

Most studies underestimate overall prevalence by assessing it in a specific timeframe; to the contrary, head lice infestation is a dynamic process that can spread hypergeometrically in closed environments such as schools and in the community (7). The point-prevalence reported by Heukelbach et al (8) may represent a more accurate indicator.

Although socioeconomic status seems to be an indicator of the magnitude of lice infestation, more specific determinants are the dynamic processes of hygienic status and overcrowding. A recent study in Turkey compared 2 neighboring villages with different socioeconomic status. The only factor that was statistically significantly related to pediculosis capitis was size of the household;  $\geq 6$  inhabitants was associated with increased prevalence (9).

Another parameter that may indirectly influence overall prevalence and account for the leveling of the prevalence gradient between rich and poor is awareness of head lice and preventive and therapeutic practices. A study in Australia showed that although parents prefer to play a major role in prevention and treatment, they may lack insight into recent advances and dilemmas regarding these measures (10).

Variations in reported prevalence were found even in data from the same country. These differences can result from surveys being conducted during different seasons, various examination techniques, reporting of active infestation or presence of nits, and potential introduction of effective pediculicides.

Although head lice account for a substantial number of missed schooldays in children, among others, it is surprising that pediculosis capitis is not monitored and prevalence is not regularly reported. Although we cannot extinguish the parasite, effective monitoring and planning will enable us to limit the prevalence and distribution of this parasitosis.

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## Texas Isolates Closely Related to *Bacillus anthracis* Ames

**To the Editor:** Forensic and epidemiologic investigation of the 2001 bioterrorism-associated anthrax attacks used multiple-locus variable-number tandem-repeat analysis (MLVA) to identify the attack strain as Ames (1). Strain identity was essential for subsequent molecular epidemiologic and forensic investigations of this biocrime. To more easily identify this particular strain, comparative whole-genome sequencing (2) and phylogenetic analyses were used to identify single-nucleotide polymorphisms (SNPs) that seem highly specific for Ames strain identification (3). Because *Bacillus anthracis* is a recently emerged clonal pathogen, these SNPs represent highly evolutionarily stable markers (4) that are amenable to many rapid and cost-effective analytical techniques.

MLVA and the Ames-specific SNP assay indicate that the Ames strain has been isolated from nature only 1 time, in southern Texas, USA. Several lineages of *B. anthracis* (5) have been ecologically established in North America. The A.Br.009 clade is the most successful and widely dispersed in North America, but it is not closely related to the Ames

strain (5), which is a member of the A.Br.001 clade. Although A.Br.001 is not as successful as A.Br.009, it appears to be ecologically well established in southern Texas. Analyses of outbreaks in this region from 1974 to 2000 found 190 culture-confirmed cases clustered mainly in 5 counties (6). A major epizootic in Texas in 2001 paralleled this trend. This outbreak (6) affected many deer species, horses, and bovinds (total 1,637), which suggests that this clade is well established and not limited to cultivated areas and domesticated livestock. Previous molecular and epidemiologic analyses (3) of isolates from this region identified close, but not identical, matches to the Ames strain, which suggests that more intense surveillance in this region would likely yield more Ames and Ames-like isolates. Two recent (2006 and 2007) outbreaks in Texas confirmed this suggestion.

Isolates from the 2006 and 2007 outbreaks were initially screened by using an 8-marker MLVA system (MLVA8) as described by Keim et al.

(7). The MLVA8 genotypes were identical to the *B. anthracis* Ames strain (GT62). Additional analysis by a 15-marker (MLVA15) variable-number tandem repeats (VNTR) system (5) again generated an MLVA15 genotype that was identical to the original Ames strain (A0462) and to the 2001 bioterrorism-associated attack strain (A2012) (Figure). Given the identical MLVA genotypes, could these natural strains be differentiated from the laboratory or biocrime Ames strain by using higher resolution genotyping?

We developed 6 Ames strain-specific SNPs to address the potential that the Ames strain might reappear naturally and hinder epidemiologic and forensic investigations (3). We found that 5 of 6 SNP loci could be used to distinguish between all known natural isolates and laboratory or biocrime isolates (Figure). Results were consistent with our previous identification of a *B. anthracis* isolate from a goat kid in Texas in 1997 (A0394) as being closely related to the Ames strain (3).

However, the 2006 and 2007 isolates from Texas were even more closely related to the Ames strain because they also shared the MLVA15 genotype with Ames. In contrast, the 1997 goat isolate differed by a single mutational step at the BaVNTR16 locus (Figure). Hence, 5 of 6 SNP markers enabled differentiation among Ames and Ames near relatives even when VNTR profiles were identical.

Resolution of nearly identical genotypes might also be accomplished by using additional VNTRs (8) or hypermutable loci (9). However, we doubt that this approach would be better than whole-genome sequencing with interrogation of resultant SNPs because these markers would most likely result in topologic conflicts due to homoplasmy (10). The available epidemiologic data from other isolates included in this clade show that although the Ames clade is well established in southern Texas, no subsequently recovered natural isolates completely match the original Ames isolate.

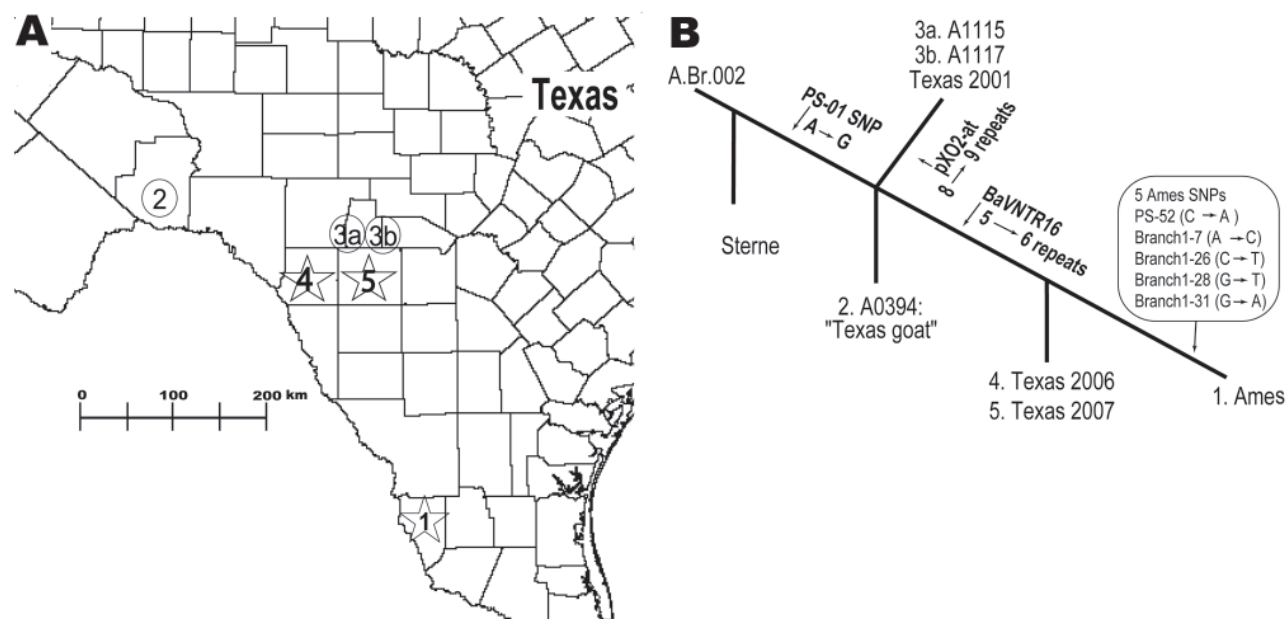


Figure. Geographic and phylogenetic relationships among strains closely related to *Bacillus anthracis* Ames strain. A) Spatial relationships among Ames-like isolates from southern Texas. 1, location of the original Ames strain, isolated from Jim Hogg County, Texas, in 1981; 2, closely related Texas 1997 goat isolate (A0394); 3a and 3b, Texas 2001 isolates; 4 and 5, most recent cases, i.e., Texas 2006 (Kinney County) and Texas 2007 (Uvalde County). B) Genetic relationships among isolates with variable-number tandem-repeat and single-nucleotide polymorphism (SNP) differences giving rise to that particular branch (arrows). The numbers at each branch terminus correlate with the numbers depicted on the map. SNP states are from ancestral to derived.

The precision of subtyping assays is a matter of importance and debate for epidemiologic and, recently, forensic investigations. Strain identity is commonly used to infer a common source even when spatial and temporal data are not congruent. Moreover, the definition of a strain is somewhat unclear and relies on analytical methods that vary widely. Therefore, isolates may be erroneously excluded or included into a strain definition and disease outbreak as illustrated with the Ames strain and 2 contrasting approaches to identification. MLVA15 ties naturally occurring isolates to bioterrorism-associated attacks, while specific SNP assays can distinguish among them.

MLVA is an unbiased approach and can be used on any set of *B. anthracis* strains, although, as in the 2006 and 2007 Texas outbreaks, it can be limited in resolving power. In contrast, our SNP assays have great resolving power but are useful only for differentiating the Ames strain, thus limiting their value to categorical inclusion or exclusion in outbreaks. Future rational use of a battery of different molecular signatures will yield far greater insights into strain identity than the application of 1 specific signature.

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## Bluetongue in Eurasian Lynx

**To the Editor:** Bluetongue is an infectious disease of ruminants; it is caused by bluetongue virus (BTV), has 24 known serotypes, and is transmitted by several species of *Culicoides* biting midges. The disease mainly affects sheep and occurs when susceptible animals are introduced to areas where BTV circulates or when BTV is introduced to naive ruminant populations. The natural host range is strictly limited to ruminants, although seroconversion without disease has been reported in carnivores (*1*). We report BTV infection, disease, and death in 2 Eurasian lynx (*Lynx lynx*) and the isolation of BTV serotype 8 (BTV-8) from this carnivorous species.

The 2 Eurasian lynx, held in the same cage in a zoo in Belgium, became lethargic in September 2007; animal 1 died after 2 days, and animal 2 died in February 2008. Both had been fed ruminant fetuses and stillborns from sur-