Avian Leukosis-Sarcoma Virus Antibodies in Wildfowl, Domestic Chickens and Man in Kenya[1] (37513)

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Earlier investigations (1) demonstrated the presence of antibodies for avian leukosis-sarcoma viruses (ALV) of subgroup A in chickens, wildfowl, and man in three different areas of Kenya. The present investigations were conducted to determine if antibodies to viruses of the other subgroups, B, C and D, also occur in the same fowl and human populations by retesting these sera with additional viruses. This is of interest not only because of the various origins of these viruses but because the sites studied in Kenya presented three different ecological situations with reference to the opportunities for contact between domestic chickens, wildfowl and man. In addition the wildfowl studied differed in their natural tendency to form closely associated flocks.

Materials and Methods. Serum specimens from wildfowl and human subjects were collected in three different areas of Kenya: (a) Longonot Farm, a commercial poultry farm near Lake Naivasha; (b) Makindu health center district and (c) Mashuru health center district—Selengai game preserve area. The human subjects were laborers at Longonot farm, persons coming to the Makindu and Mashuru health centers for minor injuries and members of the collecting crew who were from various areas of Kenya. No obviously ill or febrile patients were included.

Rous sarcoma viruses (RSV) used for detection of antibodies to subgroups A, B, C and D were respectively: RSV (RAV-1), RSV (RAV-2), Bratislava RSV-C and RSV (RAV-50).

Sera were tested for neutralizing capacity by the tissue culture technique previously described (2) using tissues of embryonated eggs derived from a flock of leukosis-free chickens maintained by Dr. R. E. Luginbuhl and supplied by the research resource program of the National Cancer Institute. All sera were tested at a final dilution of 1:20. The neutralization index was calculated by subtraction of the log of the average number of focus forming units (FFU) per petri dish inoculated with 0.1 ml of the test virus-serum mixtures from the log of the average number of lesions produced with RSV and normal serum (ca 100 FFU). The neutralization index was considered significant only if it was 0.7 or greater (2).

The tissue culture dishes were examined at 48 hr and 10 days for evidence of toxic effects on the cells due to the particular serum–virus mixture added, and two sera which showed such properties were discarded.

Results. Antibodies for subgroups A, B, C and D in various sera. Antibodies for subgroup A were more prevalent than subgroup B in the chickens. Antibodies to D subgroup were less common and only one bird had antibodies in subgroup C (Table I). About one fifth of the chickens had no antibodies to any subgroup. There were no significant differences in results between the various areas where the chickens were penned or allowed to run free. The guinea fowl, francolin and bustards had an even higher incidence of antibodies with a predominance like the chickens for subgroups A and B, several with D antibodies and only one bird, a guinea

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TABLE I. Antibodies for Groups A, B, C and D Rous Sarcoma Viruses in East African Chickens, Wildfowl and Man.

Area	Serum	Subgroup A[a]	B	C	D	None
		No. with RSV antibody / No. tested				
Naivasha (Longonot farm)	Human	0/25	7/25	0/25	2/25	18/25
	Domestic chickens (commercial farm)	8/13	9/13	0/13	3/13	4/13
Makindu	Human	0/21	1/21	0/21	2/21	18/21
	Chickens (village-free)	9/10	1/10	1/10	1/10	1/10
	Guinea fowl	8/9	7/9	1/9	5/9	0/9
	Francolins	8/8	8/8	0/8	2/8	0/8
	Bustard	1/1	1/1	0/1	1/1	0/1
Mashuru (Selengai)	Human	0/15	2/15	0/15	1/15	12/15
	Chickens (local store-penned)	5/6	4/6	0/6	3/6	1/6
	Bustards	4/4	3/4	0/4	1/4	0/4
	Ostrich	1/7	1/7	0/7	0/7	6/7
Various areas (collecting crew)	Human	0/7	1/7	0/7	1/7	6/7
		Summary of results				
All areas	Human	0/68	11/68	0/68	6/68	54/68
	Chickens	22/29	14/29	1/29	7/29	6/29
	Guinea fowl	8/9	7/9	1/9	5/9	0/9
	Francolin	8/8	8/8	0/8	2/8	0/8
	Bustards	5/5	4/5	0/5	2/5	0/5
	Ostrich	1/7	1/7	0/7	0/7	6/7

[a] Neutralization indices for sera diluted 1:20 were considered negative if less than 0.7 log.

fowl, with C antibodies. The ostrich showed only one bird positive which had antibodies for A and B subgroups.

The unexpected finding was the considerable number of human subjects with antibodies to subgroups B and D. The individuals working on the poultry farm showed the largest number of positive reactions but three Masai villagers from Mashuru–Selengai had antibodies to subgroups B or D though this tribe does not keep poultry in their villages and no chickens were in the nearby area which was a large game preserve.

Antibodies for subgroups A, B, C and D in single individuals. It was of interest to examine the incidence of antibodies to the various subgroups in a single individual (Table II). More than half of the chickens with antibodies were positive for more than one subgroup with A+B and A+B+D being predominant but only one chicken was positive for all four subgroups. This pattern of distribution of antibodies was also the most common for the gamefowl as well. Subgroup C and D antibodies were found only in association with antibodies to other subgroups but only one bird of all the gamefowl was positive for A, B, C and D.

In contrast with the fowl, the distribution of antibodies in man revealed that most had antibodies to only B or D with only 3 individuals showing antibodies to both.

Discussion. The results of this study and those of two previous investigations (1, 2) establish that infection with avian leukosis-sarcoma viruses is widespread in the wildfowl of East Africa. In Malaya investigations of Weiss and Biggs (3) have shown that antibodies to ALV of subgroups A and B occur in both feral red jungle fowl and domestic

TABLE II. Antibodies for Groups A, B, C and D Rous Sarcoma Viruses in a Single Individual.

Serum	A[a]	B	C	D	Virus subgroup AB	BD	ABC	ABD	ABCD	None
	No. with antibody / No. tested									
Human	0/68	8/68	0/68	3/68	0/68	3/68	0/68	0/68	0/68	54/68
Chickens	9/29	1/29	0/29	0/29	6/29	0/29	0/29	6/29	1/29	6/29
Guinea fowl	2/9	0/9	0/9	0/9	2/9	0/9	0/9	4/9	1/9	0/9
Francolin	0/8	0/8	0/8	0/8	6/8	0/8	0/8	2/8	0/8	0/8
Bustards	1/5	0/5	0/5	0/5	2/5	0/5	0/5	2/5	0/5	0/5
Ostrich	0/7	0/7	0/7	0/7	1/7	0/7	0/7	0/7	0/7	6/7

[a] Neutralization indices for sera diluted 1:20 were considered negative if less than 0.7 log.

fowl. These findings indicate that these viruses maintain themselves in natural settings and are not dependent on the special conditions of commercial poultry husbandry including housing, diet, immunizations and breeding control.

Close contact in closed quarters was not related to the prevalence of ALV infection since though all chickens were forced to roost in coops at night in the areas studied to protect them from predators, the guinea fowl and francolin roosted in trees and the bustards lived a solitary existence coming together only briefly at the nesting season. Antibodies to ALV were comparable in all these birds. In sharp contrast, infection with Marek's disease virus, which is spread by contact, was found in other studies on the same fowl populations in Kenya (4) to occur only in the domestic chickens. Similar findings were reported by Weiss and Biggs (3) who found that less than 10% of feral red jungle fowl in Malaya had antibodies for Marek's disease virus while all domestic breeds were positive.

The finding that 14 of 68 human subjects had antibodies to subgroup B or D or B+D was unexpected even though a previous study (1) had shown that sera of two individuals had antibodies for subgroup A (these two sera were not available for retesting in the present study.) Other investigations (5–7) have failed to identify ALV antibodies for subgroup A in sera of adults or children in the United States, some of whom had received live virus vaccines contaminated with ALV. The present studies have eliminated the possibility that the neutralizing effect of these human sera could be due to toxic properties for cells used in the assay (3, 7) since the sera did not cause such an effect and in 11 instances the neutralization was limited to a single virus in a combined test. This latter finding also eliminates the possibility of other nonspecific virus neutralizing activity and shows that the antibodies are specific.

If antibodies in these human subjects are acquired as a result of contact with infected fowl, those on Longonot farm could have had significant contact with domestic chickens and they had the greatest number with antibodies. However, antibodies also were found in individuals in Makindu where exposure was limited to the few chickens in the village as well as in three Masai who lived in a large game preserve (Selengai) in which no chickens were kept. The inhabitants of Makindu and Selengai were living in areas where francolin and guinea fowl were found in significant numbers which could provide a source of infection.

The occurrence of antibodies to subgroups B and D in the same serum specimen could be due to cross reactions (3, 8) but since 3 human sera neutralized only subgroup D, this does not account for the presence of antibodies in human sera to subgroup D. Serological cross reactions could be responsible for the neutralizing action of the fowl sera for group D but 17 fowl sera that were capable of neutralizing subgroups A and B

at high titers (neutralization index > 1.0) did not neutralize subgroup D virus.

No sera neutralized subgroup C alone so that the two sera which neutralized both C and the three other viruses are suspect. Subgroup C ALV is probably not present in Kenya and the studies of Weiss (3) also failed to find evidence for its presence in Malaya. This raises interesting questions about the occurrence of subgroup C viruses in nature.

These data and those of Weiss (3) suggest that ALV are of ancient origin in nature and have evolved and spread with the domestication of wildfowl. Viruses of subgroups A and B appear to be the most common in the wild in Kenya as they are in Malaya (3) but the evidence also suggests that subgroup D may occur in Kenya as well. Subgroup C appears to be absent in Kenya as well as Malaya.

Summary. The occurrence of infections with one or more subgroups of avian leukosis-sarcoma viruses (ALV) was indicated by the demonstration of neutralizing antibodies for these viruses in wildfowl and domestic chickens. These infections persist under natural conditions in the African bush among wildfowl as well as in chickens maintained in isolated villages or a commercial poultry farm. Infections with ALV subgroups A and B were common and some evidence was obtained for infection with subgroup D but subgroup C viruses appeared to be absent. Antibodies for ALV were also found in 14 of 68 human subjects living in the areas studied.

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