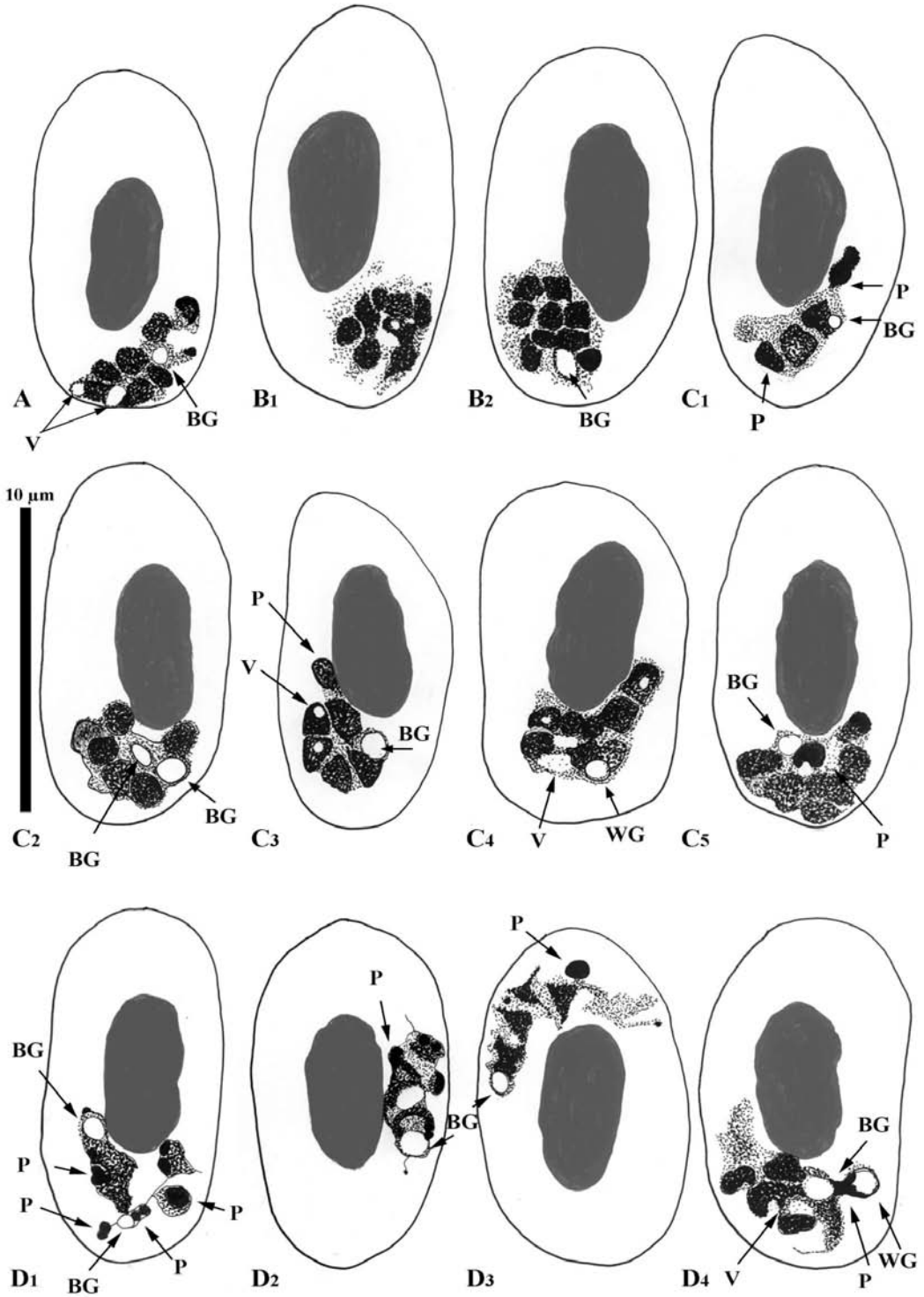


Fig. 2. A, *Plasmodium lusciniae* Paperna & Landau sp. n. from *Luscinia svecica* S16441; A_1, other configuration of differentiated schizonts containing conspicuous blue globule; BG: blue globule, V: vacuole. B, *Plasmodium* sp. from *Luscinia svecica* W84173; B_1, seven-nucleus schizont; B_2, eight-nucleus schizont with blue globule; BG: blue globule. C, *Plasmodium phoenicuri* Paperna & Landau sp. n. from *Phoenicurus phoenicurus* S16299; C_1, three-nucleus schizonts; C_2 & C_3, five-nucleus schizonts; C_4 & C_5, mature schizonts; BG: blue globule, P: pigment, V: vacuole, WG: white globule. D, *Plasmodium reticulatus* Paperna & Landau sp. n. from *Phoenicurus phoenicurus* S16299; D_1-D_4, differentiating schizonts; BG: blue globule, P: pigment, V: vacuole, WG: white globule; Scale bar = 10 µm.

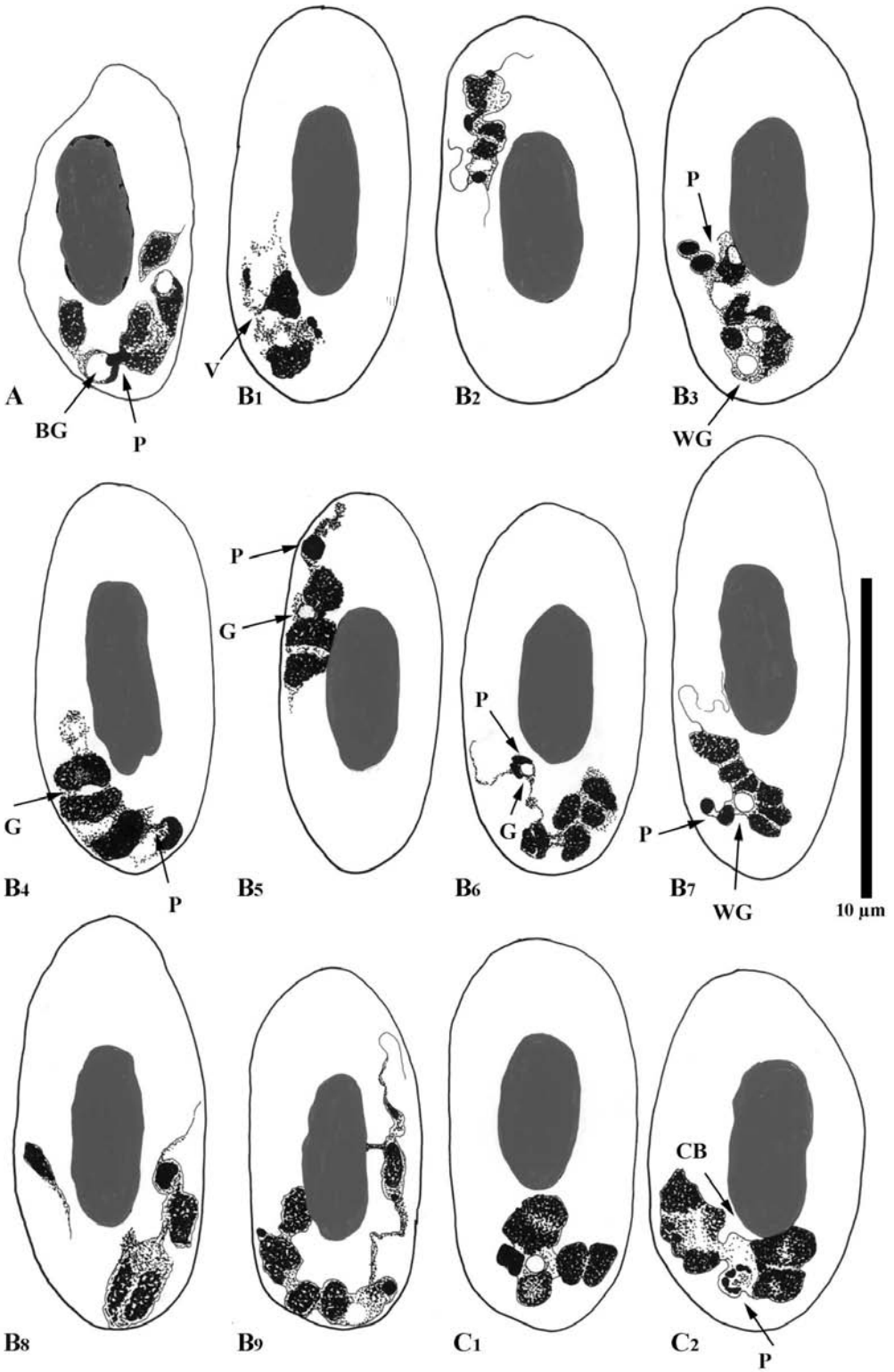


Fig. 3. A, *Plasmodium reticulatus* Paperna & Landau sp. n. from *Phoenicurus phoenicurus* S16299; A_1, schizont at onset of segmentation; BG: blue globule, P: pigment. B, *Plasmodium alloreticulatus* Paperna & Landau sp. n. from *Luscinia svecica* W84173; B_1, schizont of two nuclei; B_2-B_5, schizonts of three nuclei; B_6 & B_7, very reticulated segmenting schizonts with five or six nuclei; B_8 & B_9, schizonts completing differentiation to segmenters; G: globule, P: pigment, V: vacuole, WG: translucent globule. C, *Plasmodium bilobatus* Paperna & Landau sp. n. from *Acrocephalus schoenobaenus* S07832; C_1 & C_2, branching schizonts; CB: cytoplasmic "bridge", P: pigment aggregate; Scale bar = 10 µm.

spicuously large pigment granule; the four nuclei are arranged either longitudinally (Fig $2D_3$) or in a scattered order (Figs $2D_4$; 3A).

Remarks. Young stages and gametocytes could not be related to one particular species of the mixed infections. However, the growing schizonts of *P. reticulatus* are clearly distinguishable from the other species in the mixed infection by their reticulated cytoplasm, and their smaller number of nuclei (4 *vs* 6 in *P. phoenicuri and P. synnuclearis*). *P. synnuclearis* becomes attached to the erythrocyte nucleus.

The *Plasmodium* species described can be differentiated from the other tetranucleate species of *Plasmodium* by its scattered-elongate irregular shape and longitudinally positioned nuclei. In the other tetranucleates, *Plasmodium rouxi* Sergent, Sergent & Catanei, 1928 and the recently described new species (Paperna *et al.,* 2008) the nuclei are much smaller and are arranged in opposing doublets or in a fan shape. In *Plasmodium stellatum* Paperna & Landau, 2008, the parasite from *Muscicapa striata* (Pallas, 1764), the nuclei are star-shaped (Paperna *et al.,* 2008).

Plasmodium alloreticulatus Paperna & Landau sp. n. (Fig. 3B)

Type material. Holotype: blood smear from *Luscinia svecica*, ring number W84173, adult, male, 15.5 g, Kfar Rupin, Jordan Valley, Israel, 07.11.2002, (MNHN, 14GS, PI-220). Paratypes: blood smear from *L. svecica*, ring number S16380, age 0, female, 12.2 g, Eilat, Israel, 27.10.2005; blood smear from *L. svecica*, ring number S16441, adult, male, 14.8 g, Eilat, Israel, 04.11.2005; blood smear from *L. svecica*, ring number S17142, age 0, female, 13.9 g, Eilat, Israel,10.11.2005.

Etymology. The name is given to differentiate this parasite from *Plasmodium reticulatus*.

Distribution. Known only from Israel: Kfar Rupin (type locality) and Eilat.

Host. *Luscinia svecica* (type host).

Recovered from mixed infections: this species with *P. lusciniae* (described above) and in some cases with *P. paranuclearis* sp. n. (described below) (Table 1). The spindle-shaped trophozoites with apical appendages could not be affiliated with any of the co-infecting species.

Description. The shape of the schizonts is very variable, but they are usually elongate (Figs $3B_{1-7}$). The nuclei are generally positioned longitudinally (Fig. $3B_{1,5,7}$) along the scant, often reticulated cytoplasm (Fig. $3B_{2-7}$). The numbers of nuclei range from 2 (Fig. $3B_1$) to 6 (Fig. $3B_7$). Some carry 1 or 2 filaments at their tips (Fig. $3B_{2,6,7}$); in some, the cytoplasm is reduced into an attenuated strip (Fig. $3B_9$). One to three transparent vacuoles and a tiny colourless or pale blue globule are present, sometimes alongside pigment granules. The pigment granules either aggregate on the attenuated tips or are enclosed in distal or lateral extensions (Fig. $3B_{3,6,7}$). Merozoites from fully mature schizonts may be seen in intact erythrocytes (Fig. $3B_8$).

Remarks. Because of the mixed infection with other species of the subgenus *Novyella* the trophozoites could not be affiliated with either of the other species. *P. alloreticulatus* differs from *P. reticulatus* by its larger size and the more numerous merozoites (6 instead of 4). Some schizonts of *P. alloreticulatus* are reminiscent of the chain-like schizonts of *P. bilobatus* sp. n. described below. However, *P. bilobatus* possess much more cytoplasm and is less irregularly shaped than *P. alloreticulatus.*

Plasmodium bilobatus Paperna & Landau sp. n. (Figs 3C, 4A)

Type material. Holotype: blood smear from *Acrocephalus schoenobaenus*, ring number S07832, age 1+, sex cannot be determined, Eilat, Israel, 13.3.2004, (MNHN, 17GS, PI-218). Paratype: blood smear from *A. schoenobaenus*, ring number S16156, adult, Eilat, Israel, 11.9.2005; blood smear from *A. schoenobaenus*, ring number S19977, adult, Eilat, Israel, 07.9.2006.

Etymology. The name is derived from the main morphological trait: the bilobate shape.

Distribution. Known only from Eilat, Israel (type locality).

Host. *Acrocephalus schoenobaenus* (type host).

The infection was mixed with *Plasmodium ashfordi* Valkiūnas, Zehtindjiev, Hellegren, Ilieva & Iezhova, 2007 and with two other species of *Plasmodium*: in bird S07832, one ressembling *P. polare* Manwell, 1934, and in bird S16156, one resembling *P. hexamerium* Huff, 1935 (Table 1).

Description. The schizonts are comprised of two segments connected through a cytoplasmic area which usually contains aggregates of pigment granules and one to several globules, some colorless while others assuming faint-blue tint. A few vacuoles may also be present. The cytoplasm is abundant, and the nuclei are distributed between the two segments with a progeny of six, exceptionally 7 or 8 (Fig. $4A_{5,6}$). The schizont shape is very variable. Some are ribbon-shaped, with scant cytoplasm and contain nuclei in a single row (Fig. $4A_{1,2,4}$) or in branching sets (Figs $3C_{1,2}$; $4A_{3,6}$). A few schizonts retain apical filament and exceed the length of the erythrocyte nucleus' long axis (Fig. $4A_{1,2}$).

In a few infections there was deformation of the erythrocyte and/or slight displacement (or rotation) of its nucleus (Fig. $4A_{5,6}$).

Remarks. The amoeboid trophozoites, the spindle-shaped schizonts of two nuclei and spindle shaped gametocytes, carrying one to several flagella-like appendages could not be affiliated to any of the spe-

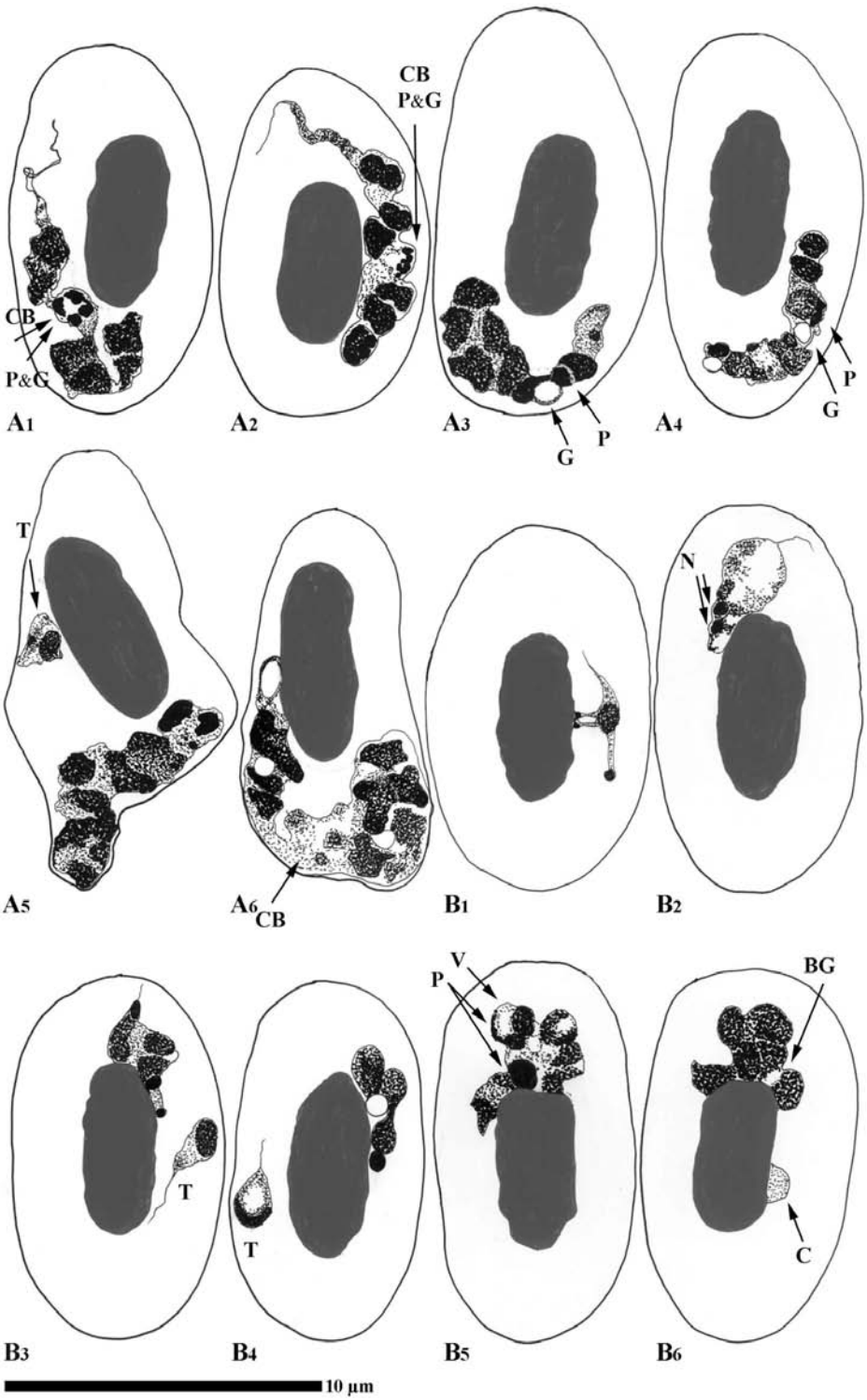


Fig. 4. A, *Plasmodium bilobatus* Paperna & Landau sp. n. from *Acrocephalus schoenobaenus* S07832; A_1 & A_2, chain-like schizonts with a filamentous tip; A_3 & A_4, chain-like schizonts without extensions; A_5 & A_6, large schizonts in deformed erythrocytes; CB: cytoplasmic "bridge", G: globule, P: pigment aggregate, P&G: pigment adjoining globule, T: trophozoite. B, *Plasmodium paranuclearis* Paperna & Landau sp. n. from *Luscinia svecica* S20559; B_1, trophozoite attached to the nucleus; B_2, filamented young binucleate schizont; B_3 & B_4, young trinucleate schizonts and filamented trophozoites; B_5 & B_6, schizonts of five nuclei, extensions of cytoplasm (C) suggest partial wrapping of the host cell nucleus; BG: blue globule, N: nuclei, P: pigment aggregate, T: trophozoites, V: vacuole; Scale bar = 10 µm.

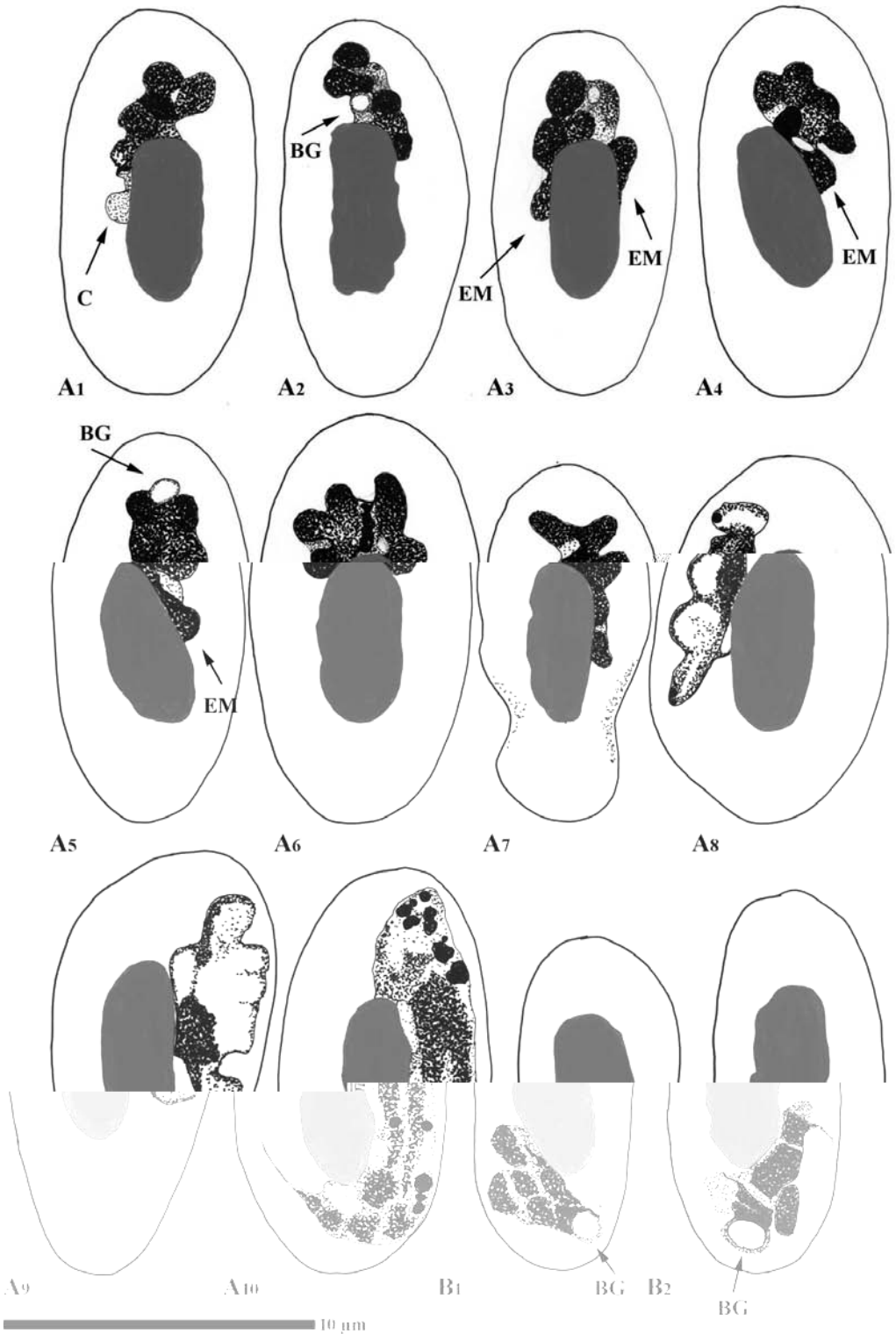


Fig. 5. A, *Plasmodium paranuclearis* Paperna & Landau sp. n. from *Luscinia svecica* S20559; A_1-A_5, schizonts of five and six nuclei, extensions of cytoplasm (C) and merozoite nuclei emerging on the erythrocyte side (EM) suggest partial wrapping of the host cell nucleus; A_6 & A_7, mature segmenting schizonts with merozoites emerging like digits above the schizont perimeter; A_8 & A_9, premature macrogametocytes; A_{10}, mature microgametocyte; BG: blue globule. B, *Plasmodium synnuclearis* Paperna & Landau sp. n. from *Phoenicurus phoenicurus* S16299; B_1 & B_2, growing schizont with limited attachment to the host nucleus; BG: blue globule; Scale bar = 10 µm.

cies co-infecting the hosts: *P. alloreticulatus*, *P. ashfordi*, *P. polare*-like and *P. hexamerium*-like.

Although this species can appear to be grossly allied with *P. reticulatus* and *P. alloreticulatus,* its chain-like schizont, interrupted by a cytoplasmic link containing globules and pigment are unique features, as yet undescribed. Some schizonts diverge from this scheme, with a double merozoite chain, or with the pigment aggregates positioned distally.

Plasmodium paranuclearis Paperna & Landau sp. n. (Figs 4B, 5A)

Type material. Holotype: blood smear from *Luscinia svecica*, ring number S20559, adult, female, 18.2 g, Eilat, Israel, 23.10.2006 (MNHN, 16GS, PI-219). Paratypes: blood smear from *L. svecica*, ring number S16380, age 0, female, 12.2 g, Eilat, Israel, 27.10.2005; blood smear from *L. svecica*, ring number S17142, age 0, female, 13.9 g, Eilat, Israel,10.11.2005.

Etymology. The name is derived from the main morphological trait: the relation with the host cell nucleus.

Distribution. Known only from Eilat, Israel (type locality).

Host. *Luscinia svecica* (type host).

The species is described in a bird (the type host specimen) in which it was not mixed with other species. Paratype hosts have mixed infection (Table 1).

Description. The trophozoites (Fig. $4B_{1-4}$) and young schizonts (Fig. $4B_3$) form an apical filamentous tip; some trophozoites (Fig. $4B_1$) and immature schizonts of the two and three nucleus stage (Fig. $4B_{3-4}$) have already established attachment to the erythrocyte nucleus. The forming nuclei of the differentiating schizonts and the budding merozoites remain clamped, holding only residual cytoplasm (Figs $4B_{5-6}$; $5A_{1-5}$). Scattered cytoplasmic pockets and emerging merozoites bulging around the host nucleus (Figs $4B_6$; $5A_{1,3-6}$) suggest that the schizonts partly (or fully) wrap around the top of the erythrocyte nucleus. Differentiated schizonts form up to 6 merozoites, emerging like digits above the schizont perimeter (Fig. $5A_{4,6,7}$). Schizont size does not exceed half that of the erythrocyte nucleus. The cytoplasm contains a conspicuous blue-staining globule; pigment aggregates are present, but not readily discernible. Young gametocytes form an apical filament (Fig. $4B_2$). The gametocytes are situated on the side of the erythrocyte and either reach or exceed its length. The two macrogametocytes (apparently premature) observed form lateral swellings (Fig. $5A_{8,9}$); the microgametocyte exceeds the erythrocyte nucleus in length and forms a spine at one end (Fig. $5A_{10}$). Neither schizogonic stages nor the gametocytes cause displacement of the erythrocyte nucleus.

Remarks. The adherence of the schizont to the erythrocyte nucleus is also common among the following American species: *Plasmodium nucleophilum* Manwell, 1935, parasite of *Dumetella carolinensis* (Linnaeus, 1766). *P. nucleophilum toucani* Manwell & Sessler, 1971, parasite of *Ramphastos swainsonii* Gould, 1833, and *Plasmodium paranucleophilum* Manwell & Sessler, 1971 from *Tachyphonus* sp. None, according to the authors, posses a refractile globule except perhaps *P. n. toucani* showing a "tiny bluish vacuole", while in our parasite there is a conspicuous blue refractile globule.

The average number of nuclei in the *P. nuclearis* from *L. svecica* ranges between 5 and 6, while it averaged 4.4 in parasites from the wild-caught toucan and 6.8 in subinoculated toucans.

The filamentous tip seen in trophozoites, early schizonts and gametocytes is not mentioned in the original above-quoted descriptions.

Plasmodium synnuclearis Paperna & Landau sp. n. (Figs 5B, 6A)

Type material. Holotype: blood smear from *Phoenicurus phoenicurus*, ring number S16299, age 0, female, 12.8 g, Eilat, Israel, 18.10.2005, (MNHN, 19GS, PI-218). Paratype: blood smear from *Phoenicurus phoenicurus* ring number S16471, age 0, male; 12.6 g, Eilat, Israel, 05.11.2005.

Etymology. The name is derived from the main morphological trait: the relation with the host cell nucleus.

Distribution. Known only from Eilat, Israel (type locality).

Host. *Phoenicurus phoenicurus* (type host).

Both host specimens had mixed infections (present species with the above-described *P. phoenicuri* sp. n. and *P. reticulatus* sp. n.).

Description. A reliable description is limited to the schizogony stages; the identity of the trophozoites, some with filamentous appendages and the gametocytes remains uncertain. The attachment of some schizonts to the erythrocyte nucleus (Figs $5B_{1-2}$; $6A_{1-2}$) is limited while the others spread their attachment and form extended adherence by wrapping over half or more of the erythrocyte nucleus perimeter (Fig. $6A_{3-5}$). The emerging merozoites either cluster (Fig. $6A_{2-3}$), or spread, with the budding merozoites emerging above the cytoplasmic core of the schizont (Fig. $6A_5$). Most schizonts contain a conspicuously large blue-staining globule; the pigment material forms one or two aggregates, often adjoining the globule (Fig. $6A_{4-5}$). The pigment aggregate is sometimes refractile (Fig. $6A_3$). In none of the forms of attachment do schizonts cause displacement or deformation of the erythrocyte nucleus.

Remarks. This species, similarly to *P. paranuclearis*, shows superficial affinity with *P. nucleophilum* and *P. paranucleophilum*. However, the extreme degree of association with the erythrocyte nucleus, reached by some and the presence of a conspicuous blue globule differentiate the parasite described here from the two American species.

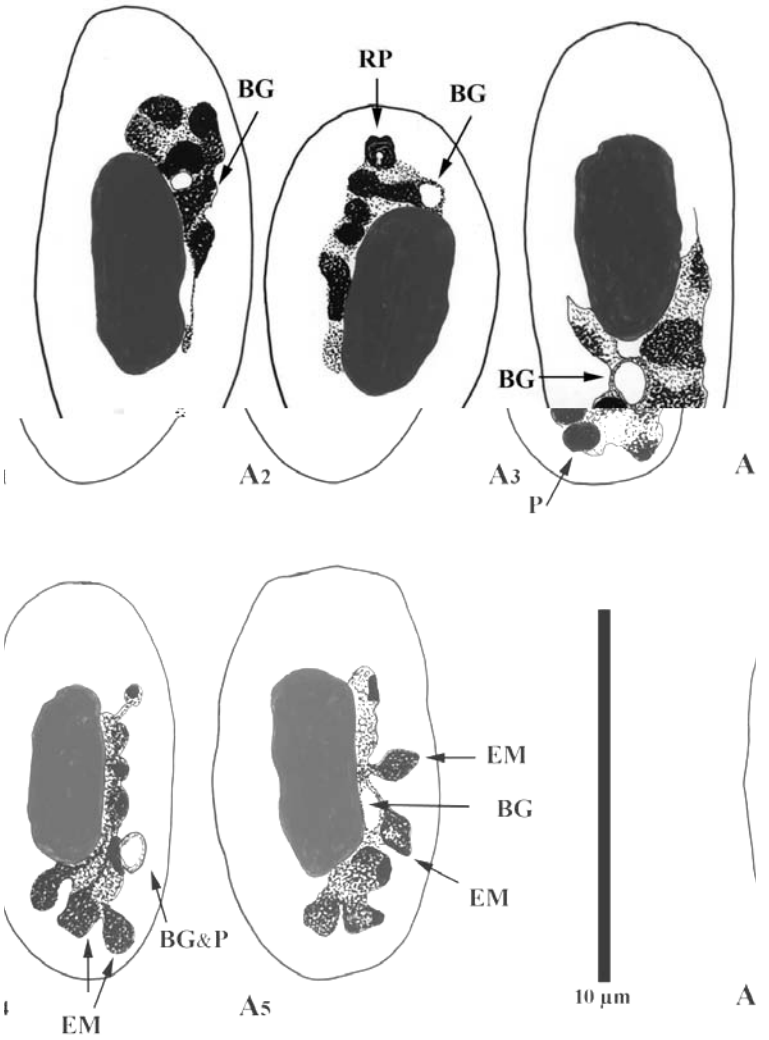


Fig. 6. A, *Plasmodium synnuclearis* Paperna & Landau sp. n. from *Phoenicurus phoenicurus* S16299; A_1-A_3, schizonts expanding over the host nucleus; A_4 & A_5, mature (segmenting) schizonts with surfacing merozoites; BG: blue globule, BG&P: blue globule and associated pigment, EM: emerging merozoites, P: pigment, RP: refractile pigment; Scale bar = 10 µm.

Discussion

Plasmodium vaughani was described by Novy & MacNeal in 1904 from an American robin, *Turdus migratorius* L., 1766, with the following characters: four nuclei, a refractile globule and a large, sometimes refractile, pigment granule. Sergent *et al.* (1929, 1931) reported a personal communication by Novy on the presence of up to eight nuclei as differentiating character from *P. rouxi*, which always has four nuclei. *P. vaughani* was re-described from the same host and locality by Manwell (1935), who confirmed the presence of schizonts of more than four merozoites (unlike in *P. rouxi*), together with a refractile globule and the refractile pigment granules. A *Plasmodium* sp. with similar features, i.e. with six clustered merozoites and a refractile globule linked to pigment aggregate, reported from *Galerida cristata* (Linnaeus, 1758), was regarded as conspecific with *P. vaughani* (Mohammed, 1958); others were assigned as subspecies of *P. vaughani*: *P. vaughani tenuis* and *P. vaughani merulae* (Manwell, 1935; Corradetti & Scanga, 1972). In a later communication, Corradetti & Scanga (1973) regarded all the above-listed species as belonging to the "*P. vaughani* complex", and elevated the *Plasmodium* from *T. merula* to the rank of species: *P. merulae*. *P. lusciniae* and *P. phoenicuri,* presently described may be added to the species allied to *P. vaughani*.

A blue-staining refractile globule has been found in all the *P. vaughani*-affiliated species. It has also been found to stain conspicuously blue also in *P. merulae* from *Turdus merula* from France (Landau

& Chavatte, unpublished). This globule is the main character to define the subgenus *Papernaia* Landau, Chavatte, Peters & Chabaud, 2010.

In addition to *P. vaughani*-affiliated species, we can recognize two additional groups that are morphologically related among the newly described species. These include *P. reticulatus*, *P. alloreticulatus* and *P. bilobatus*: forming fragile chain of four to six nucleated plasmodia, and species which wrap around the erythrocyte nucleus: *P. paranuclearis* and *P. synnuclearis*. All of these species posses a blue-staining globule and thus belongs to the subgenus *Papernaia* as defined by Landau *et al.* (2010). Note that the host birds, *Luscinia svecica* and *Phoenicurus phoenicurus,* which are related members of the bird family Muscicapidae, each act hosts to a member of these groups of species affiliated to either *P. vaughani*, to *P. reticulatus* or to *P. nucleophilum*.

Molecular analyses, which might provide a powerful means to assess the affinities between the species, would be difficult to interpret because natural infections are very often mixed infections with numerous species, and in some case one or more species are likely to be present at low levels undetectable by microscopic observation.

Plasmodium infections in all the birds except *Acrocephalus schoenobaenus* were recovered from autumn migrant (age 0) birds. This proves that all of these infections were contracted in the habitats north of Israel, between the nesting area and the point of capture, and not in Africa (see Waldenström *et al.,* 2002). All *A. schoenobaenus* were adult birds, which had wintered in Africa, spent the summer in their breeding grounds, and therefore the geographical origin of their infections remains uncertain.

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Coccidiosis of wild and captive European mouflons (*Ovis aries*) living in a natural reserve of central Italy

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Abstract. Between December 2005 and November 2006, a survey on coccidiosis of wild and captive mouflons living in the Orecchiella Natural Reserve (Tuscany, Italy) was performed on faecal samples collected approximately every two months. A total of 96 samples were collected, 55 of which from wild mouflons and 41 from captive mouflons. On these faecal samples qualitative and quantitative parasitological analyses were performed; moreover, faecal cultures were made in order to identify the involved *Eimeria* species. Significantly higher prevalence (%) and mean intensity (mean OPG±SD) of coccidian infection resulted for captive mouflons (73.17% and 814.6±1297.2 OPG) comparing to wild mouflons (36.73% and 112.7±268.7 OPG). *Eimeria parva*, *E. ovinoidalis*, *E. bakuensis*, *E. ahasata*, *E. intricata*, *E. crandallis*, *E. granulosa*, *E. faurei* and an *Eimeria* species very similar to the descriptions previously reported for *E. arloingi* and *E. yakimoffmatschoulsky*, were isolated.

Key words: coccidiosis, mouflon, *Eimeria* spp., central Italy.

Ruminants coccidiosis is an important intestinal parasitic disease caused by Eimeriid protozoans; the infection occurs directly through the ingestion of sporulated oocysts spread in the environment (Taylor *et al.*, 2007). Wild ruminants coccidiosis is prevalent in captive populations. In captivity the concentration of animals into small enclosures may result in a build up of oocysts and allow clinical parasitism to occur. However, overcrowding, malnutrition, and climatic stressors may exacerbate parasitism also in free-ranging populations (Fowler and Miller, 2003).

In the European mouflon (*Ovis aries*) (Giusti, 2005), oocysts shedding seems to be affected by stressing conditions and by age (Gomez-Bautista *et al.*, 1996). Higher intensity of coccidian infections are observed in young animals, from one to two months of age, afterwards intensity decreases and stabilizes at six months of age (Gomez-Bautista *et al.*, 1996).

Most of the coccidian species reported in the mouflon in previous studies (Pellerdy, 1974; Levine and Ivens, 1986; Gomez-Bautista *et al.*, 1996) are the same reported in the domestic sheep, except for *E. caprovina* that infects domestic goats and an *Eimeria* sp. observed only in adult mouflons by Gomez-Bautista *et al.* (1996). A further species, *E. arloingi*, has been reported in the mouflon (Pellerdy, 1974); however, Levine and Ivens (1986) believe that it can be a synonym of *E. ahsata*.

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Despite the relevance of mouflon as a wild and a captive species in different areas of Tuscany and other Italian territories (Pedrotti and Toso, 2002; Riga *et al.*, 2005), few data are available about mouflon *Eimeria* spp. infection in Italy (Arru *et al.*, 1987; Magi *et al.*, 2005; Verin *et al.*, 2006).

Our objective was to evaluate prevalence and intensity of coccidian infections in wild and captive mouflons living in the Orecchiella Natural Reserve (Tuscany, central Italy) and the involved *Eimeria* species.

Study area: the Orecchiella Natural Reserve

The Reserve occupies a vast area (approximately 52 square kilometres) and it is located in the North-West part of Tuscany, between the Apuane Alps and the Tusco-Emilian Apennines (Pieroni, 1992).

Materials and methods

Animals

In the Orecchiella Natural Reserve there are either wild mouflons and mouflons held in captivity into an enclosure of 60×40 square meters.

At the beginning of this survey (December 2005), there were 12 captive mouflons (eight males and four females); then five offspring (two males and three females) were born in April 2006. Concerning wild mouflons, in May 2006 census 147 mouflons were sighted.

Sampling

Between December 2005 and November 2006, mouflon faecal samples were collected approxi-

mately every two months. A total of 96 samples were collected, 55 from wild mouflons and 41 from captive mouflons. Each sample was labelled with the date of collection and preserved inside a disposable plastic bag for laboratory analysis.

Parasitological analysis

For coccidian oocysts isolation, qualitative parasitological analyses were performed by flotation test with a low density (s.g. 1.2) salt solution. The number of oocysts per gram of feces (OPG) was determined by using a modified McMaster technique (Permin and Hansen, 1998). For all sampling periods, except for the May sampling, and for wild and captive mouflons separately, positive faecal samples were pooled and incubated in a shallow layer of 2.5% (w/v) aqueous potassium dichromate solution to allow sporulation of the oocysts. In order to identify involved species, mature oocysts were examined to evaluate their morphometric characteristics that were compared with those described for the *Eimeria* species reported in the mouflon in previous studies (Levine and Ivens, 1986; Pellerdy, 1974; Gomez-Bautista *et al.*, 1996).

Statistical analysis

JMP® 6 software, SAS Institute Inc. (2005) was used for statistical analysis of data. Qualitative data were evaluated using χ^2 test and quantitative data (OPG) were converted using $y=\log(OPG+25)$ formula in order to obtain a normal distribution of data. Variance analysis was used to make a comparison between wild and captive mouflons and among samples collected in different periods of the examined year.

Results

Considering the qualitative analysis of the 96 faecal samples of both wild and captive mouflons, 53.33% of them resulted positive for the presence of coccidian oocysts. However, the prevalence was found to be 73.17% for captive mouflons and 36.73% for wild mouflons and this difference resulted highly significant ($P<0.01$). On the contrary, no statistical differences were found with regard to the prevalence values observed in the different periods in which samples were collected.

Concerning quantitative analysis, in the whole study period the mean coccidian intensity resulted to be 412.5 ± 933.4 OPG but, consistently with prevalence data, mean coccidian intensity resulted highly different ($P<0.01$) between captive mouflons (814.6 ± 1297.2 OPG) and wild mouflons (112.7 ± 268.7 OPG). Significant differences ($P<0.05$) were found also among the different periods of samples collection, with higher values found in December 2005 and from July to November 2006 (Table 1).

Table 1. Mean (± standard deviation) coccidian intensity observed in the period December 2005-November 2006 in the mouflons living in the Orecchiella Natural Reserve (Tuscany, Italy).

Date of sample collection	Mean coccidian intensity (OPG)
December 2005	652.9 ± 1572.4
February 2006	91.6 ± 174.2
May 2006	100.0 ± 181.2
July 2006	508.3 ± 1189.6
September 2006	678.5 ± 867.5
November 2006	657.1 ± 870.6
P	0.0330

By their morphological and metric characteristics, nine species of *Eimeria* were identified (Table 2): *Eimeria parva*, *E. ovinoidalis*, *E. bakuensis*, *E. ahasata*, *E. intricata*, *E. crandallis*, *E. granulosa*, *E. faurei* and an *Eimeria* sp. with a partial similarity to *E. ahasata*, but clearly distinct from this latter species, and very similar to *E. arloingi* as described by Pellerdy (1974) and to *E. yakimoffmatschoulsky* reported in the chamois by Restani (1968). *E. granulosa* and the *E. arloingi-E. yakimoffmatschoulsky* type were isolated respectively only from wild and from captive mouflons. Through all the study period and considering all examined samples, from wild and captive animals, the frequency of isolation of each identified coccidian species from faecal cultures resulted highly different ($P<0.01$). Globally, *E. parva* was the dominant species (90.91%), followed by *E. ovinoidalis* (72.73%), *E. bakuensis* (63.64%), *E. ahasata* (60.00%), *E. intricata* (40.00%), *E. crandallis* (30.00%), *E. granulosa* (20.00%), *E. faurei* (20.00%), and the *E. arloingi-E. yakimoffmatschoulsky* type (10.00%). *E. parva* (54.55%) and *E. ahasata* (40.00%) resulted the most frequent species in captive and wild mouflons, respectively (Table 3). The mean number of pools in which each identified *Eimeria* sp. was isolated was 2.4 for the captive population and 2.6 for the wild population, respectively.

Although the richness of coccidian community in the two examined host populations was the same (8 *Eimeria* species), in the captive mouflons a dominance of some species (i.e. *E. parva*, *E. ovinoidalis*, *E. ahasata* and *E. bakuensis*) emerged. On the contrary, coccidian community of the wild population was characterised by a higher relative eveness of the isolated species. In particular, the number of species isolated from the captive population ranged from 2 to 6 in each period, with a mean number of 3.8 species per pool, while in the wild population the number of species ranged from 3 to 6, with a mean of 4.2 species per pool.

Table 2. Morphometric characteristics of *Eimeria* species isolated from faecal samples of mouflons living in the Orecchiella Natural Reserve (Tuscany, Italy).

Species	Oocysts (µm)	Shape index	Micropyle	Micropyle cap	Sporocyst (µm)
Eimeria parva	21.2 × 17.9 (20-24.3 × 15-21.8)	1.3	–	–	7 × 6 (7-10 × 5-9)
Eimeria bakuensis	33.5 × 21.8 (26-36 × 16-25.5)	1.3	+	+	14.5 × 7 (10-16 × 6-9)
Eimeria intricata	50.5 × 40.6 (48-54 × 38-44)	2.8	+	+	19 × 10 (17-21 × 8-11)
Eimeria granulosa	30 × 21 (27-32 × 17-25)	1	+	+	13 × 8 (12-14 × 7-9)
Eimeria faurei	33.5 × 24.2 (26-35 × 19-27)	1.4	+	–	14.2 × 9.8 (11-16 × 7-10.4)
Eimeria crandallis	22 × 19.5 (20-24 × 16.3-20)	1.2	+	+	10 × 7 (9-12 × 6-9)
Eimeria ahasata	40 × 25 (39-41 × 22-27)	1.3	+	+	15 × 8 (18-22 × 6-10)
Eimeria ovinoidalis	25.3 × 20.2 (23.4-26 × 18.9-22.4)	1.2	+	–	12.3 × 7.8 (10-13 × 6-8)
E. yakimoffmatschoulsky/ E. arloingi type	38 × 27 (35-40 × 25-30)	1	+	+	15 × 8 (11-17 × 6-10)

Table 3. Prevalence of isolated *Eimeria* species observed in the period December 2005-November 2006 in wild and captive mouflons living in the Orecchiella Natural Reserve (Tuscany, Italy).

Species	Prevalence (%)		
	Captive mouflons	Wild mouflons	χ^2: p-value
Eimeria parva	54.55	36.36	*0.1926*
Eimeria ovinoidalis	36.36	36.36	*0.6179*
Eimeria ahasata	20.00	40.00	*0.1889*
Eimeria bakuensis	45.45	18.18	*0.1307*
Eimeria intricata	20.00	20.00	*1.0000*
Eimeria crandallis	10.00	20.00	*0.4870*
Eimeria granulosa	0.00	20.00	*0.0702*
Eimeria faurei	10.00	10.00	*1.0000*
Eimeria yakimoffmatschoulsky/Eimeria arloingi type	10.00	0.00	*0.2210*
χ^2: p-value	*0.0018*	*0.0545*	

Discussion

This survey represents the first study on coccidiosis of mouflons living in the Orecchiella Natural Reserve. In previous studies coccidian infections were reported in mouflon populations of the Italian territory and of several European countries (Euzeby and Hugonnet, 1980; Arru *et al.*, 1987; Gomez-Bautista *et al.*, 1996; Gottschalk *et al.*, 1999; Magi *et al.*, 2005; Verin *et al.*, 2006). Comparing to the studies previously performed in Italy, in this study it was possible to evaluate *Eimeria* spp. infections in captive and wild mouflons, both living in the Orecchiella Natural Reserve.

The total prevalence of coccidiosis resulted to be quite high (53.33 %) and similar to the prevalence found by Verin and collegues (2006) in the mouflons living in the Apuane Alps Park (58.2 %), an area close to Orecchiella Park, but it resulted lower than the prevalence found by Magi and colleagues (2005) in the mouflons living in the Monti Livornesi Park (67%).

In the two examined mouflon populations living in the Orecchiella Natural Reserve, wild and captive, the prevalence of coccidiosis resulted higher in captive mouflons as far as the mean intensity of coccidiosis itself. These data confirm results obtained in previous studies according to which in wild animals kept in captivity, the concentration of animals into small enclosures may result in a build up of oocysts with a consequent increase of prevalence and intensity of infection (Fowler and Miller, 2003; Martella *et al.*, 2003). Further, higher intensity values were observed in December 2005 and from July to November 2006. Similar trends were observed also in others studies and linked with the higher presence of young animals in these months, that are more sensitive to coccidiosis than adult animals (Gomez-Bautista *et al.*, 1996; Magi *et al.*, 2005). In their study, Gomez-Bautista *et al.* (1996) observed that in young animals oocysts intensities >10,000 OPG resulted associated with transient diarrhoea; OPG number resulted always much lower than 10,000 OPG in all samples examined in the present study.

Finally, nine different species of *Eimeria* (Table 2), eight of which already previously reported in the mouflon both in Italy and in other countries (Pellerdy, 1974; Levine and Ivens, 1986; Gomez-Bautista *et al.*, 1996; Verin *et al.*, 2006), were isolated from mouflons living in the Orecchiella Natural Reserve: eight species from captive and eight species from wild mouflons. Only a species, very similar to *E. arloingi* (Pellerdy, 1974) and to *E. yakimoffmatschoulsky* (Restani, 1968), is reported for the first time in the mouflon in the present study. Differently by what observed by Gomez-Bautista and collaborators (1996) in mouflons of central Spain, in the present study *E. caprovina* was not isolated and *E. bakuensis* did not result the predominant species.

As previously observed (Bush *et al.*, 1997), from data obtained in this study a higher degree of diversity of parasite community emerged in the wild population. Diversity has been related to the stability of the ecosystems but this relationship has aroused considerable controversy during the past years (Loreau *et al.*, 2001), and it is questionable if the wild coccidian community should be considered more stable than the captive one. However, it is interesting to observe that actually in the present study the latter community was less diverse and that this result is consistent with the observation that in domestic ruminants helminths diversity is related to management with extensive and organic farms showing a more diverse helminth fauna and low levels of infection comparing to intensive farms (Silvestre *et al.*, 2000; Cabaret *et al.*, 2002). Data about the abundance of each *Eimeria* species in each individual host should be collected in order to deeply analise *Eimeria* community diversity in wild and captive host species.

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Population fluctuation of soil and plant parasitic nematodes at Khangabok Wangbal Government silkfarm, Wangbal, Thoubal district Manipur, India

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Abstract. The population fluctuation of nematodes around the rhizospheric regions of mulberrry plants at Khangabok Wangbal Government Silk farm, Wangbal, Thoubal District, Manipur, India was studied in relation to environmental factors like soil moisture content, soil pH, soil temperature, rainfall and moisture content of air for a consecutive period of three years, 2006-2008. During 2006, nematode population was highest in the month of May with very high rainfall (174.2 mm). Positive correlation of nematode population was found with soil temperature, soil pH, rainfall and relative humidity and negative correlation with soil moisture. During 2007, nematode population was highest in the month of May with least soil moisture and highest rainfall (15.1 p.c. and 190.6 mm). Nematode population had positive correlations with soil moisture, temperature, pH, rainfall and relative humidity of air. During 2008, nematode population was highest in the month of April with highest soil temperature of 24.8°C, 66.0 p.c. moderate relative humidity and 21.0 mm rainfall. There were positive correlation with soil temperature and pH, and negative correlation with soil moisture, rainfall and relative humidity. Lowest nematode population was found during January (2006), and during December (2007, 2008) there were negligible rain and sometimes no rainfall at all. Among all dorylaimids, tylenchids, aphelenchids and mononchids, *Helicotylenchus* sp. pertain the most numerous nematode species in all the three years and seasons.

Understanding life cycles and the factors governing changes in nematode populations is basic to understanding nematode distribution and plant disease. In many respects, nematodes are no different from other organisms in that their populations fluctuate during the year and in different environments. As expected, population fluctuations vary with the geographical location. The least recovery of nematodes in cultivated fields is often in winter or early spring in the northern latitudes where cold temperatures provably are limiting. Fluctuations within the main growth curve occur during a growing season and often are correlated with major divergences in temperature and moisture. Populations may differ in a season depending on the degree of host susceptibility as demonstrated by Johnson *et al.* (1974) and Minton *et al.* (1960).

The population fluctuation of nematodes around the rhizospheric regions of mulberry plants, one of the magic plants for sericulture was studied with relation to five main environmental factors, i.e. soil moisture content, soil pH, soil temperature, rainfall and moisture content of air. The site of study, Khangabok Wangbal Government Silk farm, Wangbal lies in Thoubal District with an altitude of 23°45′N to 24°45′N, longitude 93°45′E to 94°15′E and altitude of 781.0 (above MSL). Since no defined and precise work on mulberry plants from the state of Manipur, a particular area was selected to find out more precise information which will be useful to further effective control measures.

Such type of works was also carried out on coconut and areconut by Koshy and Sosama (1978), on jute by Chaturvedi and Khera (1979), on cabbage and cauliflower by Siddiqi *et al.* (1973), on strawberry and cherry by Lownsbary and Maggenti (1963), Krishnamurthy and Elias (1971) on root knot nematodes, Khan *et al.* (1971) on mango, Salih and Shahia (1987) on the effect of pH on growth and reproduction of free living nematodes, on blackpepper by Mohandas and Ramana (1988) and on grass pasture by Azmi (1990).

From the state Manipur, such work had carried out by Dhanachand *et al.* (1997) on sugarcane, Solitary *et al.* (1997) on *Dolichos lablab* and Mohilal and Dhanachand (1998) on comparison between nematode fauna of two hilly districts.

Materials and method

Collection of soil samples

In the locality 40 subareas are selected where mulberry plants were growing in a zigzag manner. Discarding the top layers of the soils, the subareas were digged up to a depth of 20 cm. Soil was collected from those areas in equal quantity, which served as the subsample of the area and were mixed thoroughly in a bucket. From the mixture, 500 gram of soil samples were collected in separate polythene bags which served as the sample of the area. The polythene bags were brought to the laboratory and kept undisturbed for at least 2 days before further processing.

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Rainfall

Data for rainfall for three consecutive years for the area were collected from meteorological data, Rice Research Station, Wangbal, Thoubal District for the year 2006-2008.

Moisture content

The amount of water present in soil was usually expressed in terms of percentage on dry weight basis. A known amount of soil was taken from the locality for each month and it was oven dried at 80ºC for 48 hours and re-weighed. The percentage of moisture content was calculated by using the formula:

Moisture p.c. = Loss of weight on drying / Dry weight of soil × 100

Soil pH

10 mg of air dried powdered soil was mixed with 50 ml of distilled water (1:5) and the suspension was shaken thoroughly for half an hour using an electric shaker. The solution was allowed to stand for about 30 minutes. Then the upper supernatant liquid was decanted from the suspension and transferred to a 250 ml beaker. The pH values of this clear soil solution were measured by using a pH meter.

Soil temperature

While collecting the soil samples the soil temperature reading was recorded by inserting a soil thermometer inside the soil at a depth of 5-20 cm below the ground.

Processing of soil samples

The collected soil samples were brought to the laboratory and processed for the extraction of nematodes by Cobb's sieving and decanting method followed by Baermann's modified funnel technique. The nematodes were fixed in warm F.A. (4:1). The most commonly encountered nematodes were identified up to generic level.

Counting nematode population

The collected nematodes were put in a 100 ml conical flask and the volume was made up to 100 ml. The nematode suspension was homogenized by blowing with the help of a pipette. 10 ml of the homogenized suspension was taken in a Syracuse counting dish and then the nematode population present on it was recorded. At least three readings were taken to avoid the chances of error. This value multiplied by 10 represents the nematode population in the soil sample.

Results and discussion

Record of nematode population during the months of the years from 2006-2008 is given in the Tables 1, 2, 3, 4, 5 and 6. Tylenchid nematodes include *Basiria* spp., *Caloosia* spp., *Criconemella* spp., *Ditylenchus* spp., *Helicotylenchus* spp., *Hemicriconemoides* spp., *Scutellonema* spp. and *Tylenchus* spp.

The dorylaimid nematodes include *Axonchium* spp., *Dorylaimellus* spp. and *Dorylaimoides* spp. The predatory or mononchid nematodes include *Iotonchus* spp. and *Mylonchulus* spp. Only one aphelenchid nematode, *Aphelenchus* spp., is frequently encountered. A tabular form of the results that was recorded in the present study is shown in tables separately.

During the year 2006, regarding the nematode species as a whole, it was found that highest population of nematode 74,500/500 ml of soil was recorded in the month of May where there was moisture content of 16.1%, soil pH 4.6, soil temperature 25.2ºC, relative humidity of air 81.0% and a rainfall of 174.2 mm which is followed by that of the month June with nematode population of 37,500/500 ml of soil, moisture content of 21.2%, soil pH 4.6, soil temperature 23.2ºC, relative humidity of air 93.0% and a rainfall of 107.8 mm.

The least nematode population 9500/500 ml soil was recorded during the month of January with a moisture content of 56.6%, soil pH 4.4, soil tem-

Table 1. Chart showing different ecological factors and nematode population during the 2006.

Month	Soil moisture	Soil pH	Soil temperature	Relative humidity	Rainfall	Total nematode
Jan	56.54	4.4	20.6	75.5	1.0	9500
Feb	23.73	4.4	23.0	67.5	17.8	14800
Mar	21.38	4.5	24.12	79.5	18.8	27140
Apr	15.35	4.5	25.1	80.0	42.0	15300
May	16.15	4.6	25.2	81.0	174.2	74500
Jun	21.21	4.6	23.2	93.0	107.8	37500
Jul	22.28	4.3	24.2	84.0	177.2	24100
Aug	31.27	4.3	24.6	76.0	107.4	13430
Sep	22.42	4.3	25.8	83.0	95.0	17600
Oct	22.95	4.7	28.9	76.0	65.0	15700
Nov	35.07	4.6	25.7	75.0	31.0	12800
Dec.	50.77	4.4	23.5	82.0	Nil	10800

Table 2. Month-wise individual nematode distribution during 2006.

Month	*Aphelenchus* spp.	*Axonchium* spp.	*Basiria* spp.	*Caloosia* spp.	*Criconemella* spp.	*Ditylenchus* spp.	*Dorylaimellus* spp.	*Dorylaimoides* spp.
Jan	500	700	400	0	300	200	500	900
Feb	700	900	500	0	400	500	700	1100
Mar	1500	6200	500	3200	700	3300	1000	1000
April	2700	16000	900	11500	900	2500	2000	2700
May	2100	4800	1000	1800	9900	1000	3500	1500
June	1700	4200	700	1000	10000	800	2700	1000
July	1100	2000	900	1000	700	800	700	1800
Aug	700	100	300	400	130	700	500	1000
Sep	500	1400	500	400	400	800	300	700
Oct	900	1500	400	500	500	700	200	500
Nov	500	1300	700	300	300	300	500	900
Dec	700	900	500	0	500	500	0	700

Table 3. Month-wise individual nematode distribution during 2006.

Month	*Helicotylenchus* spp.	*Hemicriconemoides* spp.	*Iotonchus* spp.	*Mylonchulus* spp.	*Paratylenchus* spp.	*Scutellonema* spp.	*Tylencholaimus* spp.	*Tylenchus* spp.
Jan	1500	0	900	700	400	1100	500	900
Feb	5900	0	700	800	500	1300	600	200
Mar	1140	1500	600	700	400	4400	900	100
Apr	28100	1400	1500	1600	600	9900	2900	1900
May	27800	1600	1400	1300	700	12000	3000	1100
Jun	6000	700	1100	900	400	1800	3800	700
Jul	4000	500	1900	1000	700	2200	3900	900
Aug	3900	600	700	800	500	1500	1300	300
Sep	5000	800	1500	800	600	1700	1700	500
Oct	4900	300	900	1000	300	1300	1100	700
Nov	39000	0	700	900	500	1100	900	0
Dec	2300	0	900	1000	400	1000	700	700

Table 4. Chart showing different ecological factors and nematode population during the year 2007.

Month	Soil moisture	Soil pH	Soil temperature	Relative humidity	Rainfall	Total nematode
Jan	23.22	4.7	21.2	70	Nil	14200
Feb	33.41	4.7	22.4	77.5	89	12800
Mar	17.56	4.6	22.5	74	8.2	49100
Apr	15.15	4.5	23.4	74.5	176	48480
May	15.1	4.5	25	77	190.6	68100
Jun	15.4	4.4	21	80	199.5	31350
Jul	21.35	4.4	20	84	189.2	23210
Aug	33.26	4.3	21	78.5	59.2	13100
Sep	22.75	4.5	22	70.5	118.8	16600
Oct	26.84	4.5	30.5	70.5	106.8	13300
Nov	39.2	4.3	22.8	70	52	12700
Dec	47.97	4.4	22.4	76.5	Nil	10100

Table 5. Month-wise individual nematode distribution during 2007.

Month	*Aphelenchus* spp.	*Axonchium* spp.	*Basiria* spp.	*Caloosia* spp.	*Criconemella* spp.	*Ditylenchus* spp.	*Dorylaimellus* spp.	*Dorylaimoides* spp.
Jan	700	700	500	4000	200	1000	400	700
Feb	700	900	700	0	900	700	500	1300
Mar	1300	5200	1000	4000	1000	2900	700	1300
Apr	1500	4700	950	2400	1500	3200	3500	1500
May	2900	3900	1100	2000	2500	1300	1300	1700
Jun	1900	4900	900	1300	1700	1100	750	1900
Jul	1300	1600	700	1100	1300	900	800	1700
Aug	400	1200	500	500	200	600	200	700
Sep	900	1700	400	300	100	500	500	600
Oct	700	1300	300	600	800	300	300	700
Nov	400	500	900	400	500	400	700	900
Dec	300	900	700	300	200	500	200	700

Table 6. Month-wise individual nematode distribution during 2007.

Month	*Helicotylenchus* spp.	*Hemicriconemoides* spp.	*Iotonchus* spp.	*Mylonchulus* spp.	*Paratylenchus* spp.	*Scutellonema* spp.	*Tylencholaimus* spp.	*Tylenchus* spp.
Jan	1000	0	900	400	400	1100	1500	700
Feb	2700	300	500	200	300	1500	700	900
Mar	22500	500	700	500	700	5000	1100	700
Apr	22700	700	500	250	780	2400	1300	600
May	31100	700	1100	900	900	12700	2700	1300
Jun	7900	200	700	900	500	2100	3500	1100
Jul	4900	110	1100	700	900	2300	2700	1100
Aug	3500	200	900	400	700	1300	1300	500
Sep	4900	500	1300	700	500	1500	1500	700
Oct	3900	0	600	800	500	1100	900	500
Nov	3500	0	900	700	400	900	900	700
Dec	1900	0	700	900	300	1000	1000	500

perature 20.6ºC, relative humidity of air 75.5% and a rainfall of 1.0 mm.

Individually as represented in Table 4, during the year 2006, among all the four kinds of nematodes viz. Dorylaimids, Tylenchids, Aphelenchids and Mononchid, *Helicotylenchus* spp. had the greatest population during all months of the year showing its peak in the month of April with moisture content of 15.3%, soil pH 4.5, soil temperature 25.1ºC, relative humidity of air 80.0% and a rainfall of 42.0 mm followed by *Scutellonema* spp. showing its peak in the month of May with 16.1% soil moisture content, 4.6 soil pH, 25.2ºC soil temperature, 81.0% relative humidity and 174.2 mm rainfall.

Tylenchus spp. had least population during the year showing its peak in the month of April with the same ecological parameters mentioned for *Helicotylenchus* spp.

Among the Dorylaimid nematodes *Axonchium* spp. had highest population showing its peak in the month of April with ecological parameters same with *Helicotylenchus* spp., while *Dorylaimellus* spp. had lowest population showing its peak in the month of May with ecological parameters same as that of *Scutellonema* spp. and population is lowest in the month of October with 22.9% soil moisture, 4.7 soil pH, 28.9 soil temperature, 76.0% relative humidity of air and 65.0 mm rainfall while it was absent in the month of December as there was no rainfall and had 50.7% soil moisture, 4.4 soil pH, 23.5ºC soil temperature and 82.0% relative humidity.

The analysis of linear correlations between the five environmental parameters taken into consideration during the year 2006 indicated that there were significant positive correlation of the nematode population [significant at 0.01 (95%) level of significance and above] with soil temperature (rX2y=

0.113), soil pH (rX3y=0.110), rainfall (rX2y=0.351) and relative humidity of air (rX5y=0.297) and negative correlation with the soil moisture content (rX1y= –0.725).The results were represented in the Table 10.

During the year 2007, the nematode population was highest in the month of May 68,100/500 ml of soil with least soil moisture content of 15.1%, soil pH 4.5, greater temperature of soil 25.0ºC, relative humidity of 77.0% but highest rainfall of 190.6 mm. It was followed by the month of March, 49,100/500 ml soil with a 17.5% soil moisture content, 4.6 soil pH, 22.5ºC soil temperature, 74.0% relative humidity and 8.2 mm rainfall, while nematode population was least 10,100/500ml of soil during the month of December with greatest soil moisture content of 47.9%, 4.64 soil pH, 22.4ºC soil temperature, 76.5% relative humidity and with no rainfall.

Individually as represented in Table 6, among all the four nematode groups during the months of the year 2007, *Helicotylenchus* spp. had greatest population showing its peak in the month of May with 15.1% soil moisture content, 4.5 soil pH, 25.0ºC soil temperature, 77.0% relative humidity and 190.6 mm rainfall, followed by *Scutellonema* spp. showing its peak in the month of May with ecological parameters same as those given for *Helicotylenchus* spp. *Hemicriconemoides* spp. had the least population showing its peak in the months of April and May with same ecological parameters for *Scutellonema* spp. and *Helicotylenchus* spp. and showing totally absent during the months of January, October, November and December with same population (January: 23.2% soil moisture content, 4.7 soil pH, 21.2ºC soil temperature, 70.0% relative humidity and no rainfall; November: 39.2% soil moisture content, 4.3 soil pH, 22.8ºC soil temperature, 70.0% relative humidity and 52.0 mm rainfall; October: 26.8% soil moisture content, 4.5 soil pH, 30.5ºC soil temperature, 70.5% relative humidity and 106.8 mm rainfall and December: 47.9% soil moisture content, 4.4 soil pH, 22.4ºC soil temperature, 76.5% relative humidity and no rainfall).

Among the Dorylaimid nematodes, *Axonchium* spp. had greatest population showing its peak in the month of March with 17.6% soil moisture content, 4.6 soil pH, 22.5ºC soil temperature, 74.0% relative humidity and 8.2 mm rainfall, while *Dorylaimellus* spp. had least population showing its peak in the month of April with 15.15% soil moisture content, 4.5 soil pH, 23.4ºC soil temperature, 74.05% relative humidity and 176.0 mm rainfall; its population reach lowest in the month of August and December with ecological parameters same as those given for *Hemicriconemoides* spp. (August: 33.2% soil moisture content, 4.3 soil pH, 21ºC soil temperature, 78.5% relative humidity and 59.2 mm rainfall). Among the mononchid nematodes, *Iotonchus* spp. showed greatest population during the month of September with 22.7% soil moisture content, 4.5 soil pH, 22.0ºC soil temperature, 70.5% relative humidity and 118.8 mm rainfall, while *Mylonchulus* spp. showed least population during the month of February with 33.41% soil moisture content, 4.7 soil pH, 24.2ºC soil temperature, 77.50% relative humidity and 89.0 mm rainfall. The aphelenchid nematode *Aphelenchus* spp. showed its peak in the month of May with ecological parameters same as those given for *Helicotylenchus* spp. and least in the month of December with 47.9% soil moisture content, 4.4 soil pH, 22.4ºC soil temperature, 76.5% relative humidity and no rainfall.

In the analysis of linear correlations between the five environmental parameters taken into consideration during the year 2007, there were significant positive correlations between the nematode population with soil moisture (rX_1y=0.467), soil temperature (rX_2y= 0.144), soil pH (rX_3y=0.139), rainfall (rX_4y =0.467) and relative humidity of air (rX_5y= 0.183) at 0.01 level of significance. The results are represented in the Table 11.

Table 7. Chart showing different ecological factors and nematode population during the 2008.

Month	Soil moisture	Soil pH	Soil temperature	Relative humidity	Rainfall	Total nematode
Jan	39.2	4.5	23.2	72	35.8	12150
Feb	23.22	4.5	24.1	62	12.8	22400
Mar	17.56	4.5	23.8	68.5	26.2	53000
Apr	15.1	4.6	24.8	66	21	108600
May	16.15	4.7	21	70.5	37.2	60200
Jun	21.35	4.5	21.5	78.5	125.7	27320
Jul	22.75	4.5	20.5	74.5	133.8	22980
Aug	33.26	4.6	23.5	84	188.4	17500
Sep	26.84	4.6	22.5	77.5	94.7	18200
Oct	33.2	4.4	22	75.5	64.6	17300
Nov	33.41	4.4	21.8	78	40	13000
Dec.	47.97	4.4	21	78.5	Nil	8

Table 8. Month-wise Individual nematode distribution during 2008.

Month	*Aphelenchus* spp.	*Axonchium* spp.	*Basiria* spp.	*Caloosia* spp.	*Criconemella* spp.	*Ditylenchus* spp.	*Dorylaimellus* spp.	*Dorylaimoides* spp.
Jan	500	900	500	300	450	300	500	1100
Feb	500	100	700	500	300	300	700	900
Mar	1000	7200	700	4500	100	3500	800	1200
Apr	2900	17100	1000	21000	200	3000	7000	2900
May	2300	3900	900	1700	400	1100	1100	2000
Jun	1900	4500	900	1200	500	900	120	1100
Jul	1000	2500	500	1500	780	700	500	1500
Aug	900	2300	700	500	800	900	200	1100
Sep	700	1900	300	700	200	700	500	900
Oct	700	1700	700	500	0	700	900	900
Nov	500	900	500	500	0	500	500	900
Dec	600	700	500	0	0	500	300	900

Table 9. Month-wise individual nematode distribution during 2008.

Month	*Helicotylenchus* spp.	*Hemicriconemoides* spp.	*Iotonchus* spp.	*Mylonchulus* spp.	*Paratylenchus* spp.	*Scutellonema* spp.	*Tylencholaimus* spp.	*Tylenchus* spp.
Jan	2500	700	700	700	300	1700	300	700
Feb	7900	1500	1500	500	700	5000	400	900
Mar	12900	1800	1800	10000	500	5500	1000	500
Apr	29300	2000	2000	5000	700	10300	2100	2100
May	28000	800	800	500	900	11700	3100	1000
Jun	6900	700	700	900	500	1900	3700	900
Jul	4900	0	0	1500	900	2500	3500	700
Aug	3500	200	200	900	600	1700	2500	500
Sep	5000	500	500	1500	700	1500	1900	700
Oct	4300	900	900	1000	700	1300	1500	600
Nov	4300	200	200	900	500	900	1000	700
Dec	1900	0	0	600	400	900	500	500

Table 10. Values of correlation coefficients (r) and allometric equations for the relationship between nematode population and environmental factors during the year 2006.

Environmental factors	Correlation coefficients	Allometric equation
Soil moisture X_1	–0.725	$Y=60093.397–1309.665\ X_1$
Soil temperature X_2	0.113	$Y=8211.371+784.112\ X_2$
Soil pH X_3	0.110	$Y=26962.168+ 234.989\ X_3$
Rainfall X_4	0.351	$Y=18773.669+144.035\ X_4$
Relative humidity X_5	0.297	$Y=–68254.7+1223.019X_5$

Significant at 0.01 level of significance.

Table 11. Values of correlation coefficients (r) and allometric equations for the relationship between nematode population and environmental factors during the year 2007.

Environmental factors	Correlation coefficients	Allometric equation
Soil moisture X_1	0.467	$Y=62233.886–1112.216\ X_1$
Soil temperature X_2	0.144	$Y=–16455.3+1848.704\ X_2$
Soil pH X_3	0.139	$Y=–62998.8+19879.661X_3$
Rainfall X_4	0.467	$Y=14637.230+145.945\ X_4$
Relative humidity X_5	0.183	$Y=–32176.4+774.814X_5$

Significant at 0.01 level of significance.

During the year 2008, nematode population was highest 108,600/500 ml of soil in the month of April with least moisture content of 15.1%, 4.6 soil pH, highest soil temperature of 24.8ºC with moderate relative humidity of 66.0% and rainfall of 21.0 mm followed by March, 53,000/500 ml of soil with 17.5% soil moisture content, 4.5 soil pH, 23.8ºC soil temperature, 68.5% relative humidity and 26.2 mm rainfall. Nematode population was lowest during the month of December 8300/500 ml of soil

with 47.9% soil moisture content, 4.4 soil pH, 21.0ºC soil temperature, 78.5% relative humidity and no rainfall.

Here in this year also, among all the nematode groups, *Helicotylenchus* spp. shows the highest population reaching its peak during the month of April with same ecological parameters as those given above for the highest nematode population which was followed by *Scutellonema* spp. during this year too reaching its peak in the month of May with 16.1% soil moisture content, 4.7 soil pH, 21ºC soil temperature, 70.5% relative humidity and 37.2 mm rainfall. *Criconemella* spp. had the least nematode population showing its peak in the month of August with 33.2% soil moisture content, 4.6 soil pH, 23.5ºC soil temperature, 84.0% relative humidity and greatest rainfall of 188.4 mm., while was absent during October, November and December (October: 33.2% soil moisture content, 4.4 soil pH, 22.0ºC soil temperature, 75.5% relative humidity and 64.6 mm rainfall; November: 33.4% soil moisture content, 4.4 soil pH, 21.8ºC soil temperature, 78.0% relative humidity and 40.0 mm rainfall; December: same ecological parameters as those given for least total nematode population).

Among Dorylaimida, *Axonchium* spp. was most populous showing its peak in the month of April with same ecological parameters given for highest nematode population, while *Dorylaimellus* spp. had least population given showing its peak in the month of May (16.1% soil moisture content, 4.7 soil pH, 21.0ºC soil temperature, 70.5% relative humidity and 37.2 mm rainfall) and lowest in the month of June (21.3% soil moisture content, 4.5 soil pH, 21.5ºC soil temperature, 78.5% relative humidity and 125.7 mm rainfall).

Among the predatory nematodes *Mylonchulus* spp. had greatest population during the month of March with 17.5% soil moisture content, 4.5 soil pH, 23.8ºC soil temperature, 68.5% relative humidity and 26.2 mm rainfall. *Iotonchus* spp. had least population during the month of January with 39.2% soil moisture content, 4.5 soil pH, 23.2ºC soil temperature, 72.0% relative humidity and 35.8 mm rainfall.

The aphelenchid nematode *Aphelenchus* spp. showed its peak in the month of April with ecological parameters same as those given for greatest nematode population, while had lowest population in the months of January, February and November (January: 39.2% soil moisture content, 4.5 soil pH, 23.2ºC soil temperature, 72.0% relative humidity and 35.8 mm rainfall; February: 23.2% soil moisture content, 4.5 soil pH, 24.1ºC soil temperature, 62.0 % relative humidity and 12.8 mm rainfall; November: 33.4% soil moisture content, 4.4 soil pH, 22.8ºC soil temperature, 78.50% relative humidity and 40.0 mm rainfall).

In the analysis of linear correlations between the five environmental parameters taken into consideration during the year 2008, there were negative correlations of the nematode population with soil moisture (rX_1y = –0.173), rainfall (rX_4y = –0.491) and relative humidity (rX_5y = –0.56068). There were positive correlations between soil temperature (rX_2y = 0.117) and soil pH (rX_3y = 0.5394).The results were represented in the Table 12.

Table 12. Values of correlation coefficients (r) and allometric equations for the relationship between nematode population and environmental factors during the year 2008.

Environmental factors	Correlation coefficients	Allometric equation
Soil moisture X_1	–0.173	$Y=52951.551–530.495\ X_1$
Soil temperature X_2	0.117	$Y=–20031.5+2598.178\ X_2$
Soil pH X_3	0.5394	$Y=–259621+ 65974.138X_3$
Rainfall X_4	–0.491	$Y=55318.287–260.791\ X_4$
Relative humidity X_5	–0.56068	$Y=146667.4–1467.712\ X_5$

Significant at 0.01 level of significance.
Where X_1 = Relation of total soil nematode population with soil moisture
X_2 = Relation of total soil nematode population with soil temperature
X_3 = Relation of total soil nematode population with soil pH
X_4 = Relation of total soil nematode population with rainfall
X_5 = Relation of total soil nematode population with relative humidity of air.

All these results indicated that among the five environmental parameters considered for the study the moisture content of soil was the main parameter which cause the fluctuation of nematode population. The results were in conformity with those of Wallace (1963), Tikyani and Khera (1969), Simons (1973), Dhanachand *et al.,* (1997) and Solitary *et al.* (1997), who reported that moisture plays a key role in population dynamics. Koshy and Sosamma (1978) inferred that there were definite periods of occurrence of maximum and minimum for *Radopholous similis* in a year. Chaturvedi and Khera (1979) found that it was rainfall rather than temperature which played a direct role in the population fluctuation of nematodes. Mohandas and Roman (1988) found that rainfall increases soil moisture which helps the nematodes to migrate from infesting roots to healthy roots.

Conclusion

The population curve in the present study also showed that there was an irruptive change in the population of nematodes showing up and down but not in a cyclic order. This shows that the population of the nematode fluctuates in different months of the year being correlated with different environmental factors.

Here, in the study during the year 2006, the population of nematode is highest in the month of June, having high rainfall of 107.8 mm with soil moisture content 21.2 p.c., soil pH 4.6, soil temperature 23.2ºC and relative humidity of 93.0 p.c. This increase in the population could be due to high

temperature of soil with rapid loss of moisture, though rainfall is high. The population was lowest in the month of January with low rainfall of 1.0 mm but highest soil moisture content of 56.5, soil pH 4.4, soil temperature 20.6ºC and relative humidity 75.5 p.c. This may be due to the high moisture content and low temperature of soil. During the year 2007, the nematode population was highest in the month of April with least soil moisture content of 15.1 p.c., soil pH 4.5, soil temperature 23.4ºC, relative humidity 74.5 p.c. but high rainfall of 176.0 mm. This may be due to the fact that there was less soil moisture content combined with moderate temperature and rainfall. The population is lowest in the month of December with highest soil moisture content of 47.9 p.c., soil pH 4.4, soil temperature 22.4ºC, high relative humidity of air 76.5 p.c. but no rainfall. This may be due to the high moisture content of soil.

During the year 2008, the population of nematode was highest in the month of April with lowest moisture content of 15.1 p.c., soil pH 4.6, soil temperature 24.8ºC, relative humidity 66.0 p.c. and lower rainfall of 21.0 mm. It was obvious that increase in the population is due to the low moisture content of soil combined with moderate soil temperature and rainfall. Population was lowest in the month of December with highest soil moisture content of 47.9 p.c., soil pH 4.4, soil temperature 21.0ºC, high relative humidity 78.5 and no rainfall. This may be due to high soil moisture and relative humidity of air.

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Cutaneous myiasis in a geriatric patient

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Abstract. Cutaneous myiasis in humans involving fly species endemic in Italy are uncommon and are not often present in the literature. In the present article we describe the case of cutaneous myiasis in a woman brought to the Emergency Department (ED) of the S. Croce e Carle General Hospital in Cuneo, northwest Italy. The patient was in precarious hygienic condition, and was suffering from localized ulcerated foot ulcers with significant inflammation extending up to the knee. Fly larvae, subsequently identified as *Lucilia sericata* (Meigen, 1826), were found in the lesions. The patient was admitted to the Department of Internal Medicine where she was treated with antibiotics and the lesions were medicated, resulting in full recovery.

Key words: cutaneous myiasis, *Lucilia*, ulcerations, poor hygiene, parasitic diseases, geriatric.

The term *myiasis*, first used by Hope in 1840, refers to human and animal parasitic diseases originating with fly larvae. The definition in use today is by Zumpt (Zumpt, 1965).

Myiases can be both ectoparasitic and endoparasitic depending on the relationship with the host, and result in external infestations or internal in which the parasite can migrate within the host's body. Parasitic diseases can be caused by various species of Diptera, Cyclorrapha and may be classified according to the species' parasitic relationship with the animal host and the location and typology of myiasis which occurs in the host's body.

Classification of parasitic relationship of the Diptera species with the host subdivides myiasis as follows:

- specific/obligatory, induced from species which must at least partially complete their development inside a living host (obligatory parasites species);
- semi-specific/facultative, in which the species is free-living, i.e. do not require a host as part of the life cycle but may be attracted to humans through wounded tissue;
- accidental/non-specific, referring to eggs or larvae normally free-living which are ingested via food or water and live in the host's digestive tract, possibly continuing their life cycle or not, and with or without producing symptoms.

In Italy myiasis by obligatory species in humans is mainly caused by *Oestrus ovis* (Linné, 1758) (a species which more commonly afflicts sheep and shepherds) (Pampiglione, 1958a,b; Fazii *et al.*, 2006), *Wohlfahrtia magnifica* (Schiner, 1862) (Lombardo *et al.*, 2002), *Dermatobia hominis* (Linné, 1781), and *Cordylobia anthropophaga* (Blanchard and Berenger-Feraud, 1872). The latter two species are not endemic in Italy and are brought in by tourists and their pets upon returning from abroad (Bongiorno *et al.*, 2007; Calderaro *et al.*, 2008; Ferroglio *et al.*, 2003; Veraldi *et al.*, 1993).

Development of semi-specific myiases is closely linked to host's hygienic, physiomorphological, and clinical conditions which in Italy are known to be sustained mainly by *Lucilia*, *Calliphora*, *Musca* and *Sarcophaga* which are attracted to hosts with infected or necrotic tissue. We report here our experience with one case involving *Lucilia*.

Clinical case

On May 26, 2009 a 78 y.o. woman presented at the ED of the S. Croce and Carle general hospital in Cuneo, northwest Italy. She was brought in by ambulance on her family physician's recommendation due to her poor general condition, hyporexia, and general neglect. Due to poor personal hygiene the patient was quarantined upon arrival and was washed and changed into a hospital gown prior to being examined. Her vital signs were normal (BP: 130/70 mm Hg; HR: 90/min.; SaO_2: 97%; respiration rate: 18 breaths per minute); the body temperature was 37.8°C.

Physical examination revealed chronic venous insufficiency in the lower extremities associated with necrotized lesions at each hallux and the top of the right foot (Figs 1, 2), most likely due to badly fitting footwear. The presence of fly larvae in the ulcerations was noted, a medical entomologist was consulted, and a total of 5 larvae were extracted. The lesions were subsequently washed with abundant hydrogen peroxide. X-rays of the feet showed the presence of liquid between the cutaneous and subcutaneous layers of the dorsal area without osteolytic lesions. Blood tests indicated increased markers for phlogosis (C-reactive protein 185 mg/dl, fibrinogen 850 mg/dl); neutrophilic leukocytosis

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Figure 1. Necrotic ulceration, top of tight foot. (Photo M. Dutto).



Figure 2. Necrotic ulceration, left hallux. (Photo M. Dutto).

(white cells 15.4×10^3/µl, neutrophils 85%, lymphocytes 6.9%); and normochromic anemia (Hb 9.9 g/dl).

The patient was admitted to the Internal Medicine Department where she received intravenous amoxicillin (2.2 g/day × 3 days) and clindamycin (900 mg/day × 3 days) with low molecular weight heparine (LMWH) prophylaxis due to suspected anaerobic infection; in addition the patient received continuous therapy for dehydration and nourishment. Blood tests the next day showed hypoprotidemia (total protein 5.5 g/l, albumin 2.3 g/l) and low reticulocyte count. Liver, renal and thyroid functions were within normal values and erythrocyte sedimentation rate (ESR) was elevated (98 mm); glycemia after fasting was in the norm (90 mg/dl).

The patient's cutaneous ulcerations were treated daily with silver nitrate and polyurethane foam dressings. The second day more larvae were removed. Venous ultrasound of the patient's legs excluded thrombosis but confirmed chronic venous insufficiency.

The patient was transferred to a nearby long-term care facility where good improvement continued and over a three-week stay the patient's ulcerations were nearly completely healed. Similarly, the indices for phlogosis had returned to normal as had those for malnutrition and hemoglobin. Upon discharge a follow-up examination showed she was in good general condition and her lower extremities showed only slight dyschromia where the ulcerations had been.

Results and discussion

The larvae removed from the patient's cutaneous ulcerations were placed in saline solution; in the laboratory they were observed with a stereomicroscope to determine the stage of development. Four were third stage (L3) and one was second stage (L2).

Two of L3 larvae were placed in a Petri dish using canned moist cat food as a protein substrate and kept in an appropriate container maintained at environmental temperature; the other three larvae were placed in 70% ethanol. Subsequently one of the L3 larvae was sectioned (anterior and posterior) and the two portions were placed in 10% potassium hydroxide for 24 hours to prepare the stigmatic plates and the cephalo-pharyngeal skeleton which allow at least general identification of the larva.

Both the cephalo-pharyngeal skeleton and the stigmatic plates were mounted in glycerin and observed with an optical microscope (Leitz Diaplan) at 4× and 10×. Morphological analysis resulted in positive identification of the larvae as *Lucilia*.

The L3 larvae which were grown in the laboratory entered the pupal stage after 131 hours and after another 216 hours the flies emerged from the pupal case. The adult flies were identified as *Lucilia seri-*

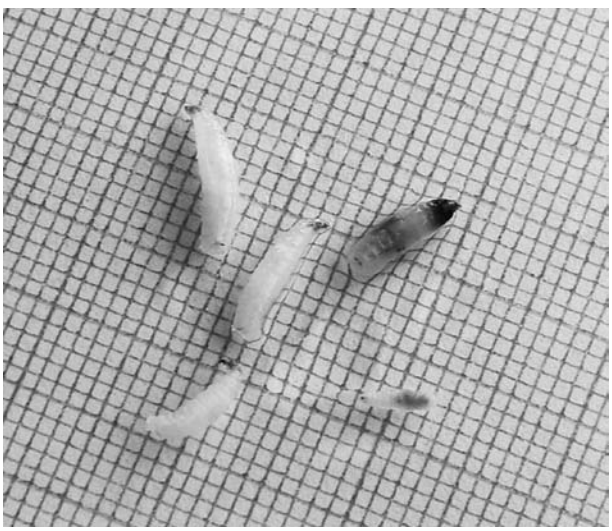


Figure 3. *Lucilia sericata* larvae removed from necrotic ulceration. (Photo M. Dutto).

cata (Meigen, 1826) (Diptera, Calliphoridae). Based on the patient's body temperature upon arrival at the ED (37.8°C ear reading) and the temperature in which the harvested larvae were grown it can be deduced that the least time for colonization in the woman's cutaneous lesions was an 50-62 hours prior to her arrival at the hospital.

Conclusions

Species in the genus *Lucilia*, particularly *Lucilia sericata*, are facultative myiasis-causing species and are well-represented in the literature. Most of these concern cutaneous myiasis (Chigusa *et al.*, 2002; Ioli *et al.*, 1997; Talari *et al.*, 2004) occurring chiefly in wounds or cutaneous ulcers though there have also been cases of myiasis afflicting the eye, ear (Cetinkaya *et al.*, 2008; Cho *et al.*, 1999; Yaghoobi *et al.*, 2005) and tracheostomy (Franza *et al.*, 2006). Many of these involve patients with poor hygiene (Talari *et al.*, 2004) which can result in the presence of malodorous exudates and secretions in anatomical openings, or infected and/or necrotic wounds of varying traumatic origins which have been inadequately treated. However there have been cases which involve healthy newborn patients who enjoy good hygiene (Cetinkaya *et al.*, 2008). In nature, *L. sericata* in its larval stage is typically found in biological substrates in a state of decay, preferably those which are moist and rich in lipids.

Female *Lucilia* are oviparous and present a serious parasitical problem in veterinary medicine for myiases in farm animals (cows, sheep, goats) which can cause decreases in production and even death, especially in sheep.

In the case presented, myiasis was most likely caused by the patient's poor hygiene and cutaneous ulcers probably over several months' time by badly fitting footwear. Infestation by *Lucilia* is chronologically secondary to the lesions and the initial infection. The eggs are deposited on the wounds by female flies attracted by the fluids in the infection.

The presence of fly larvae in a wound represents a clear sign of poor hygiene and myiases can be a good medico-legal indicator of unhealthy living conditions or abandonment. This can be seen in the rapid clinical improvement reported in the case presented here following the patient's adequate nutrition, medicinal therapy and appropriate management of the cutaneous lesions.

From a clinical point of view, *Lucilia* larvae are not responsible for serious damage to healthy tissue since they develop mainly in necrotic tissue and is notable their bacteriostatic activity against *Streptococcus* A, B, *Pseudomonas* sp. and *Escherichia coli* (Tanyuksel *et al.*, 2005). Healthy tissue can be involved in cases where the quantity of larvae is so great that it is disproportionate to the necrotic area. Obviously in conditions of decay the presence of adult flies which can access the ulcerations serves to increase the bacterial count in the wounds and consequently likewise increase the risk of infection. In treating facultative myiasis in necrotized wounds the first task is removal the parasites with anatomical tweezers together with thorough cleansing of the wound with saline solution followed by hydrogen peroxide once all parasites have been removed. In cases concerning large areas, surgical debridement must be employed to completely remove all necrotic tissue. As happened in the case we described, in the days following removal of the parasites it is possible to find others in the wound. Medication of these wounds includes silver nitrate covered by light dressing. It should be noted that bandages do not afford protection and infestation can reoccur in the hospital in rooms lacking proper screens on windows as well as fly traps used with UV-A lights.

Apart from the pathological interest most commonly generated by *Lucilia* larvae, specifically *L. sericata*, it is necessary to mention the use of maggot debridement therapy (MDT) which is utilized as a biosurgery technique for removal of dead, damaged or infected tissue of chronic ulcerations resistant to traditional treatment (Gupta, 2008; Tanyuksel *et al.*, 2005). These include lesions due to diabetic foot, burns, chronic infections, etc. In MDT disinfected L1 larvae are placed in the wound where they remain for 24-72 hours and they selectively eat only the necrotic tissue. The quantity of larvae is proportional to the size of the lesion and the cycle may be repeated until satisfactory debridement has been performed (Tanyuksel *et al.*, 2005).

In conclusion the myiasis case described here underscores how poor hygiene and the psychophysical isolation of many elderly people today can lead to health problems which, unchecked, can develop into more serious situations requiring several types of public health intervention, from medical to socio-psychological. Fortunately it is a medical condition which is rather uncommon but preventive measures can be taken to diminish it further in humans.

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First record of *Paradiplozoon amurensis* (Monogenea: Diplozoidae) in Iraq from gills of the cyprinid fish *Cyprinion macrostomum*

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Abstract. A survey of gill parasites was conducted in 164 *Cyprinion macrostomum* (Cyprinidae) collected from the Tigris River at Tikreet city, Salah Al-Deen province, in central Iraq, in 2009-2010. The monogenean *Paradiplozoon amurensis* Akhmerov, 1974 (Diplozoidae) was observed in the gills of 26.2% of these fishes with a mean intensity of 2.3. This is the first report of *P. amurensis* in Iraq. A description and morphometrics of *P. amurensis* specimens are presented.

Key words: *Paradiplozoon amurensis*, Monogenea, ectoparasites, fishes, Iraq.

Monogeneans of the family Diplozoidae are common, widespread gill parasites of cyprinid fishes (Höglund and Thulin, 1989; Pečínková *et al*., 2005). Diplozoids cause a decrease in gill respiratory function (Amlacher, 1970). Infected gills appear pale and damaged (Al-Nasiri, 2003).

According to Mhaisen (personal communication), a total of ten species of diplozoids are known to occur in freshwater fishes of Iraq. These include seven species of *Paradiplozoon*, two species of *Diplozoon* and one species of *Eudiplozoon*.

Many reports are available on the parasitic fauna of fishes of Iraq, but only eight surveys have been conducted on fishes in the Tigris River in the province of Salah Al-Deen (Abdul-Ameer, 1989; Nawab Al-Deen, 1994; Muhammed, 1995; Al-Jawda *et al*., 2000; Al-Nasiri, 2008, 2009; Al-Nasiri and Mhaisen, 2009a,b). Therefore, the parasite fauna of this region river is probably incompletely known. The present study was conducted to further knowledge of the fish parasites occurring in this area.

Materials and methods

By using gill and cast nets, 164 *Cyprinion macrostomum* (Cyprinidae) were collected from a reach of the Tigris River passing through the city of Tikreet in Salah Al-Deen province between November 2009 and August 2010. Fishes were chilled on ice and transferred to the laboratory where they were necropsied. All gill arches were thoroughly examined. Monogenean were spread on glass slides, fixed in 70% ethanol and stained with acetocarmine (Al-Nasiri, 2003). Identifications followed Gussev (1985) and Pugachev *et al*. (2010). Measurements were made with a slide micrometer. Drawings were made with the aid of a drawing tube.

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Results

Specimens of *P. amurensis* were found on gills of 26.2% of 164 *C. macrostomum*. The mean intensity of infection was 2.3 parasites. Infected gills were pale, bloody and with excessive mucous secretion.

As *P. amurensis* is recorded here for the first time in Iraq, I provide the following short account of its morphology and morphometrics, based on ten specimens (Fig. 1). All measurements are given in millimeters, with the range of values followed by the mean in parentheses.

The total body length is 2.8-6.1 (4.2), anterior part 1.6-3.8 (2.2), posterior part 0.9-2.1 (1.4). Clamp dimensions are I: 0.06-0.08 (0.07) × 0.08-0.14 (0.11); II: 0.06-0.08 (0.07) × 0.13-0.17 (0.15); III: 0.07-0.09 (0.08) × 0.12-0.18 (0.15); IV: 0.06-0.10 (0.08) × 0.12-0.18 (0.15). The anterior end of the median

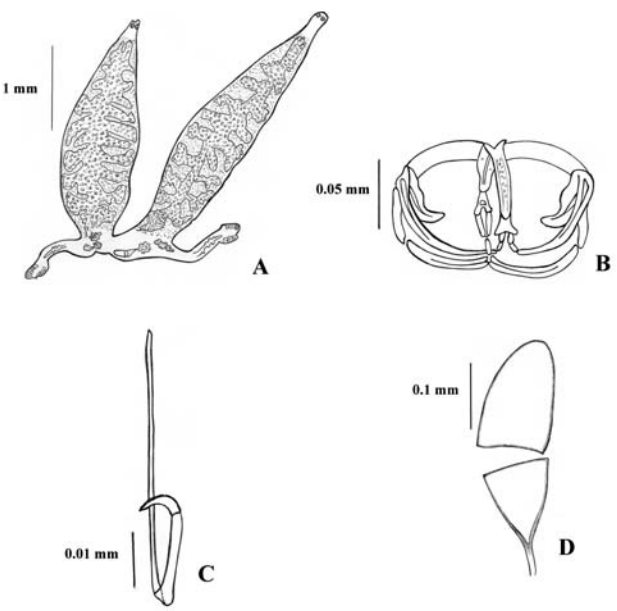


Fig. 1. *Paradiplozoon amurensis*. A: Whole mount; B: Chitinoid clamp; C: Median hook; D: egg.

sclerite has two lateral projections that are connected to the clamp jaws by two sclerites. The edge of anterior part of median sclerites is serrated. The length of the hook anchors is 0.018-0.021 (0.020), that of the handle 0.040-0.045 (0.043). The diameter of suckers is 0.06-0.10 (0.08). In the posterior part of the body, the intestine has short lateral projections. The testis is lobulate. Eggs are 0.23-0.32 (0.28) × 0.08-0.16 (0.11).

Discussion

The measurements of *P. amurensis* encountered in the present investigation are in general agreement with those of Gussev (1985) and Pugachev *et al.* (2010). Some of the slight differences observed may have arisen from differences in fish species and size (Prost, 1991; Matejusová *et al*., 2002) or from differences in environmental temperatures (Höglund and Thulin, 1989).

In the unpublished data of F.T. Mhaisen (personal communtication) on parasites and disease agents of fishes of Iraq, ten diplozoid species are known from fishes of Iraq. These include two species of *Diplozoon* (*D. kasimii* and *D. paradoxum*), one species of *Eudiplozoon* (*E. nipponicum*) as well as seven species of *Paradiplozoon*. The first reports of *Paradiplozoon* spp. in fishes of Iraq are as follows: *P. pavlovskii* (reported as *D. pavlovskii*) from *Aspius vorax* by Khamees (1983); *P. barbi* (reported as *D. barbi*) from *Chondrostoma nasus*, *C. regium* and *Cyprinus carpio* by Rasheed (1989); *P. homoion* from *B. xanthopterus* by Al-Saadi (2007); *P. megan* from both *A. vorax* and *B. xanthopterus* by Al-Saadi (2007); *P. vojteki* from *B. xanthopterus* by Al-Saadi (2007); *P. bliccae* from *Cyprinion macrostomum* by Al-Nasiri (2009), and *P. cyprini* from *Barbus grypus* by Al-Nasiri and Mhaisen (2009a).

The present record of *P. amurensis*, brings the total number of paradiplozoids recorded in Iraq to eight species. It is likely that additional diplozoid species from fishes of Iraq will be recorded in future surveys of parasite of fishes from different water bodies of Iraq.

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SOCIETÀ PER GLI STUDI DELLA MALARIA

Sulla patogenesi delle recidive nelle febbri malariche.

per il prof. A. BIGNAMI.

Come è noto, dopo le ricerche sul ciclo anofelico dei parassiti malarici, il problema della cura dei malarici ha assunto un'importanza sociale grandissima, poichè la cura metodica dell'uomo malato rappresenta uno dei mezzi di profilassi sociale, al quale molti si affidano più che a qualunque altro.

Ma chiunque abbia conoscenza di malarici, sa quali difficoltà s'incontrino non di rado nel còmpito di guarire gl'infermi ostinatamente recidivanti. Non è raro il caso di malati i quali, dopo una lunga serie di recidive sono divenuti così scettici intorno all'efficacia della chinina, che rifiutano di continuare a prendere il rimedio.

E poichè vari fatti mi portano a ritenere che la relativa resistenza delle febbri recidive al prezioso alcaloide sia in intimo rapporto con il meccanismo patogenetico di quelle, così non credo inutile, anche per l'importanza pratica dell'argomento, esporre in proposito alcune considerazioni e vari fatti, pur sapendo di non dire, in gran parte, cose nuove.

È quasi inutile notare che una delle questioni ancora più controverse ed oscure nella patologia della malaria, è appunto quella che riguarda la genesi delle recidive, le quali, come è notissimo, si verificano in tutte le specie di infezione malarica, talora separate da brevi intervalli di apiressia, talora da lunghi periodi di latenza.

È anche noto che le recidive possono succedersi con una certa regolarità. Così nei malati che prendono chinina soltanto quanto basta per far cessare la febbre e poi ne sospendono l'uso, spesso si vedono le recidive ritornare con intervalli un po' minori di due settimane,

intervalli che si avvicinano, come si è già notato, alla durata media comune del periodo di incubazione delle infezioni primitive. Non è raro anche vedere febbri malariche che recidivano con una certa regolarità ad intervalli di circa un mese. Ma è meno comunemente noto il fatto su cui, per quanto io sappia, solo recentemente si è richiamata l'attenzione, che anche dopo lunghi periodi di latenza le recidive si presentano in determinate stagioni, a quanto pare, con una certa legge. Così pare non sia raro vedere le terzane recidivare in primavera dopo essere rimaste latenti nell'inverno; e vi sono anche casi di febbri estive, le quali, dopo essere rimaste completamente latenti durante l'inverno e la primavera, recidivano al principio dell'estate.

Ad esempio ho avuto occasione di seguire un malato, il quale aveva preso le febbri nell'estate del 1901 a Trinitapoli, poi stette completamente bene durante l'inverno e la primavera del 1902 e recidivò il 25 giugno dello stesso anno con attacchi malarici gravi, durante i quali si trovarono nel sangue i parassiti estivo-autunnali, ma non semilune. Naturalmente in tutto il periodo di latenza non si era mai esposto a prendere una nuova infezione dall'ambiente. Non mi sarebbe difficile riferire altri casi simili bene osservati.

Già da un certo tempo, alcuni studiosi di cose malariche hanno creduto di poter affermare che queste recidive delle febbri estive, dopo lunghe latenze in principio di estate, siano un fenomeno regolare che si verifichi in un gran numero di ammalati; tanto che essi credono che questi recidivi siano i depositari dei gameti, dai quali si infetterebbero gli anofeli produttori delle febbri estive nuove. Così si inizierebbe il nuovo anno epidemico. Io non potrei dire, per esperienza mia, se al fatto cui alludo possa attribuirsi tanta importanza dal punto di vista epidemiologico; infatti è estremamente difficile negli individui abitanti in luoghi malarici distinguere le infezioni recidive dalle infezioni nuove; e tutti i criteri che sono stati suggeriti per raggiungere l'intento mi sembrano arbitrari più che altro. L'andamento clinico, ad esempio, su cui alcuni si poggiano, non ci dà alcun criterio sicuro: è vero che le infezioni primitive sono di regola più gravi che non le recidive, hanno attacchi più lunghi, ecc., ma ciò avviene negli individui che non hanno mai avuto febbre, mentre in un soggetto, che sia stato già altre volte malarico, un'infezione nuova può non essere distinguibile per alcun carattere da una recidiva. Mentre dunque anche per mia esperienza posso affermare l'esistenza di recidive dopo lunghi periodi di latenza a principio dell'anno epidemico, non posso dire se il fatto si verifichi con quella regolarità che sarebbe necessaria,

perchè ad esso potesse attribuirsi una importanza grande dal punto di vista epidemiologico. Ad ogni modo è questione, sulla quale mi limito ad un breve accenno, non essendo scopo del presente scritto il trattarla a fondo.

Quanto alla genesi delle recidive, è mestieri riconoscere che non ci è ancor noto con precisione in quali forme si trovino i parassiti malarici negli intervalli di apiressia tra un gruppo e l'altro di attacchi febbrili, e a quali proprietà biologiche di essi parassiti sia legato il fatto del recidivar delle febbri.

Merita di esser ricordata innanzi tutto l'ipotesi sostenuta da vari autori, i quali ammettono che i gameti possano moltiplicarsi nell'uomo per partenogenesi. Sebbene tale concetto sia stato accolto in generale con favore (alcuni, ad esempio, credono che l'anno epidemico, in principio di estate cominci con un gruppo di recidive determinate nei vecchi malarici da una *riproduzione partenogenetica estivale*, come dicono, dei gameti), io ritengo che questa ipotesi non solo non abbia base sufficiente nei fatti, ma neppure si presti a dare una spiegazione soddisfacente delle recidive.

L'opinione, che alla partogenesi dei gameti siano legate le recidive, ha come fondamento positivo soltanto l'osservazione di Schaudinn (1), il quale in un caso di terzana, all'inizio di una recidiva, vide e disegnò una serie di modificazioni dei macro-gameti, che credette di poter interpretare quali forme di moltiplicazione asessuale. Egli riproduce in una tavola le varie forme ritrovate, disponendole come in una serie ordinata di sviluppo (..... indem ich alle gefundenen Stadien zu einer Entwicklungsreihe combiniere). Aggiunge poi che anche in altri casi ha veduto alcuni stadii simili; ma sta il fatto che una sola volta ed in una sola malata, all'inizio di una recidiva, ha seguìto tutta la serie delle forme in questione Malgrado ciò egli, dopo averne dato una descrizione accuratissima del suo reperto, esprime la *convinzione* che le recidive, dopo lunghi intervalli, debbano la loro origine ai macro-gameti, i quali vivono lungamente ed hanno la capacità, a suo avviso, di subire una reversione allo stato di schizonti.

Sebbene la pubblicazione di Schaudinn risalga al 1903, il reperto, che egli ha descritto, non ha avuto che scarsissime ed insufficienti conferme con osservazioni nuove. L'opinione invece, che sopra esso reperto si poggia, è stata accolta con favore e sostenuta da alcuni come fosse un dato di fatto oramai indiscutibile.

Cito le osservazioni di alcuni autori olandesi, i soli che, per quanto io sappia, hanno riveduto le forme già descritte da Schaudinn.

Il dott. G. I. Van der Hilst Karrewij ha ripreso lo studio dell'argomento, non avendo trovato altre pubblicazioni dopo quella di Schaudinn. Egli in *un solo* caso di terzana ha trovato una serie di

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(1) J. Schaudinn. *Studien über Krankheitserregende Protozoen.* Vedi il capitolo: *Rückbildung und Schizogonie der Makrogameten bei einem Recidiv.* Arbeiten aus dem Kaiserl. Gesundheitsamte, 1903, p. 230 e seg.

forme che fanno pensare ad un ciclo evolutivo dei macrogameti, e propriamente all'inizio di un attacco, e descrive le forme vedute precisamente come Schaudinn. Si tratta quindi di una semplice conferma dell'osservazione di questo autore, i cui disegni, si noti, sono più nitidi e dimostrativi di quelli che accompagnano la memoria di Karrewij. È poi da osservare che il terzanario studiato da Karrewij aveva già avuto alcuni attacchi di febbre, quando sopravvenne quello all'inizio del quale questo autore trovò le forme descritte come dimostranti la partenogenesi dei gameti. Ora è evidente che, a spiegare la genesi delle recidive, noi ci aspetteremmo di trovare le dette forme all'inizio del primo attacco della recidiva stessa, mentre tutto ci porta a pensare che gli attacchi successivi si producano, come di regola, per la moltiplicazione regolare delle forme appartenenti al ciclo febbrigeno. L'osservazione quindi di Karrewij, a rigor di termini, non ha importanza per la genesi della recidiva; ma porterebbe a supporre che anche nell'ordinaria successione degli attacchi i gameti si moltiplicassero in luogo od insieme alle altre forme.

Non posso poi convenire con l'autore, quando dice che la divisione partenogenetica è stata varie volte osservata nelle semilune, e cita a sostegno le osservazioni di Grassi, Mannaberg e Ziemann: si tratta, a mio avviso, soltanto di reperti incompleti ed isolati, i quali accennano ad una divisione trasversale delle semilune, che non è stata seguìta nelle fasi ulteriori, e mancano di ogni valore dimostrativo in rapporto alla questione che ora ci occupa.

Osservazioni simili per la terzana sono state fatte da G. J. Merz e M. Blüml nelle Indie Olandesi (1). Ciò che vi è di nuovo nei risultati di questi autori, si è che hanno trovato insieme forme che interpretano come di macrogameti in divisione asessuale, e schizonti parimenti in divisione, in sei preparati di sangue provenienti da cinque malati di terzana. È degno di nota che si trattava in questi casi di infezioni molto intense, tanto che gli autori hanno trovato regolarmente infezione doppia di uno stesso globulo rosso, più di una volta quattro parassiti terzanari nella stessa emazia, ed in un globulo rosso fino a 6 schizonti: ricchissimi erano gli schizonti in divisione (da 140 a 300 per un solo preparato!); nè mancavano infezioni doppie di gameti e schizonti.

Per la diagnosi dei macrogameti in divisione e per distinguere questi dagli schizonti parimenti in divisione che si trovavano, come si è detto, numerosi negli stessi preparati, gli autori suddetti si valsero della descrizione data da Schaudinn, ammettendo senz'altro come già accertati ed in modo definitivo acquisiti i caratteri distintivi dei macrogameti in scissione.

Questo reperto contemporaneo di macrogameti e schizonti in scissione ed il fatto che in due dei loro casi non solo non potevano escludere l'ipotesi che si trattasse, anzichè di una recidiva, di una nuova infezione, ma anzi tendevano ad ammetterla, portò naturalmente Merz e Blüml a pensare che la divisione dei macrogameti non avesse soltanto il còmpito, come pensava Schaudinn, d'iniziare le recidive, ma fosse un fatto a cui dovesse darsi un valore ed una estensione più grandi. Ma senza seguire per ora gli autori nel loro tentativo di dare una interpretazione soddisfacente di tali fatti, io mi limito a notare che essi non hanno veduto la serie delle forme

(1) Geneeskundig Tijdschrift voor Nederlandsch-Indië, 1908.

in questione all'inizio di una recidiva, ed ammettono il rapporto tra recidiva e moltiplicazione di gameti soltanto poggiandosi sull'unica osservazione di Schaudinn. La stessa complessità, che costituisce il dato nuovo ed interessante del loro reperto, ne diminuisce, in rapporto alla questione delle recidive, il valore dimostrativo.

Le osservazioni di H. M. Neeb (1) sono ancor meno concludenti di quelle che precedono. Egli riferisce nel suo lavoro di aver veduto nella terzana alcune forme simili a quelle descritte da Schaudinn; ed aggiunge la descrizione ed i disegni di tre forme parassitarie che interpreta come semilune in partenogenesi, trovate in due preparati di un malato di febbre tropicale, del quale non dice da quanto tempo febbricitasse. Ma sta il fatto che i giudizî espressi da varî competenti, i quali esaminarono i preparati di Neeb, furono discordi intorno a due di tali forme: mentre Prowazek le interpretò come forme partenogenetiche di semilune, Le Dantec rilevò la somiglianza di esse forme con l'ordinaria schizogonia dei parassiti estivi, e fu riservato nel giudizio, ed altri, dell'Istituto di malattie tropicali di Amburgo, espressero l'avviso che si trattasse di microgameti con cromatina già divisa nella fase che precede la formazione dei microgametociti.

Quanto alla terza forma in questione, lo stesso Neeb riconosce di non poterne dare un giudizio preciso.

Questi fatti, se io non erro, sono ben lontani dall'esser sufficienti a darci il convincimento che le recidive si debbano alla moltiplicazione schizogonica dei macrogameti. Si tratta di osservazioni isolate, le quali potranno, tutto al più, dimostrare la possibilità di una partenogenesi dei macrogameti, ma non ci permettono di affermare che tale processo si compia regolarmente all'inizio delle recidive. Manca infatti la prova della costanza del reperto e del regolare prodursi del fenomeno in esatta corrispondenza del primo accesso delle recidive a lunghi intervalli. Ora non è fuori luogo ricordare che molti studiosi hanno pazientemente ricercate le fasi di moltiplicazione delle semilune all'inizio delle recidive, senza mai riuscire a metterle in evidenza, e che tale ricerca è stata anche fatta da me e da Bastianelli prima che si conoscesse il significato biologico di tali forme, quando cioè pareva necessario che la fase di moltiplicazione delle semilune dovesse esistere nel sangue dell'uomo. Se si considera tutto ciò, credo si dovrà concludere ammettendo che prima di ritener risoluta la questione della genesi delle recidive nel senso di Schaudinn (2), sono per lo meno necessarie nuove ricerche metodiche che tutt'ora ci mancano, e che sarà bene riprendere, usando i metodi di colorazione

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(1) Geneeskundig Tijdschrift voor Nederlandsch-Indië. 1909.

(2) Ritengo si debba mantenere questa riserva, sebbene Ruge (*Handbuch der pathog. Microrganismen*, herausg. von Kolle und Wassermann. Ergänzungsband, Zweites H., pag. 416) consideri la questione della patogenesi delle recidive già chiarita, dalla osservazione di Schaudinn per la terzana.

più adatti, soprattutto per i gameti delle febbri estivo-autunnali, i quali, come è noto, possiedono forme morfologicamente meglio differenziate e più facilmente distinguibili, che non i gameti delle altre specie di parassiti malarici (1).

Sono notissimi fatti analoghi, ossia di riproduzione partenogenetica, in altri parassiti; ma, se l'analogia può essere guida alla ricerca, non possiamo continuare a valercene come di base ad una ipotesi, quando la ricerca diretta, in un determinato gruppo di parassiti, non fornisce dati sufficienti a convalidarla. Che l'ipotesi non abbia base adeguata nei fatti risulta dalle considerazioni già svolte e, per l'infezione estivo-autunnale in ispecie, anche dalle osservazioni compiute or sono molti anni da me e da Bastianelli sul comportamento delle semilune nell'uomo, osservazioni che le nostre ricerche posteriori hanno confermate. Che poi, anche ammessa per ipotesi la moltiplicazione partenogenetica delle semilune nell'uomo, non se ne avvantaggi la interpretazione delle recidive, risulta dai fatti seguenti.

Innanzi tutto, come già si è notato in altri lavori, vi sono non di rado recidive di febbri estive a principio delle quali non è possibile trovare semilune, anche se si fa la puntura della milza. In alcuni casi ho eseguito varie volte in malarici cronici l'esame del succo splenico estratto per puntura nell'intervallo di apiressia tra una recidiva e l'altra, senze riuscire a trovar semilune. D'altra parte nelle quartane, nelle quali le recidive sogliono ripetersi per mesi e mesi, sono estremamente scarsi i gameti, come risulta dai risultati negativi, che si hanno spessissimo, quando si tenta di infettare gli anofeli con parassiti quartanari. Ora appare poco verosimile, che la provvista dell'infezione venga mantenuta in questo caso appunto dalle forme che si trovano soltanto molto di rado.

Stando ai fatti osservati, si può ammettere che una parte delle recidive siano in rapporto col risvegliarsi dell'attività patogena dei parassiti (schizonti) compienti il loro ciclo di vita regolarmente per un periodo di tempo più o meno lungo senza dar febbre. Ciò si può osservare non difficilmente nella quartana: vi sono infatti quartanari, nei quali si può seguire il ciclo di vita dei parassiti, prendendo il sangue dal dito, fino alla scissione, anche per varie generazioni, senza che si producano i corrispondenti attacchi febbrili. Ho ricordato già altrove questo fatto parlando della immunità; ed ho proposto di interpretarlo, ammettendo che il paziente in questi casi abbia acquistata una immunità transitoria di fronte all'azione febbrigena degli

(1) Tali ricerche sono state intraprese quest'anno nel mio laboratorio.

emosporidî. Quando questa immunità si sospende, per lo più in seguito ad una causa occasionale, come il raffreddamento, gli strapazzi, ecc., abbiamo la recidiva.

Fatti simili a quello citato (ossia presenza di parassiti nel sangue senza fenomeni morbosi salienti) sono stati osservati recentemente da vari autori, specie nelle razze più resistenti alla malaria. (A. Plehn).

In questi casi non si può dubitare che la recidiva si debba agli stessi parassiti del ciclo pirogeno, che hanno seguitato a vivere nel sangue senza sviluppare attività patogena manifesta, ossia senza produrre accessi febbrili, per le particolari condizioni del soggetto.

Un altro gruppo di recidive si può pensare che sia dovuto allo sviluppo progressivo di quegli schizonti, che in seguito a chinizzazione non abbastanza prolungata ed intensa sfuggono all'azione del rimedio. Esempi analoghi ci sono forniti da altre infezioni, come la sifilide, in cui una parte del virus sfugge alla cura specifica, ed è causa delle ulteriori manifestazioni della malattia, nelle tripanosomiasi, ecc. Così si deve verosimilmente intendere la genesi di quelle recidive ad intervalli piuttosto brevi e più o meno regolari, che si presentano con molta frequenza in quegli ammalati che non si curano in modo adeguato. Come si è detto altrove, il tempo che intercede tra un gruppo e l'altro di attacchi febbrili ha una durata che si avvicina a quella delle incubazioni delle infezioni primitive; ed in questo intervallo dobbiamo pensare che avvenga una moltiplicazione progressiva dei pochi parassiti residuali, fino a che abbiano raggiunto la quantità necessaria per dare la febbre. Ma anche alcune recidive, che avvengono dopo un periodo più o meno lungo di latenza, si può pensare che abbiano la stessa genesi. Nulla infatti ci costringe ad ammettere che i parassiti residuali sopraviventi all'azione della chinina, percorrendo regolarmente e tipicamente il loro ciclo di vita, debbano aumentare di numero ad ogni moltiplicazione in modo progressivo. Come tutti sanno, si vedono quartane e terzane, le quali non curate seguitano con monotonia per settimane e mesi senza aggravarsi, anzi non di rado attenuandosi, mentre la quantità dei parassiti non solo non aumenta, malgrado la moltiplicazione che avviene con i soliti regolari intervalli, ma può anche diminuire; la qual cosa si spiega ricordando il fatto che non tutte le forme giovani risultanti dalla moltiplicazione seguitano regolarmente a vivere e a svilupparsi. Nello stesso modo, nulla vieta di pensare che una quantità minima di parassiti sopravvissuti all'azione del rimedio specifico seguiti a percorrere il proprio tipico ciclo di vita, senza aumentare di numero, senza raggiungere anche per molto tempo i limiti di quantità necessari per dare la

febbre e per potere essere praticamente ritrovabili col comune esame diagnostico del sangue. La moltiplicazione con aumento progressivo e rapido della carica parassitaria potrà avvenire, quando il paziente soggiace ad una delle tante cause debilitanti, che sono occasione ovvia del ritorno degli attacchi febbrili. Ammesso questo modo di vedere, la genesi del maggior numero delle recidive sarebbe sostanzialmente identica a quella del gruppo di recidive, di cui si è parlato avanti. In altre parole, sia che negli intervalli di latenza si trovino nel sangue circolante le comuni forme del ciclo pirogeno, sia che non si trovino, la recidiva sarebbe sempre dovuta alla presenza di queste, le quali, nel maggior numero dei casi, si troverebbero in quantità così scarsa da essere inaccessibili all'esame.

Ma, anche tenendo nel debito conto le precedenti considerazioni, vi è un certo numero di fatti, dei quali non si riesce a dare una interpretazione soddisfacente; così non si riesce agevolmente a comprendere perchè, se si seguita, negli intervalli di apiressia, ad amministrare chinina tutti i giorni, ed anche in forte dose, in un certo numero di casi si ha la recidiva. Ciò non si comprende facilmente, ripeto, se si ammette che nel sangue vi siano le stesse forme che si svolgono durante la febbre in minima quantità e in esse non si sia verificata alcuna modificazione biologica; a meno che non si voglia supporre che durante i periodi di latenza i parassiti siano più resistenti al rimedio, o ancora che, durante la febbre, avvengano delle modificazioni per cui i parassiti siano più sensibili all'azione della chinina; la qual cosa certo non si può negare *a priori*.

Varie volte ho somministrato il solfato di chinina nella dose di un gramma al giorno ed anche in dosi più forti, per circa due settimane e più, in alcuni malarici cronici, eppure ho visto manifestarsi la recidiva con parassiti estivo-autunnali, pochi giorni dopo di avere sospesa la somministrazione del rimedio. D'altra parte ho veduto che nei casi di malaria sperimentale, curati subito energicamente dopo il primo accesso di febbre, le recidive si riducono al minimo, e qualche volta si sopprimono affatto. Risulta inoltre dall'esperienza comune che il medico tanto più facilmente può dominare il decorso di una infezione malarica, quanto più presto inizia la cura; mentre i malati, che già hanno febbricitato a lungo, sono più ribelli alla cura.

Neppure si riesce a comprendere in modo soddisfacente, dato che le recidive avvengano sempre col meccanismo di cui sopra si è parlato, il succedersi di recidive date da diverse specie parassitarie, nelle infezioni miste. Così ad es., vi son casi non rari, nei quali ad una serie di recidive estivo-autunnali succedutesi durante l'autunno

e l'inverno e curate opportunamente, segue nella primavera una recidiva di terzana comune, la quale insorge in tali condizioni di ambiente, che è possibile escludere la eventualità di una infezione terzanaria nuova. Perchè i parassiti della terzana hanno resistito alla azione del rimedio, che pure ha vinto i parassiti estivo-autunnali, se i parassiti terzanari si trovavano nel sangue, in minima quantità, nelle note forme del ciclo pirogeno?

Queste considerazioni ed altre analoghe mi hanno portato, molto tempo fa, a formulare un'ipotesi che direi a base morfologica, secondo la quale alcune spore (sporozoiti monogonici) si depositerebbero nei visceri interni, dove, rivestendosi di membrana, potrebbero mutarsi in forme resistenti e manterrebbero, per così dire, la provvista dell'infezione.

È noto che non tutte le forme, le quali risultano dalla moltiplicazione a principio di un accesso, seguitano a svilupparsi: infatti (e ciò è manifesto specialmente nella quartana) se tutte le forme risultanti dalla moltiplicazione si sviluppassero regolarmente, la quantità dei parassiti ad ogni accesso andrebbe regolarmente crescendo; la qual cosa non è. Alcune di tali spore ed anche intere forme di scissione si depositano nei visceri interni, specialmente nella milza e nel midollo osseo, dove (e in ciò sta l'ipotesi) potrebbero convertirsi in forme resistenti.

Questa ipotesi si adattava, a mio avviso, a spiegare anche i fatti di cui sopra si è parlato, e segnatamente il fatto che le recidive sono tanto meno probabili e, se avvengono, tanto meno numerose ed ostinate, quanto meno numerosi sono stati gli accessi febbrili sofferti, prima dell'inizio di una cura chininica rigorosa e metodica.

Ma poichè le ricerche più accurate eseguite tutte le volte che mi si presentava l'occasione, specialmente sulla milza e il midollo osseo di malarici cronici, non mi hanno mai dato un risultato positivo, non mi hanno mai permesso cioè di riconoscere le supposte forme resistenti, morfologicamente differenziate, nè altri ha avuto miglior successo, ritengo che la detta ipotesi debba essere oramai almeno provvisoriamente abbandonata (1); tanto più che ad essa se ne può sostituire un'altra, che ha a fondamento il sostegno della ana-

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(1) Sarebbe utile studiare di nuovo la questione, applicando all'esame degli organi ematopoietici dei malarici recidivanti, che muoiono di malattie intercorrenti come la polmonite, tutti i nuovi metodi introdotti negli ultimi anni nella tecnica istologica e nello studio dei protozoi. È appunto in vista della necessità di tale studio che io, a proposito della mia vecchia ipotesi, non mi esprimo in modo reciso.

logia: alludo alle osservazioni eseguite negli ultimi anni dalla scuola di Ehrlich intorno al comportamento dei tripanosomi di fronte a vari rimedi.

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Come è noto, nell'Istituto di Francoforte (1) è stato dimostrato che gli insuccessi della cura protratta per lungo tempo delle tripanosomiasi coi vari agenti chimici dipendono da ciò, che si formano a poco a poco *varietà* di tripanosomi resistenti contro le dette sostanze. Così Ehrlich con Browning e Röhl hanno ottenuto uno stipite di tripanosomi resistenti alla fucsina e ad una serie di altre sostanze tossiche (Triphenylmethanfarbstoffe), uno stipite resistente contro il Trypanroth e le sostanze affini — Trypanblau e Trypanviolett — ed infine una razza resistente contro l'Arsanil.

La resistenza all'Atoxyl nei topi si osserva di regola dopo lungo tempo; ma Ehrlich ha avuto casi, nei quali era anche riconoscibile dopo 14 giorni. La quale osservazione è importante, perchè ci dà la possibilità di riferire a questo stato refrattario i risultati negativi che si osservano nella cura della malattia del sonno, malgrado la somministrazione del rimedio continuata a lungo.

Questa proprietà una volta acquistata può rimanere invariata molto a lungo. Così lo stipite resistente all'Arsanil (*Arsanilfest*) è stato passato 300 volte per l'animale normale, ciò che richiede circa 700 giorni, e malgrado questo lungo tempo ha conservato intera la sua resistenza; cioè l'ha ereditata.

Ehrlich ha trasportato lo stipite di tripanosomi *Atoxylfest* per più di 125 passaggi senza osservare alcuna diminuzione della sua resistenza.

È importante anche il fatto che la resistenza di queste razze o stipiti è specifica. Così gli stipiti resistenti alla fucsina non resistono all'Atoxyl e al Trypanrot e viceversa. La resistenza però si estende ai corpi chimicamente differenti dello stesso gruppo.

Nè mancano fatti analoghi ai precedenti.

Così Franke nel laboratorio di Ehrlich, ha trovato che anche contro le sostanze protettive, che si formano nel corso di una infezione, esistono razze o stipiti (*Stämme*) di parassiti resistenti.

Levaditi ha osservato lo stesso fatto rispetto agli spirilli della febbre ricorrente.

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(1) Ehrlich. *Chemotherapeutische Trypanosomen-Studien*. Leipzig, 1909.

Allo stesso gruppo di fatti appartiene l'osservazione di Effront, il quale già da molti anni è riuscito ad ottenere un ifomiceto resistente all'acido fluoridrico, e dotato biologicamente di proprietà diverse da quelle degli ifomiceti normali; ed ha anche constatato che questo ifomicete conservava per molte generazioni questa specifica resistenza al veleno, acquisita per adattamento.

Si tratta dunque, secondo Ehrlich, di una legge generale, che è di altissima importanza per il fatto che la modificazione acquisita appartiene alle proprietà ereditarie.

Mentre gli autori suddetti nelle loro ricerche sperimentali, procedendo con metodo opportuno, hanno ottenuto razze o stipiti di massima resistenza, non c'è da aspettarsi, secondo Ehrlich, che si raggiunga lo stesso grado di resistenza nel corso del trattamento terapeutico dell'uomo e degli animali; ma in questo caso si avranno di solito degli stipiti di mezzana resistenza (*halbfeste*): sotto l'azione degli atoxili in dose opportuna, i tripanosomi in questi casi spariscono, ma dopo un certo tempo ricompaiono, onde la difficoltà della cura.

Le esperienze che possediamo riguardo all'uomo sono scarsissime. Ehrlich conosce un solo caso comunicatogli dal prof. Plimmer, riguardante uno stipite di tripanosomi proveniente da un caso di malattia del sonno curata per lungo tempo con Arsanil, il quale stipite sperimentato sugli animali si dimostrò fortemente refrattario a detta sostanza (*arsanilfest*).

Aggiungerò che stando alle ricerche di Morgenroth e Halberstaedter (1), anche tra chinina e tripanosomi avverrebbe un fatto simile. Questi autori, i quali hanno ricercato se la chinina eserciti un'azione profilattica nelle infezioni sperimentali da tripanosomi e ritengono di averla dimostrata (la chinina agirebbe sui tripanosomi inibendo la loro attività riproduttiva), ammettono la possibilità che, quando vi sono tripanosomi latenti, si stabilisca una specie di assuefazione di essi alla chinina (una certa *chininfestigkeit*).

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Ho citato lungamente i risultati di questi studi per conchiudere che mi pare giustificato, allo stato attuale delle nostre conoscenze, un'ipotesi simile per spiegare la resistenza ostinata alla chinina che presentano i malarici recidivi, specie nelle forme cliniche della infezione malarica cronica.

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(1) *Zur Kenntniss der Chininwirkung*. Berl. klin. Woch., 1910, n. 14.

Tale resistenza è comunemente conosciuta dai medici che esercitano nei luoghi malarici. Ed è la ragione per cui gli stessi ammalati non di rado rifiutano di seguitare a prendere la chinina, dalla quale non risentono più, in modo manifesto, gli effetti benefici; e preferiscono ricorrere alle specialità, che inondano il mercato e trovano facilmente credito, specialmente quando il nome non indica chiaramente il loro contenuto in chinina; onde gli ingenui non pensano di continuare, sotto altra etichetta, a prendere il prezioso alcaloide.

Ho già ricordato i risultati della mia esperienza. Non soltanto anche l'amministrazione della chinina *per os* tutti i giorni a dosi forti non impedisce, in alcuni casi, la recidiva, ma lo stesso insuccesso si può avere dopo una cura intensa di iniezioni ipodermiche. Per non limitarmi a citare osservazioni mie, ricorderò, ad esempio, un caso descritto da Torti e Angelini (1), i quali ad un loro ammalato iniettarono un gramma di chinino al giorno ipodermicamente e un gramma di liquore di Fowler per 15 giorni, poi somministrarono il rimedio *per os* durante un mese circa, e, malgrado ciò, osservarono la recidiva. Fecero di nuovo iniezioni ipodermiche ancora per 15 giorni (gr. 0.50 *pro die*), ed il giorno dopo la sospensione del rimedio, osservarono la febbre con reperto ematologico positivo.

Ma è inutile citare ancora fatti singoli, tanto è nota la resistenza che offrono non di rado i malarici cronici, abbiano essi o non abbiano recidive febbrili, alla cura chininica intensa e continuata.

Ho notata già la facilità onde si riesce a sopprimere le recidive in casi di infezioni sperimentali con gli anofeli, nei quali, appena manifestata la febbre e veduti i parassiti nel sangue, si interviene prontamente con la chinina e si seguita a somministrarla a lungo. Pei fatti che sono a mia conoscenza, sebbene non numerosi, sarei portato a ritenere che non cedano altrettanto facilmente le febbri ottenute per iniezioni di sangue malarico. La differenza del comportamento può forse dipendere dal fatto che nel secondo caso si iniettano parassiti già abituati nel primo ospite umano all'azione dell'alcaloide, al quale sono divenuti più resistenti anche nella fase di schizonti.

Tutti questi dati di osservazione sono suscettibili di una interpretazione unica che io propongo come l'ipotesi più conforme alle conoscenze attuali: ammetto cioè che i parassiti malarici sottoposti all'azione della chinina si selezionino, in alcuni casi, formando degli sti-

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(1) *Infezione malarica cronica con i sintomi della sclerosi a placche.* Riforma medica, 1891.

piti resistenti, onde la difficoltà della cura radicale in talune febbri ostinatamente recidivanti. Non è possibile certo dire, con qualche approssimazione, con quale frequenza ciò si verifichi. Ma i casi resistenti, anche se rari, sono evidentemente quelli di cui si deve tenere il maggior conto nei riguardi della profilassi, in quanto ad essi in principal modo si deve la persistenza dell'infezione e la continua diffusione di questa nell'ambiente. Sono noti anche fatti i quali possono far pensare che vi siano alcuni stipiti di parassiti originariamente dotati di grande resistenza all'alcaloide. Varie volte ad esempio è occorso a Marchiafava ed a me, di vedere all'autopsia casi di perniciosa ricchissimi di parassiti accumulati nei vasi dei centri nervosi, della milza, del fegato, del midollo delle ossa, ecc., malgrado la energica cura chininica continuata assiduamente anche per oltre due giorni. Trattandosi di infezione recente, non sembra ammissibile in questi casi che lo stipite resistente si sia per così dire, selezionato in conseguenza dell'uso dell'alcaloide. Con queste fanno contrasto quelle perniciose nelle quali la chinina usata largamente produce la completa scomparsa dei parassiti; malgrado ciò può avvenire la morte, verosimilmente in conseguenza delle alterazioni dei visceri, secondarie all' invasione parassitaria, e all'autopsia non si trovano emosporidî, o se ne rinvengono scarse forme all'esame più accurato (1).

La conclusione che scende logicamente da tutto ciò non è già la sfiducia nell'efficacia della cura chininica (tutti sanno che persistendo nella cura opportuna si riesce a vincere l'infezione), ma è il consiglio di curare i malarici quanto più presto si può, intensificando la nostra azione all'inizio della malattia; poichè è tanto più probabile la guarigione radicale e rapida, quanto più precoce è la somministrazione del rimedio (2).

È quasi inutile aggiungere che della formazione di stipiti resistenti o semi resistenti (*halbfeste*) alla chinina non ci è possibile dare la prova sperimentale, come si è fatto per le tripanosomiasi. Non essendo la malaria umana trasmissibile agli animali, si dovrebbe inoculare all'uomo sano il sangue di malarici recidivanti e resistenti, per vedere se le stesse proprietà biologiche dei parassiti fossero ricono-

(1) Vedi: MARCHIAFAVA e BIGNAMI, *L'infezione malarica*, 1902.

(2) Naturalmente alla stessa conclusione pratica si dovrebbe venire, se si ammettesse l'origine delle recidive dalla partenogenesi dei macrogameti. A tutti è nota infatti la singolare resistenza di queste forme di fronte ai sali di chinino.

scibili nell'inoculato. Ma se ciò, per ragioni ovvie, non è eseguibile, non ne è scossa però la solidità dell'ipotesi, tante sono le osservazioni che la sostengono.

Per conchiudere, mi sembra di potere affermare in ordine alla genesi delle recidive:

1. Che i dati di fatto sino ad ora a nostra conoscenza non permettono di affermare con sicurezza il rapporto delle recidive con la persistenza e la moltiplicazione partenogenetica dei gameti.

2. Che verosimilmente si debbono considerare le recidive, siano a brevi, siano a lunghi intervalli, siano separate da grandi periodi di latenza, da un punto di vista unitario, cioè come aventi fondamentalmente la identica genesi in rapporto con la persistenza di parassiti del ciclo pirogeno. In un gruppo di recidive il materiale parassitario che mantiene la infezione è rappresentato da una minima quantità di forme del comune ciclo febbrigeno, qualche volta riconoscibili all'esame microscopico accurato, almeno in alcuni momenti del decorso, le quali anche per molto tempo possono non raggiungere la quantità o la virulenza necessaria per dare la febbre. Specialmente nei casi nei quali i gruppi di recidive si seguono a distanze più o meno lunghe di tempo, senza che vi sia stata l'influenza della terapia specifica, si deve anche tener presente la possibilità che dopo una serie di attacchi febbrili l'organismo acquisti una certa immunità rispetto all'azione pirogena dei parassiti; la quale immunità essendo, come accade in altre infezioni, transitoria, si attenui o cessi dopo un certo periodo di apiressia, onde l'insorgere della recidiva.

Ma c'è anche un gruppo di recidive nelle quali il materiale, che mantiene l'infezione durante i periodi di latenza, è rappresentato da forme resistenti alla chinina selezionatesi sotto l'azione dell'alcaloide in opportune condizioni; non è necessario supporre che tali forme si differenzino dalle altre anche dal punto di vista morfologico come, molto tempo fa, io stesso avevo pensato.

Tale modificazione nelle proprietà biologiche dei parassiti viene ammessa da me per la difficoltà che si incontra a voler spiegare tutti i fatti riguardanti le recidive, e specialmente l'inefficacia della cura specifica durante i lunghi periodi di latenza accompagnati da perfetto benessere, e il rapporto che vi è tra la frequenza e l'ostinazione del recidivare, da una parte, e il numero delle febbri sofferte senza cure o con un incompleto successo terapeutico dall'altra.

Se si ammette ciò che son venuto esponendo, pur concedendo che sia rara la formazione di stipiti veramente resistenti alla chinina, è

ragionevole pensare come cosa verosimile che nella malaria inveterata si incontrino con una certa frequenza degli stipiti di parassiti dotati di una media resistenza all'alcaloide, analogamente a quanto pensa Ehrlich pei tripanosomi della malattia del sonno rispetto ai nuovi preparati arsenicali; la cura non abbastanza energica e protratta potrà in questi casi fare praticamente sparire i parassiti dal sangue, ma non evitarne la ricomparsa dopo un tempo più o meno lungo.