THE BLOOD SOURCES OF SOME AUSTRALIAN MOSQUITOES

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Summary

The results of over 1400 precipitin tests on the stomach contents of engorged mosquitoes to determine the source of their blood meals are recorded. Fifteen species of mosquitoes are included in this survey, but three species, *Anopheles annulipes, Culex fatigans,* and *C. annulirostris,* dominated the collections.

Engorged mosquitoes have been collected from three types of situation.

(i) Restricted animal habitats such as human dwellings, fowl houses, horse stables, dog kennels, and rabbit warrens;

(ii) The general farmyard environment; and

(iii) River flats and creek banks.

The collections from restricted animal habitats have indicated that for the species under consideration the site of collection is indicative of the blood source of the mosquitoes therein collected. Farmyard collections have given valuable information on the range of hosts attacked by nocturnally active mosquitoes. River flat collections have been most useful in disclosing rabbit-feeding species. Collections have also been made of mosquitoes attracted to man in the field and a small but significant percentage of these have been found to be already engorged. Such collections have been most interesting in that species feeding on marsupials have been revealed.

To the information derived from these precipitin tests has been added our accumulated observations of biting activity in the field, on man and other animals. In this way it has been possible to prepare a statement of the range of blood sources known to be utilized by each species and to give some indication of the preferences exhibited by certain mosquitoes for particular blood sources.

Anopheles annulipes has been shown to feed particularly on cattle and rabbits although other animals, man, and poultry are also attacked. *Culex fatigans* bites man in the domestic environment but is equally attracted by fowls in fowl-runs, or by horses in stables or dogs in kennels. Biting by this species is almost exclusively under shelter. *C. annulirostris* attacks man and most domestic animals but is most active out of doors. Rabbits are frequently attacked by this species. It is well known as biting man but man is not a preferred blood source. *Culex pipiens australicus* has been shown to attack rabbits and poultry. Species which have been revealed as attacking marsupials are *Anopheles stigmaticus, Aedes notoscriptus, A. alboannulatus,* and *A. queenslandis.*

Finally the significance of the feeding habits is discussed in relation to possible disease transmission by the various species. *Anopheles annulipes, Culex annulirostris,* and *C. pipiens australicus,* because of the frequency with which they attack rabbits, have an important role in myxomatosis transmission. *Culex fatigans* and *C. annulirostris,* attacking both poultry and man, are likely suspects for the transmission to man of Murray Valley encephalitis. *Anopheles annulipes,* on the other hand, because it attacks man only casually, is unlikely to assume any importance as a vector of malaria.

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I. Introduction

Little precise information has so far been available as to the usual sources of the blood meal for the majority of Australian mosquitoes, nor has existing knowledge of the range of hosts attacked been adequately reported. On only one previous occasion have precipitin tests been used to determine blood sources of wild-caught mosquitoes in Australia. This was in the studies undertaken by Dr. G. A. M. Heydon on north Queensland and New Guinea anophelines and reported under the species concerned in Lee and Woodhill (1944).

The present work has been based on precipitin test determinations of species of mosquitoes, both anopheline and culicine, found in southern Australia, and has been stimulated by the need for knowledge of rabbit-feeding species in connection with myxomatosis transmission, and of the range of hosts utilized by mosquitoes likely to be in any way involved in the cycle of transmission of Murray Valley encephalitis.

Initially, collections of mosquitoes, obviously blood fed, were made from typical animal habitats, houses, stables, poultry runs, and the like, in order to determine whether the site of collection had any relation to the source of the blood meal. Later these collections were extended to areas where one would be unable to presume the source of a blood meal owing to the variety of animals available. Typical of such areas were farmyards and the banks of rivers and creeks. Finally it was also found possible to collect engorged mosquitoes attracted to man in the field. Here again it was scarcely possible to even guess the blood source.

Over 1400 precipitin tests have now been carried out and the results of these tests are reported in full in Table 1. In all, tests of the blood meals of 15 species of mosquitoes are incorporated although only three species have been collected in large numbers.

II. Methods

Blood-fed mosquitoes for precipitin tests were usually captured in suction tubes. As far as possible, collections have been made in the morning, so that digestion would not have proceeded too far, but afternoon collections representing the previous night's feeding activity have still yielded quite useful results. After collection the gut contents were smeared with a needle on to filter paper, and identification of the mosquito was either made simultaneously or, if not obvious, the insect was pinned for later examination. The filter paper strips with dried blood spots (usually sun dried) were stored in glass bottles over silica gel and as soon as possible placed in a refrigerator for storage. The actual precipitin tests were then carried out from a fortnight to over 12 months later.

In preliminary tests antisera were obtained from various sources, but it was soon found expedient and more satisfactory to prepare our own antisera, and stocks of antisera for man, ox, sheep, horse, dog, marsupial (*Trichosurus*), rabbit, fowl, duck, and lizard (*Varanus* and *Tiliqua*) have been prepared. With few exceptions species specificity has been maintained. Anti-fowl sera proved

relatively non-specific and gave reactions with the blood of duck, rosella (*Platycerus eximius*), and dove (*Streptopelia suratensis*). Anti-duck, however, appears to be specific at least to the degree that it does not react with fowl, rosella, or dove. As expected, the anti-possum serum is more correctly an anti-marsupial serum. The anti-goanna serum has not been as non-specific as we hoped as it has proved unsuitable for the blood of the water lizard (*Sphenomorphus quoii*), the water dragon (*Physignathus leseurii*), and the blue-tongue (*Tiliqua scincoides*). Similarly, an antiserum prepared from the blue-tongue failed to react with the other species.

All antisera have been prepared in rabbits, with the exception of anti-rabbit, for which hens have been used. A variety of methods have been employed in preparing the various antisera. In the early work the intravenous method described by Arnold *et al.* (1946) was followed, but later Proom's (1943) technique was exclusively used. This involved the intramuscular injection of 10 ml of alum-precipitated serum of the appropriate animal at 30-day intervals until a satisfactory titre has been obtained. This was usually after the second injection. Testing for titre, or bleeding for antiserum preparation, is carried out 12-14 days after each injection. At the commencement of the work it was decided that titres of no less than 1 in 8000 were required for this type of testing and in the majority of cases it has been found possible to maintain or better this standard.

Individual tests have been carried out in open capillary tubes of 1·3 mm bore and 0·75 mm wall thickness. The pattern followed has usually been to test each blood spot against appropriate antisera for the one or two most likely blood sources and only when negative results are thus obtained to test against the range of available antisera. In this way the procedure has been less complicated and a considerable economy in the use of antisera has also resulted. From time to time known blood spots have been included with the unknown series as a check against any breakdown in the specificity of the antisera in use.

It is considered that, for the tests reported, positive results have been recorded for the majority of suitable spots collected. In early collections rather high (up to 50 per cent.) negative results have been obtained, but, as experience indicated that certain types of spots seldom gave a positive reaction, later collections were more selective, and positive results approaching 100 per cent. of the total collected were recorded. Actually, of the total, 84 per cent. have given positive reactions and very little significance is attached to the 16 per cent. negative results. Most of these negatives did come from blood-fed mosquitoes but at too late a stage of digestion for a positive reaction to be recorded. Admittedly we have been faced with series giving consistently negative results, and for these we strongly suspect that the range of antisera is not sufficiently wide, but these particular tests have not been included in the detailed results recorded below. They will, however, be mentioned again at a later stage.

III. Details of Precipitin Tests

Table 1 gives the results obtained from the present series of blood-fed mosquitoes. It has been arranged, not chronologically, but in increasing order of complexity of the environments from which the test mosquitoes have been collected, with the exception of a few limited series appearing at the end of Table 1.

IV. Discussion of Results Recorded in Table 1

The localities from which these mosquitoes have been collected are all in south-eastern Australia, with the exception of a very few from Moree in north-western New South Wales and Texas in southern Queensland. Those from Hornsby were from the outer fringe of the metropolitan area of Sydney, in rather sparsely populated bushland. The rest have been from country townships or the outlying areas of such townships in the Goulburn Valley in Victoria or in various parts of the Murray River Valley, or rural areas at Canberra, Kangaroo Valley, or Colo Vale, near Mittagong. Bearing this in mind, it is considered that any general conclusions may well apply to southern Australia, but this does not mean that similar results will necessarily be recorded in northern Australia.

(*a*) *Collections from Resting Place Habitats*

In the greater part of our work emphasis has been placed on the collection of fed mosquitoes from their daytime resting places. This has proved easy in some instances where the resting places of particular species have been both known and readily accessible. There are, however, other species of which the resting places are either unknown to us or present serious difficulties to the collector. The difficulties which are known to us are those associated with resting in dense vegetation where the movement of the collector continually stimulates movement of the mosquitoes away from him. In other cases, where resting is presumed to be vegetational on limited circumstantial evidence, other techniques have recently been developed which appear to be relatively successful (see (*b*) below). In our consideration of resting places we exclude the obvious resting of recently emerged individuals in the immediate vicinity of breeding grounds since such situations seldom yield engorged specimens.

(i) *Domestic and Restricted Animal Habitats.*—It will be seen that there is a strong correlation between the result recorded and the most readily available blood source in all domestic and semi-domestic collections. Mosquitoes caught resting inside houses have invariably fed on man, those taken from stables are almost all positive for horse, those from fowl-runs are similarly positive for fowl, and those from dog kennels have all fed on dogs. On the other hand mosquitoes taken from beneath houses are more likely to have fed on animals other than man.

Such an observation is in itself important for it means that we are at liberty to assume that, within the region outlined, similar results are likely to be obtained, and site of collection is a strong indication of the source of the blood meal in those environments wherein there is an obvious and predominating blood

TABLE 1

DETAILED RESULTS OF PRECIPITIN TESTS

Series	Details of Collection	Species	No.	Results	No.	Positive (%)
1	Residence, Hornsby, 8.iv.1952, collected 10 a.m., smeared 10.15 a.m.	*Culex fatigans*	11	+Man	9	
				Negative	2	82
2	Residence, Hornsby, 16.iv.1952, collected 8 a.m., smeared a.m.	*Culex fatigans*	19	+Man	9	
				Negative	10	47
3	Residence, Hornsby, Jan. 1952, collected 9 a.m., smeared 10 a.m.	*Culex fatigans*	12	+Man	12	100
4	Residence, Hornsby, 1.viii.1951	*Aedes alboannulatus*	1	+Man	1	100
5	Fowl house, Hornsby, 8.iv.1952, collected 9 a.m., smeared 9.30 a.m.	*Culex fatigans*	11	+Fowl	9	
				Negative	2	82
6	Fowl house, Hornsby, 16.iv.1952, collected 9 a.m., smeared a.m.	*Culex fatigans*	22	+Fowl	19	
				Negative	3	86
7	Fowl-run, Tuppal, 9.iv.1951, collected, smeared 8-9 a.m.	*Culex fatigans*	7	+Fowl	7	
		Anopheles annulipes	2	Negative	2	78
8	Fowl-run, Tuppal, 10.iv.1951, collected, smeared 8-9 a.m.	*Culex fatigans*	3	+Fowl	2	
				Negative	1	
		Culex annulirostris	1	+Fowl	1	
		Anopheles annulipes	2	+Fowl	1	
				Negative	1	83
9	Fowl-run, Mooroopna, 7.iv.1951. Horses close by, dog next door, man a possibility, ? cat. Collected during afternoon, smeared 2-4 hr later	*Culex fatigans*	159	+Fowl	105	
				+Man	1	
				Negative	53	67
10	Fowl house, Cobram, 5.ii.1952, 1-2 p.m.	*Culex fatigans*	14	+Fowl	14	100
11	Fowl houses, Albury and Wodonga, Dec. 1951 and Jan. 1952. Collected by K. Myers	*Anopheles annulipes*	20	+Fowl	13	
				Negative	7	
		Culex annulirostris	7	+Fowl	4	
				Negative	3	
		Culex fatigans	18	+Fowl	18	
		Culex pipiens australicus	33	+Fowl	31	
				Negative	2	85
12	Horse stable, Cobram, 5.ii.1952. Fowls present, one horse temporarily; 12-1 p.m.	*Culex fatigans*	8	+Fowl	8	100
13	Stable, Mooroopna, 7.iv.1951. Horses only obvious animals, man and dogs in immediate vicinity. Collected afternoon	*Culex fatigans*	34	+Horse	15	
				+Fowl	1	
				Negative	18	48
14	Dog kennels, Cobram, 5.ii.1952, 11-12 a.m.	*Culex fatigans*	11	+Dog	11	100

TABLE 1 (*Continued*)

Series	Details of Collection	Species	No.	Results	No.	Positive (%)
15	Resting and flying around dog and man. Cobram, 2.ii.1952, 8.30-9.0 p.m.	*Aedes theobaldi*	1	+Man	1	
		Aedes vittiger	2	+Man	2	
						100
16	Septic tank trap, Cobram, 21.ii.1952	*Culex fatigans*	2	+Fowl	1	
				Negative	1	50
17	Small isolated shed, Hornsby gully. Rabbit warren close by, 8.iv.1952, collected 11.30 a.m., smeared 2.00 p.m.	*Culex fatigans* (or possibly another member of this complex)	1	+Rabbit	1	
						100
18	Haystack, farm, Cobram 29.i.1952	*Anopheles annulipes*	1	+Fowl	1	
						100
19	From rabbit warrens, various localities in the Albury area, various dates late 1952 and early 1953. Collected by K. Myers	*Anopheles annulipes*	523	+Rabbit	487	
				+Man	1	
				Negative	35	93
20	Taken in trap over exposed rabbit on river flat, Albury, 2.ii.1953. K. Myers	*Culex annulirostris*	97	+Rabbit	96	
				Negative	1	
						99
21	General shelter collection, dairy, Curlwaa, 22.i.1952. All farm animals and man. Collected 6-7 a.m., smeared 3-4.30 p.m.	*Anopheles annulipes*	45	+Fowl	27	
				+Ox	10	
				+Dog	2	
				+Horse	1	
				Negative	5	
		Culex annulirostris	47	+Fowl	28	
				+Ox	11	
				+Dog	3	
				+Man	2	
				Negative	3	
		Culex fatigans	12	+Fowl	11	
				Negative	1	
		Aedes vittiger	3	+Ox	1	
				+Man	1	
				+Dog	1	
		Aedes alternans	1	+Ox	1	
		Aedes theobaldi	1	+Fowl	1	
		Mansonia linealis	1	+Fowl	1	91
22	House, Wentworth, 22.i.1952. Edge of billabong. From W.C., screen wires, outhouses, etc. Dogs, fowls, ducks, cattle, man, etc. present. Collected 10 a.m., smeared 2.30-3.30 p.m.	*Culex fatigans*	27	+Fowl	21	
				+Man	3	
				+Ox	2	
				+Dog	1	
		Culex annulirostris	4	+Fowl	2	
				+Man	1	
				Negative	1	
		Anopheles annulipes	15	+Ox	10	
				+Man	1	
				Negative	4	91

TABLE 1 (*Continued*)

Series	Details of Collection	Species	No.	Results	No.	Positive (%)
23	Shelters around dairy, West Merbein, 22.i.1952. Cattle, man, fowls, dogs, etc. possible. Early morning collection, smeared 3.0 p.m.	*Anopheles annulipes*	6	+Man	2	
				+Ox	2	
				+Fowl	1	
				Negative	1	83
24	General shelter collection, cow bail, chicken run, wire screens. North Tuppal, 27-31.i.1952	*Anopheles annulipes*	13	+Ox	5	
				+Fowl	3	
				Negative	5	
		Culex annulirostris	7	+Ox	4	
				Negative	3	60
25	Dairy grounds, Curlwaa, 23.i.1952, collected 3.30 p.m., smeared 5.0 p.m.	*Aedes vittiger*	8	+Man	3	
				+Fowl	1	
				Negative	4	
		Aedes theobaldi	1	+Rabbit	1	55
26	River flat, Merbein, 22.i.1952. From stumps. Collected 11 a.m., smeared 2 p.m.	*Culex annulirostris*	1	+Rabbit	1	
		Anopheles annulipes	7	+Rabbit	7	100
27	Sylvan environment, near river bank, Merbein, 21.ii.1952; tree stumps, hollow logs, and in shelter trap. A few cattle and horses, rabbits not obvious owing to earlier myxomatosis epizootic. Collected 9-11.30 a.m., smeared 2-4 p.m.	*Anopheles annulipes*	46	+Rabbit	40	
				Negative	6	
		Culex annulirostris	9	+Rabbit	9	
		Culex pipiens australicus	6	+Rabbit	6	90
28	Tree stumps, river flats, Cobram, 14, 17, 21, and 30.i.1952	*Anopheles annulipes*	55	+Horse	15	
				+Rabbit	4	
				+Man	1	
				+Fowl	1	
				Negative	34	
		Culex annulirostris	10	+Rabbit	2	
				Negative	8	
		Culex pipiens australicus	3	Negative	3	34
29	Kangaroo Valley (largely ground débris and tree stumps for *A. annulipes* and grasses for *C. p. australicus*) Dec. 1952	*Anopheles annulipes*	9	+Rabbit	8	
				Negative	1	
		Culex annulirostris	1	+Rabbit	1	
		Culex pipiens australicus	17	+Rabbit	16	
				Negative	1	92
30	Colo Vale, 17-19.ii.1953 and 9-12.ii.1953. The great majority taken attracted to human bait	*Anopheles annulipes*	1	Negative	1	
		Anopheles stigmaticus	1	Negative	1	
		Aedes vigilax	2	+Man	1	
				Negative	1	
		Aedes notoscriptus	3	+Marsupial	3	
		Aedes alboannulatus	3	+Marsupial	2	
				+Man	1	

TABLE 1 (*Continued*)

Series	Details of Collection	Species	No.	Results	No.	Positive (%)
		Aedes queenslandis	32	+Marsupial	24	
				+Man	5	
				Negative	3	
		Aedes subauridorsum	1	+Man	1	
		Culex pipiens australicus	2	+Rabbit	1	
				Negative	1	84
31	Tidbinbilla, A.C.T., 4.iii.1953 (Mykytowycz)	*Anopheles stigmaticus*	4	+Marsupial	2	
				Negative	2	50
32	Tree boles, Texas, 26.iii.1952 (Dyce)	*Anopheles annulipes*	5	+Rabbit	5	100
33	Rabbit warrens, Moree (Dyce)	*Anopheles annulipes*	7	+Rabbit	7	
		Aedes theobaldi	1	+Rabbit	1	100
34	Canberra, rabbit warrens, 29.i.1953 (Dyce)	*Anopheles annulipes*	12	+Rabbit	11	
				Negative	1	91

source and no great variety of blood sources in the immediate vicinity. It also gives evidence of the range of hosts utilized by particular species of mosquitoes but does not really indicate any degree of preference since the collections in such habitats cannot be considered a random sample to any significant degree. We have simply exploited the opportunities available to us for collecting such samples and have followed a plan of attempting to elucidate obscurities rather than to emphasize what now appears obvious.

Another special environment giving a consistent result has been rabbit warrens. A series of *Anopheles annulipes* collected from such warrens by K. Myers in the Albury area, totalling 523 spots, has given 93 per cent. positive for rabbit, the rest being negative recordings except for one positive for man. The details of these collections in relation to myxomatosis epidemiology will be reported elsewhere (Myers, Marshall, and Fenner, unpublished data).

(ii) *Farmyard Environments.*—Having disposed of these specific man and animal environments, collections have also been made on a number of farms with a considerable variety of blood sources. These collections have been made in and around the various outbuildings, stables, fowl-runs, dairies, etc. and from the exterior of the residence itself but not from inside the residence. As might be expected, these results are more varied both in range of mosquito species and in blood meal sources, and do perhaps give some indication of actual preference although these still require to be regarded with considerable caution.

Table 2 combines the results of three such collections (21, 22, and 23 of Table 1) for the three common species.

From these figures it is obvious that all three species, *Culex fatigans, C. annulirostris,* and *Anopheles annulipes,* feed readily on domestic avian blood sources and at least a degree of preference is shown for such blood by *C. fatigans.* With the other two, *C. annulirostris* and *A. annulipes,* cattle are also

freely attacked. There is also a suggestion, but only a suggestion, that *C. annulirostris* is rather more partial to avian blood when it is free to choose between domestic poultry and cattle.

The low figures for horse are due at least in part to the scarcity of these animals. Again, all three species are predominantly night biting, and since man would not commonly be present in the extra-residential environment during the biting period few positives for man could be expected.

TABLE 2
RESULT OF FARMYARD COLLECTIONS

Species	Total Spots	Fowl	Ox	Dog	Horse	Man	Negative
Culex fatigans	39	32	2	1	—	3	1
C. annulirostris	58	30	15	3	—	3	7
Anopheles annulipes	79	31	27	2	1	3	15

(iii) *River Flat Environments.*—Collections from river flats would be expected to reveal species commonly feeding on rabbits but other blood sources are usually available, e.g. cattle, sheep, horses, and waterfowl of varied species. River flat collections were made specifically in the hope that species feeding on water-birds would be revealed. This hope remained unfulfilled but other interesting information was gleaned.

TABLE 3
RESULTS OF RIVER FLAT COLLECTIONS

Species	Total Spots	Rabbit	Horse	Fowl	Man	Ox	Negative
Anopheles annulipes	108	51	15	1	1	—	34
Culex annulirostris	20	12	—	—	—	—	8
C. pipiens australicus	9	6	—	—	—	—	3

Series 27 (Table 1) taken from a river flat approximately 2 months after the major myxomatosis epizootic with a seriously depleted rabbit population still gave overwhelming positives for rabbit in the three species collected, *A. annulipes, Culex pipiens australicus,* and *C. annulirostris.* This was our first indication of a blood source for *C. pipiens australicus.*

The combined results of the river flat collections (series 26, 27, and 28 of Table 1) are recorded in Table 3.

These results indicate that the three species listed, *A. annulipes, C. annulirostris,* and *C. pipiens australicus,* all find rabbit a very satisfactory blood source.

(iv) *The Hill Stream Environment.*—The vicinity of smaller running streams in hilly or mountainous country provides collecting sites for a variety of species of mosquitoes. Some of these collections cannot be reported here as the common blood source has not yet been revealed, particularly for species such as *Culex pseudomelanoconia.* The blood source possessed nucleated red cells, and, as no reactions occurred with our relatively non-specific anti-fowl serum, we tend to eliminate the possibility of birds. Further, since the common lizard of these environments is *Sphenomorphus quoii,* we suspect this but as yet have no suitable antisera with which to test this suspicion.

However, one such collection is reported in Table 1 (series 29) and it is interesting to note that here again we have strong evidence of rabbit feeding by *A. annulipes* and *C. pipiens australicus.*

(*b*) *Collections from Human Bait*

We have been particularly interested in the blood sources of such species as *Aedes alboannulatus* and *A. queenslandis* but have so far been unsuccessful in locating any significant numbers of engorged resting females. In collections carried out for recovery of myxomatosis virus from wild caught mosquitoes at Colo Vale by D. J. Lee, R. L. Dyce, R. Mykytowycz, and others, it was noticed that a small percentage of the mosquitoes attracted to human bait were already blood fed. These might have been interrupted feeds on nearby collectors, but the specimens were kept for blood smears, and yielded the most interesting information that a considerable proportion actually fed on marsupials (see series 30 of Table 1).

V. Discussion of Results for Individual Species

In assessing the blood preferences, or strictly the frequency of attack on particular blood sources by individual species of mosquitoes, the results of precipitin tests should be linked with other observations, particularly of observed biting of man or other animals under natural conditions.

Under each species listed below an attempt is made to summarize our existing knowledge of their blood-feeding habits. For the collateral field observations we have drawn only on our own observations. When published, the analysis of the various collections made by W. C. Reeves* and others at Mildura in 1951-52 should give far more information on the activity of many of the species dealt with herein.

In Table 4 is recorded an analysis of all precipitin test results for each species. This table does not take into account the other field data of observed

* This has since appeared: Reeves, W. C., French, E. L., Marks, E. N., and Kent, N. E. (1954).—Murray Valley encephalitis: a survey of suspected mosquito vectors. *Amer. J. Trop. Med. Hyg.* 3: 147-59.

biting on man and other animals which is incorporated in the following discussions.

(*a*) *Anopheles annulipes* Walker

There is no question that this species does bite man, but it has also been reasonably obvious that man is not a preferred blood source, and this probably has some bearing on its lack of importance as a vector of malaria (Roberts 1943). That it is widespread in Australia has long been known, and, although it occurs in urban and rural areas, surveys along hundreds of miles of roadways tended to associate its distribution with cattle rather than with man.

TABLE 4

TOTALS FOR INDIVIDUAL SPECIES

Species	Total Tests	Positive for Man	Positive for Fowl	Positive for Rabbit	Positive for Ox	Positive for Dog	Positive for Horse	Positive for Marsupial	Negative Tests
Anopheles annulipes	778	6	47	576	27	2	16		104
A. stigmaticus	5							2	3
Culex fatigans	372	54	197	1*	2	12	15		91
C. pipiens australicus	62		31	24					7
C. annulirostris	184	3	35	109	15	3			19
Aedes alboannulatus	5	2		1				2	
A. queenslandis	32	5						24	3
A. notoscriptus	3							3	
A. subauridorsum	1	1							
A. theobaldi	4	1	1	2					
A. vittiger	13	6	1		1	1			4
A. alternans	1				1				
A. vigilax	2	1							1
A. bancroftianus	1	1							
Mansonia linealis	1		1						
Totals	1464	80	313	713	46	18	31	31	232

*Accurate identification of this specimen was not made at time of capture. It might have been another member of the *fatigans-pipiens* complex.

Field observations have amply demonstrated a decided propensity for cattle biting on the part of *A. annulipes*. At times of low population density of this species it can usually be detected coming to cattle bait during its biting period, and at Cobram, given the choice of man or cow it attacked the cow 15 times more frequently than man. No attempt has been made to substantiate these field observations with special collections for precipitin tests although in general farmyard collections ox was one of the two most favoured blood sources.

The precipitin test results would suggest that rabbit is the most favoured blood source, but there is a definite bias, in that most positives for rabbit came

from collections made in rabbit warrens. There is no doubt, however, that rabbits form a major source of the blood supply for *A. annulipes*.

Whether or not a preference exists for either cattle or rabbit is of little practical significance since both are so strongly attacked by this species. One point of interest is that the range of this mosquito is practically Australia-wide, as is the range of cattle, whereas rabbits, though widely distributed, are not available in quite large areas of Australia populated by *A. annulipes*.

Another observation of significance is that *A. annulipes* is very frequently found in small numbers resting in fowl sheds. That poultry are a blood source is evidenced by the precipitin tests where in the mixed farmyard environment biting of poultry and cattle is about equal.

Horses are also attacked and it is interesting that collections from typical river flat resting places, exclusive of rabbit warrens, still show a marked predilection for rabbit feeding but where horses have also been present a reasonable recovery of horse-fed specimens has been made.

In conclusion one would presume cattle and rabbits to be the two major sources of blood for *A. annulipes* but where poultry and horses are present within the feeding range of this species they are attacked, but to a lesser degree. Dogs and man are also attacked but to a very significantly lesser degree.

It is also worthy of comment that in a species which we must consider as an indigenous one in Australia its major sources of blood are from animals which have been introduced to the country since the time of white settlement. Whatever trend of speculation this may lead one to, at least there is a strong suggestion that the population level of this species is nowadays considerably greater than it was 150 years ago.

Excluding the close relationship of *A. annulipes* to rabbit warrens, at least in inland areas, recoveries of fed adults have been made in a variety of different places. In the semi-domestic environments, outhouses, animal sheds, and fowl-runs have all been useful. Privies, particularly when they are decidedly odorous, have also been a fairly reliable source. In the sylvan environment cavities in tree stumps, beneath logs, in ground cavities, and débris on the ground have yielded good returns. Other recoveries have been from cavities in rocky creek banks, in grasses and sedges, and under bridges.

(*b*) *Anopheles stigmaticus* Skuse

This species has been used in malaria transmission experiments by Mackerras and Roberts (1947). They recorded that it bit very reluctantly in the laboratory and that much time and patience were needed to induce even a few individuals to engorge on man. We are aware of only three records of this species biting man in the field and we have not observed it attacking any other animals. Obviously man is not a usual source of blood for this species, and, although our precipitin tests record two positives for marsupial blood, we suspect that our range of antisera is not sufficiently wide to reveal other possible blood sources.

Although by no means a common species, engorged females have been collected in rock crevices close to the water in rocky creeks in hilly country, and occasionally in ground débris.

(*c*) *Culex fatigans* Wiedemann

For an adequate understanding of this species we must emphasize its exceptionally strong tendency to bite during the night in houses or other shelters. It is undoubtedly a strongly anthropophilic species and all collections from within houses have been man-fed. Again, as the precipitin tests reveal, it has an equally strong habit of attacking poultry and it is probably even more abundant in suitable poultry sheds than it is in human habitations. Where horses are stabled overnight it will also attack these with reasonable freedom. Again, where dogs are suitably kennelled, dog-fed specimens are recovered. Cattle are not significantly attacked by this species (but we must admit that no collections have been made where cattle have been under a roof overnight) and rabbits apparently not at all.

This is undoubtedly the easiest of all species to collect in the engorged condition since it rests after its blood meal in the shelter wherein biting took place. It can be recovered inside houses, particularly in darker corners and cupboards, but also generally on walls and ceilings in the early part of the morning, and in sheds, outhouses, and especially fowl sheds and stables.

We have seldom observed *Culex fatigans* biting out of doors, or rather outside the confines of some sort of artificial shelter whether it be house, stable, lean-to fowl shed, or motor car. Such shelters are used for resting after feeding but some apparently do not provide ideal conditions throughout the day, especially where sunshine has access to the inside of the shelter. In such cases movement takes place some time after feeding to more suitable diurnal resting places. Many unengorged specimens will also be recovered from suitable diurnal resting places.

We conclude from this that *Culex fatigans*, although having a strong association with domestic birds (sparrows could also be included with these because of the association of *C. fatigans* with the transmission of bird malaria), there is little evidence to suggest that the species comes into contact with native birds and in particular the various species of waterfowl.

(*d*) *Culex pipiens australicus* Dobrotworsky and Drummond

C. pipiens australicus has only recently been differentiated as a distinct entity of the *fatigans* complex although for quite a few years it has been recognized as the sylvan counterpart of the domestic *C. fatigans* (see Dobrotworsky and Drummond 1953).

Until quite recently we had no knowledge of its blood sources, but the present precipitin tests reveal it to be both a rabbit- and fowl-biting form. For the time being emphasis should be placed on rabbit as a blood source since this subspecies is far more commonly taken in sylvan environments than in semi-domestic ones. Nevertheless further study is desirable as it is still possible that other blood sources will be revealed. Although we have recorded it attacking man on three occasions this subspecies is in no sense anthropophilic, nor does it appear to commonly attack the usual range of farmyard animals.

C. pipiens australicus has been recovered as engorged specimens quite rarely in farmyard shelters except perhaps the Albury fowl-run collections of

K. Myers. Resting in tree stumps and similar situations on river flats has been the most successful situation so far exploited. Grasses and sedges have been next but fewer fed specimens are recovered. Ground débris and ground cavities have also yielded small numbers.

(*e*) *Culex annulirostris* Skuse

The precipitin tests reveal that this species has a strong tendency to attack rabbits. They also reveal that poultry and cattle are also attacked with some frequency, but man rather less often. To interpret these results, it is necessary to record that the biting of this species is largely, but not consistently, in the early hours of darkness and usually in the out-of-door and especially the sylvan environment. It does not ignore man but attacks cattle with greater frequency, a ratio of 5 : 1 cattle to man being recorded at Cobram. In the farmyard environment, when this is close to its semi-permanent river breeding grounds, poultry are most frequently attacked, but cattle, dogs, and man are also recorded as blood sources. Field observations have recorded it as attacking cattle, man, rabbits, and cats.

Cattle and rabbits are probably the predominant blood sources, fowls are important in certain environments, and man is rather more frequently attacked than the precipitin test data suggest. Our field evidence would suggest that *C. annulirostris* is a little more anthropophilic than *A. annulipes*.

C. annulirostris is often an obvious component of the farmyard collections, but is not usually taken within dwellings. Most recoveries of engorged specimens have come from tree stumps on river flats; occasionally it has been taken from grasses and sedges and in ground débris.

(*f*) *Aedes alboannulatus* (Macquart)

Most specimens of this species are taken actually biting man when man enters its environment, especially bushy gullies. We have suspected that man is not an important blood source for this species in nature and it is most interesting to record the two tests positive for marsupial blood (Table 4).

This species very occasionally enters houses when these are in close proximity to bush, and fed adults have been recovered on one or two occasions. Otherwise we have failed to collect engorged specimens in field resting places although occasional blood-fed specimens are taken attracted to man.

One precipitin test for this species, forwarded by A. L. Dyce from Moree (not recorded in the foregoing tabulations) gave a double positive reaction to man and rabbit. This and one other are the only definite records we have of this species feeding on rabbits as revealed by precipitin tests. Double feeds have been rare in our experience but it does agree with our observations elsewhere that engorged females may still attempt to bite a second time, and are still responsive to a blood source such as man coming within their range of detection even when at least partially engorged.

This species has also been observed attacking a cow and a dog but both of these are single observations. Recently, in trapping experiments, we have observed this species attacking caged rabbits in the field.

(*g*) *Aedes queenslandis* (Strickland)

This species is similar in most respects to *A. alboannulatus*. It occupies roughly the same area of distribution, its breeding habitats are similar, and it is closely related morphologically.

It is also taken most frequently attacking man but again we have suspected that man is not its normal blood source. From our collections of this species attracted to man as bait we have quite strong evidence that it is predominantly a marsupial-feeding species. The strength of the observed reactions in our precipitin tests with the non-specific anti-possum serum and the habitat in which the specimens were collected would suggest, but by no means conclusively, that the actual blood source would be possums or phalangers. Rabbits are also attacked by this species as we have observed on caged rabbits exposed in the field.

Again we failed to discover characteristic resting places for engorged individuals of this species.

(*h*) *Aedes notoscriptus* (Skuse)

This species is well known to be anthropophilic in the domestic environment. It is interesting to record that we have also found it to be a marsupial feeder. Our field observations have also disclosed it biting dogs and cattle.

In the data before us we have no significant records of the resting places of this species. Resting inside houses after feeding does occur but is in no way comparable to the feeding activity.

(*i*) *Aedes subauridorsum* Marks

An uncommon species, *A. subauridorsum* is very occasionally taken attacking man in the bush. The one positive precipitin test recorded for this species was apparently one taken immediately after feeding on man. No resting places are known for this species.

(*j*) *Aedes theobaldi* (Taylor)

Although only a total of four fed specimens have been taken for precipitin tests on this species, giving positives for man, fowl, and rabbit, many field observations of its biting have been made.

A. theobaldi is predominantly a day-biting mosquito although in certain areas climatic circumstances tend to divert peak biting to the early evening. It attacks obvious blood sources such as man, cattle, horses, sheep, and dogs with equal avidity but although it attempts to bite poultry it is seldom successful in daylight. Cats are also attacked. In our Cobram records where significant differences were recorded for biting rates of other species as between cattle and man, there was little evidence of cattle being more attractive than man to *A. theobaldi*.

This species obviously lacks discrimination in its blood-feeding but we do not know whether it attacks birds to any significant degree.

Recently emerged specimens fly up from grasses to attack animals or man, others at times appear to descend from low trees. Blood-fed adults have

seldom been taken and we have been unable to disclose any characteristic resting places of such individuals.

(*k*) *Aedes vittiger* (Skuse)

Attacks on man by this species are well known and in many respects its behaviour is similar to that of *A. theobaldi*. It frequently rises from rank grass to attack man and other animals during the day but may also be very active after dark. It readily follows man into motor vehicles and often travels long distances in this way.

Our precipitin tests reveal that it has been recorded biting man, ox, dog, and fowl and our field observations have shown it attacking horse, sheep, and cat as well. It attacks principally in the sylvan environment and is rarely taken resting in shelters except when these are close to its breeding grounds. Recovery of fed adults in the field has not been satisfactory, most specimens taken having recently attacked the collector. Again we have no knowledge of the extent to which birds are attacked by this species.

(*l*) *Aedes alternans* (Westwood)

Only one precipitin test has been done for this species, giving a positive for ox. Nevertheless *A. alternans* is widely known as a pest of man, cattle, and horses. It attacks viciously in the vicinity of its breeding grounds during the day and in the early evening bites in the open and occasionally on open verandahs. Whether or not birds are attacked is unknown.

(*m*) *Aedes vigilax* (Skuse)

Again a pest of man, cattle, and horses wherever it is abundant and a vicious day and evening biter, it would seem likely that with both *A. vigilax* and *A. alternans* some other blood source is utilized. The possibility of the estuarine bird fauna exists but we have no evidence one way or another on this point. The single positive precipitin test for man is recorded for the sake of completeness of the Colo Vale records.

(*n*) *Mansonia linealis* (Skuse)

Until recently little has been known about this species other than that it does attack man from time to time. Our single positive precipitin test reveals that it will attack fowls and at Kangaroo Valley it was observed attacking man and dog with approximately equal avidity.

VI. Epidemiological Conclusions Related to Biting Activity

The use of precipitin tests on the stomach contents of engorged mosquitoes has certain limitations. Firstly, one does not always have as wide a range of antisera as one would desire, and secondly, some species of mosquitoes are so elusive that it is difficult to obtain satisfactory material for precipitin testing.

Nevertheless, when precipitin tests are used in conjunction with observations of biting in the field, a useful body of information is amassed. The pre-

cipitin tests themselves are most useful in proving what is otherwise suspected, in arriving at an appreciation of the range of hosts favoured by night-biting mosquitoes, and in giving clues to the unsuspected biting activity of species whose habits are otherwise imperfectly known.

To use precipitin tests as a tool in the elucidation of mosquito blood-feeding requires deliberate collecting of particular species in particular environments. In this we consider we have had some measure of success, but there are some species and some environments which so far we have not had suitable opportunities to investigate.

In particular, we have not been able to gather any useful information on the mosquitoes attacking wild birds, and especially water-birds. In this connection, we would like to record that *Culex globocoxitus,* a common, perhaps dominant, riverine species of the Murray Valley in South Australia, inhabits an environment shared equally by many waterfowl. We consider it highly probable that *C. globocoxitus* is ornithophilous, an opinion also expressed by Dobrotworsky (1953).

Another species which may have some association with birds in permanent swamp or riverine habitats is *Mansonia linealis,* which we have occasionally taken resting in willow trees on river margins.

Apart from these, the species which we have been able to collect in riverine environments, namely *Anopheles annulipes, Culex annulirostris,* and *C. pipiens australicus,* have yielded no evidence (with the exception of a single avian positive for *A. annulipes*) that birds are utilized as a blood source in this type of environment, despite the fact that all three attack domestic poultry. Precipitin testing of engorged individuals from typical waterfowl environments is required to establish whether these species do feed on such birds.

If, as seems more than probable, mosquitoes do act as vectors of the virus of Murray Valley encephalitis from bird to bird, then any of the species mentioned above might be implicated. If *C. globocoxitus* were disclosed as a vector, then the virus would have, as far as we now know, little if any contact with man and the animals or birds in his domestic environment. With the others, however, contact with the domestic environment could be established from time to time, particularly with *A. annulipes* and *C. annulirostris,* and to a considerably lesser extent, because of its more patchy distribution, *M. linealis.* Again, neither the latter nor *C. pipiens australicus* can be considered characteristic members of the farmyard or semi-rural environment, in so far as our observations have disclosed, despite the common occurrence of breeding of *C. p. australicus* in semi-rural areas, whereas *A. annulipes* and *C. annulirostris* are commonly and widely found under such circumstances. However, since McLean (1953) has shown that *A. annulipes* is not hospitable to Murray Valley encephalitis virus this species can be ignored.

The elucidation of the bird-biting complex of mosquitoes is of importance both for an understanding of the full cycle of Murray Valley encephalitis and of the transmission of various fowl pox viruses. If we can disclose the species commonly attacking birds, then selective collections for virus recovery can be made when it is suspected or known that one or other of these viruses is active.

In both cases a basic working hypothesis is that bird-to-bird transmission occurs through the agency of those mosquitoes most frequently attacking birds and that from time to time a break-through to domestic birds is occasioned by mosquitoes having opportunity to attack both wild and domestic birds. Then, since it has been shown to be capable of transmitting the virus (McLean 1953), there is no doubt that *Culex fatigans* must be the primary suspect for transmission of Murray Valley encephalitis amongst domestic poultry, and a strong suspect for the transmission of fowl pox. In Murray Valley encephalitis it must be equally the primary suspect for transmission to man.

Especially in Murray Valley encephalitis such a conclusion is not fully justified on the evidence of biting habits alone. We must also take into account certain other types of evidence, as follows:

(*a*) McLean (1953) has shown 11 species of mosquitoes to be hospitable to M.V.E. virus. These are *Culex annulirostris, C. fatigans, C. globocoxitus, C. pipiens australicus, C. pipiens molestus, Aedes camptorhynchus, A. queenslandis* (as *A. occidentalis*), *A. notoscriptus, A. theobaldi, A. vigilax,* and *A. vittiger.* Of these at least *C. annulirostris, C. fatigans, A. queenslandis,* and *A. vigilax* are also capable of transmitting the virus to chickens (McLean 1953). This is strong evidence that there is not a high degree of specificity established between virus and mosquito, at least within a wide range of culicine species. This is closely parallel to the findings for Japanese *B* encephalitis for which Reeves and Hammond (1946) found four species of *Culex,* four of *Aedes,* and two of *Culiseta* to be successful laboratory vectors. Hence the general belief that a complex of vectors is involved in the transmission of M.V.E. is not unjustified on existing evidence.

(*b*) The observations of Anderson *et al.* (1952) and Miles and Howes (1953), which both arrive at a 20 per cent. infection rate among humans in the Murray Valley, indicate a very high degree of transmission to man when one considers that the period of viraemia in birds is likely to be short and non-recurring. In fowls a period of 3 days is given by Anderson *et al.* (1952) and up to 8 days for the silver gull (*Larus novohollandiae*) by Miles (1952). Such figures lead us to believe that a 20 per cent. infection rate in man is high and can only be occasioned by a species of mosquito in close contact with domestic poultry and man rather than by species casually fulfilling this association.

C. fatigans then is undoubtedly the primary suspect, and failing the disclosure of other pertinent information in the meantime, it must be the species against which active measures should be taken in order to break the cycle of transmission to man in future outbreaks.

A further observation of interest is the even higher percentage of humans showing antibody to Murray Valley encephalitis recorded for the Northern Territory, the figure recorded by Miles and Howes (1953) being 26 per cent. It seems most unlikely that the same cycle of transmission to man would obtain in the Northern Territory aborigines as in whites in the Murray Valley area.

A final point to note is the high percentage of infection in horses within the outbreak area. A figure of 50 per cent. or more is given by both Anderson *et al.* (1952) and Miles and Howes (1953). It would appear that horses are attractive to mosquitoes likely to have fed on other hosts for the virus and hence in the choice of animal baits for collecting mosquitoes for virus recovery during the course of an outbreak, horses should perhaps be given preference over cattle.

The view expressed above, that *C. fatigans* has merited first consideration as the vector route of Murray Valley encephalitis to man, is perhaps contrary to the views expressed by Burnet (1952) and Anderson *et al.* (1952), who place more emphasis on the role likely to be played by *C. annulirostris.* This change of emphasis is due to the well-established fact that man is extensively attacked by *C. fatigans* on the one hand and that on the other we have little evidence to support a view that man is sufficiently frequently attacked by *C. annulirostris* to produce an infection rate approaching 20 per cent. Nevertheless, further study of the biting activity of *C. annulirostris* is highly desirable since important differences in possible control measures are involved.

With fowl pox so little is known of the incidence of the disease and its occurrences in birds other than fowls that there are few data on which to base speculation. An obvious possibility for fowl-to-fowl transmission is *C. fatigans* but the apparent seasonal limitation of the disease to autumn in the Sydney area would perhaps tend to implicate *A. annulipes.* Such a suggestion is contingent on the seasonal incidence being related to vector activity which in any case is a very precarious assumption, since the determining factor might just as readily be the availability of susceptible birds in late summer and autumn.

However, a study of fowl pox virus and the means by which it comes into contact with poultry may lead to observations of considerable interest in the understanding of the avian cycle of Murray Valley encephalitis.

An entirely different problem is involved in the transmission of myxomatosis. Here we are only concerned with the movement of the virus from one rabbit to another, and, since the transmission of the disease is mechanical (Fenner, Day, and Woodroofe 1952), any mosquitoes likely to bite rabbits twice are highly presumptive vectors. The evidence presented strongly implicates *A. annulipes* and also *C. annulirostris* and *C. p. australicus.*

We have already mentioned the close association of *A. annulipes* with cattle, and there is a possibility that transmission of ephemeral fever of cattle might be more closely linked with this species than was earlier suspected (Mackerras and Mackerras 1940). Nevertheless, the previous guess that sandflies (Ceratopogonidae) might be the transmitting agent still remains a distinct possibility.

Finally, the demonstration of *A. annulipes* as having a wide range of blood sources, with particular preference, where available, for cattle and rabbits, does confirm the opinion of most workers as to why it so seldom acts as a vector of human malaria despite its being a satisfactory experimental one (Roberts 1943).

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