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Incubation Period and Risk Factors Support Sexual Transmission of Bacterial Vaginosis in Women Who Have Sex with Women

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Abstract

Objective—The epidemiology of BV favors sexual transmission of BV-associated bacteria. We examined incubation period and risk factors for incident BV (iBV) in a prospective study of women who have sex with women (WSW).

Methods—Using daily self-collected vaginal swabs, WSW with normal vaginal microbiota (no Amsel criteria and a Nugent score of 0–3) were followed for 90 days or until iBV (Nugent score 7–10 on at least 2–3 consecutive days). Daily diaries of sexual activity and menses were completed. Time to iBV was estimated. Accounting for differing lengths of follow-up and age, rates of sexual activities (per 100 person-days [pd]) were compared according to iBV status. The relationship between menses and iBV was also investigated.

Results—Of 36 WSW, mean age was 30 years (SD, 8); 92% were African American. The probability of iBV at 30 and 60 days was 20% (SD, 7%) and 36% (SD, 8%), respectively; 14 (39%) developed iBV by 90 days. In WSW with iBV versus those without iBV, the relative rate of any sexual activity prior to iBV was 40% higher (20.4 vs. 14.6 per 100 pd; $p=0.010$), sex with a woman was 38% higher (14.3 vs. 10.3 per 100 pd; $p=0.038$), digital-vaginal sex was 57% higher (14.3 vs. 9.1 per 100 pd; $p=0.005$), and digital-anal sex was 5.6 times higher (2.9 vs. 0.5 per 100 pd; $p<0.001$). iBV was more likely for those WSW with menses in the prior 2 days as compared to those without recent menses (Hazard ratio, 3.4; $p=0.029$). Sexual activity occurred in 93% WSW at a median of 4 days (95% CI, 2–6) prior to iBV.

Conclusion—Incident BV was common and associated with sexual activity in this cohort of predominantly African American WSW. An incubation period of 4 days is consistent with other bacterial STIs.

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CONTRIBUTORS

Conception and design: CAM, JRS. Acquisition of data: CAM, KJA, JRS. Analysis/interpretation of the data: CAM, SYL, KJA, JRS. Drafting of the manuscript: CAM, SYL. Revising it for intellectual content: CAM, SYL, KJA, JRS. Final approval: CAM, SYL, KJA, JRS.

Keywords

bacterial vaginosis; women who have sex with women; sexual transmission; incubation period

INTRODUCTION

Bacterial vaginosis (BV) is the most common cause of vaginal discharge (1). It is associated with preterm birth, post-operative gynecological infections, and increased risk for acquisition of HIV and sexually transmitted infections (STIs) (2). The epidemiology of BV favors sexual transmission of BV-associated bacteria (3). However, the etiology and incubation period remain unknown (4). What is known is that BV is characterized by loss of lactic acid-producing lactobacilli and increases in facultative (*Gardnerella vaginalis*) and strict anaerobes (2). The trigger(s) initiating these changes are not well defined.

BV is very common among women who have sex with women (WSW), more so than in women who have sex with men (WSM) (1, 5). BV prevalence has been found to be significantly higher among women reporting a history of sex with women (45.2%) versus women who did not (28.8%) (1). High BV prevalence rates and the fact that both women in female sexual partnerships can be colonized with BV-associated bacteria makes WSW a unique population in which to study BV pathogenesis. In one study of Caucasian WSW, prevalent BV was associated with a female sexual partner with BV, shared use of vaginally inserted sex toys during the past 3 months, and vaginal lubricant use (6). Incident BV (iBV) in Caucasian WSW has been associated with presentation 14 days since onset of menses, a new female sexual partner with BV history, change in vaginal discharge, and detection of BV-associated bacteria in vaginal fluid at enrollment (7). A high level of concordance by BV status (per Nugent score) (8) has been found in women and their female sexual partners, suggestive of sexual transmission of BV-associated bacteria (9). However, risks for iBV in racially diverse populations of WSW as well as the incubation period of BV are not well defined. Substantial differences in BV prevalence based on race have been noted, with African American women at highest risk (1, 10). Risk factor differences have not explained the racial disparity in the occurrence of BV among African American women (10).

Our objective was to examine incubation period and risk factors for iBV in a prospective study of predominately African American WSW presenting to the Jefferson County Health Department (JCDH) STD clinic in Birmingham, Alabama.

METHODS

This is a secondary analysis of data collected from a prospective study on the pathogenesis of iBV in WSW (11). Recruitment methods and study procedures have been described (11). Briefly, screening inclusion criteria were female sex, age 18–45 years, history of sex with a woman during the past 12 months, and a current female sexual partner. Screening exclusion criteria were use of antibiotics within the past 14 days, HIV infection, pregnancy, and menses. WSW meeting screening criteria provided a sexual history and had a urine pregnancy test. They self-collected one vaginal swab for determination of Amsel criteria (12) and Nugent score (8). A Nugent score of 0–3 was designated normal vaginal

microbiota, 4–6 intermediate microbiota, and 7–10 BV. Enrollment inclusion criteria were absence of all Amsel criteria and Nugent score of 0–3 with no *G. vaginalis* morphotypes on vaginal Gram stain (interpreted by a research clinician and confirmed by a second reader in our laboratory; if there was disagreement, a third reader was used). Enrollment exclusion criteria were pregnancy, trichomoniasis on wet mount, and symptomatic vaginal yeast infection.

Enrolled participants completed a study questionnaire on socio-demographic characteristics, tobacco use history, STI and BV history, douching history, and current hormonal contraception use. A pelvic exam was performed with a cervical swab obtained for *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* nucleic acid amplification testing (NAAT) using the BD ProbeTec Qx CTQ/GCQ/TVQ assays (13, 14). Women were instructed to complete a one page daily diary for up to 90 days (a yes/no checklist of daily oral, vaginal, and anal sexual activities, sex toy use, gender of sexual partners, sexual partner type [regular, occasional, new], douching, and vaginal symptoms [discharge, odor, itching, and irritation]). They were also instructed on how to self-collect daily vaginal swab specimens for vaginal Gram stain (for Nugent score determination by study staff) and storage for future vaginal microbiome analysis (11). Daily diaries, vaginal Gram stains, and vaginal specimen tubes were transferred by participants on ice to the study site weekly; follow-up ended after the first episode of iBV (defined as a Nugent score of 7–10 on at least 2–3 consecutive days) or at 90 days for those not developing iBV. Women testing positive for *T. vaginalis*, *C. trachomatis*, or *N. gonorrhoeae* by NAAT were excluded.

For this secondary analysis, participant characteristics were compared according to development of iBV (per Nugent score) or not using a t-test for continuous data or Fisher's exact test for categorical data. Kaplan-Meier estimates were computed for days to iBV diagnosis or last follow-up from study entry; women lost to follow-up were censored. Accounting for differing lengths of follow-up and age group (<30, 30 years), rates of sexual activities, douching, and BV symptoms (per 100 person days [pd]) prior to iBV were compared according to iBV status using Poisson calculations. When low frequency activities were evaluated, no adjustment for age was made. Only evaluable days on the study were used in Poisson calculations; missing data were limited given that 34 (94%) of the women completed their daily diaries for approximately 95% of the days on the study. Menses was analyzed as a time-varying covariate in a Cox proportional hazards model adjusting for age group. P-values less than 0.05 were deemed statistically significant. All analyses were performed using SAS 9.4 (Cary, NC).

This study was approved by the University of Alabama at Birmingham Institutional Review Board (Protocol #F131127001) and the JCDH Research Review Committee. Written informed consent for screening and enrollment was obtained from all women.

RESULTS

Between September 2014–November 2017, 204 WSW were screened. Of those, 42 (21%) met enrollment criteria and 162 (79%) were screen failures. Reasons for the large proportion of screen failures have been previously described (11), the majority of which included an

abnormal baseline Nugent score >3 (64%). Of the women enrolled, 2 were withdrawn due to a Nugent score >3 at second read, 1 was withdrawn due to *G. vaginalis* morphotypes on enrollment Gram stain on second read, and 3 were withdrawn because of positive NAAT for *C. trachomatis* (n=2) and *T. vaginalis* (n=1); thus, 36 WSW continued in the study. Five participants were lost to follow-up at a median (range) of 21 (2–36) days. Compliance with daily diary and vaginal swab specimens was high with median (IQR) return rates of 100% (97–100%) and 97% (91–98%) while on the study, respectively.

Of the 36 WSW, mean age was 30 years (SD, 8) and 92% were African American Table 1). Regarding sexual history, 59% reported a history of sex with women only, 83% reported an STI history (47% trichomoniasis, 33% chlamydia, 17% gonorrhea), and 33% reported a BV history. Current hormonal contraception use was infrequent (14%). There were 14 episodes of iBV in 14 (39%) women by 90 days, and the probability of iBV at 30 and 60 days was 20% (SD, 7%) and 36% (SD, 8%), respectively Figure 1). Keeping in mind the small sample size, no significant differences were found at baseline between women who developed iBV versus those who did not with regards to multiple characteristics Table 1).

Sexual activity occurred in 93% of WSW at a median of 4 days (95% CI, 2–6) prior to iBV. In WSW with iBV versus those without Table 2), the relative rate of any sexual activity prior to iBV was 40% higher (20.4 vs. 14.6 per 100 pd; $p=0.010$), sex with a woman was 38% higher (14.3 vs. 10.3 per 100 pd; $p=0.038$), digital-vaginal sex was 57% higher (14.3 vs. 9.1 per 100 pd; $p=0.005$), digital-anal sex was 5.6 times higher (2.9 vs. 0.5 per 100 pd; $p<0.001$), and sex using fingers or hands on the vagina was 33% higher (16.6 vs. 12.4 per 100 pd; $p=0.048$) after adjusting for age. Unexpectedly, the rate of BV symptoms was significantly lower in those developing iBV (3.4 vs. 12.5 per 100 pd; $p<0.001$) after adjusting for age. This finding was due to differing rates of vaginal discharge, which was significantly lower in those developing iBV as compared to those who did not (1.5 vs. 9.9 per 100 pd); rates of vaginal odor, itching, and irritation were not significantly different between groups (data not shown). The relative rate of douching was extremely low in both groups and not found to be significantly different.

In a Cox proportional hazards model adjusting for age with menses as a time-varying covariate, iBV was significantly more likely among women menstruating in the past 2 days compared to women who were not (HR=3.4, 95% CI, 1.1–10.3, $p=0.029$). This finding was consistent with the observation that at the end of follow-up, 43% of those developing iBV had menstruated in the past 2 days compared to 14% of those who did not develop iBV.

Since sexual activities often overlap, we sought to better understand the contribution of digital-anal sex performed by study participants or their partners in the development of iBV (given the highly significant relationship found with regards to digital-anal sex and iBV). We explored rates of sexual behaviors during follow-up according to groups defined by reported digital-anal sex (any vs. none).. Eight of 36 women reported digital-anal sex during follow-up; 4 of these developed iBV. Among these 8 women, the unadjusted rate of digital-anal sex was significantly higher among those developing iBV as compared to those who did not (10.1 vs. 2.5 per 100 pd; $p=0.001$). For the entire cohort, women reporting digital-anal sex during follow-up also reported significantly higher rates of any sexual activity (23.2 vs. 13.0

per 100 pd; $p<0.001$). They also reported significantly higher rates of sex with a man (8.4 vs. 1.6 per 100 pd; $p<0.001$), receptive oral sex (10.0 vs. 4.9 per 100 pd; $p<0.001$), sex toy use (5.0 vs. 2.5 per 100 pd; $p=0.006$), inconsistent dental dam use (17.9 vs. 9.7 per 100 pd; $p<0.001$), inconsistent condom use (3.8 vs. 0.5 per 100 pd, $p<0.001$), and digital-vaginal sex (19.8 vs. 6.9 per 100 pd; $p<0.001$). Other sexual activities in Table 2 did not significantly differ according to digital-anal sex status.

DISCUSSION

In this sample of predominantly African American WSW, iBV was common and associated with multiple sexual activities. It is important to note, however, that sexual activities often overlap. We did not collect data on the concurrency of these sexual activities during the study and it is difficult to determine the relative contribution of each to the risk of BV acquisition. Nevertheless, our data suggest that iBV is significantly associated with a number of sexual activities and support the hypothesis that BV-associated bacteria are sexually transmitted. Sexual exchange of infected vaginal secretions may be a possible mechanism for the acquisition of BV-associated bacteria among WSW (6).

It has been suggested that some BV-associated bacteria are acquired vaginally from preexisting reservoirs at extravaginal sites. In a prospective study of WSW, detection of *G. vaginalis* in the oral cavity or anus and of *Leptotrichia/Sneathia* spp. in the anus was significantly more common at enrollment among women who acquired BV (15). However, our findings are in contrast to a study of predominantly Caucasian WSW which found no association between sexual activities and BV acquisition (7). This study did find a dose-response relationship between the risk of BV acquisition and greater number of episodes of receptive oral sex however, this association was not observed when receptive oral sex was analyzed using other measures. In another study of WSW, colonization with *G. vaginalis* (present in 95–100% cases of BV) was associated with >20 digital-vaginal sex acts or >10 toy-vaginal acts in the past 3 months (16). Oral-vaginal and anal sexual behaviors were not significantly associated with *G. vaginalis* colonization. Reasons for the inconsistencies between predisposing factors for iBV in our study and prior WSW studies are unknown.

In our study, women with menses during the past 2 days were significantly more likely to develop iBV than women without menses. Others have demonstrated increased variance of the vaginal microbiota during menses (17). In a study of microbiological changes in the vaginal microbiota based on Nugent score, women who reported menses in the previous day were 2.15 times more likely to demonstrate a change in their Nugent score the following day compared with women who did not have menses ($p<0.001$) (18). In a study of women with daily vaginal Gram stains, increases in *Gardnerella/Bacteroides* morphotypes and reduced quantities of lactobacilli were significantly correlated with the onset of menses (19). A qPCR study of the temporal variability of the vaginal microbiota found that women without BV had vaginal microbiotas dominated by lactobacilli, whose levels fluctuated with menses. With the onset of menses, quantities of *L. jensenii* and *L. crispatus* decreased and were inversely related to *G. vaginalis* concentrations ($p<0.001$) (20). Others have found similar fluctuations in vaginal bacterial community composition during menses using 16S rRNA gene sequencing data (21). Menses is known to promote the temporary expansion of BV-

associated bacteria that are normally present in low levels (17). Iron favors the growth of proteolytic organisms such as *G. vaginalis* (22). Free iron is found in limited amounts in humans and is sequestered in compounds such as ferritin, heme, and hemoglobin or bound by high-affinity iron-binding proteins such as lactoferrin and transferrin (23). *G. vaginalis* can directly bind iron-containing compounds, including heme, hemoglobin, and catalase (23). It also produces high-affinity iron chelators, known as siderophores (23). However, BV does occur in post-menopausal women not on hormone replacement therapy, although at a lower prevalence (6%) than in women of reproductive age (9.8%) and peri-menopausal women (11%) (24). Thus, the exact role of menses in the pathogenesis of iBV is unclear.

Sexual activity occurred in 93% of WSW at a median of 4 days prior to iBV in our study, and at a 40% higher rate in women who developed iBV compared to those who did not. If BV-associated bacteria are sexually transmitted, these data suggest that their incubation period may be around 4 days, which is consistent with other bacterial STIs such as *N. gonorrhoeae* (25). This timeframe is corroborated by data from a case report of a woman diagnosed with BV who began to experience vaginal irritation 3 days after unprotected penile-vaginal sex with a male partner whose other female sexual partner also had BV (26).

Surprisingly, we found that the rate of reported BV symptoms was significantly lower in those developing iBV compared to those who did not. This is similar to a large prospective study of women without chlamydia, gonorrhea, or trichomoniasis in which 58% of those with BV denied vaginal discharge, 75% denied vaginal odor, 88% denied vaginal itching, and 91% denied vaginal irritation (27). This was despite direct, explicit, and detailed questioning of participants. The authors concluded that common BV symptoms are only reported by a minority of women. However, questions on vaginal symptoms covered a 6 month timeframe and the correlation between vaginal symptoms and BV may have been stronger if women were queried during a shorter timeframe. In contrast, Amsel et al reported that the prevalence of symptoms among women with BV increased substantially upon direct, specific questioning (12). In our study participants reported on vaginal symptoms on a daily basis on their daily diaries. Although the data were not collected by direct questioning, recall bias should not have played a major role in the results. Additional research should determine factors among women with BV that result in the reporting (or non-reporting) of vaginal symptoms.

Douching was uncommon in our study, making it difficult to adequately evaluate the association with iBV, which was not found to be significant. Other studies have found a positive association between douching and BV. In a prospective study of 182 women attending an STI clinic, women who douched for cleanliness or hygiene had a 2.1-fold increased risk for iBV than women who did not (hazard ratio, 2.1; 95% CI, 1.0–4.3) (28). In a study of 250 adolescent women who regularly douched enrolled in a randomized douching intervention trial, douching after menses had the strongest association with BV in multivariate analysis (odds ratio, 5.11; 95% confidence interval, 1.99–13.15) (29). In contrast, Marrazzo et al found that BV was more common among participants who did not douche within the past 30 days (9). Additional prospective studies are needed to determine the role of douching in the development of iBV.

Our study has several limitations. First, survey questionnaire and daily diaries were based on self-report and subject to social-desirability and recall bias. Second, despite the use of experienced Nugent score readers in this study (CAM and JRS), Nugent score sensitivity can be as low as 65% (using Amsel's criteria as the gold standard) (30), and some cases of iBV may have been missed. Third, as our study only focused on WSW, the results are not generalizable to WSM. We chose to study WSW as they are at high risk for BV and present a unique opportunity to study a variety of sexual activities. Finally, this study had a small sample size. While there were no statistical differences between groups at baseline, this could be due to a lack of power as there were qualitative difference between the groups, such as the difference for a history of BV (21% vs 41%), chlamydia history (43% vs 27%), and current use of hormonal contraception (7% vs 18%). Future studies should include larger samples. Despite these limitations, our study has multiple strengths including the collection of prospective daily diary and Nugent score data from a cohort of predominantly African American WSW, a demographic with the highest risk for BV (1, 5, 10). The results provide additional evidence supporting the sexual transmission of BV-associated bacteria among women. Interventions that promote safer sex practices and avoidance of sex during or in close proximity to menses may provide opportunities to reduce subsequent episodes of BV in this high-risk population.

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COMPETING INTERESTS

Christina A. Muzny, MD, MSPH is a consultant for Lupin Pharmaceuticals and BioFire Diagnostics. She has also received honoraria from Roche Diagnostics and Cepheid. Jane R. Schwebke, MD has been a consultant for and received research support from Lupin Pharmaceuticals, Hologic/GenProbe, BD Diagnostics, Cepheid, Quidel, Sybmimix, StarPharma, and Toltec. The other authors have no potential conflicts of interest.

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KEY MESSAGES

1. The epidemiology of BV favors sexual transmission among WSW.
2. Participation in any sex, receptive digital-vaginal sex, and receptive digital anal-sex was associated with iBV among WSW in this study.
3. Menses is known to promote the temporary expansion of BV-associated bacteria and was a risk factor for iBV among WSW in this study.
4. The incubation period of BV may be around 4 days, which is consistent with other bacterial STIs.

Figure 1. Time to incident BV.

Kaplan-Meier estimates of BV probability over a 90-day time-frame among a cohort of predominately African American women who have sex with women. The dashed lines represent the 95% confidence intervals for the Kaplan-Meier estimates.

Table 1.

Characteristics of Women Who Have Sex with Women Participating in a BV Pathogenesis Study in Birmingham, AL (n=36) *

Characteristic	Incident BV (n=14)	No Incident BV (n=22)	Total (n=36)	p-value [€]
Age, years				0.571
Mean $\pm$ SD	29 $\pm$ 8	31 $\pm$ 8	30 $\pm$ 8	
Range	19–44	20–48	19–48	
African American race	13 (93)	20 (91)	33 (92)	0.999
Education				0.703
Some high school	1 (7)	3 (14)	4 (11)	
High school / GED	5 (36)	9 (41)	14 (39)	
Some college / Degree	8 (57)	9 (41)	17 (47)	
Sexual behavior history				0.478
Women only	9 (69)	11 (52)	20 (59)	
Women and men	4 (31)	10 (48)	14 (41)	
Smoked 30+ packs, lifetime	9 (64)	9 (41)	18 (50)	0.305
History of STIs ^β	13 (93)	17 (77)	30 (83)	0.371
Trichomoniasis	8 (57)	9 (41)	17 (47)	0.500
Chlamydia	6 (43)	6 (27)	12 (33)	0.472
Gonorrhea	3 (21)	3 (14)	6 (17)	0.658
History of BV	3 (21)	9 (41)	12 (33)	0.293
Current hormonal contraception use ^{**}	1 (7)	4 (18)	5 (14)	0.628
Most recent douching				0.678
Within 72 hours	0 (0)	0 (0)	0 (0)	
Within 30 days	0 (0)	1 (5)	1 (3)	
Within 3 months	0 (0)	1 (5)	1 (3)	
Greater than 3 months	6 (43)	12 (55)	18 (50)	
Never	8 (57)	8 (36)	16 (44)	

* Data presented as n (column %) unless otherwise specified; there was missing data for education (n=1), sexual behavior history (n=2)

[€] T-test (age) or Fisher's exact test

^β No woman had a history of syphilis, genital herpes, genital warts, HIV, or pelvic inflammatory disease

^{**} Included pill (n=2), injection (n=1), not specified (n=2)

SD=standard deviation; GED=General Equivalency Diploma; STIs=sexually transmitted infections; BV=bacterial vaginosis

Table 2.Rate of Sexual Activities^β and Douching per 100 Days of Follow-Up (95% CI), Adjusting for Age Group

Sexual Activity or Symptoms	Incident BV[€] n=14	No Incident BV[€] N=22	Relative Incidence	p-value*
Any sexual activity	20.4 (16.4–25.5)	14.6 (12.8–16.6)	1.40 (1.08–1.81)	0.010
Sex with a woman	14.3 (11.0–18.5)	10.3 (8.9–12.0)	1.38 (1.02–1.87)	0.038
Sex with a man	3.4 (2.0–5.86)	2.8 (2.0–3.8)	1.23 (0.66–2.28)	0.511
Sex with a new female partner	0.5 (0.2–1.38)	0	-	-
Sex with a new male partner	1.3 (0.3–5.4)	0.4 (0.2–0.8)	3.71 (0.62–22.20)	0.151
Receptive oral sex	8.1 (5.7–11.4)	9.2 (7.8–10.8)	0.88 (0.60–1.29)	0.515
Direct genital-genital contact	3.9 (2.3–6.4)	5.3 (4.2–6.5)	0.73 (0.42–1.28)	0.277
Sex toy use	3.5 (2.1–6.0)	3.0 (2.3–4.0)	1.17 (0.64–2.16)	0.605
Inconsistent dental dam use	14.2 (11.0–18.5)	11.3 (9.7–13.1)	1.26 (0.93–1.71)	0.132
Inconsistent condom use	2.1 (0.9–5.3)	1.3 (0.8–2.0)	1.65 (0.59–4.63)	0.343
Any digital-vaginal sex	14.3 (11.0–18.7)	9.1 (7.8–10.7)	1.57 (1.15–2.15)	0.005
Any digital-anal sex	2.9 (1.6–5.3)	0.5 (0.3–1.0)	5.56 (2.4–12.8)	<0.001
Any finger or hand on vagina	16.6 (13.0–21.1)	12.4 (10.8–14.3)	1.33 (1.00–1.77)	0.048
Douching**	0	0.2 (0.1–0.7)	-	-
BV Symptoms	3.4 (1.9–6.3)	12.5 (10.9–14.3)	0.27 (0.15–0.51)	<0.001

^βSex with a female or male partner was defined as any oral, vaginal, and/or anal sexual contact. Direct genital-genital contact refers to the genital regions of each partner touching during sex (could be between two female sexual partners or female-male sexual partners). Sex toy use could include shared use however this data was not specifically captured. Data on vaginal insertion of sex toys or external use only was also not captured.

* p-values calculated from a Poisson model adjusting for age group (<30, 30 years)

[€]There were 428 days of follow-up in the incident BV group (mean, 30.6 days) and 1614 days of follow-up in the non-incident BV group (mean, 73.4 days).

** Unadjusted estimates presented since the age-adjusted model would not converge.