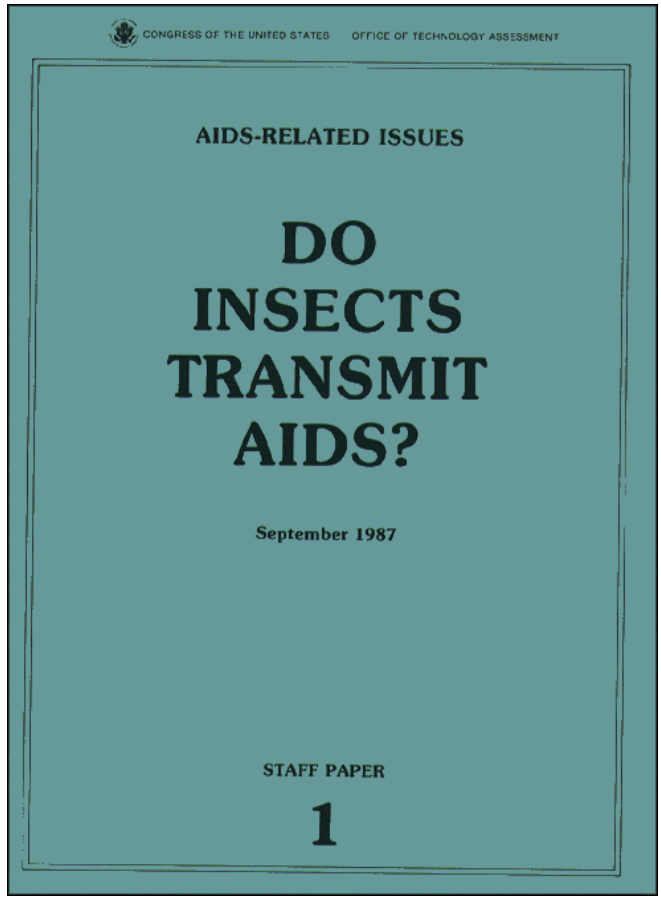
Do Insects Transmit AIDS?

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DO INSECTS TRANSMIT AIDS?

by

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SUMMARY

The AIDS virus, or HIV (Human Immunodeficiency Virus), can be transmitted through sharing of contaminated needles by intravenous drug users. Can HIV infections therefore be transmitted by bloodsucking insects, such as biting flies, mosquitoes, and bedbugs?

The conditions necessary for successful transmission of HIV through insect bites, and the probabilities of their occurring, rule out the possibility of insect transmission of HIV infection as a significant factor in the way AIDS is spread. If insect transmission is occurring at all, each case would be a rare and unusual event.

There are theoretically two mechanisms through which biting insects might transmit HIV infections. First, when bloodsucking insects feed on HIV-infected persons, the virus might be ingested with blood, reproduce and multiply in the insect, migrate to the insect's salivary glands, and be injected into uninfected persons on whom the insect subsequently fed. This is known as biological transmission and is the mode through which such diseases as malaria are transmitted.

The second possible mechanism through which bloodsucking insects might transmit HIV is known as mechanical transmission. The insect might begin to feed on an HIV-infected person and be interrupted for one reason or another. Instead of returning to its original host, the insect might move on to another, uninfected person to complete its meal. The insect might then transfer part of the fresh blood remaining on its mouthparts from its previous feeding attempt to the uninfected person, and might also regurgitate some of its previous bloodmeal.

Experiments in which insect cells were cultured with HIV and in which insects were artificially fed with high concentrations of HIV-infected blood have shown that the AIDS virus does not multiply in insects. If transmission of HIV infection by bloodsucking insects is occurring, it would have to be through mechanical transmission during interrupted feeding.

The probability of HIV transmission from an insect bite would be calculated by multiplying (not adding, because each event's probability is independent of each other) the following factors: 1) how frequently interrupted feeding occurs, 2) the probability that the insect had bitten an HIV-infected person prior to biting an uninfected person, and 3) the probability that the insect bite contained enough HIV to transmit infection.

The frequency of interrupted feeding depends on the type of insect; in general, the larger the insect and the more painful the bite--such as with horse flies--the greater the probability that interrupted feeding will occur. Other bites, such as from mosquitoes and bedbugs, are usually unnoticed and therefore usually uninterrupted. With others, such as ticks, if their feeding is interrupted, the probability of quickly transferring to another person is extremely low.

In mechanical transmission, the maximum amount of HIV that insects would be able to transfer would be the amount of virus in the blood they had ingested prior to biting an uninfected person. Experience with viruses actually transmitted in this manner has shown that the amount of blood that might be transferred is limited to the amount of blood on the insect's mouthparts (on the order of 1/100,000 of a milliliter of blood). An uninfected person would also have to be bitten within an hour of the insect's biting an infected person; and both infected and uninfected persons would have to be in close proximity to each other (a few hundred feet for mosquitoes and biting flies, in the same household for bedbugs), or else the insect will not have an opportunity to transfer to another person if its feeding was interrupted.

Most HIV-infected persons (70 to 80 percent) do not have detectable levels of infectious virus in their blood. Those that do have measurable HIV have very low levels, much below the levels that are needed for insect transmission of other viral diseases. Only rarely does an HIV-infected person have a blood virus level that might contain enough infectious HIV for insect transmission.

The question of insect transmission as an issue has persisted only in the case of Belle Glade, Florida, one small community in the United States. Of 76 AIDS cases diagnosed in western Palm Beach County (which includes Belle Glade) between 1982 and 1985, 13 (17 percent) had no identifiable risk factors, but 10 of the 13 had died before epidemiological investigations could be completed. Seventeen additional cases were labeled as having known risk factors on the basis of being born in Haiti. If these 17 cases are added to the 13 with no official risk factors, then 39 percent (30/76) had no known risk factors. However, it does not necessarily follow that insect transmission must be the cause. Of 736 persons who were tested for HIV infection in Belle Glade, only 26 were positive (3.5 percent prevalence). Eighty-eight percent were ages 18 to 49. The percent of positive adults decreased with age, and no positive results were found in 121 children ages 2 to 10, nor in 94 adults over 60 years of age. There was no clustering of HIV-infected persons within households, except for two pairs of sexual partners.

These findings are consistent with sexual transmission. More importantly, they do not support the hypothesis that insect transmission of HIV infections is occurring in Belle Glade, because of the absence of infection in both the younger and older age groups. The absence of infection in persons over age 60 is also consistent with recent introduction of HIV. Although epidemics of HIV infection are occurring in parts of Africa, similar patterns have been found there in areas endemic for HIV infections.

There is one preliminary finding from French researchers who collected various species of insects from areas in Africa with high rates of HIV infection, and who found HIV-like nucleic acid sequences in the DNA of some of these insects. There was no evidence that these insects could transmit infections. The HIV-like nucleic acid sequences may be some other virus in the DNA of these insects (which is known to occur) or may be artifacts resulting from the identification methods used. These findings need to be confirmed by other laboratories and in further field studies of insects in areas endemic for HIV infections. If these preliminary findings are confirmed, insects will be proven capable of becoming infected with HIV, but not proven to be capable of transmitting HIV infections. However, the potential of insects to act as reservoirs for future HIV infections would then need to be investigated further.

Public concerns that insect transmission of HIV infections might be occurring could be addressed through studies of selected human populations. Surveillance activities could be carried out in areas where conditions most conducive for insect transmission occur, such as in densely populated urban areas in the tropics and subtropics, with high percentages of HIV infections in the exposed population. Such studies might look for insect transmission within households (through bedbugs and mosquitoes), in small neighborhoods (through mosquitoes and biting flies), and among populations heavily exposed to biting flies, such as migrant farm workers.

INTRODUCTION

Infections of HIV (Human Immunodeficiency Virus), the virus that causes AIDS, are spreading rapidly among intravenous drug users, and there have been rare occasions in which health care workers became infected after being accidentally stuck with contaminated needles. If infection can occur by these routes, is it possible for the AIDS virus to be transmitted from an infected to an uninfected person by biting insects? What evidence is there for actual transmission by insects? If insect transmission is possible, how serious is the threat, and what research is being conducted and could be conducted that could answer these questions?

Inquiries into whether insect transmission of HIV could occur fall into two general categories. First, field and laboratory studies of insects can determine: 1) whether their feeding/biting habits are compatible with their possible role as vectors of HIV transmission, 2) whether these insects are capable of drawing up and transmitting enough virus to cause infection, and 3) whether the microbiology of the virus in the insect is conducive to transmission. Second, epidemiological studies can be conducted among populations who live in environmental conditions favorable to insect transmission, to see if there are HIV infection patterns: 1) that cannot be explained by established risk factors (such as sexual transmission or intravenous drug use), and 2) that are consistent with transmission through insect vectors.

The insects of primary interest as possible vectors in the spread of HIV infections are biting flies, mosquitoes, and bedbugs. Other possible insect vectors include lice and fleas.

In this Office of Technology Assessment (OTA) Staff Paper: 1) the evidence for the possibility of insect transmission of HIV infection is summarized, and 2) areas for further investigation are identified.

IS INSECT TRANSMISSION POSSIBLE?

Transmission of some types of infectious diseases through bites of bloodsucking insects is well known to occur when the disease agent is able to reproduce and multiply in the insect vector itself and concentrates in the insect's salivary glands. Under these conditions of biological transmission, the agent not only is subsequently available in much greater quantities than the amount originally taken up by the insect, but also is concentrated in fluids (saliva, which in the insect contains anti-clotting substances to make feeding easier) that will be injected into the next person or animal that the insect bites.

There is no evidence that the AIDS virus multiplies in insects. Nor is HIV known to naturally infect other animals besides humans, so the "reservoir" of infection that insects might dip into to transfer HIV to uninfected humans is limited to humans infected with HIV. (The chimpanzee is so far the only animal that can be infected experimentally, although it will not develop the AIDS disease, so a search is still being conducted to find an appropriate animal model for AIDS.)

Mechanical transmission theoretically could occur when the biting insect is interrupted while feeding on an infected person and shortly thereafter completes its feeding on an uninfected person. The maximum amount of virus that could theoretically be transmitted would be the amount of virus previously ingested, but in all likelihood the amount would be limited to the quantity of virus left on the insect's mouthparts. For example, the common bedbug imbibes approximately l/100th of a milliliter (1×10^{-2} ml) of blood in a full bloodmeal, and approximately 3/1 ,000th of a milliliter (0.3×10^{-2} ml) in a partial, interrupted bloodmeal (44). However, the amount of blood on a bedbug's mouthparts is estimated to be only 7/100,000 of a milliliter (0.007×10^{-2} ml) (56). Comparable levels (0.001×10^{-2} ml) of residual blood on the mouthparts of horse flies have been quantified (24). If 10 percent of the residual blood on the mouthparts of horse flies are deposited when biting the second person or animal,

the range of potential deposition would be between 10^{-6} and $1O^{-5}$ ml (0.0001 x 10^{-2} to 0.001 x 10^{-2} ml). These estimates are well correlated with transmission experiments among animals (37). The insect could also regurgitate some of its previous bloodmeal when feeding on its next victim, but the extent to which this occurs is not known and probably depends on the feeding habits of each type of insect.

Whether infection will occur from insect bites by mechanical transmission depends primarily on the amount of virus injected into an uninfected person. The injected dose depends on the following factors: 1) the amount of virus ingested by the insect and present on the insect's mouthparts after feeding on an infected person, 2) the number of bites from infected insects that an uninfected individual incurs, 3) the time interval between biting an infected person and a subsequent uninfected person (the amount of virus remaining in the insect decreases over time), and 4) the amount of virus remaining in and on the insect that will actually be injected into the uninfected person. The amount of virus ingested depends on the concentration of virus in the blood and the amount of blood withdrawn by the insect from an infected person. The number of bites from infected insects depends on the number of insects that bit infected persons (which depends on the prevalence of HIV-infections in the population within the range of the insect), but were interrupted in their feeding and moved on to bite uninfected persons. And as noted above, not all of the remaining virus is expected to be injected into a subsequent victim. Only a part of the blood that is on the insect's mouthparts (and the amount in regurgitated blood, if regurgitation occurs) is usually injected.

Mechanical Transmission of Other Viruses

Mechanical transmission, if it occurs, would be expected to play a relatively minor role in transmission, as compared to the recognized routes of HIV transmission, and so would be difficult to detect in natural conditions (i.e., through field and epidemiological studies). Through field experiments, mechanical transmission has been shown to occur with a few animal

diseases, such as equine infectious anemia virus (EIAV) in horses and bovine leukemia virus (BLV) in cattle, both transmitted by horse flies. There is evidence that several other viral diseases of humans and/or domestic animals might be transmitted mechanically. In the case of Rift Valley fever, a hamster model has been used to demonstrate mechanical transmission experimentally. Dengue fever virus has been shown to be mechanically transmitted by mosquitoes in human volunteer studies. In addition, hepatitis B virus (HBV) has been mechanically transmitted to rabbits and guinea pigs by bedbugs that have fed on HBV-infected blood.

With experimental transmission of EIAV, when horse flies ingested a partial bloodmeal containing approximately 1 million infectious doses per milliliter (108/ml) from an infected horse and the bloodmeal was completed on an uninfected horse, transmission of infection could occur: 1) with one horse fly, if the uninfected horse was bitten immediately to complete the bloodmeal (33), or 2) with 25 horse flies, if bitten within 30 minutes (34). However, when mosquitoes were used instead of horse flies and the same concentration of virus-containing blood was ingested by the insects, transmission of EIAV infection did not occur with up to 200 mosquitoes (80).

With experimental transmission of BLV, when blood containing more than 1,000 infectious doses per milliliter (10305/ml) was ingested by horse flies, infection was transferred from infected cattle when uninfected goats and sheep were bitten immediately 50 or more times (24). While the virus level in BLV is relatively low, BLV causes an increase in the number of lymphocytes (the type of white blood cell to which the T-cells in humans belong). Although the proportion of infected cells remains low, the absolute number of infected lymphocytes increases greatly.

A hamster animal model has been developed for insect transmission of Rift Valley fever, a disease of humans. When blood containing 100 million infectious doses per milliliter (108/ml) was ingested from one animal by tsetse flies and the bloodmeal completed on an

uninfected animal within 1 hour, 70 percent of the individual transfers resulted in infection. When mosquitoes instead of tsetse flies were used under similar conditions, 15 percent of the individual transfers resulted in infection when one strain of Aedes aegvp~ti was used as the vector (36), and 75 percent (3 of 4) of individual transfers resulted in infection when another strain of Aedes aegvpti was used (42).

Finally, there is evidence that some insects can harbor the hepatitis B virus (HBV) in situations that could result in human infection. In artificial feeding and transmission studies of the common bedbug, HBV survived in the bugs for at least 7.5 weeks but was undetectable after 18 weeks. In these experiments, infection was successfully transferred to rabbits and guinea pigs on which HBV-infected bugs had recently fed (40). HBV is also excreted in the bedbug's feces (41). In a non-experimental study, one group of investigators isolated the hepatitis B virus in mosquitoes and bedbugs they had collected in areas with high rates of hepatitis B infection in the human population (81,82).

HIV Survival in Insects

Experiments with HIV in insects have centered on the following questions. With cell cultures (in vitro experiments): 1) can HIV bind to insect cells, and 2) can HIV replicate (multiply) in insect cells? With live insects (in vivo experiments): 1) how long can HIV survive in the insect, 2) does the virus multiply in the intact insect, and 3) can insects transmit HIV through interrupted feeding? From field studies: is there evidence for HIV infection in insects collected in areas endemic for HIV infections?

In humans, HIV has an affinity for T-helper cells (a type of white blood cell) and binds to the surface of the cell at the site of the "CD4" molecule that characterizes a specific subset of T-helper cells. Experiments have shown that, despite the lack of the CD4 molecule, HIV can bind to some types of insect cells, such as cells from some species of mosquitoes and fruit flies (3,1 1).

Further experiments have shown that HIV does not replicate in these cells. HIV can be integrated into the insect cell's genome--i.e., in the proviral or DNA form of HIV, which is an RNA virus in its free form--a condition that is necessary for replication. However, no replication of HIV occurs; i.e., no "free" virus is present. This lack of replication in insects appears to be due to ineffectiveness of the part of HIV (the "long terminal repeat" sequences, or "LTR") that allows the virus to take control of its host cell's genes in order to promote replication in human T-helper cells (72) and is probably due to an undetermined intracellular mechanism that is specific to insect cells (1 1). Fruit fly, tick, moth, and mosquito cells have been examined, and none have shown replication of HIV (1 1,65,72).

The finding that HIV could integrate into the genome of some insect cells in cell culture experiments led Chermann and his colleagues at the Pasteur Institute to inquire into "the possibility of finding, in endemic zones, insects which would carry genomic sequences homologous to those of the HIV virus" (1 1). They therefore collected bloodsucking and non-bloodsucking insects from areas endemic for AIDS in central Africa, as well as insects from the Paris area (for control purposes) (3,10,1 1,12).

In African insects, HIV-like nucleic acid sequences were found in the DNA of some mosquitoes, tsetse flies, bedbugs, and ticks, but also in cockroaches and antlions (antlions eat other insects, primarily ants). Termites, crickets, wasps, and dragonflies were negative. All insects from the Paris area were negative. The positive results were found in urban and suburban areas, but not in rural areas, reflecting the distribution of HIV infections in the African areas from which the insects had been collected.

HIV-like nucleic acid sequences were found in both male and female mosquitoes (male mosquitoes do not take blood meals), suggesting that reproductive cells had been infected in prior generations (i.e., transovarian transmission). However, as with the laboratory experiments, there was no evidence of free virus.

These findings are puzzling for a number of reasons. First, the HIV genome appeared to be present in all cells of positive insects, although the method used could not specifically identify which and how many cells contained the HIV-like nucleic acid sequences. HIV is able to enter only a few specialized human cells, so a possible explanation is that the findings are artifacts resulting from the assay methods used.

Second, HIV was found in the genome of cockroaches and antlions, insects that would not be expected to be positive. Cockroaches could ostensibly have eaten HIV contaminated materials, but why antlions?

Third, 30 percent of mosquitoes (male as well as female) in areas endemic for HIV infection apparently contained HIV-like nucleic acid sequences, a rate that is higher than the prevalence of HIV infection in the human population of the collection area. This infection rate among mosquitoes is high even for severe epidemics of known insect-transmitted infectious diseases. On the other hand, variable prevalence rates for hepatitis B virus (HBV) infection in mosquitoes have been found. In high prevalence areas of Africa, one study found only 12 of 1,658 mosquitoes infected with HBV (81), but other studies have found that 131 of 247 (25) and 10 of 42 mosquitoes (6) contained HBV. In the case of wild-caught bedbugs in South Africa, HBV infection rates of 157 and 138 per 1,000 bedbugs have been recorded (41).

The existence of HIV-like nucleic acid sequences in the DNA of insects in areas endemic for HIV infection is consistent with the laboratory experiments that have shown that such integration of HIV is possible. However, the validity of these findings must be confirmed, because the positive results may be due to non-specific reactions with the assay reagents used, contamination of the specimens, or reactions with other RNA viruses that can be found integrated in insects' DNA (4). Insects and other species are known to sometimes contain pieces of DNA that are homologous with viruses. Thus, one explanation of the presence of HIV-like nucleic acid sequences in these insects may be that other retroviruses had been integrated into the insects in previous generations.

Taken together with the lack of replication of HIV in insects, Chermann and his colleagues at the Pasteur Institute emphasize that their findings "suggestt that insects might be contaminated by infected human material and thus could be carriers of HIV genes but not~ vectors as clearly evidenced by previous epidemiological studies" (their emphasis) (12). If some insects are natural reservoirs for the viral genome, further research would be needed to determine how HIV replication is suppressed in insect cells, and to assess the possibilities for activation of HIV replication in these insects.

Live mosquitoes and bedbugs have also been fed blood with high concentrations of HIV or have had HIV-contaminated blood injected into their thoraxes (44,49,50,51,55,65,69). At these high concentrations, HIV persists in mosquitoes for a few hours up to 48 hours (50,51,55,69), and in bedbugs, for 1 to 4 hours (43) up to 72 hours (55).

Survival time may be related to the amount of virus initially ingested. A feeding dose of between 10,000 to 100,000 tissue culture infectious units per ml (104-106/ml) resulted in no survival in mosquitoes but 1 to 4 hours survival in bedbugs (39,43,44). Forty-eight hour survival in mosquitoes was accomplished with a feeding dose of approximately 1 million tissue culture infectious units per ml (106/ml) (51). Bedbugs fed on approximately 10 million tissue culture infectious units per ml (107/ml) retained detectable virus for 72 hours (55).

Survival time of HIV in insects after feeding thus appears to depend on the type of insect and on the concentration of HIV in the bloodmeal. There is also a suggestion that survival may depend on the virus. When HIV-2 (a variant of HIV found in parts of Africa) (13) was used instead of HIV (which would be HIV-l), survival for several hours was found in mosquitoes with a feeding dose of 10,000 tissue culture infectious units per ml (104/ml) (69), a dose that resulted in no survival when HIV-1 was used (see above).

These experiments demonstrated that, while virus was detectable for varying periods after feeding, it eventually disappeared. Moreover, the concentration did not increase, indicating that no virus replication occurred. Similar results--i. e., no virus replication--have

been found for HTLV-I (66), a virus that is closely related to HIV (HIV was identified as either "HTLV-111" or "LAV" prior to its present name).

Experiments have also been conducted to see whether insects could transmit HIV from infected blood to uninfected blood through a membrane during interrupted feeding. Mosquitoes fed on blood infected with 1 million tissue culture infectious units of HIV per ml (l O^{6}/ml) did not transfer virus, even though HIV survived in the mosquitoes for up to 48 hours (51). Control experiments had shown that as few as 100 infected lymphocytes could transmit the virus, suggesting that very little blood was being transferred by the mosquitoes. No transmission occurred with mosquitoes or bedbugs with 10^{4}-10^{5} infectious units per ml (recall that in this experiment, no virus was recovered in the feeding mosquitoes, but virus was recovered in bedbugs for 1 to 4 hours post-feeding) (39,43).

Thus, the feeding experiments show that detectable levels of HIV are present in mosquitoes and bedbugs for a short period of time and are primarily related to the concentration of HIV in the blood meal. However, transfer of infection has not taken place despite the presence of detectable levels of HIV in these insects. These findings tend to confirm the hypothesis that only a very small amount of the ingested blood is transferred by insects in interrupted feeding. Furthermore, the longer the time interval between feedings, the less virus will be available for transfer. It should also be noted that the feeding experiments generally involved HIV concentrations much greater than would be achieved under natural conditions of feeding on HIV-infected humans.

How Probable Is Insect Transmission?

Among the major factors affecting the probability of insect transmission of HIV infection are: 1) the prevalence of HIV-infections in the population within the range of the suspect insects, 2) the frequency of interrupted feeding among these insects, 3) the time interval between feeding on an infected person and feeding on an uninfected person, 4) the

concentration of HIV in infected blood, and 5) the amount of HIV-containing blood that is injected (and perhaps regurgitated) into the uninfected person.

In HIV infections, the amount of virus in blood is often very low or not detectable. The pattern that seems to be emerging is as follows. HIV is found in blood soon after infection but usually disappears from blood as antibodies are produced (1,26). The virus then reappears in blood prior to the onset of developing clinical disease (29), with HIV appearing in the blood as much as 12 months (46) to 32 months (59) prior to the development of clinical disease. These observations are supported by recent evidence from studies of the wives/female sexual partners of hemophiliacs with HIV infections, in which the level of infectivity of the hemophiliacs increased with loss of their immune functions (28). The presence of HIV without the development of antibodies can also occur (17), especially in infants and young children, in whom perhaps as many as 10 percent may lack antibodies but are infected with HIV (7).

However, at any point in time, there will be many antibody positive, HIV-infected persons who will have no detectable virus in their blood. For example, various investigators have been able to detect HIV in the serum of antibody positive persons in only 1 of 13 persons (46), 6 of 30 persons (59), 40 of 198 persons (16), and 20 of 78 persons (54), or in approximately 20 percent of HIV antibody-positive persons. When lymphocytes from peripheral blood were cultured, 59 of 284 (77) and 18 of 39 (54) HIV antibody-positive persons had detectable HIV. In the latter study, one-third, or 6 of the 18 persons with positive lymphocytes, also had detectable HIV in their serum (54). Levy estimates that HIV can be cultured from the serum of about 30 percent of HIV antibody positive persons, and that HIV is present in about 1 of every 50,000 ($5x10^{-4}$) white blood cells (lymphocytes constitute between 20 and 30 percent of white blood cells), or in about 1 of every 10,000 circulating lymphocytes (10-4), ranging between 1 of every 1,000 to 1 million (10^{-3} to 10^{-6}) circulating lymphocytes (47). Piot and Schofield estimate that only 1 in 1 million (10^{-6}) circulating lymphocytes are likely to

be infected with HIV (63). Gallo and his colleagues estimate that less than 1 in 10,000 (10^{-4} to 10^{-5}) circulating lymphocytes are infected (32,35).

Furthermore, the availability of drugs to treat clinical AIDS may significantly alter the presence of virus in the blood of HIV-infected persons, especially if anti-HIV drugs are used to treat viremia and not just those who have already progressed to clinical disease. The first available drug, AZT (now known as zidovudine, or Retrovir^{R}), has been shown to significantly decrease the level of HIV in blood (9,19).

The amount of HIV circulating in blood is also very small compared to the amounts of virus in other diseases in which mechanical transmission through insect vectors has been shown in field or laboratory experiments. Serum contains less than 10 tissue culture infectious doses per milliliter; when infectious serum is diluted more than 10 times, evidence of HIV activity usually cannot be obtained (56). Others have found that only undiluted serum (not a tenfold dilution) yielded infectious virus, although in one study, 1 of 78 persons with antibodies to HIV had 25,000 (2.5×10^{4}) tissue culture infectious doses per milliliter (54).

In contrast, the amount of blood on a horse fly's mouthparts is only on the order of 10^{-5} ml; if a 10 percent deposition of this bloodmeal residue is taken as a minimum, the range of potential deposition would be between 10^{-6} and 10^{-5} ml (23). This range of potential deposition correlates well with actual transmission trials of EIAV and BLV (37). In the human HIV situation, such a deposition of blood would have a low probability of containing even one tissue culture infectious dose of HIV.

If a person is bitten by a bloodsucking insect, what are the chances that infection with HIV will occur? The probability of infection will be determined by numerous factors, all of which must converge through a chain of events that makes insect transmission of HIV infection extremely improbable. If it does occur, each case of insect transmission would be a rare and unusual event.

The following chain of events must take place: 1) the insect must start to feed on one person, be interrupted for one reason or another, and then complete its feeding on another person within 1 hour; 2) the first partial feeding must take place on an HIV-infected person, and the insect must complete its feeding on an uninfected person; and 3) the blood that the insect transfers must contain enough HIV to infect the second person. The probability of the occurrence of this chain of events is not just the sum of the probability of each of these events; instead, each event's probability is independent and therefore must be multiplied by the probability of the others. For example, suppose that each event's probability was 1 in 10. Then the probability of the entire chain of events would be 1 in 1,000. One in 10 bites would be interrupted, but only 1 in 10 of those interrupted would have bitten an HIV-infected person and then bitten an uninfected person, and only 1 in 10 of those insects would have transferred enough HIV to infect the second person.

How often does interrupted feeding take place in the normal feeding behavior of bloodsucking insects? In general, the larger the insect and the more painful the bite, the greater the probability that their feeding will be interrupted. Horse flies and other large biting flies would therefore be among the insects whose initial feeding attempts would most often be interrupted. Other bites, such as from mosquitoes and bedbugs, are mostly noticed afterwards. Interrupted feeding does not usually occur with bedbugs, and when it does occur, they usually return to the same individual. Mosquitoes are usually relatively rapid feeders, often being three-fourths to fully fed before the person being attacked is aware of them (8). Still others, such as ticks, virtually never have the chance to engage in interrupted feeding. Thus, on the basis of interrupted feeding alone, mechanical transmission of HIV infections by ticks can be ruled out.

If interrupted feeding does take place, what are the chances that the insect had first fed on an HIV-infected person and then completed its feeding on an uninfected person? The probability will be determined principally by the following factors: 1) the probability that an

insect that has been interrupted in its feeding will move on to another person to complete its feeding rather than attempting to bite the first person again, 2) the distance between the HIV-infected person and the uninfected person, and 3) the prevalence of HIV-infected persons in the population within the feeding range of the insect (a few hundred feet for flying insects, in the same household for bedbugs).

How often will an insect that has had its feeding interrupted move to another person to complete its bloodmeal? Experiments conducted with horse flies resulted in the following findings. Four horses were placed in a square of 9 meters to a side and allowed to be bitten by 750 horse flies of eight species. Only those flies that transferred from the original horse to another were captured. There appeared to be a correlation between horse fly size and transfers; i.e., the larger the horse fly, the greater the chances of the fly transferring from its original host to another horse. Only about 2 percent of the time did the horse flies transfer to another horse instead of refeeding on the original host horse (20,37). Thus, even when interrupted, horse flies, which are among the insects most prone to engage in interrupted feeding, seldom do. These experiments were also conducted in a very small area, and the probability that the horse flies would have transferred between animals when their feeding was interrupted would decrease rapidly as the distance between horses increased.

How would the prevalence of HIV infections in the population within the feeding range of bloodsucking insects affect the probability of transfers between HIV-infected and uninfected persons? If 1 percent of the population was HIV-infected, and if interrupted feeding actually occurred, the chances of an insect first feeding on an HIV-infected person and then completing its feeding on an uninfected person would be 0.0099 (0.01 x 0.99), or approximately 1 in 100. If we assume a2 percent probability that the insect will actually transfer between hosts (i.e., that infected and uninfected persons are within a few yards of each other), the probability increases to O.000198 (0.0099x0.02), or about 1 in 5,000. Using similar assumptions but varying the prevalence of HIV infections, the probability when the prevalence

of HIV infections is 5 percent would be 0.00095, or approximately 1 in 1,000; for a prevalence of 10 percent HIV infections, the probability would be 0.0018, or approximately 1 in 500; if 50 percent of the population is already infected with HIV, the probability would be 0.005, or 1 in 200. (See appendix A.) These probabilities are based on experiments with horse flies, with horses in close quarters. The probabilities would decrease significantly as the distance between infected and uninfected persons increased. Furthermore, the risks should be considerably lower with other insects, such as mosquitoes and bedbugs, for which interrupted feeding occurs much less frequently.

If all of the prior events occurred, what are the probabilities that enough HIV-infected blood will be transmitted to cause infection? Recall that, on average, only 20 to 30 percent of HIV-infected persons will have detectable levels of HIV in their blood, and almost all of those with detectable levels will have very small amounts of HIV in their blood.

Researchers have been working for several years to develop a simple predictive model for mechanical transmission of other pathogenic agents. While use of currently available information results in a wide range of probabilities, the chances of mechanical transmission occurring under defined circumstances are predictable. The amount of blood transferred from an infected person to an uninfected person either contains an infectious dose or it doesn't. When estimates are made to predict how many insects bites it would take to reach a probability of one for transmission, it means that at least one insect would be expected to transfer infection.

Two examples of probability estimates are presented in appendix A for transfer of infection by bloodsucking insects that have recently fed on an HIV-infected person and that still contain some HIV-infected blood. While the probability estimates are low, they vary quite widely. For mosquitoes, the probability ranges from 1 in 33,000 bites for mosquitoes (based on the amount of tissue culture infectious units of HIV in the blood of an HIV-infected person, the amount of blood transferred, and comparisons with the rate of infection from needlestick

injuries) to 1 in 10 million bites (based on mechanical transmission experiments with Rift Valley fever virus).

Thus, when the probabilities for each of the necessary chain of events--interrupted feeding, feeding on an HIV-infected person before feeding on an uninfected person, and transfer of sufficient blood to infect the second person with HIV--are multiplied by each other, the resulting probability of insect transmission will be extremely remote.

Finally, the risk of HIV infections through needle-stick injuries may provide some perspective on the probabilities of transmission through bloodsucking insects. HIV infections through needle-stick injuries occur approximately 0.3 percent (0.003)of the time (48,56,75). In contrast, hepatitis B virus (HBV) infections through needle sticks occur 6 to 30 percent of the time (15), with an average transmission rate of 12 percent (30,75,76). There is also one reported case of a needle-stick injury from a needle that had been used on an AIDS patient who was also infected with HBV. The person who was accidentally stuck became infected with HBV, but not with HIV (27). The volume of blood that would be inoculated with a needle-stick injury (with a 25 gauge needle) has been estimated at one-thousandth of a milliliter (10^{-3} ml) (57), or about 100 times the amount on a mosquito's proboscis and 14 times greater than in the mouthparts of a bedbug (56).

Conclusion

Experiments with mechanical transmission of other viral diseases have shown that, under the right conditions, transmission through insect vectors can occur. Experiments designed to answer the question of whether HIV can survive in bloodsucking insects long enough to be transmitted if interrupted feeding occurs have shown that it is theoretically possible. However, based on the conditions necessary for successful transmission of other viral diseases, and on the biology of HIV infections in humans, the probability of insect transmission of HIV is extremely low.

A chain of unlikely events must occur if mechanical transmission will be at all possible, and the probability that a bloodsucking insect's bite will transmit HIV infection will be determined by multiplying the probabilities of each of these events.

The probability of mechanical transmission by insects would be increased if the following conditions were met: 1) a high percentage of HIV-infected persons in the population within the range of bloodsucking insects, 2) continuous, high virus concentrations in the blood of HIV-infected persons, and 3) very high concentrations of bloodsucking insects, so that the exposed population is literally bitten hundreds or even thousands of times by insects that have bitten HIV carriers and then bitten susceptible individuals within a short period of time (an hour or less).

Some environmental situations may exist in which persons will suffer literally hundreds of bites from bloodsucking insects, but it is highly improbable that most of these insects will have bitten an HIV carrier immediately before. This situation may change as the prevalence of HIV infection increases, but the majority of the exposed population will have to be already infected before insect bites can become even a plausible route of transmission of any consequence to the remaining uninfected population. Even if the prevalence of infection increases significantly, there is no indication that the current pattern of low or undetectable virus concentrations in the blood of most HIV-infected persons will change. If there is a change, hopefully it will be toward even lower levels of HIV in the blood, as more drugs to treat AIDS and HIV infections are developed.

These conclusions are based on the available evidence, and there undoubtedly will not be unanimous agreement as to whether this evidence is sufficient or all-inclusive. For example, other infections may enhance a person's susceptibility to being infected with HIV (64). However, even under conditions where HIV infection may be enhanced, there is no reason to believe that insect transmission would be preferentially enhanced over other modes of transmission. People with other infections may be more vulnerable to HIV infection than

persons without these other infections, when all are exposed to the same route of transmission. In other words, different natural histories of HIV infection may occur in a given population that is exposed to HIV infection in the same manner. The point, however, is that further investigations on the possibilities and probabilities of insect transmission of HIV may be needed to confirm (or refute) the conclusions that can be made on the available evidence. What these investigations might consist of will be addressed later in this Staff Paper.

However, it is agreed that further investigations are needed of the tentative findings of Chermann and his colleagues at the Pasteur Institute that some insects in areas endemic for HIV infection contain HIV-like nucleic acid sequences in their DNA. These findings need to be confirmed by other researchers, especially because some of the tentative findings--such as the apparent presence of the HIV genome in antlions--cannot be reconciled with what is known about HIV. If confirmed, the most important issue from the standpoint of insects as possible transmitters of HIV is to investigate the possibility of changes in the intracellular mechanisms of these insects that might make biological transmission--replication and expression of HIV--possible.

IS THE EPIDEMIOLOGY OF HIV INFECTIONS CONSISTENT WITH INSECT TRANSMISSION?

Two questions can be directed at the epidemiological information: 1) are there HIV infection patterns that cannot be explained by established risk factors (such as sexual transmission or intravenous drug use), and 2) are these HIV infection patterns consistent with transmission through insect vectors?

The major routes of transmission in the United States are clearly related to sexual practices and intravenous administration (i.e., intravenous drug use, blood-clotting factors for hemophiliacs, and blood transfusions), and in infants, through perinatal transmission from HIV-

infected mothers. The conclusions that can be reasonably drawn from related insect-borne viral diseases and the biology of HIV infections support these findings and make it clear that insect transmission, if occurring, is not a significant cause of HIV infections.

Proponents for insect vectors as a major cause of HIV infection in parts of the United States have alleged that 22 percent of AIDS cases in Florida, 33 percent in Miami, and 50 percent in the Belle Glade community of south Florida have no identifiable risk factors (79).

On the other hand, the Centers for Disease Control (CDC) have reported that only 4.8 percent of adult cases of AIDS in Florida had no reported risk factors. Of the 76 adult cases of AIDS (3 pediatric patients were born to HIV-infected mothers) reported in western Palm Beach County, Florida (including Belle Glade), between July 1982 through September 15, 1986, 13 (17 percent; 11 men and 2 women) had no reported risk factors; but 10 had died before epidemiological investigations could be completed. Twenty-seven of the risk factor cases were attributed to heterosexual contact. However, only 10 had heterosexual contact with a person with AIDS or at increased risk for AIDS; the other 17 were categorized as heterosexual contacts solely on the basis of being born in Haiti, where heterosexual transmission is believed to play a major role (67).

If the 17 persons born in Haiti were assigned to the “no risk factor” group, a much larger percentage (39 percent vs. 17 percent) of AIDS cases in western Palm Beach County would have no identifiable risk factors. However, can insect transmission account for these cases?

Proponents of insect transmission state: “In Belle Glade, the absence of antibody to HIV in 134 children tested has been used as an argument against insect transmission... children have far less exposure to insects than adults (they go to bed early, go to school, and do not work in the fields). By virtue of their tender age children have had less time to be exposed to agents found in a very small percentage of mosquitoes” (78). No evidence is available to support this hypothesis.

Moreover, CDC has reported a broader picture of HIV infection prevalence rates for Belle Glade residents. Of the first 736 persons for whom data entry had been completed, antibodies to HIV were found in: 1) none of 121 children ages 2-10 years; 2) 14 (8.9 percent) of 157 persons ages 18-29; 3) 7 (4.4 percent) of 160 persons ages 30-39; 4) 2 (1.8 percent) of 113 persons ages 40-49; 5) 3 (3.2 percent) of 91 persons ages 50-59; and 6) none of 94 persons over 60 years of age. Eighty-eight percent of HIV antibody-positive adults were ages 18-49 years; 90 percent of adult AIDS patients reported in the United States are in that same age group (67). More importantly, in Belle Glade the percent of adults positive fo rHIV antibody decreased with age, and none of 94 persons over age 60 were positive. These findings are not consistent with insect transmission, because if insects were responsible for causing HIV infections in this population, why are there no HIV infections in people under 18 years of age or over the age of 60? Furthermore, the rationale stated above for absence of antibodies to HIV in children does not explain the decreasing prevalence of HIV antibodies with age and the absence of antibodies in those over age 60.

The most striking difference between HIV infections and AIDS in Americans and Europeans versus Africans (and Caribbean) is the ratio of male to female cases. In Africa the male-female ratio is 1:1, while the American and European ratios are 13:1.

The American and European ratios are explained by male homosexual contact as being a major mode of transmission of HIV, and the number of women began to rise only after they had sexual contact or shared intravenous drugs with HIV-infected men. The African (and Caribbean) ratio has suggested heterosexual transmission of HIV and exposure to contaminated needles for medical purposes, transfusion with unscreened infected blood, cultural rites such as scarification (5,62), and affliction with a wide variety of diseases that result in immunologic changes which would make a person more susceptible to HIV infection (64).

HIV infections in Africa have been associated with sexually active age groups (52,53), and female prostitutes (45,61) and their clients (14). For example, in a study of 1,078 subjects

in Lusaka, Zambia, the prevalence of infection was low in subjects less than 20 years of age and more than 60 years of age. The peak prevalence occurred in men between the ages of 30-35, and in women, between the ages of 20-25. The infection rate between men and women was not significantly different. None of 12 subjects under the age of 15, and none of 21 subjects above the age of 70, were infected (52). These types of findings have been used to support the hypothesis of heterosexual transmission, and the lack of HIV infection among the older population has been interpreted as reflecting recent introduction of HIV infection. Even if these findings are not considered persuasive for heterosexual transmission, they nevertheless do not support the hypothesis that insect transmission is the operative cause, because of the lack of HIV infections in the younger and older age groups. Insect transmission would also require a large reservoir of infected persons in order to transmit HIV to uninfected persons, and a large reservoir of infected persons would not exist if HIV were recently introduced.

In an evaluation of 2,464 Haitians in 1985-86 and 191 Haitians for whom blood had previously been collected during a 1977-79 dengue fever outbreak, it was concluded that HIV infections were recently introduced into Haiti and were more prevalent in urban areas and in lower socioeconomic groups (60). The finding of HIV infections being more prevalent in lower socioeconomic groups in urban areas of a tropical country might be taken as a proxy for crowded, unsanitary conditions conducive to high concentrations of biting insects, and hence, supportive of insect transmission as a possible cause for HIV infection. But recent introduction of HIV into Haiti would again argue against insect transmission because of the lack of a large reservoir of infected persons. Furthermore, in Africa, higher education levels (in contrast to the Haiti findings of a correlation with lower socioeconomic standing) have been correlated with HIV infection (52,61,62). One explanation for the African pattern of infection is that more highly educated persons have greater opportunities than more poorly educated persons for travel and social interactions, and thus exposure to HIV. This could also explain the higher prevalence

of HIV infections in urban versus rural areas that have been found in Africa (53), Haiti (60), and the United States.

These findings do not reflect insect transmission as a major factor in HIV infection, even in tropical countries, but they do not rule out the possibility that some HIV infections might be caused by insect transmission. One such possibility arises in households containing HIV-infected persons and extremely large numbers of bloodsucking insects, such as bedbugs.

In one study of 45 Florida households containing persons with clinical AIDS and/or HIV infections, none of 90 uninfected children and none of 29 household contacts became infected. Uninfected persons who did become infected became so through sexual contact (18). In contrast, in an African household (Rwanda), at the time of testing for HIV infection, the mother, father, and three sons ages 6 years, 5 years, and 18 months, were all HIV antibody positive (38). The mother and all three sons had clinical symptoms, while the father did not. The investigators hypothesized that bloodsucking insects, among other causes, might have accounted for the infections among all three brothers, because the children's symptoms started developing several months to years afterbirth. However, in children with AIDS reported to the Centers for Disease Control from 1982 to 1985, the estimated incubation period for AIDS in children has increased each surveillance year, with the longest incubation exceeding 7 years (68). Moreover, the mother developed clinical signs of AIDS 3 months after the birth of the first child, which means that she was likely to have been already infected (but without clinical disease) during the pregnancy. Thus, it is more likely that perinatal transmission was the cause of HIV infections in all three children.

Conclusion

The major routes of transmission of HIV infection in the United States (and Europe) are firmly established; i.e., sexual practices of homosexual (and bisexual) men and sharing of contaminated intravenous drug paraphernalia. Methods to inactivate the virus in blood clotting

factors (for hemophiliacs), and exclusion of high risk persons and use of the HIV antibody test in blood and plasma donations have reduced infection through therapeutic use of blood and blood products. Perinatal HIV infections in infants from infected mothers and spread from HIV-infected males to females are increasing. In tropical countries (e.g., in Africa and the Caribbean), heterosexual transmission seems to be the dominant mode.

The nature of epidemiological studies is to look for associations--i. e., risk factors that can be correlated with HIV infections and with progression in HIV-infected persons to the clinical disease, AIDS. These types of studies cannot be expected to identify risk factors in all cases because of statistical limitations. Moreover, eliciting a possible cause of infection from HIV-infected persons is difficult in some cases because the greatest known risk factors are sex and drug practices that many persons morally object to and/or that are illegal.

Uncertainty about the cause of HIV infections in a proportion of the cases, however, is clearly not proof that another transmission route, such as insect transmission, is involved. A plausible causal explanation must exist. In surveys of various populations, wherever in the world these have been conducted, infected persons have been concentrated in the sexually active age groups. Doubts may exist that heterosexual transmission is the explanation for the equal prevalence rates of HIV infection in men and women in Africa (and the Caribbean), but there is no reason to conclude that insect transmission must therefore be operating.

While the data from insect studies indicate insect transmission of HIV infection as extremely improbable, situations may exist in which some insect transmission might occur. If so, insect transmission is likely to occur in limited areas with unusual environmental characteristics. For example, in field studies with equine infectious anemia virus (EIAV), transmission through horse flies can be significantly reduced by keeping infected animals more than 200 yards away from uninfected animals (21). Conditions conducive to insect transmission would be: 1) extremely crowded living conditions, 2) a very high biting insect population, with persons routinely suffering hundreds to thousands of bites (dependent on the vector potential of

the biting insects; i.e., the types of insects), and 3) a high rate of HIV infection among the persons within the range of the insects. Even if these conditions were satisfied, the laboratory evidence shows that: 1) at any given time, most HIV-infected persons do not have infectious virus in their blood, and 2) when infectious virus is present, it is at a much lower concentration than the levels that have been necessary for transmission with other insect-borne viral diseases.

Environments meeting the criteria for potentially sustaining insect transmission could be investigated in a more purposeful manner to determine if insect transmission in fact occurs. If insect transmission can occur, then it would be in very localized neighborhoods (through flying, biting insects) that satisfy the three conditions identified above, or in households containing HIV-infected persons (e.g., through bedbugs or mosquitoes). Thus, the place to look for evidence of insect transmission is in densely populated neighborhoods in proximity to large numbers of suspect vectors, in households in densely populated urban areas in the tropics with a high prevalence of HIV infections, and among migrant farm workers exposed to large numbers of biting flies.

In sum, there is no evidence that insect transmission causes HIV infections in temperate zones or even in tropical climates. This conclusion is consistent with the HIV insect experiments and with experience with other insect-borne viral diseases. Insects as an unusual cause of HIV transmission in specific situations such as those described above are a possibility, but are still improbable. These situations, however, could be investigated in a more purposeful manner, as described in the next section.

WHAT FURTHER INFORMATION IS NEEDED?

Biology of HIV

Insect studies indicate that the amount of infective HIV in the blood of HIV-infected persons is inadequate to support insect transmission. Data on HIV blood levels depend on the sensitivity of the assay system used to measure HIV in blood. If the test systems in use detect only a fraction of the true concentration of infectious virus particles, conclusions regarding the quantity of virus ingested by a vector will have to be revised. On the other hand, cell culture techniques currently in use to measure infectivity may not measure all the HIV that is present, such as viral particles bound up in antigen-antibody complexes in the serum, but they likely measure all infectious(unbound) particles (47} Thus, the tissue culture techniques may just as likely be identifying HIV at some blood levels that may not be clinically infectious, because the body's defenses may successfully sequester and eliminate some HIV before a threshold viral load is reached that would overwhelm the body's defenses. So even if insects do transmit some HIV, the amount transferred may not be enough to result in infection.

Nevertheless, better assays need to redeveloped in order to measure more accurately: 1) the amount of HIV and its various forms in blood, and 2) the infectivity of the various forms of the virus that is found in blood--i.e., intact virus, partial virus particles, free virus/virus particles in the serum or liquid portion of blood, and intracellular virus (virus in lymphocytes). Second, fluctuations in the concentration of virus in the blood of HIV-infected persons over time need to be more clearly understood. From current research, we know that virus is usually present in blood after initial infection but before a substantial amount of antibody is produced, then disappears and appears again as clinical disease develops. Third, variations in the infective potential of HIV need to be examined. Persons with HIV-2 infection may have higher

concentrations in their blood than persons with HIV-1 infections. One feeding experiment has also shown that HIV-2 is recoverable in mosquitoes for a short time at bloodmeal concentrations that do not result in recoverable virus when HIV-1 has been used, and it is known that HIV-1 (and probably HIV-2) can frequently change its envelope (the outside surface of the virus), the part of the virus that is crucial to infection of cells.

Insect Studies

HIV is recoverable in some species of mosquitoes and bedbugs for a few hours to 3 days only after feeding on blood that has been artificially infected with very high concentrations of virus. Different species often have different feeding patterns, behavior, and physiology. Some species regurgitate blood, others seldom or never do. Not only do different species of mosquitoes often vary in behavior, so may subspecies and different geographic and genetic strains of a species (31,73). Because of possible variations in the biological relationship between HIV and different species and strains of mosquitoes and bedbugs, similar feeding experiments could be conducted on more types of mosquitoes and bedbugs, not only to confirm the previous findings regarding survival time, but also to check further for the possibility of biological transmission (i.e., whether HIV can replicate and multiplying these insects). Because of the recent finding that HIV is harbored by the Langerhans cells of the epidermis, studies could be conducted as to whether HIV could be picked up from these cells while the insect probes for a blood capillary (8). Other insects that could also be tested include lice, fleas, horse flies, and sand flies.

When mosquitoes and bedbugs are artificially fed on highly infected blood, interrupted in their feeding, and allowed to complete feeding on uncontaminated blood, no transfer of HIV has been found. This implies that transfer of infection between living sources does not occur, but the only animal model for HIV infection that is available is the chimpanzee (which can become infected but does not develop disease). Since chimpanzees are in very short

supply and are already inadequate to meet the needs of higher priority HIV-related research, new animal models need to be found. Existing, relevant models might include monkeys and STLV (the monkey counterpart of the human HIV virus).

A unified model for human retrovirus mechanical transmission could be established using EIAV and BLV infections, which range in measurable titers between 1 and 10^8 infectious particles per milliliter of blood. The potential for a variety of insect species to act as vectors within a range of retrovirus concentrations in ingested blood could then be evaluated (22).

It is known that Rift Valley fever virus (RVFV) ingestion by mosquitoes and subsequent transmission can be enhanced if mosquitoes are infected with another organism (microfiliariae) (74). In these experiments, 64 percent of mosquitoes without the other organism took up detectable levels of RVFV, with 5 percent of refeeding mosquitoes transmitting the virus. These numbers were increased to 88 percent and 31 percent, respectively, when infected with microfiliariae. The presence of this other organism, however, only enhances the ability of mosquitoes to transmit RVFV and does not turn mosquitoes from ineffective to effective transmitters of RVFV. Furthermore, the way in which microfiliariae are thought to enhance transmission of RVFV is by puncturing the midgut wall, thereby allowing RVFV to infect the salivary glands, which is reflected in the observation that injected virus becomes more rapidly transmissible than does ingested virus. No HIV has been recovered in mosquitoes that have been directly injected with HIV-contaminated blood (see earlier discussion on “HIV Survival in Insects”).

The effect on transmission of concurrent feeding of mosquitoes and bedbugs with HIV and hepatitis B virus (HBV) could also be investigated.

The preliminary findings of French researchers that field insects in areas endemic for HIV infection contain HIV-like nucleic acid sequences in their DNA need to be confirmed. The finding of HIV-like nucleic acid sequences in both male and female mosquitoes (male mosquitoes do not take blood meals) reflects the possibility of transovarian transmission; i.e.,

integration into DNA such that it is inherited by subsequent generations. This is known to occur with other viruses (2,7 1). However, the French findings may represent artifacts or other endogenous insect nucleic acid sequences related to HIV but unrelated to active HIV infection of these insects. Insects found to be positive by Chermann and his colleagues could be provided to other groups to see if the findings are reproducible on the same materials. Bloodsucking and other types of insects could be collected in neighborhoods at high-risk for HIV infections, and a search made for free virus (RNA form) as well as integrated virus (DNA form).

The reasons for HIV binding to insect cells in the absence of the CD4 molecule (the marker and entry point of HIV in human cells) also need to be determined, as well as the mechanism in insect cells that inhibits the ability of the integrated form of HIV to take over cell production processes in order to replicate and multiply. Researchers will be confirming and pursuing these findings (if confirmed) in the search for new methods to inhibit HIV replication, which could theoretically help in the search for new drugs against HIV. But these preliminary findings should also be investigated for the potential of insects to act as reservoirs and future sources of HIV infection.

[n the experiments showing that HIV could be integrated into insect cells, cultured cells were placed in direct contact with HIV and incubated in the laboratory. No experiments have been performed in which insects have been fed or injected with HIV-infected blood, and their offspring and subsequent generations examined for HIV integration into their DNA. Laboratory studies using insects that are fed HIV-infected blood or in whom HIV was injected intrathoracically, such as the types of studies conducted to determine HIV survivability in mosquitoes and bedbugs, could also be used to look for HIV integration into these insects' DNA. Electron microscopy studies could also be undertaken to look for virus in virion or lymphocyte form after artificial feeding.

Parallel investigations could also be conducted among domestic animal populations in the United States for EIAV and BLV. Insects associated with infected and virus-free herds

could be screened for incorporated retrovirus genomes. Laboratory studies using insects fed directly on infected animals or insects injected intrathoracically with purified virus could also reconducted.

Epidemiological Studies

The epidemiological patterns of infection in Africa are especially difficult to decipher at this time, and there is widespread agreement that prospective studies are needed in different population groups to examine both transmission factors and those factors that make people who are HIV-infected more prone to develop clinical disease (Quinn etal., 1987). However, even though transmission factors are not well understood, the information that is available is not supportive of insect transmission as a major factor in infection, even in Africa. Prospective studies of the kinds needed to identify methods of transmission could also be broadened to address the possibility of insect transmission.

If insect transmission is occurring, then the logical place to conduct these epidemiological studies is in neighborhoods and households with high prevalence rates for HIV infection and with superabundant concentrations of bloodsucking insects. Household studies of the kinds conducted in the United States to examine the issue of whether HIV infections can be transmitted through casual contact (18) can be easily adapted to look for insect transmission under the conditions just described. Although more difficult to design, neighborhood studies could be similarly conducted.

Epidemiological studies with intervention strategies built into them could also be conducted. Insect control programs could be instituted in appropriately identified neighborhoods, and the incidence and cumulative prevalence of HIV infections compared with similar neighborhoods without insect control programs. Various intervention strategies could

also be compared; i.e., strategies designed to decrease sexual contact and/or intravenous drug use could be instituted in some neighborhoods, while insect control programs could be instituted in other neighborhoods.

Appendix A:

PROBABILITY OF INSECT TRANSMISSION

I. PROBABILITY OF MOSQUITO TRANSMISSION OF HIV, BASED ON HAMSTER ANIMAL MODEL FOR RIFT VALLEY FEVER VIRUS (RVFV):

It is known that inoculation of less than 1 infectious unit of RVFV will infect hamsters 100 percent of the time.

In mechanical transmission experiments, when 10^8 units of virus per ml of blood was present in the first animal and the blood meal completed on a susceptible animal within 1 hour, actual RVFV transmission rates to hamsters by mosquitoes were approximately 15 percent.

Therefore, if $1/10^9$ milliliter (10^{-9}, or 0.000,000,001 ml) of blood had been inoculated, 100 percent of the hamsters would have become infected.

Thus, mosquitoes must have inoculated less than 10^{-9} ml, or approximately 10^{-10} ml of blood during an interrupted feeding.

If we assume that an HIV-infected person has an HIV concentration of 10^3 units per ml of blood (which is about 100 times the concentration usually found in an HIV-infected person without symptoms of disease), the probability of a mosquito that began feeding on an HIV-infected person and completed its feeding on a susceptible person inoculating one unit of virus would be 103/1010, or 10^{-7}, or 1 in 10 million.

(Provided by Char!es Bai!ey, Chief, Department of Arboviral Entomology, U.S. Armv Medical Research Institute, Ft. Detrick, Maryland)

II. PROBABILITY OF BEDBUG TRANSMISSION OF HIV, BASED ON AMOUNT OF BLOOD ON THE MOUTHPARTS OF A BEDBUG:

Volume of blood on bedbug mouthparts = 7×10^{-5} ml

Number of lymphocytes on bedbug mouthparts:

= (number of lymphocytes in blood) x (vol. in bedbug mouthparts)

= (2.5×10^6 lymphs/ml) x (7×10^{-5} ml)

= 175 lymphocytes/bug

(Continued...)

Concentration of virus in HIV-infected person's blood:

serum = usually less than 10 tissue culture infectious doses per ml

lymphocytes = usually about 1 in 1 million (10^{-6})

= (2.5×10^{6} lymphs/ml) x (1×10^{-6} infected lymphocytes)

= 2.5 tissue culture infectious doses per ml

Total tissue culture infectious dose per bedbug bite:

From serum: 1 to 10

From lymphocytes: 2.5

= (3.5 to 12.5 tissue culture infectious doses/ml) x (7×10^{-5} ml)

= *(24.5* to *87.5)* $\times 10^{-4}$ tissue culture infectious doses per bite

# of bites required so that at least one bite will transfer 1 tissue culture infectious dose:

= $1/((24.5 \text{ to } 87.5) \times 10^{-4})$

= 4,082 to 1,143

Therefore, at each bite, there is a 1/1,143 to 1/4,082 chance that 1 tissue culture infectious dose of HIV will be transmitted.

These estimates are based on the assumptions that serum usually contains less than 10 tissue culture infectious doses per milliliter of HIV (54,56) and that only about 1 in 1 million (10^{-6}) lymphocytes are infected (56,63). However, an occasional HIV-infected person has been shown to contain up to 25,000 tissue culture infectious units of HIV per ml (54), and lymphocyte infection has been estimated by others to range from 1 in 1,000 to 1 in 1 million (47), and averaging less than 1 in 10,000 ($10^{-4}$to 10-5). On the other hand, only 20 to 30 percent of HIV-antibody positive persons have recoverable virus in their serum at any one time (see text).

The volume of blood injected through an accidental needle-stick injury has been estimated at 0.001 ml (10^{-3} ml) (57), and the rate of HIV infection through needle-stick injuries has been estimated at 0.3 percent (1/333)(48,56,75). The volume of blood injected through a needle-stick injury is about 14 times greater than the amount of blood on the mouthparts of a bedbug and 100 times greater than on a mosquito's proboscis (56). Therefore, the risk of infection following a single mosquito transfer would be 0.003 percent (1/33,000), and the risk following a bedbug transfer would be 0.02 percent (1/4,667). The bedbug estimate is similar to the estimates derived above from the amount of blood on a bedbug's mouthparts, when the assumptions are that serum contains less than 10 tissue culture infectious doses of HIV per ml, and that only about 1 in 1 million lymphocytes are infected.

(Modified from estimates provided by Thomas Monath, Director, Division of Vector-Borne Viral Diseases, Centers for Disease Control, Ft. Collins, Colorado)

III. PROBABILITY OF A HORSE FLY TRANSFERRING FROM AN HIV-INFECTED HOST TO AN UNINFECTED HOST DURING INTERRUPTED FEEDING:

When horse flies are interrupted in feeding, they will usually return to the original animal to continue feeding rather than move to another animal. Even when the animals are close together, horse flies move to another animal only about 2 percent of the time (20,37). If we assume that horse flies will move to another host 2 percent of the time if interrupted in their feeding and that horse flies and HIV-infected humans are randomly distributed, what are the probabilities of an uninfected person being bitten by a horse fly that had been feeding on an HIV-infected person when the horse fly was interrupted in its feeding? The probabilities would vary with the prevalence of HIV infection in the target population.

In the first example, the prevalence of HIV infection is 1 percent. If a horse fly first bit an HIV-infected person, the probability of its then completing feeding on an uninfected person would be: 0.01 x 0.99 x 0.02 = 0.000198, or about 1 in 5,000. The probabilities associated with different prevalence rates of HIV infection are also presented.

1 Percent Prevalence of HIV Infection:

Bite #1	Bite #2
1% HIV positive	1°/o HIV + = (0.01 x 0.01 x 0.02) = 0.000002
	99% HIV - = (0.01 x 0.99 x 0.02) = 0.000198
99% HIV negative	1% HIV + = (0.99 x 0.01 x 0.02) = 0.000198
	99% HIV - = (0.99 x *0.99* x *0.02)* = *0.0196*

5 *Percent Prevalence of HIV Infection:*

Bite #l	Bite #2
5% HIV positive	5% HIV + = (0.05 x 0.05 x 0.02) = 0.00005
	95% HIV - = (0.05 x 0.95 x 0.02) = 0.00095
95% HIV negative	50/0 HIV + = (0.95 x 0.05 x 0.02) = 0.00095
	95% HIV - = (0.95 x 0,95 x 0.02) = 0.018

(Continued...)

10 Percent Prevalence of HIV Infection:

Bite #l	Bite #2
10% HIV positive	10% HIV + = (0.1 x 0.1 x 0.02) = 0.0002
	90% HIV - = (0.1 x 0.9 x 0.02) = 0.0018
90% HIV negative	10% HIV + = (0.9 x 0.1 x 0.02) = 0.0018
	90% HIV - = (0.9 x 0.9 x 0.02) = 0.016

50 *Percent Prevalence of HIV Infection:*

Bite #l	Bite #2
50% HIV positive	50% HIV + = (0.5 x 0.5 x 0.02) = 0.005
	50% HIV - = (0.5 x 0.5 x 0.02) = 0.005
50% HIV negative	50% HIV + = (0.5 x 0.5 x 0.02) = 0.005
	50% HIV - = (0.5 x 0.5 x 0.02) = 0.005

These calculations show that even if the prevalence of HIV infection was 50 percent, if horse flies are interrupted in their feeding, only 5/1,000 of the time would the horse fly have bitten an infected person first and an uninfected person second. The chance of transmitting HIV would be much less, because only 20 to 30 percent of those infected would have HIV in their blood. Furthermore, most bites would involve an insufficient amount of blood to transmit an infectious dose of HIV when in fact the virus was present in the blood of the first person bitten. These probabilities are also only for interrupted feeding and do not account for the times, which would probably be in the (great) majority of cases, that insects did not bite two persons in quick succession.

Appendix B:

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Can the AIDS Virus be Transmitted by Insects?

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