Laboratory and epidemiologic studies have established human herpesvirus 8 (HHV8) as an etiologic agent of Kaposi's sarcoma. With strong evidence linking HHV8 infection with the number of sexual partners among homosexual men, the challenge now is to determine the specific sexual acts associated with HHV8 transmission. Initial studies of specific practices, however, have differed in their conclusions; the paper by Dukers et al. in this issue of the Journal is the first to associate penile-oral intercourse with HHV8 transmission. Many sources of bias may contribute to the conflicting findings of studies reported to date: HHV8 research still lacks an adequately specific and sensitive serologic assay; identification of relevant exposure periods and measurement of sexual practices are imperfect; and sufficient adjustment for confounding is problematic. These numerous potential biases may be particularly important when trying to detect underlying associations that may be of low-order magnitude. The study by Dukers et al. (Am J Epidemiol 2000;151:213-24) is an important contribution to research on HHV8 transmission, but we do not yet know enough about the possible sexual routes of transmission to recommend avoiding any single behavior. For now, the best prevention advice is to reinforce the more general safe sex practices that have been promoted to prevent human immunodeficiency virus and other sexually transmitted diseases.

Herpesviridae infections; herpesvirus, Kaposi sarcoma-associated; homosexuality, male; risk factors; sarcoma, Kaposi; sex behavior; sexual partners; sexually transmitted diseases, viral

Epidemiologic arguments provided the impetus for the enormous effort put forth by basic scientists to discover the infectious etiologic agent of Kaposi's sarcoma. As the acquired immunodeficiency syndrome (AIDS) epidemic unfolded, the substantially higher frequency of Kaposi's sarcoma in homosexual men led to the hypothesis that a sexually transmitted agent, other than human immunodeficiency virus (HIV), was an etiologic factor (1). Over a decade of work produced numerous candidate agents (2-4), but the evidence was convincing for none until human herpesvirus 8 (HHV8), or Kaposi's sarcoma-associated herpesvirus, was discovered (5). Now that HHV8 has been described and its causal role in Kaposi's sarcoma confirmed (6-10), epidemiologists are again called back to the field, this time to determine the virus's mode of spread. Just as the original search for the "KS agent" proved to be painstaking, determining the specific routes of transmission for HHV8 appears to be a substantial challenge as well.

Considerable groundwork has already been completed. In the United States and northern Europe, initial work postulated that HHV8 was sexually transmitted because the seroprevalence was highest in groups at highest risk for sexually transmitted disease, particularly homosexual men (11-13). More direct evidence for sexual transmission came from studies in which individual-level sexual behavior was measured (8, 10, 14-18). For example, among homosexual men in San Francisco, HHV8 seroprevalence increased linearly with the number of male sexual partners in the previous 2 years (8). In contrast, in parts of Africa, infection appears to occur to a substantial degree before adulthood and is age dependent prior to puberty (14, 19). This pattern argues for predominantly nonsexual horizontal (and perhaps vertical) transmission. We will not consider further HHV8 transmission in Africa other than to note that, in addition to determining specific practices responsible for sexual transmission of HHV8, the other major challenge for epidemiologists will be to explain the reasons for this geographically disparate pattern of transmission.

The strong association between the number of sexual partners and HHV8 seropositivity among homosexual men focuses the search for a specific transmis-
sion route to a form of intimate human contact. However, because researchers have defined sex broadly to include a variety of practices, the specific act or acts responsible for transmission have not been pinpointed. The detection of HHV8 by polymerase chain reaction in both semen (20, 21) and saliva (21–23) likewise does not narrow the biologically plausible routes and, in fact, also puts forth kissing as a potential mode of spread. Furthermore, oral-anal sexual practices (rimming) must also be considered, based on earlier work examining risk factors for Kaposi’s sarcoma (24–26). Therefore, seven practices must be considered: insertive and receptive penile-anal, penile-oral, and oral-anal contact and kissing.

In this issue, Dukers et al. (27) propose that HHV8 is transmitted by penile-oral intercourse. Although they were unable to discern the relative importance of receptive versus insertive activity, their finding that some aspect of penile-oral intercourse was associated with transmission differs from other studies to date and indicates that reaching consensus as to how HHV8 is sexually transmitted may not be easy. Including the present study, epidemiologic studies focusing on homosexual men published or presented at conferences have reported associations of HHV8 seropositivity with three groups of sexual practices—penile-anal, penile-oral, and oral-anal. In a Danish cohort, receptive penile-anal intercourse was the only act associated with seroprevalent HHV8 infection (15). Receptive penile-oral intercourse, receptive oral-anal contact, and insertive oral-anal contact were not associated; insertive penile-anal intercourse and insertive penile-oral intercourse were not examined. No acts were associated with seroincident infection. Three other groups, presenting in abstract form, have also found an association with receptive penile-anal intercourse (10, 16, 28). One of these groups additionally found an association with both insertive penile-anal intercourse and insertive oral-anal contact (10). The absence of an association with receptive penile-anal intercourse, reported by Dukers et al., was also reported by Grulich et al. (29).

In comparison with HHV8, it is of interest to note that consensus was reached much more readily when determining the sexual practices associated with HIV infection. The earliest studies of HIV were unanimous in finding substantially stronger associations with receptive penile-anal intercourse than with insertive penile-anal intercourse or receptive penile-oral intercourse (30–33). Why might inconsistency exist between studies examining sexual transmission of HHV8? We believe this inconsistency is most likely explained by an imposing list of potential biases coupled with underlying associations that may be of low magnitude.

Error in the measurement of the outcome, HHV8 antibody, has received the most attention thus far. No single serologic assay has satisfactorily demonstrated high specificity and sensitivity, and agreement between assays is poor (34). Regardless of study design, assays with suboptimal specificity result in attenuation of associations between a given sexual practice and HHV8 infection. In particular, the specificity of some immunofluorescence assays measuring antibodies to HHV8 replicative-phase antigens has been questioned (35, 36). Lack of sensitivity, by itself, produces no bias in cross-sectional studies when prevalence ratios are estimated, but it can bias associations in longitudinal analyses (i.e., cohort studies and case-control studies) in two ways. The first, occurring in case-control studies, is the commonly appreciated mechanism whereby underascertainment of HHV8 infection, even if equally pervasive among exposure groups, will result in attenuation of associations. The second is less recognized and can occur in either case-control or cohort studies. In these designs, investigators define the period of relevant exposure as that occurring between the last negative and first positive antibody test. Use of a suboptimally sensitive assay (even if highly specific) may induce the investigator to identify a period of time in which to measure a subject’s sexual practices that is after the actual time when infection occurred. To the extent that sexual behavior changes over time, misclassification of the principal exposures of interest (i.e., specific sexual practices) can result. Here, the bias is not necessarily always toward the null hypothesis and may result in spurious associations.

Other pathways leading to exposure misclassification may also occur. In cross-sectional studies, the actual time when HHV8 infection occurred is not known, and the short-term measurements available may not capture the sexual practices relevant to the occurrence of transmission in the past. As noted above, depending upon how sexual behavior within an individual changes over time, this shortcoming could result in bias in either direction. Although not unique to HHV8 research, even when the relevant exposure period is identified, measurement of sensitive sexual behaviors may not be accurate. While the accuracy of measuring these behaviors would not be directly influenced by HHV8 status (which was not known by participants during the time the available studies of HHV8 transmission were conducted), participants’ awareness that certain sexual acts were responsible for transmitting HIV increased during the time many of the available studies on HHV8 were performed. This may have resulted in “socially desirable” misreporting of sexual behaviors that is differential with respect to HIV sta-
Failure to account for potential confounding may also create differences between studies. Among homosexual men, all of the sexual acts of potential importance for HHV8 transmission are commonly practiced. Therefore, before declaring that one act is responsible for HHV8 transmission, investigators must control for the influence of all other acts, which may be the true culprits in transmission. Because studies have been retrospective, investigators have often not had available measurements of all potentially relevant practices. In particular, kissing was not routinely measured. As such, differences in the comprehensiveness of adjustment across studies may account for different conclusions. Accurate measurement of confounders is also important. Just as underreporting of sensitive behaviors results in difficulties in determining the role of such behaviors per se in transmission, it also results in the inability to completely control for these behaviors when evaluating the independent role of other behaviors. Even if all relevant practices are measured accurately, there may be so much collinearity between certain acts that statistical estimation of the independent role of any one act may be impossible. For example, the high correlation, found by Dukers et al., between insertive and receptive penile-oral intercourse precluded estimation of their independent effects. Finally, inappropriate adjustment may also result in differences between studies. Because HIV infection has been shown to be a risk factor for HHV8 infection (8–10), it is often included in models looking at the role of specific sexual acts in HHV8 transmission. Because there is no evidence suggesting that HIV increases susceptibility to HHV8 infection, we believe that HIV is acting as a marker for a particular sexual activity that is associated with both HIV and HHV8 transmission. Adjusting for HIV infection status, as done by Dukers et al., does not threaten the validity of an observed association (such as penile-oral activity found by Dukers et al.) but could diminish the association for other acts that are associated with both HIV and HHV8. This is especially true for receptive penile-anal intercourse because it is strongly associated with HIV infection.

Associations of large-order magnitude can withstand a certain degree of bias and still be detected. What perhaps makes the aforementioned biases so influential in the study of HHV8 transmission is that the underlying associations that investigators are trying to uncover may be of low-order magnitude. Even if all relevant sexual practices could be measured, not all partners with whom an individual practices an act are necessarily HHV8 infected. Furthermore, not all infected partners shed virus. Although HHV8 has been detected in semen, the most recent carefully performed studies indicate that the prevalence of HHV8 in semen of infected men is very low (21, 37). This finding suggests that the absolute risk per partner for HHV8 infection associated with sexual acts in which semen is transferred between partners may be very low. Among infected persons, HHV8 appears to be more prevalent in saliva than in semen, but given that practices in which saliva is transferred between homosexual men (e.g., kissing and penile-oral intercourse) are nearly universal and overall HHV8 seroprevalence is not, saliva must be at best an inefficient conduit for sexual spread and acts that transfer saliva must have a low absolute risk for HHV8 transmission. If more than one specific type of intimate behavior is capable of transmitting HHV8 and if most individuals practice more than one of these behaviors, then, to the extent that the absolute risks associated with these acts are small and close in magnitude, the relative risk for any one act may also be small. Not only are intrinsically small relative risks prone to being obscured into nondetectability by bias, but they also present statistical limitations. Even a relatively well-powered study like that by Dukers et al. had confidence intervals that could not exclude an odds ratio of 2 for several sexual practices.

At this time, are there sufficient data to proclaim that any one sexual practice is associated with HHV8 transmission and hence to develop a public health prevention message around avoiding this act? We believe there are not. Given the methodological limitations we have described, the number of available well-conducted studies is limited, and no one specific act has been found to be important by a preponderance of well-conducted studies. Although the study by Dukers et al. is among the most carefully reported to date, additional studies that observe an independent role for penile-oral practices in HHV8 transmission will be needed to confirm this route of spread. As Dukers et al. remark and we agree, even in studies where one route of sexual transmission is convincingly demonstrated, failure to detect evidence for other routes is not tantamount to proving there is no risk. For this reason, promoting the avoidance of one act now may be counterproductive if additional or entirely different acts are later deemed more important. Nevertheless, our current inability to determine a specific route of spread should not detract from disseminating the evidence that HHV8 can be spread by intimate contact, especially between homosexual men. In fact, at a time when high-risk sexual activity is increasing in some communities (38), we might take advantage of our current inability to pinpoint one or two
specific acts to reinforce the more general message about safe sex practices that has been promoted to prevent HIV and other sexually transmitted diseases.

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REFERENCES

34. Rabkin CS, Schulz TF, Whitby D, et al. Interassay correlation

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