GEL DIFFUSION ANALYSIS OF *ANOPHELES* BLOODMEALS FROM 12 MALARIOUS STUDY VILLAGES OF ORISSA STATE, INDIA

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ABSTRACT. In Orissa State, India, the double gel diffusion technique was used to analyze 97,405 bloodmeals of all fed anophelines that were caught during standardized monthly surveys in 12 malarious study villages, from 1982 through 1988. *Anopheles culicifacies* contributed the highest number of smears from the 19 *Anopheles* species recovered. It was observed that a pronounced predilection to take mixed bloodmeals attenuates the vector potential of the species concerned. Consequently, prevalences based only upon "pure" (unmixed) primate bloodmeals provide the most accurate way to assess the intensity of feeding contact that actually occurs between a given species and man. By this method, the ranking order is *Anopheles fluviatilis*, *An. culicifacies* and *An. annularis* (N); a sequence which concurs with current knowledge on the vector status of malaria mosquitoes in Orissa.

INTRODUCTION

Anopheles sundaicus Rodenwaldt was described as a coastal vector of malaria by Senior White (1937) and as a vector in the Chilika Lake area of Orissa by Covell and Singh (1942). Subsequently, early DDT malaria sprays appear to have eliminated *An. sundaicus* from coastal Orissa, but it is still found in seashore areas north and south of the state. Further observations by Senior White and Das (1938) in the Jeypore Hills of southwestern Orissa revealed that *An. fluviatilis* James, *An. minimus* Theobald and *An. varuna* Iyengar are primary vectors, while *An. jeyporiensis* James is a secondary vector. *Anopheles annularis* Van der Wulp was revealed as the main, if not the only, vector in the plains of Orissa by Panigrahi (1942) and Senior White et al. (1943). *Anopheles culicifacies* Giles was not regarded as a malaria vector in Orissa by these early workers. It was first incriminated in 1980 by our workers in Dhenkanal district of Orissa, and thereafter several additional positive *An. culicifacies* were recovered by our study teams in Sambalpur, Mayurbhanj and Phulbani districts, Orissa. These teams also proved that *An. fluviatilis* is a perennial transmitter by the dissection for sporozoites every month of the year. In a review of Orissa anophelines, Guha et al. (1981) reported 21 species in rural Orissa, but they said no reports were available from Dhenkanal, Phulbani, Puri, Bolangir or Kalahandi districts, thus showing that entomological data were very scanty up to 1980. The National Malaria Eradication Program recognized the pressing need to upgrade entomology, so 3 field studies were established in Orissa, with primary objectives to incriminate or reincriminate vector species and to study larval and adult bionomics, particularly to improve control strategies. By the time a double gel diffusion (DGD) mosquito bloodmeal identification system was developed, the 3 study teams already had been conducting weekly routine field collections for more than a year, so it required very little extra effort for the teams to make blood smears and record data on all fed specimens being caught. The smear workloads were far less than the number the Bhubaneswar laboratory could process and costs involved were relatively negligible. Consequently, bloodmeal determinations were incorporated as a special adjunct to the baseline studies and were carried out as a routine procedure, like making identifications, evaluating mosquito midguts or dissecting salivary glands, ovaries, etc. Our objectives were to explore the parameters of the DGD method under field conditions and to contribute detailed data on adult feeding bionomics to the study team objectives.

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MATERIALS AND METHODS

Identical, standardized collections were conducted by 3 Entomology Field Investigation Units (FIUs) in 3 physiographically distinct areas of Orissa State, India (Fig. 1). Each FIU used the same work schedule in each of its 4 study villages, where blood smears were made on strips of Whatman no. 1 filter-paper from *all* fed anophelines collected. A separate filter-paper was used for each species, each collection biotope, each village and each year. A single filter-paper held up to 25 smears. For all-night, 10-min per hour collections, each smear was labeled to show the day and hour of collection. Regarding indoor-resting collections, code numbers linked each smear to its routine mosquito

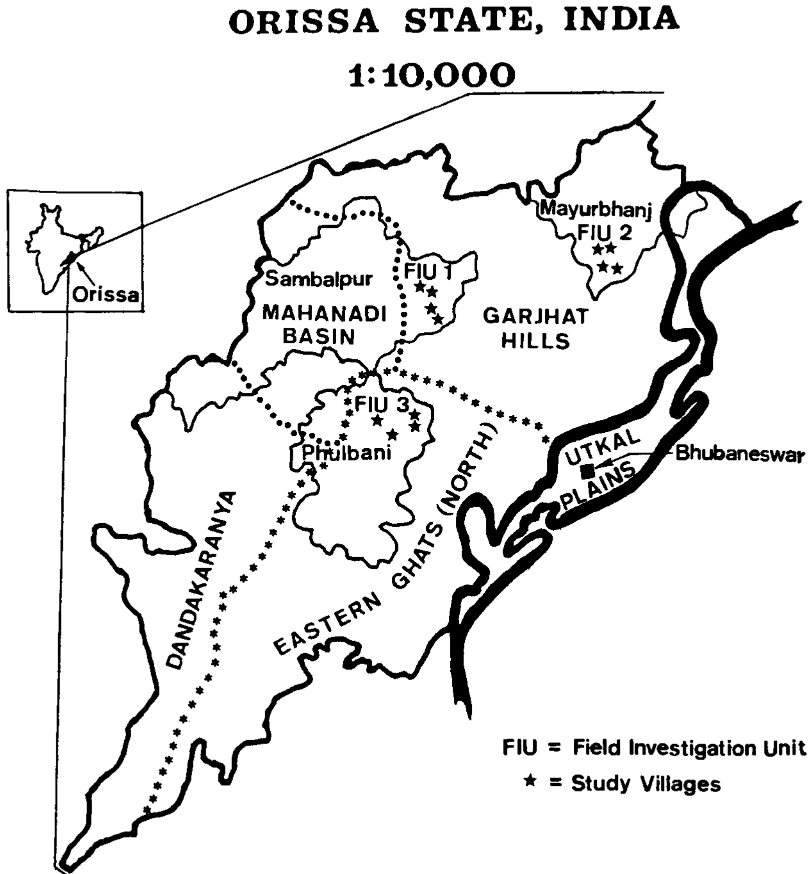

Fig. 1. Map of Orissa State showing the major physiographic categories, the location of Field Investigation Units and their respective study villages (from Das Gupta 1977, plate 41).

collection form, on which the house code number, type of resting surface, abdominal condition and the estimated height from the floor, were recorded for each mosquito caught. Smears were kept in sealed plastic bags and stored inside screw-cap plastic containers which were held in as cool a place as possible, until being sent (about every 3 or 4 months) to the PfCP[3] Zone II Entomology Gel Diffusion Laboratory at Bhubaneswar, Orissa State. This laboratory was established according to a manual by Collins and Bang (1988), and smears were processed according to the methodology outlined in this manual.

General guidelines for collecting smears: The best way to assess *Anopheles* feeding risk is to analyze the bloodmeals of all fed females collected in areas of stable malaria, where periodic high-endemic transmission is known to be actually autochthonous to those villages which have been selected for study. If it is not possible to collect mosquitoes every month of the year, then surveys should be conducted at least during months when peaks of transmission occur. If all major biotopes cannot be sampled, then collections should be focused on human-biased biotopes, such as human dwellings; and whenever substantial numbers of humans are observed sleeping outdoors, collections should include all fed anophelines found resting in the immediate vicinity of the sleepers. The collections mentioned are designed to distinguish which species are feeding most extensively on humans during periods of intense transmission, thus exposing the most likely vector species. An analysis of their bloodmeals reveals the proportion of the total fed specimens (fed density) that contain

[3] *Plasmodium falciparum* Containment Programme: a project funded by the Swedish International Development Agency (SIDA), which supplements the National Malaria Eradication Programme through WHO/SEARO.

Table 1. Gel diffusion results on anophelines of Orissa, India, from 1982 through 1988, rated by anthropophilic index (A.I.), human blood prevalence (H.B.P.) and "pure" human blood prevalence (P.H.B.P.).

Anthropophilic index (A.I.)	Human blood prevalence (H.B.P.)	Pure human blood prevalence (P.H.B.P.)
1. *An. fluviatilis*[a]	1. *An. fluviatilis*[a]	1. *An. fluviatilis*[a]
2. *An. theobaldi*	2. *An. culicifacies*[a]	2. *An. culicifacies*[a]
3. *An. pallidus*	3. *An. vagus*	3. *An. annularis* (N)[b]
4. *An. varuna*[c]	4. *An. annularis* (N)[b]	4. *An. vagus*
5. *An. annularis* (N)[b]	5. *An. hyrcanus* group	5. *An. subpictus*
6. *An. jeyporiensis*[c]	6. *An. subpictus*	6. *An. hyrcanus* group
7. *An. culicifacies*[a]	7. *An. pallidus*	7. *An. pallidus*
8. *An. vagus*	8. *An. theobaldi*	8. *An. theobaldi*
9. *An. aconitus*	9. *An. varuna*[c]	9. *An. varuna*[c]
10. *An. maculatus*	10. *An. jamesi*	10. *An. jamesi*
11. *An. hyrcanus* group	11. *An. aconitus*	11. *An. aconitus*
12. *An. jamesi*	12. *An. maculatus*	12. *An. maculatus*
13. *An. barbirostris*	13. *An. barbirostris*	13. *An. barbirostris*
14. *An. subpictus*	14. *An. tessellatus*	14. *An. tessellatus*
15. *An. tessellatus*	15. *An. jeyporiensis*[c]	15. *An. jeyporiensis*[c]
16. *An. splendidus*	16. *An. splendidus*	16. *An. splendidus*
17. *An. annularis* (A)	17. *An. annularis* (A)	17. *An. annularis* (A)

[a] Proven vectors of study villages.
[b] Suspected vector of study villages.
[c] Local vectors of Jeypore hills, according to previous studies; do not appear to be vectors in study villages.

human blood, and from these a further refinement may be made by considering only the "pure" (unmixed) feedings. Such data clearly confirms and quantifies the degree of man-mosquito contact occurring when the survey was made.

However, besides feeding behavior and population density, there are additional characteristics that enhance vector competence, such as short gonotrophic cycles, high levels of mosquito-parasite compatibility and prolonged longevity.

RESULTS

Definition of terms and interpretation of feeding behavior: In this paper, the anthropophilic index (A.I.) is the percentage of blood smears from a single *Anopheles* species, which is positive for human blood. The A.I. usually combines data from several different biotopes, and these may be strongly biased toward one host or another. Quite often the biotopes involved are not named or described at all by the compiler, so the A.I. should be regarded with caution and used for general comparisons only.

The human blood index (H.B.I.) is the proportion of blood smears from a single *Anopheles* species, which is positive for human blood and which was collected in a particular biotope which is identified by location and time of day. Such smears may include the mixed blood of 2 or even more types of hosts, as long as one of them was human. The H.B.I. is more precise than the A.I. for assessing specific feeding behavior patterns. However, blood prevalences appear to be more accurate than either of these 2 indices.

The human blood prevalence (H.B.P.) is similar to the H.B.I. except that it refers to the proportion of the total smears containing human blood (mixed and unmixed) from *all* species combined, which are comprised by a particular species.

The pure human blood prevalence (P.H.B.P.) is similar, except that only smears that contain "pure" (unmixed) human blood are compiled. Such blood consists of one or many feedings on human blood, upon one or several human hosts, but the total number of human feedings is cryptic. The focus of the P.H.B.P. on unmixed feedings, especially those from indoor-resting, human dwelling (AM) collections seems to provide the most accurate means of assessing man-mosquito contact in Orissa.

Review of data on feeding behavior: Table 2 shows the general feeding proclivities for the anopheline fauna of Orissa. From 1982 through 1988, a total of 97,405 blood smears, collected in 15 biotopes from 19 *Anopheles* species, were processed against human and cow antisera. Of these, 94,371 smears were positive for primate or bovine blood, and only 3.1% were negative. Among the species in Table 1, *An. annularis* appears as 2 forms: *An. annularis* (N) is an arbitrary name coined locally for "normal" specimens and *An. annularis* (A) designates "abnormal" winter forms, marked by additional dark bands on the tarsi or palpi, that appear in about

10% of the population at Mayurbhanj District, from November to March. Anophelines in Table 1 are listed in descending order, according to total blood smears collected. *Anopheles culicifacies*, *An. vagus*, *An. subpictus* Grassi and *An. hyrcanus* group [4] have relatively high fed-densities, while that of *An. annularis* (N) is intermediate. All other fed-densities are considerably lower. *Anopheles fluviatilis*, the most egregious vector in the study villages, ranked only 7th in fed-density prevalence. Species contributing less than 1,000 smears are probably too low in fed-density to be regarded as dangerous in the study villages. The data for human positives show details on smears positive *only* for primate blood ("pure") and those containing both primate and bovine blood. Table 2 includes 3 assessment methods for ranking species according to their anthropophagic tendencies. Table 1 simply lists the *Anopheles* species in ranking order by each of the 3 methods, and it indicates the proven or suspected vectors of the study villages. According to Table 1, an appraisal based on pure human blood prevalences (P.H.B.P.) seems to provide the most accurate assessment method.

Table 3 contains results of the 3 paramount species, from catches in each of 15 biotopes. The catch-rates of one biotope usually cannot be compared directly with those of another, because the collecting time and effort expended usually varies from one biotope to another. For example, all-night, 10-min indoor-wall collections soon proved to be unfeasible because villagers objected to being disturbed hourly throughout the night. Consequently, these collections had to be discontinued after a trial period lasting only a few months. In addition, the experimental hut and human dwelling, space-spray collections were implemented only in FIU 3, Phulbani. Even for the same type of collection, such as indoor-resting, twice as many human dwellings were surveyed as were cattle-sheds or mixed dwellings, because the former are more strongly biased to reveal man-mosquito contact.

Indoor-resting, cattle-shed (AM) collection is the most productive technique for all species, except *An. fluviatilis*. Due to its very pronounced anthropophagic behavior, *An. fluviatilis* usually is caught in highest numbers by indoor-resting, human dwelling (AM) collections, where it characteristically registers a much higher Human Blood Index (H.B.I.) than any other species. Unsurprisingly, collections involving human dwellings are strongly biased and yield the highest H.B.I.s regardless of species. In comparing various biotopes, the ease of conducting the collection involved, the total number caught and the magnitude of the H.B.I. for each species are primary considerations. In general, indoor-resting, human dwelling (AM) collections perform best, except when large numbers of people sleep outside. However, indoor-resting, cattle-shed (AM) collections offer a promising alternative under certain conditions because they are considerably easier to carry out, achieve higher catch-rates (except for *An. fluviatilis*), are relatively unaffected by outdoor sleeping activities and they often register H.B.I.s that are adequately high to reveal man-mosquito contact, especially when high levels of transmission or epidemics occur.

[4] A key for *Anopheles* of the Cuttack Region (Wattal and Kalra 1961) was used for routine identifications. Although *An. hyrcanus* appears in this key, this species is not known to occur in India and specimens labeled as *An. hyrcanus* represent closely related species of the *Anopheles hyrcanus* group. In Orissa, *An. nigerrimus* Giles is probably the commonest member of the group (Ramachandra Rao 1984).

DISCUSSION

Background information on double gel diffusion: Wharton (1953) and Boreham (1975) attested that identifying the hosts of hematophagous arthropods from bloodmeals determines whether an anopheline species may be an efficient vector of malaria. Tempelis (1975) named the precipitin test as the basic serological tool for identifying bloodmeals, but Najera (1974) pointed out that its use has been limited by high costs, which were estimated by the World Health Organization (WHO 1985) to be U.S. $4.00 per blood smear. However, in 1982 an adaptation of the double gel diffusion (DGD) technique was developed by Collins et al. (1986) specifically for processing anopheline bloodmeals. This provided a simple, rapid, sensitive, reliable and inexpensive method, whereby one person can test over 300 smears a day against human and cow antisera, for under ½¢ (U.S. $0.005) per blood smear. In 1987 this DGD was compared with other available blood identification techniques in an interlaboratory trial, and the DGD provided the best results (Pant et al. 1987). If the DGD specifications for preparing the substrate and cutting wells are followed very scrupulously, and precise titers at 1:20,000 of human and bovine antisera are used, acutely sensitive reactions result, revealing many mixed feedings that would not be detected by other serological techniques. Since mixed feedings give visible testimony that all bovine feedings are noninfective for human malaria, any predilection for a given species such as *Anopheles vagus* Dönitz, to take a high proportion of mixed feedings attenuates its vector potential. Because

Table 2. Ranking order of anophelines in Orissa, India, according to their feeding habits, as determined by 3 different methods of assessing gel diffusion results.

			Details on smears with human blood							
Species in order by total smears taken	Total smears processed	Total smears positive	No reaction %	Total smears with human blood	Positive for human blood only	Positive for mixed blood	Mixed blood %	A.I.[c]	H.B.P.[c]	P.H.B.P.[c]
1. *An. culicifacies*[a]	22,553	21,897	2.9	670	500	170	25	3.1	0.19	0.19
2. *An. vagus*	18,104	17,459	3.6	506	169	337	67	2.9	0.14	0.07
3. *An. subpictus*	16,948	16,373	3.4	194	161	33	17	1.2	0.06	0.06
4. *An. hyrcanus* group	12,116	11,921	1.6	206	116	90	44	1.7	0.06	0.04
5. *An. annularis* (N)[b]	8,717	8,444	3.1	322	177	145	45	3.8	0.09	0.07
6. *An. jamesi*	3,978	3,773	5.2	63	51	12	19	1.7	0.02	0.02
7. *An. fluviatilis*[a]	2,434	2,390	1.8	1,143	1,084	59	5	47.8	0.33	0.42
8. *An. pallidus*	2,274	2,221	2.3	122	112	10	8	5.5	0.04	0.04
9. *An. aconitus*	2,028	1,971	2.8	47	36	11	23	2.4	0.01	0.01
10. *An. varuna*[b]	1,752	1,677	4.3	85	72	13	15	5.1	0.02	0.02
11. *An. maculatus*	1,470	1,435	2.4	25	20	5	20	1.7	0.01	0.01
12. *An. splendidus*	1,302	1,239	4.8	0	0	0	0	0.0	0.00	0.00
13. *An. barbirostris*	1,289	1,273	1.2	20	12	8	40	1.6	0.01	0.01
14. *An. theobaldi*	1,120	1,054	5.9	92	87	5	5	8.7	0.03	0.03
15. *An. tessellatus*	988	937	5.2	10	8	2	20	1.1	0.00	0.00
An. annularis (A)	116	107	7.8	0	0	0	0	0.0	0.00	0.00
16. *An. jeyporiensis*[b]	106	94	11.3	3	3	0	0	3.2	0.00	0.00
17. *An. karwari*	97	95	2.1	1	1	0	0	1.1	0.00	0.00
18. *An. stephensi*	9	8	11.1	0	0	0	0	0.0	0.00	0.00
19. *An. moghulensis*	4	3	25.0	0	0	0	0	0.0	0.00	0.00
Total	97,405	94,371	3.1	3,509	2,609	900	26	3.7	—	—

[a] Known vectors in Orissa.
[b] Vectors of local importance in certain parts of Orissa.
[c] A.I. = anthropophilic index, H.B.P. = human blood prevalence, P.H.B.P. = pure human blood prevalence.

Table 3. Gel diffusion results by various biotopes of collection for known malaria vectors in 12 study villages of Orissa, India, from September 1981 through December 1987.

	An. culicifacies				*An. fluviatilis*				*An. annularis* (N)			
Collection biotopes	Total smears	Total pos.	Pos. H.[1]	H.B.I.[2]	Total smears	Total pos.	Pos. H.	H.B.I.	Total smears	Total pos.	Pos. H.	H.B.I.
Indoor-resting, human dwelling (a.m.)	2,743	2,651	173	0.07	868	851	682	0.80	499	465	54	0.12
Indoor-resting, human dwelling (p.m.)	743	714	29	0.04	20	19	11	0.58	122	115	4	0.04
Indoor-resting, cattle-shed (a.m.)	4,166	4,056	96	0.02	119	113	37	0.33	1,931	1,888	87	0.05
Indoor-resting, cattle-shed (p.m.)	2,145	2,090	50	0.02	24	24	8	0.33	833	797	29	0.04
Indoor-resting, mixed, human part (a.m.)	483	467	14	0.03	12	12	4	0.33	212	204	9	0.04
Indoor-resting, mixed, human part (p.m.)	156	147	6	0.04	1	1	0	0.00	48	47	6	0.13
Indoor-resting, mixed, cattle part (a.m.)	1,058	1,035	19	0.02	23	23	3	0.13	775	740	28	0.04
Indoor-resting, mixed, cattle part (p.m.)	594	576	16	0.03	9	9	1	0.11	263	260	5	0.02
All-night, 10 min/h cattle-shed	5,772	5,622	93	0.02	211	207	24	0.12	2,362	2,318	24	0.01
All-night, 10 min/h outdoor-resting	735	713	45	0.06	24	24	11	0.46	390	386	13	0.03
All-night, 10 min/h indoor-wall	2	2	0	0.00	0	0	0	0.00	0	0	0	0.00
Exit window-trap (a.m.)	135	118	28	0.24	14	12	6	0.50	38	31	17	0.55
Pit-shelter collection (a.m.)	72	61	4	0.07	30	26	13	0.50	6	6	0	0.00
Experimental hut (a.m.)	11	10	3	0.30	8	8	3	0.38	0	0	0	0.00
Human dwelling, space-spray (a.m.)	138	136	1	0.01	5	5	2	0.40	2	2	0	0.00
Total	18,953	18,398	587	A.I.[3] = 3%	1,368	1,334	805	A.I. = 60%	7,481	7,255	276	A.I. = 4%
No reactions and mixed feedings	No reaction = 2.9%, mixed feeding = 16.8%				No reaction = 2.5%, mixed feeding = 4.7%				No reaction = 3.0%, mixed feeding = 28.2%			

[1] H. = primate blood (unmixed + mixed).
[2] H.B.I. = human blood index.
[3] A.I. = anthropophilic index.

the DGD technique clearly reveals such mixed feedings, it is much more valuable than any alternative technique which does not.

Comments on gel diffusion data: One of the most difficult aspects of interpreting blood smear results is trying to evaluate the etiological importance of patent mixed bloodmeals, by estimating the number of cryptic multiple partmeals that may have been taken on human hosts. Our gel diffusion results can reveal only unmixed bovine, unmixed primate or mixed bovine-primate bloodmeals. A mixed bloodmeal consists of a multiple meal, which is defined by Boreham and Garrett-Jones (1973) as any bloodmeal resulting from 2 or more feeds, the last of which has been taken before the first feed has been digested sufficiently to prevent identification of its origin. In this same article the authors provide a manner of computing an index on the proportion of multiple meals (including cryptic meals) in a sample.

Previously, before bloodmeal analysis was being done, it was generally assumed that human dwellings were so highly biased to provide mosquitoes which fed on human blood that each specimen caught in a house was presumed to represent a contact between that mosquito and man. However, Table 3 shows very clearly that this is not true, especially for *An. culicifacies* and *An. annularis* (N), which show that only 6.5 and 11.6%, respectively, of the fed specimens recovered from the indoor-resting, human dwelling (AM) collections contained primate blood. Only *An. fluviatilis* consistently provided smears showing a high percentage (80% in Table 3) that were positive for primate blood. All other *Anopheles* smears from this very strongly human-biased biotope characteristically ranged from only about 25%, down to 10% or even less, in positivity for primate blood. Consequently, irrespective of the place where mosquitoes are captured, it is essential that their blood be analyzed before even a rudimentary understanding of their actual feeding behavior can be ascertained.

Anopheline feeding deportment: According to Bidlingmayer (1985), a resting mosquito embarks upon an appetential (searching) flight in response to a physiological stimulus, such as the need for a bloodmeal. During the flight her appropriate sense organs, whether olfactory, visual, thermal, auditory or humidity receptors, indicate the presence of her objective. The moment such a cue is encountered, the appetential flight ends and the target or consumatory flight begins. The consumatory flight is direct and brief, since visual and biochemical cues do not operate over long distances. Therefore, it is likely that most patent mixed bloodmeals involve 2 types of spatially separate biotopes, and if so, presumably they would entail at least 2 appetential and 2 consumatory flights. Most cryptic multiple feedings probably occur within a single biotope containing several individuals of a single type. This is supported by Washino and Tempelis (1983), who state: "It is reasonable to assume that a high proportion of multiple meals will be cryptic in a habitat containing predominantly one host (e.g., chicken house)." This assumption also applies to cattle-sheds or human dwellings, etc., where several individuals of the same type inhabit the same structure. The proximity of many available hosts precludes the need for additional appetential flights, so if feeding is interrupted, only very short successive consumatory flights from one host to another within the biotope are necessary for the mosquito to become fully engorged. Such cryptic feedings would not be revealed by gel diffusion results.

One weakness of most bloodmeal identification techniques is an insufficient sensitivity to reveal mixed feedings. Each patent mixed bloodmeal provides proof that one or more feedings taken on bovine were noninfective for human malaria. It seems quite plausible that any propensity for a species to switch readily from one type of host to another type seriously dilutes its vector potential. For example, as shown in Table 2, *An. vagus* was caught in very high fed-density and it showed relatively high evidence of feeding on primate blood. However, 67% of its human positives actually were of mixed primate and bovine origin. Of course, all bovine feedings were harmless. Perhaps this attenuated feeding behavior helps explain why *An. vagus* is not recognized as a primary or a secondary vector.

The tendency to take multiple meals could possibly be genetically defined, but it seems more probable that it is either an expression of preferred feeding, or (even more likely) is caused by interrupted feeding due to anti-mosquito actions (aggressive or evasive) taken by the intended host. An example of preferred multiple feeding is provided by Smith and Weitz (1959), who observed that *Anopheles gambiae* Giles interrupted its feeding to seek out a preferred host, if the initial feeding was made on a "subsidiary" host (animals other than man and cattle, the preferred hosts). Interrupted feeding due to anti-mosquito behavior by several ciconiiform birds is described in detail by Edman and Kale (1971) and by Webber and Edman (1972). It seems reasonable that the majority of appetential and consumatory flights end in a biotope frequented by the host upon which the mosquito feeds, although they may seek harborage elsewhere.

For example, in many malarious villages *An. fluviatilis* is caught in relatively high numbers only in pre-dawn collections inside human dwellings. For this biotope, *An. fluviatilis* shows a very high H.B.I. (0.80) in Table 3, and only 3% of these human-positives involved mixed feedings. Any interrupted feeding of *An. fluviatilis* on a human inside a house most likely would result in a second feeding, either on the same person or upon another nearby human within the same house. Since humans are fairly excitable hosts, before a mosquito becomes fully engorged, several interrupted part-meals may occur. If an infective mosquito takes a series of part-meals that involve several uninfected persons, many new malaria cases may be generated. The danger posed by this type of feeding activity is clear and may explain why *An. fluviatilis* is such an effective vector, even in very low population density.

The extreme contrast between feeding habits of *An. fluviatilis* and *An. vagus* is clearly depicted in a graph of bloodmeal results on anophelines of Orissa by Collins et al. (1989). This graph takes into account species fed-density, H.B.I. and "pure" (unmixed) human bloodmeals. *Anopheles fluviatilis* is in the upper right corner of the graph, indicating very dangerous feeding behavior, while *An. vagus* is near the lower left corner, showing relatively harmless feeding habits.

Significance of the study: The comprehensive data garnered in this study engendered confidence when selecting priorities and sample sizes of bloodmeal collections in subsequent studies, according to the location, season, species, collection methods and major biotopes involved. In addition, the study results show the possibility of evaluating the effectiveness of mosquito controls that aim to reduce vector-man contact, by analyzing vector bloodmeals in appropriate biotopes before and after the controls are applied.

A very important potential reward from this study is the possibility of using the DGD to establish an entomologically based early warning system for malaria epidemics. This seems to be technically quite possible, wherever the epidemic vector is both anthropophilic and zoophilic. The senior author submitted a protocol indicating how field trials implementing an entomology-based early warning system could be set up in Orissa, India. If successful, tocsins of an ensuing epidemic would appear far in advance of those provided by a parasitologically based early warning system which usually has been advocated and precipitate anti-epidemic measures that would offer preventive rather than remedial rewards.

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