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Diagnosis of *Taylorella equigenitalis* by culture or by real time PCR?

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Infection with *Taylorella equigenitalis*, the etiological agent of contagious equine metritis (CEM), may cause an endometritis in mares and temporary infertility. It is most frequently transmitted by sexual contact with carrier stallions, which are always asymptomatic (Heath and Timoney, 2008). Spread of this infection is prevented by excluding infected horses from breeding. Identification of carriers depends on the detection of *T. equigenitalis* on swabs from the clitoral fossa, and uterus of mares and from the urethral fossa, urethra and the external surface of the penis and prepuce of stallions (Heath and Timoney, 2008). Diagnosing infected horses by culture takes at least 7 days and identifying suspicious colonies in the mixed flora of external genitalia is difficult. In 2006, a real time PCR has been proposed (Wakeley et al., 2008) to aid the control of CEM. Nowadays a commercially available kit facilitates the introduction of this test into the routine diagnostic laboratory. The goal of this study was to evaluate the use of this commercially available real-time PCR kit under field conditions as a measure of reducing the time from sampling to result.

In the present study stallions were checked by taking charcoal swabs (Transwab® with Charcoal Medium, mwe medical wire, Corsham, Wiltshire, England SN13 9RT) from the urethral fossa, urethra and penis. Samples were streaked onto Columbia agar plates enriched with 10 % horse blood. For each sample two agar plates were used, one containing 200 mg/L streptomycin and 100 mg/L cycloheximide, and the other containing 1g/L trimethoprim, 5g/L clindamycin, and 5mg/L amphothericin B. After incubating for 5 days at 37 °C in 5 % CO_2, Gram negative, catalase and oxidase positive colonies were subcultured on the same media at 37 °C under aerobic conditions as well as in a 5 % CO_2 atmosphere. Isolates meeting the criteria mentioned above and growing only under microaerophilic conditions were sent to the national reference laboratory for confirming the identity of the bacteria by PCR for the *rrs* gene coding for the 16s ribosomal RNA (Miserez et al. 1996).

Once the result was confirmed the swabs were stored at –20 °C for up to 4 years and used for a concordance study with the cador T.equigenitalis PCR Kit (QIAGEN). Extraction of bacterial DNA was performed directly from swabs using the QIAamp® DNA Mini Kit according to the manufacturer's instructions (Qiagen, Hombrechtikon, Switzerland). Real-time PCR reactions were performed on a ABI 7500 (Applied Biosystems, CA, USA). Ten microliter of extracted DNA were added to 40 µl of master mix according to the kit instructions. Data were analyzed at 40 cycles using the Sequence Detection Software version 1.4 of Applied Biosystems. A result was considered positive if a threshold value of 0.2 was reached at 40 or fewer cycles. The swabs used in this study originated from 60 stallions. Since three swabs (fossa, urethra, penis) were taken at each occasion and five stallions were sampled repeatedly a total of 216 swabs were tested (Tab. 1). In addition, a sample of *T. asinigenitalis* isolated from a sample of a stallion was included to check for cross-reactions.

The results are summarized in Table 2. Using the result of culture as standard, specificity and sensitivity would be 57/61 = 93 % and 11/11 = 100 %, respectively; and predictive values for a positive and a negative PCR result 11/15 = 73 % and 57/57 = 100 %, respectively. The perfect sensitivity and predictive value of a negative result demonstrate that this test allows identifying infected stallions reliably. Therefore, a horse with a negative test result can safely be used for breeding. Positive PCR

Table 1: Number of stallions included in the study and number of swabs analyzed

	number of stallions	number of swabs analyzed*
culture-negative sample	53	159
single culture-positive sample	2	6
culture positive sample with two follow-up checks	4	36
culture positive sample with four follow-up checks	1	15
total	60	216

* of each stallion and at each sampling swabs of fossa, urethra and penis were collected and tested

Table 2: Test characteristics based on the number of results: A positive test result means that at least one of the three samples (fossa, urethra, penis) indicated the presence of *Taylorella equigenitalis*.

		culture		
		positive	negative	
PCR	positive	11	4	15
	negative	0	57	57
		11	61	72

results without corresponding positive culture results were obtained. However, in all but one case these swabs originated from animals from which *T. equigenitalis* had been isolated by culture in the weeks preceding the samples in question (Tab. 2). Therefore, positive PCR results are associated with an infection in all of these cases and it would be prudent to exclude such horses from breeding. Even in the one case, where no bacteria were isolated previously, a cross reaction with other bacteria is an unlikely explanation since previous studies demonstrated a high specificity for this test kit (Ousey et al., 2009). Furthermore, infections with the closely related *Taylorella asiningenitalis* also yielded a negative result. This is important because only horses infected with *T. equigenitalis* are excluded from breeding according to Swiss legislation. To determine whether swabs need to be taken from fossa, urethra and penis for testing by PCR we analyzed the results for each site individually. If only fossa, urethra or penis had been sampled 1, 2 or 7 cases, respectively, had been missed. Therefore, all three anatomical sites need to be tested even if the more sensitive real time PCR is used. Real-time PCR using a commercially available kit offers a fast and reliable alternative for identifying *Taylorella equigenitalis* infected horses by bacterial culture. Uninfected animals might be admitted to breeding within one day from obtaining the sample in the laboratory. In cases where real time PCR yields a positive result at first sampling further testing is required until the animal has eliminated the bacteria. Isolating the bacteria by culture at the time of second sampling might allow typing the bacteria for assessing the epidemiological context.

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