On the identification of some *Entamoeba* species – response to Ponce-Gordo and Martínez-Díaz

The recent comments by Ponce-Gordo & Martínez-Díaz (2007) on our paper in IJSEM (Clark *et al.*, 2006) are unfortunate as they are based on a misreading of the text and a misunderstanding of our results.

The authors are entitled to their opinion that we are not justified in attributing the name *Entamoeba equi* to our horse isolate and can use *Entamoeba* sp. if they prefer. However, we take issue with their reasoning. Their criticism of our citation comes from a misreading – the citation was for the medium, not the amoeba. Describing the morphology of the culture trophozoites would in any case have been useless as it is well known that trophozoite size in culture need not reflect size *in vivo*. Species of the genus *Colpodella* are aerobic organisms while *Entamoeba* species are anaerobic, so they cannot both derive from contamination. We did not see cysts, but we did show that the small subunit rRNA gene sequence of the *Entamoeba* clusters strongly with the 4-nucleated cyst clade in phylogenetic trees. Since the sequence represents a distinct lineage with no specific relatives and is from a unique host, and since *E. equi* is the only valid species name for a 4-nucleated cyst-producing *Entamoeba* species in horses, we feel justified in using it for this organism.

We were well aware of the genetic differences between the authors' *Entamoeba struthionis* (Ponce-Gordo *et al.*, 2004) and the three other uninucleated *Entamoeba* groups. However, like Verweij *et al.* (2001), we concluded that the comparatively small sequence divergence observed among these four genetic groups of uninucleated *Entamoeba* is not adequate justification on its own for separating them into distinct species and that the name *Entamoeba polecki* has precedence. These four groups, all of which have been found in humans, are

the variants of *E. polecki* to which we refer. The authors have suggested that the amoebae from three humans (Verweij *et al.*, 2001) and a pig that gave a match to their sequence from ostrich isolates all result from 'spurious parasitosis', by which they presumably mean ingested cysts passing through without establishing an infection. It is difficult to imagine a situation in which humans could ingest sufficient ostrich faeces to produce *Entamoeba* cysts in their stools that would be easily detectable by microscopy, yet this is how all these infections were originally identified. Four additional human samples containing this particular type of uninucleated *Entamoeba* have since been found and, in one case, both trophozoites and cysts were observed in the sodium acetate–acetic acid–formalin (SAF)-fixed portion of the faeces (J. J. Verweij, personal communication). It is notable that the nuclear morphology of these organisms is variable, even though the sequences are identical.

The studies by Blessmann *et al.* (Blessmann *et al.*, 2002, 2003) did not ignore uninucleated cyst infections, the data were simply not reported until now. Those publications focussed on *Entamoeba histolytica* and so the data on uninucleates were not relevant. However, the pig samples containing *Entamoeba suis* were acquired during those studies, which is why the papers were cited. The prevalence of human uninucleated *Entamoeba* infections in Viet Nam was reported in our IJSEM paper (3.5 %) and the relevant sequences matched the database entry for *Entamoeba chattoni* (unpublished data).

In summary, we feel the criticisms of Ponce-Gordo and Martínez-Díaz are unwarranted and we stand by our conclusions.

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