Use of the Pheasant (*Phasianus torquatus*) to Produce a Specific Anti-Chicken Serum.* (27528)

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In recent studies, the chicken was shown to be more effective than the rabbit for production of specific precipitating antisera for identification of bird bloods(1). There has been a serious need for such antisera to allow studies of the host feeding range and preference of aviophilic mosquito vectors of an increasing number of viruses for which birds serve as natural hosts(2). The frequency of mosquito feedings on suspected hosts can best be determined by identifying the source of blood in the stomachs of known suspected vectors. While satisfactory antisera for identification of bird bloods can be prepared in chickens(1) it is equally important to develop a specific antiserum for identification of chicken blood as this species is included in vector feeding ranges(3-5).

Wolfe and Dilks(6) indicated that of 7 avian species studied, only the pheasant and owl approached the chicken in its ability to produce precipitins. The present study evaluates the pheasant's ability to produce antibodies to chicken serum.

Materials and methods. Adult male and female pheasants (*Phasianus torquatus*) were obtained from the California State Fish and Game Dept. Birds were inoculated with chicken serum by one or more of the following schedules: (1) Primary series—2 intravenous injections of 3.0 ml, given 3 days apart. (2) Secondary (one month after the primary), and (3) Tertiary series (one month after the secondary)—2 intramuscular injections of 3.0 ml emulsified with an equal volume of Freund's incomplete adjuvant, given 3 days apart.

All birds were trial bled from the wing vein daily from the sixth day through the tenth day following the second primary injection, and if the sera reacted to a 1:10,000 dilution or higher of chicken serum, the pheasants were exsanguinated from the heart. If the homologous antibody titer was sufficiently high on the eighth day, it was tested for cross-reactivity with sera of other gallinaceous species. In preliminary experiments, the majority of pheasants had peak titers on the eighth day following the last primary injection.

The 1.0 ml flocculation reaction mixture contained 0.25 ml of antiserum, 0.25 ml of varying concentrations of antigen diluted in 8% NaCl, and 0.5 ml of 11.5% NaCl. The final salt concentration was 8%(7). The end point of antigen-antibody reaction was determined by observing flocculation or turbidity in the tubes after 3 hours incubation at 37°C. The flocculation test was used because of its ease. Titers correlated closely with interfacial tests which are used for the identification of arthropod blood-meals.

Results. Antibody titers to chicken serum ranging from 1:400 to 1:20,000 were obtained in pheasants (Table I). Titers of 1:10,000 and 1:20,000 were reached in some birds following the primary series of inoculations. Only 3 sera cross-reacted in low titer to heterologous serum antigens of other gallinaceous birds, regardless of the intensity of the injection schedule. No cross-reactivity was observed with a non-gallinaceous (goose) serum.

Discussion. Pheasants produce antibodies to chicken serum in sufficient titers for use in the identification of mosquito blood-meals. Lee *et al.*(5) set a minimum standard of a titer of 1:8,000. Weitz(8) reported that a titer of 1:20,000 must be obtained in rabbits to be acceptable for identification of mammalian bloods. Such titers have been obtained in this study. However, Weitz's antisera had to be absorbed prior to use and this usually resulted in a fall of titer, even though it was not "appreciable." The antisera produced in this study would not require absorption as the initial dilution of a mosquito blood-meal would be sufficient to eliminate any nonspe-

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TABLE I. Specificity Tests and Titers of Precipitating Antibodies Produced in Pheasants Inoculated with Chicken Serum.

Sex of animal	Inoculation series*	Titer†			
		Chicken	Chukar Partridge	Valley Quail	Turkey
♂	A	4,000	0‡	0	0
	B	10,000	0	0	0
♀	A	4,000	0	0	0
	B	10,000	0	100	0
♂	B	4,000	0	0	0
♀	A	10,000	0	0	0
♂	A	10,000	0	0	0
♀	A	4,000	NT	NT	NT
	B	4,000	200	100	100
♂	A	400	NT	NT	NT
	B	1,000	NT	NT	NT
	C	4,000	0	0	0
♀	A	1,000	NT	NT	NT
	B	4,000	100	0	0
♂	A	4,000	0	0	0
	B	4,000	0	0	0
♀	A	10,000	0	0	0
♂	B	1,000	NT	NT	NT
♀	A	20,000	0	0	0

* A—Primary series.
B—Secondary "
C—Tertiary "
† Reciprocals of numbers in table.
NT—No test.
‡ Titration started at 1:100.

cific reactions. The cross-reactivity observed to other gallinaceous blood sera in the pheasant was less than that observed to other avian species with antisera produced in chickens(1).

There are numerous accounts of mosquito feedings on fowl based on field observation and serological testing(4,9,10,12). However, most of these reports of serological tests can be questioned. Lee *et al.*(5) reported field observations of feedings on fowl and positive serological tests with their antifowl serum. Their antisera cross-reacted with duck, rosella (*Platycerus eximius*) and dove. Other investigators(11,12) also stated that their antisera were nonspecific when they tested for fowl feedings. The only report of the use of a chicken specific antiserum is that of Hammon and Reeves(10), which indicated mosquito feedings on this species.

There is ample evidence of the need for specific antisera to bird bloods. Use of the pheasant and chicken will allow the preparation of a complete set of antisera for identification of mosquito blood-meals of suspected avian origin. As with the absorbed antisera to mammalian blood serum(8), these sera will be specific enough to distinguish between different bird species or groups(1,13). Clarification of the bird feeding habits of mosquitoes is important in understanding the full cycle of a number of mosquito-borne diseases.

Summary. 1. Pheasants produce high titers of antibody to chicken serum. 2. This antiserum is highly specific and can be used in identification of mosquito blood-meals.

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