Authors’ reply to ‘Misidentification of Bordetella bronchiseptica as Bordetella pertussis using a newly described RT-PCR targeting the pertactin gene’

Recently, Register & Nicholson (2007) reported the occurrence of misidentification of Bordetella bronchiseptica strains as Bordetella pertussis, by the specific real-time PCR protocol targeting the pertactin gene, which we previously described (Vincart et al., 2007), in their analysis of a collection of 88 isolates of B. bronchiseptica from human and animals. Using sequence analysis of the pertactin gene, these authors showed that the region of the pertactin gene of B. pertussis chosen for our RT-PCR shows homology to the pertactin gene of B. bronchiseptica strains. Six strains of the collection (including three strains from human hosts) were tested with our protocol, four of which gave a positive result.

We agree that our pertactin RT-PCR protocol lacks specificity regarding some subspecies of B. bronchiseptica (which was not observed with the subspecies of the strain tested in our evaluation). However, we would like to place the limitation of this assay in the context of diagnostics of pertussis using PCR protocols. Cross-reactivity between Bordetella species is not a new problem: phylogenetic analysis of Bordetella species based on insertion sequences showed that all Bordetella species are susceptible to showing IS481, as a result of horizontal or vertical transmission (Diavatopoulos et al., 2005). Thus, PCRs for B. pertussis diagnostic protocols targeting IS481 are also susceptible to presenting false-positive results with some B. bronchiseptica strains, as described in published work (Mattoo & Cherry, 2005; Muylldermans et al., 2005, Register & Sanden, 2006).

Anyway, the clinical purpose of B. pertussis diagnostic PCR must be kept in mind. The probability of a false-positive test due to B. bronchiseptica must be correlated with the prevalence of respiratory infection or colonization by this organism in the population of interest. Unfortunately, there are very limited data about the incidence of B. bronchiseptica colonization and infection in humans. We suppose that this prevalence must be low as only few cases of infection due to B. bronchiseptica (sinusitis, laryngotracheitis, tracheobronchitis, pneumonia, septicemia and whooping cough) have been described in immunocompromised patients and/or patients in close contact with sick animals (Berkowitz et al., 2007; Dworkin et al., 1999; Geirard et al., 1995; Huebner et al., 2006; Woolfrey & Moody, 1991). In healthy adults and infants, B. bronchiseptica may be encountered as a colonizer of the respiratory tract (Mattoo & Cherry, 2005).

Culture is the gold standard technique for identification of B. pertussis. However, B. pertussis culture is fastidious, requires specific media and has a poor yield, and is thus increasingly replaced by rapid molecular methods such as PCR. These molecular techniques have already proved their usefulness for the diagnosis of pertussis in clinical practice (Fry et al., 2004). However, as mentioned above, all PCR protocols currently available lack specificity, due to the high homology of target sequences between Bordetella species. Consequently, Muyldermans et al. (2005) have suggested that the results of a PCR protocol for identification of B. pertussis should be reported as evidence of the presence of Bordetella species awaiting a confirmatory test, namely culture or serology. A comparative study of the sensitivity and specificity of the various RT-PCR protocols available for B. pertussis should be done on clinical specimens to evaluate their respective diagnostic performance. At the present time, the European Quality Assessment (Muyldermans et al., 2005) is the only comparative study available, in which the pertactin-based protocol obtained the best score, even if only a limited number of reference strains were used to check the specificity. In conclusion, whilst awaiting a clinical validation that could assess the clinical predictive positive value of diagnostic RT-PCR assays for B. pertussis infection, a positive result should be considered with caution, especially in patients who are immunocompromised or in close contact with sick animals.

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