Permethrin resistance in the head louse *Pediculus capitis* from Israel

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**Abstract.** Head lice, *Pediculus capitis*, were collected from children aged 3–12 years in Maale Adumin, a town near Jerusalem, after reports of control failure with the pyrethroid insecticide permethrin. A total of 1516 children were examined: living lice and eggs were found on 12.1% of the children; or another 22.8% of the children only nits were found. Twice as many girls as boys (8.1% v 4%) were infested with lice and or nits. Head lice collected from infested children were exposed to permethrin impregnated filter-papers. Log time probit mortality (ltp) regression lines were calculated for mortality data and compared to ltp lines for a similar collection of head lice made in 1989. The regression lines for the two years were significantly different, with a 4-fold decrease in susceptibility at the LT$_{50}$ level between 1989 and 1994. The slopes of the lines also suggested that the 1994 population was more heterogenous in its response to permethrin than the 1989 population. In contrast, a laboratory population of body lice (*Pediculus humanus*) tested with the same batch of permethrin-impregnated papers showed a slight but non-significant increase in susceptibility between 1989 and 1994. The results suggest that resistance to pyrethroids has developed rapidly among head lice since permethrin was introduced in 1991 as a pediculicide in Israel.

**Key words.** Head louse, *Pediculus capitis*, body louse, *Pediculus humanus*, control failure, insecticide resistance, permethrin, Israel.

**Introduction**

Resistance of the human head louse, *Pediculus capitis* De Geer (Phthiraptera: Pediculidae), to organochlorine insecticides such as DDT and lindane has been recorded in many parts of the world including Israel, Canada, Denmark and Malaysia, but there are no reports of resistance to other insecticide classes (Brown & Pal, 1971; Maunder 1971; W.H.O., 1992). The body louse, *Pediculus humanus* L., has developed widespread resistance to organochlorines (Brown & Pal, 1971; Gratz, 1977), malathion resistance in parts of Africa (W.H.O., 1992), pyrethrin tolerance (Nicoli & Sautet, 1955; Sautet & Aldighieri, 1959) and ‘low-level’ resistance to synthetic pyrethroids was reported in France (Fine, 1963).

The second-generation photostable pyrethroid permethrin was first used against the body louse in 1976 (Nassif & Osman, 1977). Large-scale clinical trials of permethrin for the control of head lice were conducted in the early 1980s in the U.S.A. (Taplin et al., 1986). Since then, several clinical trials have shown the effectiveness of this insecticide under field conditions (Brandenburg et al., 1986; Bowerman et al., 1987). In 1991, permethrin was introduced to the market in Israel as a 1% formulation (trademarks ‘Zehu Ze’ and ‘Nok’) for control of head lice. The baseline susceptibility in the Israeli populations of head and body lice was determined in 1989, prior to the introduction of permethrin for louse control (Mumcuoglu et al., 1990). The efficacy of permethrin was reflected by the significant reduction of head lice incidence in Israel after 1991, when permethrin
accounted for 90% of the market for pediculicides. In 1992 there was a 60% reduction in pediculicides sold in Israel compared to 1991, suggesting that a high level of control had already been achieved. The first reports of control failure with permethrin in Israel occurred in early 1993. To determine whether control failure was the result of resistance developing in the Israeli head louse population this study was undertaken. To ensure comparability of data obtained in different years, the permethrin sensitivity of a standard laboratory colony of body lice was also compared between 1989 and 1994.

Materials and Methods

Children from thirty-three kindergartens and two junior schools in the town of Maale Adumin, just outside Jerusalem, were surveyed for head lice infestation in mid-1994. A total of 1516 children (822 boys and 694 girls) between the ages of 3 and 12 were examined. Head lice were collected by means of a lice comb from the scalp of infested children and immediately transferred to a plastic pill box with netting (0.4 mm mesh) on two sides which contained a few strands of human hair (Buxton, 1950). The box was then placed on the abdomen of one of the authors (K.Y.M.), and maintained there, so that the lice were able to obtain a blood meal whenever required under optimum temperature and humidity conditions.

Colonies of human body lice were maintained at 30°C and 70 ± 5% relative humidity. Every second day, lice were placed on the shaved abdomen of a restrained rabbit and allowed to feed to satiety (Ben-Yakir et al., 1994). Lice fed 24h previously were used for susceptibility testing.

A 0.25% solution of permethrin (v/v) was prepared in silicone fluid (Dow Corning 556); 700 ml of the solution mixed with an equal volume of acetone was spread evenly on a rectangle (12 × 15 cm) of Whatman No. 1 filter paper. The impregnated filter papers were left for 3 h at room temperature to allow the acetone to evaporate, wrapped in aluminium foil and stored at 4°C until used. This method gives 0.25% permethrin impregnated papers as used for discrimination of permethrin susceptibility/resistance in mosquitoes (W.H.O., 1992).

The insecticide susceptibility of batches of twenty lice (adults and second and third stage nymphs) was determined by exposing them to permethrin-impregnated filter papers in a sealed 15 cm diameter petri dish at room temperature. As untreated controls, lice were exposed to papers impregnated with silicone fluid alone. The numbers of dead lice were recorded at regular time intervals (three or more replicates per stage tested) until complete mortality occurred. Log time probit (ltp) mortality regression analysis of the data (Finney, 1971) used a computer program written by Dr C. J. Schofield.

Results

Living head lice and their eggs were found on 183 (12.1%) of the 1516 children examined. On another 346 children (22.8%) only nits (i.e. hatched egg cases attached to hairs) were found, indicating that these children had been infested within the last 6 months and that they had been treated successfully. Twice as many girls as boys had lice or nits.

Fig. 1 compares the ltp regression lines calculated from mortality data for lice exposed to 0.25% permethrin, showing a significant decrease in susceptibility to permethrin of the 1994 sample compared to that collected in 1989, with no overlap in the 95% confidence intervals between the lines. At the LT95 level, a resistance ratio of 4.1-fold was observed between the lice tested in 1994 and 1989. The slope of the lines had also decreased (Table 1), suggesting that the 1994 samples were more heterogenous in their response to permethrin.

There was no mortality among controls for up to 60 min after the start of their exposure, but significant control mortality occurred by 17 h when the test of the 1994 material was terminated. Hence the calculated LT95 and the degree of heterogeneity in the 1994 samples are probably underestimated.

We attempted to reduce the time needed to obtain complete mortality of the head lice collected in 1994 by increasing the permethrin concentration to 1% on the impregnated papers. Table 1 shows that this increase in concentration did not significantly decrease the time needed to achieve >90% mortality.

Susceptibility testing in 1989 was undertaken with 0.25% permethrin-impregnated papers supplied by the World Health Organization. Papers were not available from this source in 1994, so we produced our own, as described above (cf. Hemingway, 1995). For comparability of results obtained with the papers used in different years a colony of body lice, tested with the W.H.O. papers in 1989 (Mumcuoglu et al., 1990) and subsequently maintained without insecticidal pressure, was also tested in 1994 on the new permethrin-impregnated papers. Fig. 2 shows that the mortalities were slightly, though not significantly, higher in 1994 than in 1989. Hence the reduction in susceptibility observed in the wild head louse population cannot be accounted for by differences in efficacy of the impregnated papers. When the body

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<th>Table 1. Log time probit mortality of head lice populations, collected in Israel in 1989 and 1994, compared to a susceptible colony of body lice, exposed to 0.25% or 1% permethrin-impregnated filter papers.</th>
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louse colony was exposed to 1% permethrin papers, the mortality rate rose more quickly than with 0.25% permethrin, although the two probit lines were not significantly different. At each concentration in 1994 there was no overlap between the ltp lines for the head and body lice exposed to permethrin (Fig. 3), unlike the situation in 1989 when the 95% confidence intervals of the lines overlapped for the two species exposed to 0.25% permethrin (Mumcuoglu et al., 1990).

Discussion

After our earlier investigation (Mumcuoglu et al., 1990), the LT$_{50}$ for Israeli head lice exposed to 0.25% permethrin rose about 4-fold from 1989 to 1994. During this period the LT$_{50}$ for a laboratory colony of body lice did not alter significantly. Hence the observed shift in the LT$_{50}$ for the head lice cannot be accounted for by differences in the insecticide-impregnated test papers between years.

Resistance in the head louse population has occurred within 2.5 years of the introduction of permethrin to the Israeli pediculicide market. This time period is equivalent to approximately forty generations of lice. Sporadic reports of control failure with permethrin were recorded in early 1993, although these reports were not confirmed by bioassay. We have now confirmed that permethrin resistance is present in human head lice from Israel, but the underlying mechanisms of this resistance have not yet been determined in this species.

The rapid development of permethrin resistance in Israeli head lice could have been potentiated by cross-resistance from DDT due to the knock-down resistance (kdr) mechanism. DDT resistance has been reported from R. capitis in Israel (W.H.O., 1992). Cross-resistance between DDT and the pyrethroids is well documented in other insects (Shono, 1985; Osborne & Pepper, 1992). The kdr gene, which reduces nerve sensitivity to pyrethroid poisoning, affects Na+/K+ channels which are the common target sites for organochlorines, such as DDT, and the pyrethroids (Williamson et al., 1993). Kdr-like resistance has since been documented in a range of insects, such as the cockroach, Blattella
germanica (L.) the housefly Musca domestica L., the mosquitoes Aedes aegypti (L.) and Culex quinquefasciatus Say, and various agricultural crop pests (Amin & Hemingway, 1989; Scott et al., 1986; Hemingway et al., 1993; Ahmad et al., 1989; Taylor et al., 1993; Mahmood et al., 1993). Kdr genes in houseflies have invariably been shown to be functionally recessive (Sawicki, 1975; Farnham, 1977). Therefore kdr-type resistance is difficult to detect at low frequencies by bioassay, as resistant heterozygotes are indistinguishable from susceptibles. Studies of the mechanism(s) of resistance to DDT and pyrethroids in Israeli Psocoptes have not yet been undertaken to check for the presence of nerve insensitivity. Various other mechanisms of resistance to each insecticide could occur without cross-resistance.

In Israel, head lice have previously been treated with DDT. In addition, the pyrethroid bioallethrin has been used in combination with the organophosphorus insecticide malathion for the last 15 years. It is possible that a kdr-type mechanism has been selected by the initial use of DDT, and that the mechanism has then been maintained at a relatively low frequency by low-level selection pressure from the bioallethrin treatment until it has been more strongly selected for by the use of permethrin.

Resistance may also have been rapidly selected because permethrin was used almost exclusively for louse control in Israel over a 3-year period. The two formulations of permethrin have accounted for approximately 90% of the pediculicides market in Israel since 1991. Neither formulation of permethrin contained any synergist. Addition of the synergist piperonyl butoxide has been recommended for suppression of monooxygenase (Cytochrome P<sub>450</sub>) resistance mechanisms in insects, to slow down the development of permethrin resistance (Forrester, 1988). However, the addition of piperonyl butoxide would have no significant effect on a kdr-type mechanism.

The rapid development of permethrin resistance, compared to the apparent lack of bioresmethrin resistance after use of the latter for a period of 15 years, may be linked to the contrasting residual effects of the two pyrethroid insecticides. In houseflies, non-residual pyrethroids such as bioresmethrin have not selected rapidly for resistance, yet the flies quickly developed resistance to permethrin (Denholm et al., 1983). With the head lice treatment, the levels of permethrin remaining 2 weeks after application are still sufficient to kill lice (Taplin & Meinking, 1987). The slow decay of permethrin on the hair after treatment may also result in a significant period when lice are exposed to sublethal doses of insecticide perhaps affecting their reproduction,
exerting further selection pressure for insecticide resistance. In areas where permethrin resistance has not yet developed in head lice, it may be advisable to avoid intensive coverage with this insecticide. For example, rotational or mosaic strategies for the use of pediculicides could be adopted, which should slow down the rate of development of resistance (Tabashnik, 1990). If such a strategy is to be adopted, care should be taken to use insecticides with different modes of action, e.g. pyrethroid, organophosphate and/or carbamate. Therefore mosaics or rotations of different pyrethrroids should be avoided. Further studies on the mechanism(s) of permethrin resistance found in the Israeli head lice are needed to determine whether the use of a synergized formulation of permethrin may be practical as a means of overcoming the current resistance.

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References


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Editorial commentary on pyrethroid resistance in and specific status of Pediculus capitis

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While the preceding paper by Mucmugolu et al. (1995b) was in press, the authors notified us (in lit., 8 June 1995) that permethrin-resistant Pediculus capitis had been reported also from the Czech Republic by Rupes et al. (1994), with pyrethroid cross-resistance extending to bioallethin and phenothrin, as in Israel (Mucmugolu et al., 1995a). Phenothrin treatment failure has also been reported in P.capitis from Paris (Chosidow et al., 1994), but the reason remains controversial (Burgess, 1995; Chosidow, 1995; Vander Stichele & Bogaert, 1995) and it may not be due to pyrethroid resistance.

Like many other authors (e.g. Durden & Musser, 1994), Mucmugolu and co-workers previously classified the head louse (capitis DeGeer) as a subspecies of the body louse, Pediculus humanus Linnaeus (= P.h.corporis DeGeer), implying that these two taxa belong to the same panmictic species. The obsolete terminology P.humanus var capitis also recurs in medical literature (e.g. Taplin et al., 1982; Chosidow et al., 1994) although variety has no formal status (cf. Rule 45g) under the International Code of Zoological Nomenclature (I.C.Z.N., 1982). Busvine (1978) found that P.capitis and P.humanus co-exist without interbreeding, so they have the status of separate species (Schafer, 1978; Pittaway, 1991). This distinction is relevant to studies of insecticide resistance because, if P.capitis and P.humanus were to interbreed, resistance genes could be transferred by introgression. However, their generally contrasted resistance spectra are consistent with ranking head and body lice as different non-interbreeding species. Eco-epidemiologically, infestations of head or body lice do not arise from each other (Buxton, 1939), proving that they are an allopatic pair of apomictic species.

References

See p. 447.
References (from Editorial commentary, p. 432)


