Comparison of faecal PCR with traditional methods in the detection of Syphacia obvelata and Pasteurella pneumotropica.

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Abstract.

Polymerase Chain Reaction (PCR) methodologies are increasingly used for routine Health Monitoring in animal colonies. We compare PCR on faecal material with "traditional" techniques in detecting Pasteurella pneumotropica and Syphacia obvelata to highlight that, in some cases, PCR may not be the most appropriate method. We found that on comparing PCR vs. Culture in detecting P. pneumotropica there was good correlation using the Kappa coefficient. For PCR vs. anal imprint and direct examination of caecal contents for S. obvelata there was poor correlation using the Kappa test. We propose that PCR on faeces is a good alternative to culture in P. pneumotropica's case where culture is not possible. i.e. limited animal stock. In the case of S. obvelata we would advise that the PCR test is used with caution, or along side microscopy, due to a high probability that positive animals could be missed. There is also the problem that PCR will not tell you whether the DNA detected is from a live or dead organism.

Kappa coefficient (Cohen's Kappa).

When two binary variables are attempts by two individuals to measure the same thing then 'Kappa' can be used as a measure of agreement between the two individuals. In our case the binary variables are positive or negative in the test and the 'individuals' are PCR or culture/microscopy. Kappa is always less than or equal to 1; 1 implies perfect agreement and <1 less than perfect agreement.

		Test #1		
		1	**2**	**Totals**
Test#2	**1**	**P11**	**P12**	**P1**
	2	**P21**	**P22**	**P2**
	Totals	**P1**	**P2**	**Σ Totals**

To compare Kappa, the observed **Po** proportion of agreement needs to be calculated:

$$Po = \frac{P11+P22}{\Sigma}$$

This value is then compared to the expected proportion of agreement **Pe** if the two tests were totally independent:

$$Pe = \frac{(P1P1/\Sigma + P2P2/\Sigma)}{\Sigma}$$

The Value of Kappa is defined as:

$$K = \frac{Po-Pe}{1-Pe}$$

One possible interpretation of Kappa:-

poor agreement	<0.2
fair agreement	0.2 to 0.4
moderate agreement	0.4 to 0.6
good agreement	0.6 to 0.8
very good agreement	0.8 to 1.0

Pasteurella pneumotropica: Culture vs PCR

Table 1	PCR Positive	PCR Negative	Totals
Culture Positive	17	5	22
Culture Negative	7	154	161
Totals	24	159	183

Methods:

Culture: Throat swabs are inoculated onto selective agar and incubated at 37 deg C for 24 hours.

PCR: Mouse faecal DNA extract run in a Taq-Man real time PCR on Qiagen Rota-gene Q with dual probes with a single pair of primers (One probe for each biotype).

Calculation:

Po = (17 + 154)/183 = **0.9344**.

Pe= ((24x22)/183) +((159x161)/183)/183 = (528/183)+(25599/183)/183 = (2.891+139.88)/183 = 142.77/183 = **0.7802**

Kappa =(0.9344-0.7802)/(1-0.7802) = 0.1542/0.2198 = **0.7015 (Good agreement)**

Syphacia obvelata: Microscopy vs PCR

Table 2	PCR Positive	PCR Negative	Totals
Microscopy Positive	3	17	20
Microscopy Negative	4	28	32
Totals	7	45	52

Methods:

Microscopy: Sellotape® pressed firmly onto peri-anal area of mouse, transferred to microscope slide and examined at X30. Also a wet preparation of caecal contents examined at X30 magnification.

PCR: Mouse faecal extract run in a real time PCR with Taq-Man probe on a Qiagen Rota-gene Q

Calculation:

Po = (3+28)/52 = 0.5961

Pe = ((20x7)/52 + (32x45)/52)/52 = (2.6923 + 27.6923)/52 = 30.3846/52 = **0.5843**

Kappa = (0.5961-0.5843)/(1-0.5843) = 0.0118/0.4157 = **0.0284 (Poor Agreement)**

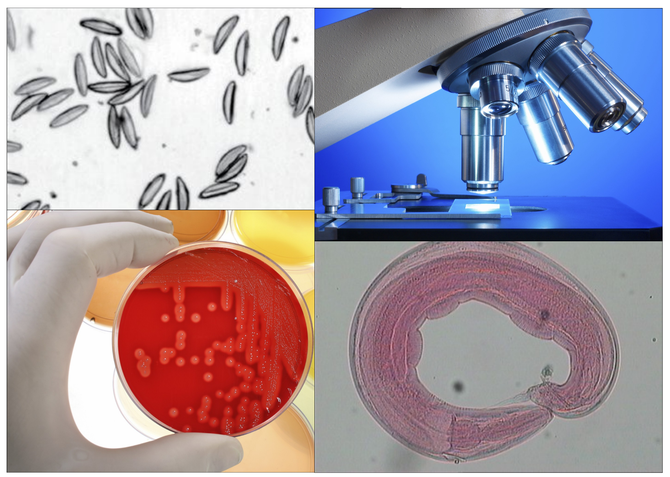
Conclusions.

The data for the Kappa coefficient presented here gives a clear indication that the PCR for Syphacia obvelata (Table 2) is not a reliable assay and we would recommend against its use as a primary method for screening animal colonies. The possible reasons for this shortfall could be due to the nature of the life cycle of Syphacia nematodes. The ova are deposited in the peri-anal region of the mouse, rather than within the gut, it is highly likely that not many ova were present in the faeces. This is consistent with our experience in faecal float tests where Syphacia is rarely found in faecal pellets, contrasting with the other common mouse pinworm, Aspiculuris tetraptera.

Further tests were performed to verify the specificity of the PCR assay. Positive control material was acquired from eggs found in anal imprint tests and also by spiking S. obvelata eggs (identified by microscopy on anal imprints) to a pool of 2 rat faecal pellets . These were easily detected by the S. obvelata PCR.

In addition to these tests, faecal DNA extractions from mice found to contain Syphacia worms by microscopy, were sent to other laboratories offering a PCR test for Syphacia obvelata and only a 50% detection rate was reported. We can conclude that although the PCR works reliably, it cannot be relied upon to work consistently in a clinical context.

The findings for the Pasteurella pneumotropica PCR (Table 1) were more favourable and indicate that this is a reliable assay, giving good agreement with the results obtained by traditional culture methods. However, it is also shown that even in PCR assays with a high proportion of agreement, there were a number of cases where the two methods did not agree perfectly. This leads us to conclude that when implementing a colony screening strategy, it would be prudent to use a combination of both traditional methods (Serology, Bacteriology and Parasitology) and PCR based methods, so increasing the probability of finding any infectious agents.



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