Discussion
6. DISCUSSION

Determination of susceptibility of vectors to the human plasmodia is important to define their vector competence and the role in malaria transmission, particularly in situations where many suspected vectors are prevalent. In the erstwhile Koraput district in Jeypore Hills in the eastern Ghats, which is known to be hyper-endemic for malaria (Rajagopalan et al., 1990), multiple vectors are considered to play role in malaria transmission. Of 25 anopheline species prevalent in the area, susceptibility of nine anopheline species to \textit{P.falciparum}, \textit{P.vivax} and \textit{P.malariae} infections have been determined. Since, the studies were carried out in the field, the results were limited by the availability of number of eligible volunteers and the mosquito species in adequate numbers. \textit{An. annularis}, in which natural infections were found in Jeypore zone could not be included in the study because of its less prevalence in Malkangiri zone. All the nine species tested were found to be susceptible to the infection with \textit{P.falciparum}. The degree of infection varied among the species with the three parasite species. Due to the less abundance of \textit{P. vivax} and \textit{P. malariae} in the study area, the feeding experiments on these two species were carried out only with \textit{An. fluviatilis}, \textit{An. culicifacies}, \textit{An. jeyporiensis} and \textit{An. subpictus}. Among the nine, natural infection was recorded, earlier, only in \textit{An. fluviatilis} and \textit{An. culicifacies} (Gunasekaran et al., 1989).

Susceptibility of the anopheline species to plasmodia

\textit{An. fluviatilis}

\textit{An. fluviatilis} was the most susceptible to all the three parasite species than the other anophelines studied. The Human Blood Index of \textit{An. fluviatilis} has also been reported to be high (Gunasekaran et al., 1994). Preference to man feeding and high susceptibility to malaria
Parasites corroborates with the conclusion drawn by Perry (1914), Senior White et al. (1938), Weeks (1951) and more recently by Gunasekaran (1991) that An. fluviatilis is the major vector in the area.

Feeding experiments with P. falciparum infected donors showed that the percentage of infection and the mean oocyst count varied between individual mosquitoes in a single experiment, as well as between experiments. Ward (1963) had suggested that the extraordinary variability in oocyst number between individual mosquito fed simultaneously on the same donor had been due to inherent differences in susceptibility. Another explanation for such variability is that the development of peritrophic membrane in mosquito which begins after the blood meal may interfere with the migration of ookinete to the gut epithelium (Ponnudurai et al., 1988). Vector susceptibility may depend on the speed of parasite development and the speed of development of the peritrophic membrane. Only in those mosquitoes, the ookinetes penetrate the peritrophic membrane before the membrane gets thickened can develop into oocysts successfully.

Parida et al. (1991) estimated the vectorial capacity of An. fluviatilis to P. falciparum, P. vivax and P. malariae for different seasons in Malkangiri zone. The estimate of vectorial capacity is based on the assumption that the physiological ability of the vector population in acquiring and transmitting the acquired infection (vector competence) was the same. In order to get the reliable data on vectorial efficiency, it is always necessary to consider its vector competence (Reisen, 1989). Assuming that all the infective mosquitoes successfully transmit the infection they acquired, the susceptibility value of An. fluviatilis determined during the present study was included in the estimation of vectorial capacity. The vectorial capacity calculated without considering the parameter of vector competence to plasmodia was an over
estimation. However, this has not changed the ranking of the anophelines species according to the importance in malaria transmission in the area.

Malaria caused by *P. falciparum* is endemic in the study area, constituting 93% of total malaria cases followed by *P. vivax* (5 to 6%). The high prevalence of *P. falciparum* could be due to the higher vector efficiency of the major vector *An. fluviatilis* in transmitting this parasite species compared to other species. Further, the longer duration of *P. falciparum* gametocytæmia in untreated persons and lesser stimulation of immune response, particularly in areas of prolonged transmission, might enhance the chances of the vector getting infected with *P. falciparum* resulting in higher rate of transmission (Bruce-Chwatt and Glanville, 1973).

The present study indicates that the susceptibility of *An. fluviatilis* is lower to *P. vivax* and *P. malariae* than *P. falciparum*. This lower susceptibility is expected to reduce the receptivity of the area for transmission of *P. vivax* and *P. malariae*, as vector competence is one of the important factors limiting the transmission and reducing the number of infection. This could also be a reason for low prevalence of *P. vivax* and *P. malariae* in the study area.

Recently, in Indian populations of *An. fluviatilis*, three sibling species have been identified and designated as S, T and U (Subbarao et al, 1994). In Malkangiri District, *An. fluviatilis* has been found to comprise of 97.1% S and 2.9% T types (VCRC unpublished data). No variations were observed in the relative abundance of the sibling species between seasons. As species S is the most abundant species, the susceptibility of *An. fluviatilis* observed in the laboratory could be considered as the susceptibility of sibling species S. Recent studies have shown that natural infections have been recorded in S and not in T and U (Subbarao, 1998).
An. culicifacies

The susceptibility of *An. culicifacies* to the infections with *P. falciparum* was higher than with *P. vivax*, similar to that observed in *An. fluviatilis*. However, there was no development of *P. malariae* in *An. culicifacies*.

*P. malariae* is generally prevalent in hill top and forested villages where the climate is cool. The prevalence of *An. culicifacies* is low in hilltop villages and during cold season (Das et al., 1990). Thus, the chances of contact between *An. culicifacies* and *P. malariae* is remote, both due to geographical and seasonal differences. Therefore, the parasite might have had lesser opportunity for adaptation to the vector. An ongoing taxonomic study in Malkangiri shows that *An. culicifacies* complex comprises of sibling species A (0.3%), B (63.7%) and C (36.0%). The species D is not found in Malkangiri (VCRC unpublished data). Both B and C are abundant in summer and rainy seasons. Since the natural population comprises of mainly two sibling species, the observed susceptibility value could be attributable to the sympatric population of *An. culicifacies* in the study area. Species B and C are known to be sympatric in North India. Distinct biological variations such as host-specificity, susceptibility to malaria parasites and response to insecticides have been noticed between the species. While species A, C, and D have been incriminated as the vectors, the role of species B in malaria transmission in North India is doubtful. Though, species B could not be incriminated in natural conditions, in Pakistan, laboratory feedings show that, it supports development of both *P. falciparum* and *P. vivax* (Subbarao, 1998). In India, species B was not found to support development of *P. vivax*. In the present study, the susceptibility of *An. culicifacies* to *P. vivax* was found to be lower. The natural infection rate in this species has also been lesser than that in *An. fluviatilis* in the study area. Further, *An. culicifacies* population was reported to be zoophagic in habit (Gunasekaran et
Therefore, its importance in transmission is lesser as compared to *An. fluviatilis*. The difference in susceptibility to the three parasite species between *An. fluviatilis* and *An. culicifacies* could be related to the distribution of sibling species.

*An. jeyporiensis*

The trend in the difference between the susceptibility of *An. jeyporiensis* to the three parasite species was similar to that observed in *An. fluviatilis* and *An. culicifacies*. Earlier, Senior White (1938) recorded oocyst infections in the natural population of *An. jeyporiensis* in Jeypore hills. This species was a known vector of malaria in China, Burma (now Myanmar) and in countries formerly included in Indo-China, (Rao, 1984). Since natural infection was not recorded during the recent studies (Gunasekaran et al., 1989), its importance in malaria transmission remained doubtful. The present study shows *An. jeyporiensis* is susceptible to the infections of the three parasite species. Its survival rate in nature is also high. However, the anthropophilic index of *An. jeyporiensis* (4.0%) is lower than that of *An. culicifacies* (7.0%), which may account for the lower vectorial status of former than the latter species.

The present study is the first experimental documentation of the susceptibility of *An. jeyporiensis* to malaria parasites in India. In China, Xu (1986) feeding of *An. jeyporiensis in-vitro* on the blood from the eight patients with *P. vivax* infection showed gland infection rates as high as 100% and mean positive gland index of 3.73.

*An. subpictus*
An. subpictus is the most abundant species in the area (Gunasekaran et al., 1989). The present study shows that while 5.3% of An. subpictus was susceptible to P. falciparum, the species was not infected with P. vivax and P. malariae. In nature, 9% of An. subpictus was found to feed on human (VCRC unpublished data). There are reports indicating high anthropophilic index for this species (Roy, 1943, Bruce Chwatt et al., 1966 and Kulkarni, 1983). However, the survival rate in nature was very low. Out of 340 wild caught females of An. subpictus, only one was found with three dilatations in the ovarian follicles (VCRC unpublished data). In the laboratory, 80% of An. subpictus did not survive up to day 9 post-infection. It has been shown that mortality in the field in nature would be considerably higher than that under laboratory conditions (Mehta, 1934; Rao, 1884). Thus, a low survival rate seems to the major limitation for this species to be an important vector.

In some parts of the country, natural infections were recorded in this species (Russell et al. 1939 and Russell and Rao, 1940). The species had been incriminated as vector in Maldives islands (Roy et al., 1978), Indonesia (Harinasuta et al., 1976) and in coastal villages of Pondicherry (Panicker et al., 1981). From the literature, it is also evident that the species shows considerable variation in the susceptibility to malaria in different geographical areas. These contrasting reports, based on laboratory as well as field observations suggest the possibility of the existence of more than one geographical strains or sibling species within An. subpictus complex. Recently, it has been shown that An. subpictus complex comprises of four reproductively distinct species viz., A, B, C and D occurring sympatrically in India (Suguna et al., 1994). Based on the morphological characters described by Suguna et al. (1994), it has been found that all the four sibling species of An.subpictus complex are present in the study area. Species D (35.7%) is the most abundant, followed by species B (30.1%), C (17.5%) and A
(16.7%). However, cyto-genetics studies could not be carried out for the identification of sibling species in the study area.

Even though the results of the present study are in agreement with some of the earlier laboratory studies, showing that *An. subpictus* can be a potential vector, its role in nature would be limited by its poor longevity.

Five other species viz. *An. varuna*, *An. splendidus*, *An. pallidus*, *An. tessellatus* and *An. theobaldi* were studied for their susceptibility to *P. falciparum* only. The susceptibility of these mosquito species to *P. vivax* and *P. malariae* could not be studied due to less prevalence of *P. vivax* and *P. malariae* in the community and as volunteers were not available.

*An. varuna*

From the earlier studies, it is evident that *An. varuna* was an important vector only in the hilly and forested areas of Central India and perhaps to a certain extent in Deltaic Bengal and Vishakapatnam coast of Andhra Pradesh (Rao, 1984). The highest sporozoite rates for this species (3.7%) were recorded in east Satpura hills followed by Jeypore hills (3.3%). However, in the study area no natural infection was observed during recent studies (Gunasekaran, 1989). The present study, however, showed that *An. varuna* was capable of developing sporozoites when fed on infected donors. Laboratory studies had earlier shown that the species was susceptible to *P. falciparum* (Russell and Mohan, 1939a). The VCRC unpublished data show that out of 100 field collected females tested, all were found to have cattle blood. Similarly out of 70 dissected, none was found with more than two dilatations in the ovarian follicles, indicating a low survival rate in nature. The present study shows that though, *An. varuna* is
susceptible, characteristics such as low anthropophagy and poor longevity seem to be the two major constraints for the species to be vector.

*An. splendidus*

*An. splendidus* is generally a hill species and except its wide prevalence, very little is known regarding its biology. There is no evidence about its role in malaria transmission throughout India and only one natural gut infection has been reported in Punjab (Rao, 1984). But, natural infections have been recorded in Hong Kong and Taiwan (Rao, 1984). This study has, for the first time, shown its susceptibility to *P. falciparum*. Although 7.8% of the females survived in nature for more than 10 days (VCRC unpublished data), which is the minimum period considered critical for the transmission of malaria parasite (Gunasekaran, 1991), the blood meal analysis of field collected specimens showed that this too is a predominantly zoophagic species. Therefore, does not seem to play any role in malaria transmission.

*An. pallidus*

*An. pallidus*, which is also common in the study area during the winter months has occasionally been found infected with malaria parasites in Orissa, east central India and Bengal at very low infection rates (Wattal, 1961). Thus, it is considered as a vector of some significance locally in these areas, although no natural infection was recorded in the present study area (Gunasekaran *et al.*, 1989).

During the present study, the species was found to be susceptible to *P. falciparum* parasites. Moreover, a considerable proportion survived more than 10 days in nature and a
small proportion (1.0%) was found in nature with human blood (VCRC unpublished data). Thus, even though no infection has been found in nature, its role as a likely vector in the area can not be completely ruled out.

*An. tessellatus*

*An. tessellatus* is a seasonal anopheline species in this area and mainly prevalent in cooler months. It is not known to be a vector in mainland of India, but is considered as a suspected vector in Lakshadweep, on epidemiological grounds (Roy *et al*., 1974 and Roy *et al*., 1978). Laboratory studies had earlier shown it to be susceptible to simian parasites (Choudhury *et al*., 1963). In the present study, out of 129 *An. tessellatus* dissected, 14 were found with oocysts, but none (of the 79 mosquitoes observed) was found with sporozoites. One mosquito, which survived for 21 days after providing the infective blood meal contained oocysts but no sporozoites. Similarly, in another study in Sri Lanka, 15% of *An. tessellatus* infected with oocyst failed to develop sporozoites in salivary glands (Gamege-Mendis *et al*., 1993). Meis and Ponnadurai *et al*., 1987 opined that in case of *P. falciparum*, formation and migration of ookinetes takes place slowly. Because of this delayed ookinite activity the peritrophic membrane becomes thicker and probably constitutes a significant barrier to the penetration of ookinete and the parasite infectivity is therefore reduced. Ramasamy *et al*.,(1996) have also given the same reason to explain the low infectivity of *P. falciparum* to *An. tessellatus*. Further, *An. tessellatus* is an exclusively zoophagic mosquito (VCRC unpublished data) and therefore this species may not pose any threat to the community of this area.

*An. theobaldi*
An. theobaldi is also a species that is abundant only during the winter months. It has not been incriminated as a vector in the past in India (Wattal, 1961). However, the present study has shown its susceptibility to *P. falciparum*. Unpublished field study in this area has shown that 12.1% of the population crossed potentially dangerous age of 10 days. Moreover, its density during the winter months is high. Therefore, the zoophagic behaviour seems to be an important factor in limiting this species from transmitting malaria.

**Mosquito feeding rate**

The feeding rate of anophelines observed in the laboratory does not corroborate with the observation on the man feeding habit of the species in nature. Even, species such as *An. subpictus* and *An. splendidus*, which have low propensity to man feeding in the study area, were found to have higher feeding rate in captivity.

**Factors influencing infectivity of gametocytes of *P. falciparum***

Different factors may be associated with variations observed in the susceptibility of vectors. These factors may be associated either with the vector, or the infected human host. Those associated with the vectors could be survival of infected mosquitoes, mosquito age, temperature and geographical strain variations. Those associated with human reservoir could be gametocyte density; sex ratio of gametocytes, asexual parasitaemia, parasite strain and age of the human host.

**Factors associated with vectors**

**Survival of infected mosquitoes**
Comparison of the survival of females within the same cohorts fed on infected and uninfected donors, showed that the parasite infection in the mosquitoes did not have any effect on its survival. In earlier studies, some (Buxton, 1935; Gad et al., 1979a, 1979b; Klein et al., 1982, 1986; Maier, 1973) have reported a higher mortality in mosquitoes due to plasmodium infection, while others (De Buck and Swellengrebel, 1935; Boyd, 1940; Wilkinson et al., 1972; Maier et al., 1987) showed no adverse effect. These differences may relate to the use of different vector-parasite systems or even to the different ranges of parasite densities as studied by Robert et al. (1990). It is generally accepted that vector's longevity is unaffected by infection and an infective vector remains so for the rest of its life span (Wernsdorfer and McGregor, 1988). In this context it would be pertinent to quote the following statement attributed to Garnham: "How utterly harmless the malaria parasite must be to the mosquito" (Russell et al., 1963). The present study is in agreement with Garnham.

Mosquito age

The experiments showed that the difference in the infection rate between the younger and the older age group of mosquitoes was not statistically significant. This observation is in disagreement with the only previous report, by Russel et al. (1963) that younger mosquitoes (like children) are more likely to be infected than older ones. It is difficult to explain the contrasting results between the present set of experiments and earlier one by Russel and his co-workers.

Temperature
The relationship between temperature and the prevalence of infection in mosquitoes is significant while considering the epidemiology of malaria in an area. In the study area, malaria incidence peaked during November-December when the temperature ranged from 22.8 to 25.6°C. In the present study, the optimum range of temperature for the development of parasite was found to be 21 and 25 °C, which compares favorably with the range of temperature in nature at which transmission was at its peak.

Geographical strain variations

*An. fluviatilis* is the major malaria vector in both Malkangiri and Jeypore zone. Recent studies have shown that infection rate in *An. fluviatilis* varied between these two zones. A higher sporozoite rate of 5.09% was recorded in Malkangiri zone compared to 0.16% in Jeypore zone (Parida et al., 1991). This could be due to the variation in the degree of susceptibility of the mosquito from the two areas. Other workers suggested that there may be an existence of two geographically/ecologically isolated forms of *An. fluviatilis* in different areas (Viswanathan, 1950; Issaris et al., 1953; Kulkarni and Wattal, 1982; Gunasekaran, 1991 and Parida et al., 1991). The taxonomic studies confirmed that the population of *An. fluviatilis* of Malkangiri zone comprises of 97.1% of S type and 2.9% of T type, while in Jeypore zone *An. fluviatilis* comprises of 70.3% of S type and 29.7% of T type (VCRC unpublished data).

It is interesting to note that *An. fluviatilis* from Malkangiri has higher susceptibility to *P. falciparum* of both Malkangiri and Jeypore. Thus, findings of the present study are in agreement with the earlier field observations.

Factors associated with human host
Gametocyte count

Among the factors concerning the susceptibility of mosquitoes to the species and strains of plasmodia, the density of gametocyte is one of the important factors (Mohan, 1955). Past studies have shown the striking variability of infection in mosquitoes fed on donors having different gametocyte densities (Wernsdorfer and McGregor, 1988). It has been shown that the count of gametocyte as low as 10 per cubic mm, which is equivalent to 0.25 gametocytes per 200 WBCs of *P. falciparum*, can infect more than 50% of the mosquitoes (Barber *et al.*, 1931; Robertson, 1945; Burgess, 1960; Bray and Burgess, 1964; Bray *et al.*, 1976; Graves, 1980; Rutledge *et al.*, 1969).

In the present study, a non-linear relationship was observed between gametocyte count in the donors and infection rate in the mosquitoes. Similar findings were reported earlier by Kliger and Mer (1937), Cantrell & Jordan (1946) and Kasap *et al.* (1987). It appears that the infection rate increases with the increase in gametocyte count and beyond 16-20, there is a limitation. This implies that even moderate gametocyte density in the community could facilitate transmission.

There was a correlation between the mean number of oocysts per infected gut in the mosquitoes and gametocyte count in the donors. Eyles (1951, 1952 a, b & c) also demonstrated a linear positive correlation in *P. gallinaceum*. In contrast, Huff (1927). Muirhead-Thomson (1954) and Cantrell & Jordan (1946) failed to detect any direct relationship for either *P. gallinaceum* or *P. falciparum*. The latter determined that gametocyte infected hosts were not of equal infectivity to the vector throughout the infection (Medley, 1993).
Sex ratio of gametocytes

Sex ratio did not have any significant correlation with the rate of infection in *An. fluviatilis*. Earlier studies of Boyd *et al.* (1935) with *An. quadrimaculatus* showed that the variation in sex ratio affected the infectivity of *P. falciparum* but not that of *P. vivax*. Even for *P. falciparum*, the only convincing correlation with infectivity was the density of male gametocytes (Wernsdorfer and McGregor, 1988). They have also suggested that the sex ratio of the gametocytes may be a factor in determining only minor variability in mosquito infectivity.

Asexual parasitaemia

No definite pattern in the relationship between the level of asexual parasitaemia and the rate of infection in mosquitoes was observed. Other workers observed a declining trend in infection rate in mosquitoes with the increase in asexual parasitaemia (Rutledge *et al.*, 1969; Carter and Gwadz, 1980; Hawking *et al.*, 1966 and Dei-Cas *et al.*, 1980 and Eyles, 1951). The asexual parasites, due to high metabolic activity, produce toxic byproducts that affect the infectivity of the gametocytes (Wernsdorfer and McGregor, 1988). In the present study, the level of parasitaemia in the donors might not be high enough to produce such an effect on the gametocytes.

Parasite strain

The study showed that *P. falciparum* from Malkangiri was more infectious to *An. fluviatilis* than the parasites from Jeypore. This could be due to the existence of two different strains of *P. falciparum*, the one in Jeypore being less infective than the one in Malkangiri.
Human age

The present study shows that the infectivity of *P. falciparum* gametocytes from the donor of 4 to 14 year age to *An. fluviatilis* was higher. There was a marked reduction in infectivity in the age categories of 15 to 25 and >25. It has been demonstrated during mass blood survey in this area that highest *P. falciparum* gametocyte rate was found in the age groups up to 10 years, with a peak in the age of 5 years (Pani, 1990). Thus, the highest contribution to the transmission of malaria is from children, who also have the capacity to infect a higher proportion of mosquitoes. Gamage Mendis et al. (1991) on the basis of a laboratory study in Kataragama (Sri Lanka) opined that infectivity of gametocytes was higher in the age group of 6 to 25 years. In contrast, Muirhead-Thomson (1957) reported that children under the age of 5 years, who constituted only 15% of the population, alone contributed to about 42% of the infectious reservoir.

Draper (1953), who found that the higher gametocyte count in 1-4 year age groups infected a greater proportion of mosquitoes, wondered whether the possibility of increased immunity in higher age groups was responsible for reduced infectivity.

Other likely reasons for a proportionately lower contribution of the higher age groups could be due to the effects of transmission blocking or enhancing antibodies as described by Mendis et al. (1987), Ranawaka et al. (1988) and Peiris et al. (1988). Mendis et al. (1990) also considered about the possibility of anti-parasitic effects of cytokines in higher age groups, resulting in lower infectivity to mosquitoes.
There was a wide variation between the susceptibility status of the anophelines determined in the laboratory and the records of natural infections. Except in *An. fluviatilis* and *An. culicifacies*, no other anophelines were found infected in nature. In the laboratory tests, all the nine species were found to be susceptible to the most abundant parasite species in the area, *P. falciparum*. The importance of these anophelines in malaria transmission or the variation in their potential as vectors seems to depend upon their behavioural characteristics such as man feeding habit and longevity rather than the variation in their susceptibility to malaria parasites. The relative susceptibility of the major malaria vector, *An. fluviatilis*, to three malaria parasite species seems to be important in reflecting the prevalence of malaria in the study area.