observed that initially inseminated females copulate again and do so repeatedly under free-flying conditions, but that following even long subsequent copulations females were not reinseminated and ejected no material from their bursae. The bursa has no muscles with which to eject semen[8]. Long after the bursa is empty, males copulate with but do not reinseminate impregnated females. We still do not know how inseminated or impregnated females prevent ejaculation by potent males.

CRAIG[6] reported that 24 h after implanting a single male accessory gland into each of a series of 86 virgins of the Rockefeller strain of *A. aegypti* none of the females were inseminated by many males. He calculated[6,9] that 1 male has enough material in his accessory glands to prevent insemination of 64 to 80 virgins. He reported that injection of 1 µl of a dilution equivalent to 5 to 62.5 individual glands in 1 ml of saline completely prevented insemination of virgin mosquitoes.

We implanted 1 male accessory gland into the abdomen of each of 10 virgin females of the Bangkok strain of *A. aegypti* and 3 days later placed the females with 20 virgin males in a 1 cu ft cage and allowed them to cohabit 24 h. One of the 9 survivors was inseminated. We separately homogenized 2, 8, 25 and 50 male accessory glands in 1 ml of mosquito saline[10] and injected 1 µl from each concentration into 10 to 20 virgin females, after which they were allowed to co-habit for 24 h with an equal number of virgin males. In almost every case, the females were found to have been impregnated. 17 out of 18 injected with 1 µl from 50 glands were impregnated. Even after injection with 1 µl from 100 glands/1 ml saline, 2 out of 7 females were impregnated in one test and 4 out of 10 in a second. We thought that our failure to prevent impregnation might be due to the fact that we had not added seminal vesicle material, so we injected 11 virgins with 1 µl of an homogenate of 12 vesicles and 24 male accessory glands in 1 ml saline and found that all 9 surviving females were impregnated after co-habitation with males. Since CRAIG[9,10] did not actually use homogenates of isolated male accessory glands but sonified the terminalia or ground-up the whole mosquitoes, we sonified 100 male terminalia (equivalent to 200 accessory glands) in 1 ml saline and injected 1 µl of the supernatant into 17 virgins. 24 h after co-habitation with 32 males, 14 females were impregnated and 3 were not inseminated.

It is roughly estimated that the average female mosquito has a hemolymph volume of 1–4 µl. Since the male ejaculates about one-fifth of his accessory gland secretion into a female, and since it is all absorbed into the hemolymph, it can be calculated that he places an amount equivalent to 25 to 100 glands in 1 ml. Our injection experiments indicate that females must contain the equivalent of at least 100 glands if they are not to be inseminated.

CRAIG et al.[6,11] thought that the male accessory glands were responsible for preventing copulation and insemination, and were responsible for increasing the number of eggs developed and laid by the mosquito. CRAIG[12] coined the term *matrone* because he believed that the secretion acted on the female to convert her from 'a maid to a matron'. It seems to us that this term is inappropriate for several reasons. It is not used by females for fertilization of the eggs and it specifically acts to prevent the male from reinseminating an already inseminated or impregnated female. The secretion does not prevent copulation. The failure to be inseminated more than once is not a characteristic of either a mother or a matron.

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Isolation of a Soldado-Like Virus (Hughes Group) from *Ornithodorus maritimus* Ticks in Ireland[1]

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Summary. Three isolations of a virus of the Hughes group were obtained from seabird ectoparasites, *Ornithodoros (Alectorobius) maritimus*, on Great Saltee Island, Ireland. The agent is closely related to Soldado virus, originally obtained from related ticks near Trinidad, West Indies, and represents the second recorded tickborne arbovirus in Ireland.

The virus reported here (RML 59972) was isolated in 1972 from ticks collected by one of us (T.C.K.) from seabird nesting areas in southern Ireland. A sample of *Ornithodoros (Alectorobius) maritimus* Vermeil and Marguet was collected beneath a stone on an east facing ledge, 2 m below a 30 m high Lewisian granite-gneiss cliff summit of Great Saltee Island (52.07 N, 6.36 W) 7 km S of County Wexford in southeast Ireland. *Uria aalge* (guillemot or common murre) is the most numerous seabird species nesting here, but kittiwakes (*Rissa tridactyla*), razorbills (*Alca torda*), and shags (*Phalacrocorax aristotelis*) also occupy ledges, crevices, and holes in the surrounding area.

Adult (64 male, 50 female) ticks were sent to the Rocky Mountain Laboratory (RML) where they were identified as *O. (A.) maritimus*, segregated by sex (5 male or 5 female per pool each, except one pool of 7 female), pooled and processed for virus isolation (55 male, 37 female) or

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Neutralization of 59972 virus by Hughes group immune fluids[a,b]. Immune mouse serum (S) or ascitic fluid (AF)

	Hughes (AF)	Sapphire II (S)	Raza (S)	Farallon (AF)	Punta salinas (AF)	Soldado (AF)	Zirqa (AF)
Virus 59972	0.6 / 2.7	0.4 / 3.0	0.5 / 3.0	0.6 / 2.0	1.7 / 5.0	1.3 / 2.8	1.5 / 4.0

[a] Log neutralization index (LNI), rounded off to nearest 0.1 log; average of 3 tests. [b] Numerator = LNI of immune serum with 59972 virus; denominator = LNI of immune serum with its own virus.

colonization (9 male, 13 female). Laboratory-reared larvae were identical to those *O. maritimus* redescribed by HOOGSTRAAL et al. (in preparation). Tick suspensions were inoculated into suckling white mice and Vero cell cultures. Virus isolation and identification procedures were performed as described by THOMAS et al.[3], CLIFFORD et al.[4] and EARLEY et al.[5].

Three virus strains were obtained in Vero cells from 3 pools of 5 female ticks each. Average survival time of suckling mice inoculated intercerebrally with 0.02 ml of tick suspension was 10–11 days. Suckling mouse brain from early passages varied in titer from $10^{1.54}$–$10^{2.75}$ LD_{50}/0.02 ml. Preliminary complement fixation (CF) screen tests with 38 representative grouped and ungrouped arboviruses demonstrated relationships only with viruses of the Hughes group. CF cross reactivity showed the isolate to be most closely related to Soldado virus[6]. We were unable to study this virus in depth by mouse neutralization test (NT) because of low virus titers.

The virus was tested in a one-way plaque NT with immune fluids prepared against 7 Hughes group agents: Hughes (Dry Tortugas), Sapphire II (52301-14), Farallon (Ar 846), Raza (5/18/64), Punta Salinas (Ar 888), Soldado (Tr 52214), and Zirqa (Por 7866). The most significant reduction of plaques was obtained only with the last three (Table). Of these three, proportionately more 59972 virus was neutralized by Soldado immune ascitic fluid than by Zirqa or Punta Salinas immune fluids.

Earlier (1970) we processed 70 nymphal *O. maritimus* collected on Great Saltee Island (G.A.W.). These ticks were negative for virus in suckling mice; however, they had not been tested in tissue culture.

Soldado virus, to which this agent is closely related, was isolated from *O. capensis* group ticks near Trinidad[6]. More recently it has been reported from *O.* (*A.*) *maritimus* on Puffin Island, Wales[7].

Further studies on viruses of the Hughes group are deemed important because at least 2 of these agents, Punta Salinas and Zirqa, have been implicated in human illness[8,9]. Punta Salinas, Zirqa, Soldado and 59972 viruses have all been isolated from closely related species of the subgenus *Alectorobius*. The association of some of these ticks with human illness emphasizes the need for further biological and epidemiological investigation of *Alectorobius* ticks and the viruses they carry.

This is the second recorded tickborne arbovirus in Ireland where, previously, only louping-ill was known to occur[10,11].

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Antibody-Induced Formation of Caps in *Toxoplasma gondii*

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Summary. Trophozoites of *Toxoplasma gondii* from mouse peritoneal exudate move their surface membrane antigens towards one pole of the cell when incubated with antibodies. The phenomenon may be induced in up to 50% of incubated parasites. It is prevented by some metabolic inhibitors and low temperatures (0–4 °C). These properties do not change in parasites subpassaged after repeated incubation with antibodies.

It has been demonstrated that immunoglobulin (Ig) molecules distributed on the cell membrane of B lymphocytes show a dramatic redistribution when subjected to the action of divalent antibodies directed against surface Ig[2–5]. The Ig-molecules first aggregate into patches, which then move towards one pole of the cell to form a 'cap'. Similar redistribution of other surface membrane components takes place in many mammalian cells after reaction with specific antibodies or concanavalin A[6,7], and it has recently been reported that capping also occurs in some parasitic protozoa such as *Leishmania enriettii*[8], *Leishmania tropica*[9] and *Entamoeba histolytica*[10]. The studies described below were aimed at demonstrating cap formation in *Toxoplasma gondii*.