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Adsorption of *Mycobacterium avium* subsp. *paratuberculosis* to Soil Particles[▽]

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Attachment of *Mycobacterium avium* subsp. *paratuberculosis* to soil particles could increase their availability to farm animals, as well as influence the transportation of *M. avium* subsp. *paratuberculosis* to water sources. To investigate the possibility of such attachment, we passed a known quantity of *M. avium* subsp. *paratuberculosis* through chromatography columns packed with clay soil, sandy soil, pure silica, clay-silica mixture, or clay-silica complexes and measured the organisms recovered in the eluent using culture or quantitative PCR. Experiments were repeated using buffer at a range of pH levels with pure silica to investigate the effect of pH on *M. avium* subsp. *paratuberculosis* attachment. Linear mixed-model analyses were conducted to compare the proportional recovery of *M. avium* subsp. *paratuberculosis* in the eluent between different substrates and pH levels. Of the organisms added to the columns, 83 to 100% were estimated to be retained in the columns after adjustment for those retained in empty control columns. The proportions recovered were significantly different across different substrates, with the retention being significantly greater ($P < 0.05$) in pure substrates (silica and clay-silica complexes) than in soil substrates (clay soil and sandy soil). However, there were no significant differences in the retention of *M. avium* subsp. *paratuberculosis* between silica and clay-silica complexes or between clay soil and sandy soil. The proportion retained decreased with increasing pH in one of the experiments, indicating greater adsorption of *M. avium* subsp. *paratuberculosis* to soil particles at an acidic pH ($P < 0.05$). The results suggest that under experimental conditions *M. avium* subsp. *paratuberculosis* adsorbs to a range of soil particles, and this attachment is influenced by soil pH.

Mycobacterium avium subsp. *paratuberculosis* is a pathogen of great significance for livestock since it causes a fatal and economically important disease called paratuberculosis or Johne's disease (JD). The significance of *M. avium* subsp. *paratuberculosis* has further increased due to speculation over its role in the causation of Crohn's disease in humans (10). Although reports trying to establish a causative association between *M. avium* subsp. *paratuberculosis* and Crohn's disease are conflicting and inconclusive, they have aroused concerns among public health authorities (13); therefore, greater attention is now being paid to understand the natural ecology of *M. avium* subsp. *paratuberculosis* (32, 34). We investigated a largely unexplored aspect of the natural ecology of *M. avium* subsp. *paratuberculosis*: its attachment to soil particles, which could influence its availability to farm animals and humans (see below).

Bacteria can become loosely associated with clay or soil particles through reversible adsorption mediated by electrostatic and van der Waals' forces or by cell surface hydrophobicity (20). An irreversible firm attachment may later occur usually mediated by extracellular bridging polymers (8). The attachment of microbiota such as *Escherichia coli*, *Arthrobacter* spp., and poliovirus to soil or clay particles has been reported previously (2, 3, 11, 22, 26), but there is only indirect evidence of the association of mycobacteria with soil particles. A study reported the recovery of only 3.5% of nontuberculous mycobacteria inoculated into soil samples and attributed this to their adsorption to clay particles (5). Later, a similar phenomenon was inferred for *M. avium* subsp. *paratuberculosis* because 99% of these organisms in feces could not be detected upon culture of feces mixed with soil, suggesting the binding of *M. avium* subsp. *paratuberculosis* to soil particles (33). An association between *M. avium* subsp. *paratuberculosis* and clay particles was also suggested by an epidemiological study conducted to investigate the risk factors for ovine JD, indicating the possibility of bacterial attachment to clay particles (6).

M. avium subsp. *paratuberculosis* is transmitted primarily by the feco-oral route. Infected animals shed huge numbers of *M. avium* subsp. *paratuberculosis* in their feces (29, 35), thus contaminating soil and the farm environment. The ability of *M. avium* subsp. *paratuberculosis* to survive for extended periods in an external environment, in spite of it being an obligate parasite (32, 34), facilitates the build-up of soil and pasture contamination levels over time. The attachment of *M. avium* subsp. *paratuberculosis* to soil particles could help retain the bacteria in the upper layers of the soil, thus further enhancing contamination levels. The contaminated farm environment thus becomes a potential source of infection for farm animals because grazing ruminants normally consume soil with pasture, and the amounts can be substantial, up to 300 or more grams per day for sheep (9, 21).

In addition, runoff from contaminated farm soils can contaminate water bodies (23), which can be a potential health hazard for humans because the routine chlorine disinfection of water is not able to eliminate *M. avium* subsp. *paratuberculosis* completely (28). The transportation of bacteria from the farm environment to water sources is influenced by their attachment to soil or clay particles (11, 12). Generally, bacterial adsorption

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to soil particles decreases the rate of transportation through soil (3), but it also helps retain bacteria in the top surface layers of the soil, thus increasing the possibility of the contamination of runoff water (24). Note that soil particles can be dislodged and moved by wind, water, and mechanical factors.

The aim of the present study was to verify whether *M. avium* subsp. *paratuberculosis* attaches to clay and other soil particles and whether this attachment is influenced by soil pH. The study findings improve our knowledge and understanding of the natural ecology of *M. avium* subsp. *paratuberculosis*.

MATERIALS AND METHODS

***M. avium* subsp. *paratuberculosis* suspension.** A clonal sheep (S) strain (Telford 9.2), with an S1 IS*900* RFLP pattern (19) and an IS*1311* S pattern (18), was reconstituted from a freeze-dried seed stock by adding 0.1 ml of sterile water and then inoculating this into radiometric culture medium, followed by incubation at 37°C. The radiometric medium consisted of 4 ml of enriched Middlebrook 7H9 medium (BACTEC 12B; Becton Dickinson, Sparks, MD) with 200 μl of PANTA PLUS (Becton Dickinson), 1 ml of egg yolk (prepared in-house), 5 μg of mycobactin J (Allied Monitor, Inc., Fayette, MO), and 0.7 ml of water (30). Growth in this medium was recorded with an automatic ion chamber (BACTEC 460; Johnston Laboratories, Towson, MD) as a growth index. When this reached 999, usually after 3 weeks, the liquid medium was subcultured onto Middlebrook modified 7H10+mycobactin J slopes, followed by incubation at 37°C for 6 weeks, by which time colonies were abundant (30, 31). Bacteria were harvested into 200 μl of phosphate-buffered saline (PBS) plus 0.1% (vol/vol) Tween 80 (PBST), vortexed for 1 min, diluted 1:10 in sterile PBST, passed through a 26-gauge needle, filtered through an 8-μm-pore-size filter, and examined by light microscopy at ×400 magnification to confirm that the majority of cells were single. Suspensions were stored at −80°C until required, with no loss of viability. Suspensions were thawed at room temperature, followed by thorough mixing by vortexing. The bacterial suspensions induced typical JD in sheep after experimental oral inoculation (data not shown). This suspension was used for the experiments described below.

Experiment 1: pilot experiment. A known quantity of *M. avium* subsp. *paratuberculosis* was pumped through three chromatography columns packed with different substrates (see below), and the organisms recovered in the eluent were quantified.

Quantification of *M. avium* subsp. *paratuberculosis* in the *M. avium* subsp. *paratuberculosis* suspension. The pure culture suspension of *M. avium* subsp. *paratuberculosis* stored at −80°C (described above) was thawed to room temperature and mixed thoroughly. A 50-μl aliquot from this suspension was serially diluted in 10-fold steps to 10^{-10}. An aliquot of 50 μl from each dilution was added to three replicate modified BACTEC 12B (Becton Dickinson) vials, followed by incubation at 37°C for 12 weeks (31). The growth index for each vial was measured every week by using an automatic ion chamber (BACTEC 460; Johnston Laboratories, Towson, MD), and the numbers of *M. avium* subsp. *paratuberculosis* in the original suspension were estimated by using the most-probable-number technique (4, 35).

Column chromatography. Three polypropylene chromatography columns 5.5 cm in length and 1.5 cm in internal diameter (9.7-ml substrate volume) (PD 10; General Electric Healthcare) were prepared, each with a filter at the bottom and at the top to retain the substrate. One column was packed with kaolinite-clay-coated silica (donated by Markus Flury, Washington State University [14]), the second was packed with silica sand (Sigma catalogue no. 274739), and the third was an empty control (containing only the two filters). The columns were mounted vertically on a stand with a peristaltic pump attached to the top lid, set to 200 ml per h and saturated with 2 ml of sterile PBS just prior to the experiment. A 0.1-ml aliquot of the *M. avium* subsp. *paratuberculosis* suspension diluted in 9.9 ml of PBS was added to the column, and the eluent was collected in a vial. Subsequently, 10 ml of PBS was passed through the column to wash off any unattached *M. avium* subsp. *paratuberculosis* and collected in the same vial.

Quantification of *M. avium* subsp. *paratuberculosis* in the eluent. The eluent was centrifuged at 2,850 × *g* for 30 min at 4°C, and the supernatant was drained off carefully to retain only a pellet, which was resuspended in 1 ml of PBS, of which an aliquot of 50 μl was diluted up to 10^{-10} as discussed above. The difference between the quantity of *M. avium* subsp. *paratuberculosis* added to a column and that in the eluent, after adjusting for the numbers retained in the blank column, was defined as the quantity of *M. avium* subsp. *paratuberculosis* attached to the substrate in the column.

TABLE 1. Composition of substrates used in the columns in experiment 2

Sample type	Substrate[a]	Substrate composition (%)		
		Clay	Silt	Sand
Soil samples	Sandy soil	6.4	6.9	86.7
	Clay soil (diluted with silica)[b]	18.5	10.7	70.8
Pure samples	Silica alone	0.0	0.0	100.0
	Clay-silica mixture	10.0	0.0	90.0

[a] Nine columns for each substrate (three per pH level) were used in this experiment.

[b] The proportions of clay, silt, and sand in the original mixture were 37.0, 21.4, and 41.6%, respectively, but this had to be diluted with an equal amount of silica for the column to operate.

Experiment 2. This experiment was performed in a similar way to the pilot experiment but quantitative PCR (QPCR) was used to estimate the quantity of *M. avium* subsp. *paratuberculosis* DNA in the *M. avium* subsp. *paratuberculosis* suspension and the eluent.

Preparation of substrates. Four substrates were tested in this experiment at three different pH levels (pH 3, 7, and 10). Nine columns were set up for each substrate with three replicates per pH level, resulting in a total of 36 columns. The composition of the different substrates used in the columns is shown in Table 1.

Sandy soil. Soil samples collected in an earlier study (6, 7) were available with known proportions of clay, silt, and sand, measured by particle size analysis at The University of Sydney Soil Physics Laboratory. These samples were sorted to select the 10 with the highest proportions of sand, and these were subsequently weighed and pooled together. The proportions of clay, silt, and sand in the final sample were estimated by calculating the weighted average of their proportions in the original samples. The final sample was sterilized by autoclaving at 121°C for 20 min.

Clay soil. A similar procedure as used for sandy soil was performed except that the 10 samples with the highest proportion of clay were identified. Further, since clay soil did not allow any fluid to pass through in a preliminary trial, 100 g of pure silica was mixed with an equal amount of pooled clay soil sample to reduce the proportion of clay in the final sample. The final mixture was sterilized by autoclaving at 121°C for 20 min.

Pure silica. Silica was procured from Sigma (catalogue no. 274739) and sterilized by autoclaving at 121°C for 20 min before use.

Clay-silica mixture. Pure kaolinite clay obtained from the Soil Sciences Laboratory, The University of Sydney, was mixed with pure silica in a ratio of 1:9 (for the same reason as for clay soil) and autoclaved as described above.

Estimation of pore volume of various substrates. The pore volume was estimated to measure the volume of *M. avium* subsp. *paratuberculosis* suspension that could be held in the column. Using a calibrated peristaltic pump, Blue dextran 2000 dye (2%; General Electric Healthcare) was passed through a silica-packed column that was saturated with 50 ml of PBS. The elution was monitored on a spectrophotometer at 280 nm, and the pore volume was measured based on flow rate and the time taken by the dye to elute. Similar to silica, the pore volume was also measured for clay-silica mixtures at ratios of 1:9 and 1.5:8.5.

The pore volume of the column containing only silica was 5 ml, and those for the columns containing clay-silica mixture in ratios of 1:9 and 1.5:8.5 were 5 and 3 ml, respectively. However, the pore volumes for the soil columns could not be estimated due to elution of soil pigments and therefore were assumed to be 4 ml for sandy soil columns (in between that for the pure silica and clay-silica mixture [1.5:8.5]) and 2 ml for clay soil columns (less than the clay-silica mixture [1.5:8.5]).

Quantification of *M. avium* subsp. *paratuberculosis* in the *M. avium* subsp. *paratuberculosis* suspension. Serial dilutions of the *M. avium* subsp. *paratuberculosis* suspension were prepared as described for experiment 1. DNA from each serial dilution was extracted and quantified by QPCR.

Column chromatography. The column experiment was performed in a similar way to the pilot experiment but the columns were maintained at one of three pH levels (pH 3, 7, or 10). All solutions and suspensions were added to the columns at that specified pH.

A 50-μl aliquot of the *M. avium* subsp. *paratuberculosis* suspension diluted with a required amount of PBS at a specific pH to make 1 pore volume was added to

the columns saturated beforehand with a sterilized PBS solution of the same pH at a flow rate of 30 ml per h. The *M. avium* subsp. *paratuberculosis* suspension was held in the columns for 30 min to allow sufficient time for binding and was then allowed to elute at 10 ml per h, the eluent being collected in a sterile vial. Unattached *M. avium* subsp. *paratuberculosis* were washed off with 5 pore volumes of PBS (at the same pH as the *M. avium* subsp. *paratuberculosis* suspension) at 10 ml per h, and the eluent was collected in the same vial. The eluent was centrifuged to obtain a pellet, which was resuspended in 1 ml of PBS and used to quantify *M. avium* subsp. *paratuberculosis* DNA by QPCR (see below).

Extraction of DNA for QPCR. DNA was extracted from the *M. avium* subsp. *paratuberculosis* suspension and the eluent, adapting a previously standardized procedure (16). Briefly, a 450-μl aliquot of the resuspended suspension was added to a 2-ml screw cap vial containing 0.3 ml of 0.1-mm zirconia/silica beads (BioSpec Products, Inc.), followed by the addition of 500 μl of detergent solution (9.6 ml of Decon90, 24 ml of 50 mM sodium acetate [pH 5.2], and 66.4 ml of pure water) and 500 μl of organic solvent (phenol-chloroform-isoamyl alcohol [25:24:1]). The vials were agitated at a speed of 6.5 m/s for 20 s (thrice) with a homogenizer (Bio 101 FastPrep, 180 Qbiogene) and then placed on ice for 5 min before centrifuging at 16,000 × *g* for 5 min. Subsequently, 500 μl of the supernatant was transferred to a new 1.5-ml vial containing 1 ml of 100% ethanol and mixed. After maintaining the tubes at −20°C for 10 min, the vials were again centrifuged at 16,000 × *g* for 30 min to obtain a DNA pellet, which was washed with 1 ml of 70% alcohol and resuspended in 50 μl of nuclease-free water. DNA samples were stored at −20°C until required.

QPCR analysis. A 2.5-μl aliquot of DNA suspension (prepared above) was added to 22.5 μl of a QPCR reaction mixture that contained 12.5 μl of 2× QuantiTect SYBR green PCR Master Mix (Qiagen), 0.125 μl of Forward primer (MP10-1; 5′-ATGCGCCACGACTTGCAGCCT-3′), 0.125 μl of Reverse primer (MP11-1; 5′-GGCACGGCTCTTGTTGTAGTCG-3′; 100 pmol), and 9.75 μl of nuclease-free water. The primers (16) targeted the IS*900* insertion sequence, considered to be an *M. avium* subsp. *paratuberculosis*-specific gene.

QPCR reactions were carried out in duplicate 25-μl reaction volumes on an MX3000*p* multiplex QPCR system (Stratagene) using the following program: a pre-heat activation phase of 15 min (95°C), and 45 cycles of 95°C for 30 s and 68°C for 1 min, with fluorescence acquisition at the end of the 68°C primer extension step. The specificity of the reaction was checked postamplification by a post-cycle melting phase of 65 to 95°C. The built-in amplification-based proprietary algorithm (Stratagene) was used to set the fluorescence threshold value for each reaction based on the efficiency as determined by standard curve generation. The value of the fluorescence threshold was used to analyze data from experimental reactions. The number of cycles at which the amplification-corrected normalized fluorescence (dRn) for each reaction crossed the threshold value was exported to Excel (Microsoft) for further analysis.

Sequence-specific standard curves were generated by using serial dilutions of genomic DNA from *M. avium* subsp. *paratuberculosis* strain 316V. The DNA quantity in the sample was compared against the standard DNA curve on the basis of the number of cycles at which the dRn value for each reaction crossed the threshold value (16). The quantity of DNA in the original samples was estimated by weighting for all dilutions. The number of *M. avium* subsp. *paratuberculosis* cells was estimated from the DNA quantity, assuming that 0.001 pg of IS*900* is equivalent to 0.2 *M. avium* subsp. *paratuberculosis* cells (25). Note that, in contrast to *M. avium* subsp. *paratuberculosis* culture, QPCR detects only *M. avium* subsp. *paratuberculosis* DNA that could originate from both dead and viable *M. avium* subsp. *paratuberculosis* cells.

Experiment 3. The second experiment was repeated using only pure silica as a substrate at five pH levels (pH 3, 4, 5, 6, and 7). All suspensions and wash solutions were allowed to pass through the columns just with the force of gravity as distinct from the previous experiments where the solutions were pumped using a peristaltic pump.

Data analysis. The total numbers of *M. avium* subsp. *paratuberculosis* added to the columns were adjusted for the numbers retained in the blank columns to account for the *M. avium* subsp. *paratuberculosis* that got stuck to the sides of the column, adsorbed to the filters, or were otherwise wasted in the experiment. The numbers of *M. avium* subsp. *paratuberculosis* recovered in the eluent in the treatment columns were divided by the adjusted numbers of *M. avium* subsp. *paratuberculosis* to obtain proportional reduction in *M. avium* subsp. *paratuberculosis*. The proportional reduction was compared between various substrates and pH levels by using the restricted maximum-likelihood (linear mixed model) approach in GenStat (version 11.1.0.1575; VSN International, Ltd., United Kingdom). The variable representing the proportional reduction was log transformed prior to analysis to normalize its distribution but was back transformed for the presentation of results. The predicted means from the linear mixed model were compared by using the least significant difference method where a *P* value of <0.05 was considered to be significant. The data for all experiments were analyzed separately.

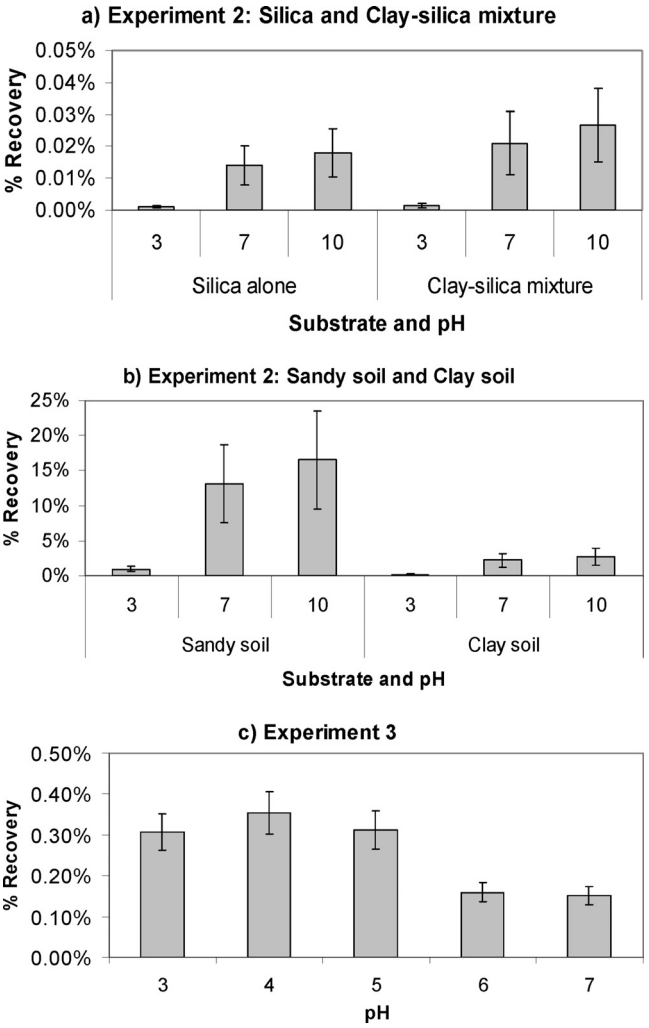

FIG. 1. Predicted means ± standard errors for the proportions of *M. avium* subsp. *paratuberculosis* recovered in the eluent (after adjusting for those retained in the blank) from columns with different substrates maintained at different pH levels. Note that four substrates were tested in experiment 2 but only silica was tested in experiment 3.

RESULTS

Experiment 1. Of the 1.5×10^4 organisms added to each column after adjusting for those retained in the blank, only 4.3×10^3 were recovered in the eluent from both the clay-silica and the silica columns indicating that ca. 70% of *M. avium* subsp. *paratuberculosis* organisms were retained in association with the substrate.

Experiment 2. Of the 5.6×10^6 *M. avium* subsp. *paratuberculosis* organisms added to the columns after adjusting for those retained in the blank, only 0.5×10^6 were recovered in the eluent from the treatment columns, indicating that 90.8% were retained in the columns. The predicted means of the proportion of organisms recovered in the eluent from columns packed with different substrates and maintained at different pH levels are shown in Fig. 1. These data suggest that a ma-

jority of the organisms were retained in the columns, irrespective of the substrate type or pH level.

However, both the type of substrate and the pH at which the substrate was maintained significantly influenced the proportion of organisms recovered in the eluent (and hence retained in the column). After adjusting for the substrate type, an increase in substrate pH significantly increased the proportion of organisms recovered in the eluent ($P = 0.015$), indicating that a lower proportion adsorbed to substrates in the columns at an alkaline pH. Specifically, the recovery of organisms was significantly higher at pH 10 than at pH 3, but differences in recovery were not significant between pH 3 and 7 or between pH 7 and 10. These differences in the recovery of *M. avium* subsp. *paratuberculosis* between pH 3 and 10 were also significant for all four substrates individually (all $P < 0.05$).

Similar to pH, the type of substrate also significantly influenced the *M. avium* subsp. *paratuberculosis* recovery rate ($P < 0.001$). Although on average 0.01% of the *M. avium* subsp. *paratuberculosis* organisms were recovered in the eluent from the columns containing pure substrates (silica and clay-silica mixture; Fig. 1a), 5.96% were recovered in the eluent from the columns containing actual soil (clay soil and sandy soil) (Fig. 1b). However, there was no significant difference in recovery rates between pure silica and clay-silica mixture or between sandy soil and clay soil, although the recovery appeared to be higher in sandy soil (10.21%) than clay soil (1.71%; Fig. 1b).

Experiment 3. Overall, of 1.5×10^7 *M. avium* subsp. *paratuberculosis* added (adjusted for the control), only 4.3×10^4 were recovered in eluent, indicating a retention of 99.7% in the columns. There were no significant differences in recovery across pH levels in the range 3 to 7 (Fig. 1c), which was consistent with experiment 2. However, the percentage recovery was slightly greater in experiment 3 than in experiment 2 for the same substrate.

DISCUSSION

A column chromatographic approach was used in the present study to quantify the attachment of *M. avium* subsp. *paratuberculosis* to soil particles. A substantial reduction in *M. avium* subsp. *paratuberculosis* recovered in the eluent even after stringent washings of the substrate implies that *M. avium* subsp. *paratuberculosis* adsorbed to soil particles in the columns. Association of bacteria and viruses with soils, particularly with clay particles, has been reported previously (1, 3, 12), but this appears to be the first report to demonstrate this for *M. avium* subsp. *paratuberculosis* under controlled laboratory conditions. Attachment of *M. avium* subsp. *paratuberculosis* to soil particles may help retain *M. avium* subsp. *paratuberculosis* particles in the superficial layers of the soil rather than allowing them to be leached to the deeper layers, thus making them easily available to grazing ruminants. It is worth mentioning here that sheep can ingest ~100 g of soil per day (ca. 10% of dry matter intake), and this amount can exceed 300 g during wet winters and at high stocking rates (9, 21).

Less attachment of *M. avium* subsp. *paratuberculosis* in columns packed with soil compared to those packed with pure silica or clay-silica complexes could be because of the greater surface area of pure samples. Clay particles are less than 2 μm in diameter, whereas coarse sand particles can have a diameter up to 2,000 μm (20). The smaller size allows around 2×10^{11} clay particles to be packed in a gram of soil, generating approximately 23,000 cm^2 of surface area/g. In comparison, there are only 5.4×10^2 coarse sand particles per g and only 21 cm^2 of surface area/g (20). Although soil samples were sieved and tightly packed between two filters, leaching of *M. avium* subsp. *paratuberculosis* suspension through undetected channels in the columns packed with coarse soil could be possible but needs further evaluation.

These experiments were initiated by a hypothesis of an association between *M. avium* subsp. *paratuberculosis* and clay particles based on knowledge of such associations between other organisms and clay particles and a significant positive epidemiological association of the soil clay percentage with JD prevalence (27). Although the retention of organisms appeared to be higher in clay soil columns compared to sandy-soil columns, these differences were not statistically significant. Similarly, there were no significant differences between pure silica and clay-silica. This could be due to low sample size or due to the limited range of soil clay percentages investigated. Blocking of columns due to the small size of clay particles precluded experiments with higher percent clay soils; therefore, further studies using an alternative approach should be undertaken.

The less retention of organisms in experiment 3 compared to experiment 2 may have been due to differences in washing protocol. Although wash solutions were pumped through the columns using a peristaltic pump in experiment 2, they were allowed to pass through the columns just with the force of gravity in the third experiment. The latter washings were quicker, and thereby *M. avium* subsp. *paratuberculosis* might have had less time for attachment with the silica.

Adsorption of bacteria to clay and other soil particles in general is mediated by electrostatic and van der Waals' forces or by cell surface hydrophobicity. These forces vary depending on the electrolyte concentration and pH (26) and considerably increase at an acidic pH of around 2 or 3 (17). This could be the reason for significantly higher retention of *M. avium* subsp. *paratuberculosis* in columns maintained at acidic pH in experiment 2. However, no significant differences in recovery across pH levels in experiment 3 were observed since only pH levels 3 to 7 were evaluated in this experiment versus the pH 3, 7, and 10 levels in experiment 2. Further detailed investigations are needed to confirm the results of this study, maintaining samples at a wider range of pH. This appears to be the first study to demonstrate the differences in attachment of *M. avium* subsp. *paratuberculosis* to soil particles at different pH levels.

The electrostatic and van der Waals' forces between soil particles and bacteria are reversible, but the association may become irreversible if they are kept together for an extended period of time (2). In the present experiments, only an immediate adsorption was studied, and further studies should be conducted by maintaining clay/soil particles and *M. avium* subsp. *paratuberculosis* together for longer periods.

Soil pH has long been considered to be a potential risk factor for JD, with a greater prevalence of disease being reported in animals maintained on soils with acidic pH (15). This has been hypothesized to be due to greater survival of *M. avium* subsp. *paratuberculosis* at acidic pH associated with greater solubility of iron, but this has not been convincingly

proved. Could the greater attachment of *M. avium* subsp. *paratuberculosis* with soil particles at an acidic pH detected in the present study be the reason for higher JD prevalence in cattle and sheep maintained on acidic soils? This needs to be investigated. Greater attachment of *M. avium* subsp. *paratuberculosis* to soil particles at an acidic pH could possibly result in increased availability of *M. avium* subsp. *paratuberculosis* to grazing cattle or sheep.

Conclusions. The results presented here provide indirect evidence that, like many other bacteria, *M. avium* subsp. *paratuberculosis* adsorbs to soil particles. This attachment appears to be dependent on soil pH, with greater adsorption recorded for soils maintained at acidic pH. Further studies are required to substantiate the findings at a range of soil types and pH levels and by direct measurement of viable organisms in the soil but, if confirmed, these results could explain prior epidemiological observations and have potential repercussions for animal and human health. *M. avium* subsp. *paratuberculosis* attached to soil particles might be retained in the upper layers of the soil rather than being leached to the deeper layers, therefore remaining available to grazing animals that normally ingest soil while grazing, thus increasing their likelihood of infection. Similarly, the leaching of *M. avium* subsp. *paratuberculosis* from soil to water supplies may be influenced by attachment of *M. avium* subsp. *paratuberculosis* to mobile soil particles.

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