studies (unpublished) showed that types 53 and 80 and some STs cross-reacted with the typing sera, and on sequencing we found that there is considerable sequence similarity within the N-terminal regions of M proteins from these types. Thus, isolates scored as M nontypeable or M53 or M80 because of this ambiguity are now identified as one or the other M type or as STs upon sequencing of their corresponding M genes.

Finally, the definitions of both M nontypeable and sequence types should be considered provisional, and as sera for new types become available or an identification with uncommon but already defined sera is made, the data base for *emm* gene sequences will vastly increase. Such knowledge will contribute to the continuous epidemiological surveillance of group A streptococci.

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Abolish *Mycobacterium paratuberculosis* Strain 18

Mycobacterium paratuberculosis is a slowly growing (12 to 16 weeks), mycobactin-dependent organism which causes paratuberculosis (Johne's disease) in ruminants (5). There has been a heightened interest in this species because of the suggestion that it may be associated with some cases of Crohn's disease in humans (3). Discussions of the biology of this species invariably result in erroneous assignment of characteristics to the species which are supported by published data. Careful review generally reveals that the erroneous data were obtained from strain 18.

Recent articles in the *Journal of Clinical Microbiology* by Coffin et al. (6) and Kunze et al. (9) continue this trend and erroneously identify the infamous strain 18 as *M. paratuberculosis*, when in fact the strain is *Mycobacterium avium* serovar 2. The continued use of this organism in *M. paratuberculosis* research erodes our knowledge and understanding of this species.

During the 1920's, W. C. Hagan at Cornell University observed that one of his laboratory strains of *M. paratuberculosis* grew faster than other strains and had lost its dependence on mycobactin. Although this laboratory strain had been passed from graduate student to graduate student and these same students were also working on *M. avium*, there were few criteria available to definitively identify this species, and the possibility of cross-contamination was not considered at the time. The availability of a rapidly growing, mycobactin-independent strain of *M. paratuberculosis* was a major boon to research around the world.

In October 1939, this laboratory-adapted strain was provided to the Regional Animal Disease Laboratory, Auburn, Ala., where it became known as U.S. Department of Agriculture (USDA) strain 18 (10). At the request of the American Type Culture Collection (ATCC), it was deposited by the USDA as the "working type" and designated ATCC 12227. Because of its relative ease of cultivation, rapid growth, and lack of mycobactin dependency, strain 18 was widely used in experimental studies, as well as in the production of antigens, mycobactin, and vaccines. ATCC requested several additional deposits, the last being October 24, 1966 (10).

In addition to rapid growth and mycobactin independence, strain 18 had many other differences from *M. paratuberculosis*. The inability of this strain to cause disease in ruminants resulted in fruitless efforts to associate mycobactin dependency and virulence. The differences between strain 18 and *M. paratuberculosis* were so great that insiders began to question if this strain was really a laboratory-adapted field strain of *M. paratuberculosis* at all. However, the technology to determine such was not readily available at the time.

Recognizing the uncertainty regarding this strain, in 1968 Merkal (10) officially withdrew strain 18 from ATCC as unrepresentative of the species and replaced it with a bona fide wild-type strain of *M. paratuberculosis* as the "neotype strain," designated ATCC 19698. Despite this withdrawal, strain 18 continued to see widespread use in paratuberculosis research.

With the advent of DNA technology, suspicions regarding the authenticity of strain 18 were confirmed. Every study in which strain 18 was used unequivocally showed that strain 18 was not a strain of *M. paratuberculosis* but rather was *M. avium* (4, 7, 12). Although *M. avium* and *M. paratuberculosis* are very closely related and often difficult to distinguish, the absence of the species-specific insertion sequence (IS*900*) in strain 18, which exists in 15 to 25 copies in all *M. paratuberculosis* strains (8), confirmed that it could not have evolved or been laboratory adapted from *M. paratuberculosis*. Even the articles of Coffin et al. (6) and Kunze et al. (9) serve to confirm the true identity of strain 18. As we had long suspected, strain 18 is actually *M. avium* serovar 2 and likely represents a 50-year-old laboratory contaminant!

These findings have a great impact on our knowledge of the biology of *M. paratuberculosis*. It is now necessary to carefully and critically review research articles on *M. paratuberculosis* to determine whether the data presented are based on *M. paratuberculosis* or strain 18. Despite published data to the contrary, we now know, to name just a few, that there are no mycobactin-independent strains of *M. paratuberculosis*, it does not express a species-specific (1) (or otherwise) peptidoglycolipid similar to that from *M. avium* (2), and mycobactin J (11) is actually mycobactin from *M. avium*. Most available antigens, both commercially available and from the USDA, and the currently employed vaccine are all made from *M. avium*. It will be years, if ever, before we can sort out the true characteristics of *M. paratuberculosis* and those of strain 18. Fifty years of research on a laboratory contaminant will be difficult to correct.

Compounding this existing problem is the fact that despite evidence that strain 18 is actually *M. avium* serovar 2, this strain continues to be widely used in *M. paratuberculosis* studies and identified as *M. paratuberculosis* 18. The excellent articles by Coffin et al. (6) and Kunze et al. (9), although both confirm the true identity of strain 18, erroneously identify this strain as *M. paratuberculosis* 18. This practice further adds to the confusion and the inaccurate description and characterization of the species and contributes to the 50-year erosion process.

For example, in the article by Coffin et al. (6), it implies that on the basis of restriction fragment length polymorphism (RFLP) analysis some *M. paratuberculosis* strains are "*M. avium*-like" while others are not. Strain 18 is further erroneously authenticated as an *M. paratuberculosis* strain by designating it as ATCC 12227, an obsolete and nonexistent ATCC number. In the article by Kunze et al. (9), it is implied that some strains of *M. paratuberculosis* contain the insertion sequence IS*901* when in fact they do not—the IS*901* insertion sequence was found only in strain 18. Although these two otherwise excellent articles were selected to raise this issue, they are by no means the primary or worse offenders. Rather, they simply represent recent publications on which to base this letter.

After 50 years of erroneous, conflicting, and confusing data, it is time to abolish strain 18 and label it appropriately if it is used at all. Since this strain is still used for vaccine, antigen, and mycobactin production, its inclusion in some studies would seem appropriate, while in others it may not be. In either event, strain 18 must be identified as *M. avium* to avoid the continued erosion of and conflicting data on *M. paratuberculosis* biology.

It is therefore recommended that authors and editors of JCM and other journals disallow the designation of strain 18 as *M. paratuberculosis*. It is further recommended that if the use of strain 18 is necessary that it be identified as "*M. avium* 18 (formerly *M. paratuberculosis* 18)."

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Authors' Replies

In his letter to the editor, Dr. Chiodini correctly points out that discussion of *M. paratuberculosis* in the literature is frequently confusing because of the common problem of strain misidentification and the resultant assignment of erroneous characteristics. Furthermore, he suggests that we have compounded this problem by identifying strain 18 as *M. paratuberculosis* in our recent publication (1). While we are in agreement with the arguments for a change in the nomenclature of strain 18 that were made by Dr. Chiodini, we would like to make the following comments.

With respect to the history, growth, and physiological characteristics of strain 18, eloquently outlined by Dr. Chiodini, we have no argument or further comment. We fully recognize *M. paratuberculosis* ATCC 19698 (5) as the neotype strain and have denoted it as such in our article (1). Our study included strain 18 for the very reasons outlined by Dr. Chiodini, that is the frequent previous and current use of this strain in vaccine, antigen, and mycobactin production. Indeed, we took considerable trouble to summarize the DNA-based analyses of *M. avium* complex strains in a lengthy introduction and stated that strain 18 gave an *M. avium* profile in these studies. Moreover, as Dr. Chiodini rightly points out, our results confirm the true identity of strain 18 and in our discussion we state, "*M. paratuberculosis* 18 gave profiles similar to those of . . . *M. avium* serotype 2. . . ." This is consistent with previous documentation in the

literature and supports the view that strain 18 is really an *M. avium* isolate (2–6). At no time did we suggest or propose that this strain is a true *M. paratuberculosis* strain. In fact, one of the major conclusions of our work is that classical forms of identification are insufficient to differentiate *M. paratuberculosis* and *M. avium*, especially with respect to primary isolates. It is hoped that one of the many benefits of an identification scheme based on DNA technology will be to prevent such anomalies from arising in the future.

We entirely agree with Dr. Chiodini's listing of the properties of true *M. paratuberculosis* strains and do not need to comment further except perhaps to reiterate that while all *M. paratuberculosis* strains are mycobactin dependent, the converse is certainly not true.

When including strain 18 in our study and our manuscript we described it as *M. paratuberculosis* 18 because this is what it has been consistently termed in the literature to date. This nomenclature and the ATCC number used reflect the description of the strain as it was received by us. Perhaps we did not provide sufficient clarification of this point, but we certainly did not intend to mislead and felt that our discussion of the results for strain 18 made this clear.

In conclusion we fully agree with and support the cumulative evidence and Dr. Chiodini's proposal that strain 18 should henceforth be identified as *M. avium* 18 (formerly *M. paratuberculosis* 18) and applaud all attempts to clarify the often murky waters of mycobacterial identification and nomenclature.

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I fully agree with Dr. Chiodini that strain 18 is indistinguishable from some strains of *M. avium*. Our own work demonstrated that the genome of strain 18 contains IS*901*, and we designated it *M. avium* RFLP type A/I (1). I therefore agree that strain 18 should henceforth be referred to as a strain of *M. avium*. However, I do not agree with Dr. Chiodini when he states that "strain 18 is actually *M. avium* serovar 2. . . ." We have examined most of the serotypes of *M. avium* by RFLP analysis (1, 2, 5) and have found that, as with other bacteria, serotyping does not correspond to genetic identity: of the 10 serotype 2 strains we examined only 6 contained IS*901* and were identical to strain 18 by RFLP analysis (1).

Dr. Chiodini goes on to object to our statement that some strains of *M. paratuberculosis* contain IS*901*, claiming that IS*901* was not found in any *M. paratuberculosis* strain (other than strain 18). However, as we described (1), we found three strains isolated from deer with Johne's disease that were identified (on the basis of initial mycobactin dependence) as *M. paratuberculosis* that contained IS*901* rather than IS*900*. We stated that "we therefore designate [these strains] *M. avium* [type] A/I"—not *M. paratuberculosis*, reserving the designation of *M. paratuberculosis* for strains containing IS*900*. This would suggest that *M. avium* A/I is able to cause Johne's disease, at least in deer, a possibility that may also be significant to human disease since we have isolated strains of *M. avium* A/I from patients with inflammatory bowel disease (4).

It may be, as Chiodini suggests, that strain 18 represents a laboratory contaminant; however, it is also possible that *M. avium* A/I may occasionally cause Johne's disease in cattle, and strain 18 may represent one such strain. Examination of a larger number of strains isolated from animals with Johne's disease with probes specific for IS*900* and IS*901* should resolve this issue.

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