IDENTIFICATION OF BLOOD MEALS OF BLACKFLIES (DIPTERA: SIMULIIDAE) ATTACKING FARM ANIMALS [1]

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The development of serological methods to determine the sources of blood meals of haematophagous insects has stimulated studies of host preference and multiple-feeding habits of biting flies in many parts of the world. Such studies have been conducted chiefly in connection with problems of disease transmission. There are, however, few records of attempts to study the host ranges or host preferences of biting flies in Canada. Rempel *et al.* (1946) reported precipitin-test studies of the feeding habits of *Aedes* mosquitoes in Saskatchewan, and Downe and West (1954) gave a brief account of the blood meals of *Aedes hexodontus* Dyar at Churchill, Manitoba. We have found no published accounts of serological studies of the blood meals of Canadian species of Simuliidae.

This report describes preliminary studies on determining the blood meals of blackfly species attacking domestic animals on a farm near Rapides des Joachims, Quebec, during the spring and summer of 1954.

COLLECTION OF BLACKFLIES. Blood-engorged blackflies were collected in window traps in a one-room barn housing horses, cattle, pigs, and chickens. The traps were patterned after the designs of various authors and have been illustrated by Peterson (1955). While the blackflies were abundant, specimens were removed from the traps early in the morning and at dusk. Later collections were made once daily. Tests had shown that blood meals of blackflies can be determined by the precipitin test when they have remained in the guts of the living insects not more than 24 hours. The specimens used in the test included only blood-engorged individuals that were collected during 24-hour periods when exposure to blackflies was about equal for the animals. Specimens removed from the traps were killed with ether or chloroform, and about half of them were mounted on insect pins, the remainder being smeared on pieces of filter paper. All pinned specimens were determined to species with the assistance of Mr. G. E. Shewell, Entomology Division, Ottawa. Smeared specimens were classified only as blackflies.

SEROLOGICAL METHODS. Preparation of Antiserum. Antisera were prepared in healthy adult rabbits by injecting the sera of various domestic animals and of humans by the method of Proom (1943). Seven to 10 days after inoculation, each rabbit was trial-bled from the ear, and the serum tested for antibody sensitivity with the homologous antigen as described by Eligh (1952). The titre was determined as the highest dilution of serum antigen that gave a positive precipitin-ring test with the constant dilution of antiserum. If titres from trial bleedings were sufficiently high (1:5,000 or higher), larger quantities of blood were collected from the rabbits by ear bleeding or cardiac puncture. After the blood had clotted, antiserum was removed, sterilized by Seitz filtration, and stored in sterile bottles at —25° C. until required for testing the biting flies. Many of the antisera were observed to be nonspecific: anti-horse serum often reacted with dilutions of pig serum and beef serum, and anti-pig and anti-horse sera gave cross reactions also (cf. Weitz, 1952). The extent of cross reactions was determined for a sample of each antiserum, and cross reactions were eliminated by absorption techniques described by Weitz (1952). The anti-human serum did not react with the antigens of species other than man. Anti-chicken

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serum produced in rabbits is nonspecific and detects all avian antigens (Downe and West, 1954; Eligh, 1952). No attempt was made to render the anti-chicken serum specific, but no cross reactions with mammalian sera were observed.

PRECIPITIN TESTING OF BITING FLIES. Extracts were prepared from the dried blackflies mounted on pins as well as from the filter-paper smears. The insects were removed from the pins and crushed with sterile forceps in 5-ml. beakers. One millilitre of physiological saline was added to each beaker and extracted for three to four hours at room temperature. The extracts were transferred in 0.05-ml. amounts to small tubes (3 mm. in diameter by 25 mm. long) so that each extract was tested for horse, beef, pig, chicken, and human blood. Antisera were dyed with safranine "O" as described by Herndon and Schubert (1951) before being layered beneath extracts. A positive reaction was indicated by the appearance of a precipitin ring at the interface between the extract and the antiserum. The smears were extracted and tested in a similar manner.

The precipitin testing of dried specimens (Amaral and Aguiar, 1950) has some advantages over the use of filter-paper smears. The smears must be prepared shortly after the insects have been trapped in the field whereas pinned specimens may be retained for identification. This is of particular value with species difficult to identify in the field. Our tests showed that the results obtained with dried specimens are as accurate as those with smears.

RESULTS AND DISCUSSION. The data in

TABLE 1.—Numbers of blackfly species that gave positive precipitin reactions for various domestic animals.* The blackflies were collected during 24-hour periods from a one-room barn housing the animals.

Species	Animals in barn: 2 Horses 1 Cow 2 Periods	2 Horses 2 Cows 4 Chickens 6 Periods	2 Horses 4 Cows 2 Pigs 14 Chickens 17 Periods	2 Horses 3 Cows 2 Pigs 14 Chickens 3 Periods	Totals
Simulium vittatum Zett.	29 H	58 H	201 H	19 H	307 H
		3 B	59 B	1 B	63 B
			2 P		2 P
S. venustum Say	17 H	59 H	164 H	26 H	266 H
		11 B	42 B	6 B	59 B
		1 X			1 X
S. parnassum Mall.		9 H	43 H	7 H	59 H
		2 B	16 B	1 B	19 B
S. decorum Wlk.			24 H	3 H	27 H
		1 B	26 B	5 B	32 B
S. tuberosum Lund.			5 H	1 H	6 H
		1 B	3 B		4 B
			1 F		1 F
S. corbis Twinn			5 H		5 H
			2 B		2 B
	1 X		1 X		2 X
S. rugglesi N. & M.	1 F				1 F
Prosimulium hirtipes (Fries)	2 H		27 H	4 H	33 H
	2 B		16 B		18 B
			1 P		1 P
Cnephia mutata (Mall.)			8 H		8 H
			6 B		6 B

* B, beef; F, bird; H, horse; P, pig; X, undetermined feeding.

Table 1 (the results for the pinned specimens) show that pigs and chickens were seldom attacked when horses and cattle were also present. *Simulium vittatum* and *S. venustum* attacked horses much more frequently than cows, and most of the other species attacked horses more frequently than the other animals. No positive reactions were obtained with anti-human serum; humans were in the barn only for short periods while handling the livestock or collecting specimens. The small number of undetermined feedings (0.3 percent) suggests that there was little possibility of other hosts, such as small game or wild birds, being involved.

None of the specimens tested contained blood from more than one host, indicating that multiple feeding may be uncommon among blackflies. Tests of blackfly smears appear to support this statement. Of 750 smears tested, 518 were positive for horse, 221 for beef, and 5 for pig; 5 gave no reaction and only one was positive for both beef and pig. Blackflies are not readily disturbed once they start feeding and tend to remain on one host until fully engorged.

The results suggest that certain blackflies have decided host preferences and that pigs and chickens are rarely attacked by any of the species listed in Table 1. For some of the species, the numbers collected were too small to attach much significance to the findings. Records of blackflies attacking pigs are found in the literature, and, in western Canada, Rempel and Arnason (1947) reported that *Simulium arcticum* Mall. will attack pigs but prefers cattle and horses.

Several of the species listed in Table 1 have been recorded as feeding on birds. *Simulium rugglesi* is a well-known bird feeder and the role of this blackfly in disseminating *Leucocytazoon simondi* M. & L. in ducks and geese in Canada has been discussed by Shewell (1955). Fallis *et al.* (1951) reported that *Simulium venustum, S. vittatum, S. decorum, S. tuberosum, S. parnassum,* and *Prosimulium hirtipes* can be induced to feed on ducks in the laboratory. The present studies show that all these species except *S. rugglesi* obtain blood meals from mammals even when birds are present.

Some approaches to studies on host preferences of blackflies are made difficult by certain habits of the insects. They appear to be very sensitive to unnatural conditions, and attempts to induce them to feed on domestic animals in cages or in bait traps were largely unsuccessful. Blackflies (particularly *Simulium vittatum, S. venustum,* and *S. decorum*) readily entered animal-baited traps through baffle entries, but once inside they ignored the host and searched for a means of escape.

SUMMARY. The blood meals of blackflies attacking domestic animals on a farm near Rapides des Joachims, Quebec, during the summer of 1954 were determined by the precipitin test. Horses and cattle were attacked by all but one of the species studied, but pigs and chickens were seldom bitten. Of 1,672 specimens tested, only one (a smeared, unidentified specimen) had fed on more than one host. Some limitations of host-preference studies of blackflies are discussed.

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