Running Rings Around Ringworm With PCR

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IDEXX LABORATORIES

WINN
FELINE FOUNDATION
Gold standard:
Fungal culture

2-3 weeks to final result

75% of RW suspects are negative (Moriello JFMS 16: 419-431 2014)
PCR

1-3 days to final result

Performance?

Interpretation?
Why does this study matter?

- Ringworm is a cosmetic disease but uses a lot of time, space and resources and leads to increased length of stay or even euthanasia.
- **Most** ringworm suspects are **negative** for ringworm.
- Cutting isolation time for negative cats increases life-saving capacity and reduces euthanasia.
IDEXX® PCR panel

- *Microsporum* sp., *M. canis* and *Trichophyton* sp.
- At time of study, *Microsporum* and *Trichophyton* sp. only
- Positive or negative
Comparison of real-time PCR with fungal culture for the diagnosis of Microsporum canis dermatophytosis in shelter cats: a field study

Linda S Jacobson, Lauren McIntyre and Jenny Mykusz

Assessment of real-time PCR cycle threshold values in Microsporum canis culture-positive and culture-negative cats in an animal shelter: a field study
Cats

- Included cats with skin lesions or suspected exposure
  - High-risk: Suspicious skin lesions
  - Exposed: Non-lesional, history of exposure
  - Low risk: Skin lesions not typical for dermatophytosis
Treatment and diagnostics

- Treatment
  - Low-risk – single dip lime sulfur 1:16
  - Exposed and high-risk – lime sulfur twice weekly, itraconazole 5mg/kg PO q24h for 21 days (14 days if first culture was negative)

- Culture and PCR: Weekly until cleared (first culture negative or two negative cultures after initial positive culture)
Tests

• Hair samples were split into two parts

• Cultures were performed at the THS. Positive initial cultures were confirmed by IDEXX®

• PCR was performed by IDEXX®
Case Definitions

• Positive case: *M. canis* was grown on the first fungal culture, regardless or presence or absence of skin lesions

• Mycological cure: Two negative cultures 1 week apart
Culture results for 132 cats (% of subgroup)

<table>
<thead>
<tr>
<th></th>
<th>Culture +</th>
<th>Culture -</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk (61)</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>Exposed (30)</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>Low risk (41)</td>
<td>5</td>
<td>95</td>
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</tbody>
</table>
## PCR pre-treatment (n=132)

<table>
<thead>
<tr>
<th></th>
<th>Culture +</th>
<th>Culture -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +</td>
<td>28</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>PCR -</td>
<td>0</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>104</td>
<td>132</td>
</tr>
</tbody>
</table>

**Sensitivity:** 100% (87.7-100)

**Specificity:** 88.5 (80.7-93.9)
“False” positives (n=12)

9 had repeat cultures:

• 2/9 - subsequent positive culture
• 5/9 - history of exposure
• 2/9 - could not explain positive PCR; very low amount of fungal DNA present
PCR for confirmation of mycological cure (n=17)

<table>
<thead>
<tr>
<th></th>
<th>First negative culture</th>
<th>Second negative culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +</td>
<td>82%</td>
<td>65%</td>
</tr>
<tr>
<td>PCR -</td>
<td>18%</td>
<td>35%</td>
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</table>
Cycle threshold (Ct) values

- Ct value is inversely and exponentially proportional to amount of DNA in the sample
- Ct 20.26 – 12,565,433 DNA copies; Ct 39.51 – 21 DNA copies
- Lab reports > 39.99 as negative
Assessment of Ct values: Goals

- In cases with a negative culture and a positive PCR, can a Ct cut-off value be found to help interpret the PCR result?
- The cut-off would differentiate true PCR positives from clinically non-significant PCR positives.
Design

- Pre-treatment (n = 132)

- Treated: Cats that had complete weekly data until the second negative fungal culture (if *M. canis* positive) or until the 14-day culture result (if negative)
  - n = 39 cats; 84 pooled time points for all
Results

- ROC curve cut-off (for sens and spec both > 90%)
- Pre-treatment – cutoff was Ct < 35.7 (DNA count approx. 300)
  - Sens 92.3, spec 95.2
- During treatment – no acceptable cut-off value
Pre-treatment Ct values - true-positive and false-positive cats
Ct values over time for positive cases
Discussion/Conclusions

Excellent agreement between PCR and culture before treatment - consistent with human and veterinary studies.

PCR not recommended for confirming mycological cure.

Many factors could cause false positives – dead organisms, cross-contamination of samples, fomite contamination.
Caution

- “NSQ”

- **Interpret all findings** – history, clinical findings, Wood’s lamp; don’t just rely on the PCR
  - We have subsequently seen initial false negatives in a litter of very young kittens in an exposed group

- Extrapolation between labs is risky

- Shelters’ experiences may differ especially based on prevalence and fungal loads
PCR cost analysis

- 92/103 culture-negative cats were PCR-negative

- Iso time = 92 x 14 = 1,288 cat care days
  - $20/day – $25,760

- Iso time if PCR had been trusted: 92 x 3 = 276 cat care days
  - At $20/day – cost of $5,520 i.e. savings of $20,240

- Cost of PCR tests – 92 x 56 = $5,152

- Savings = $20,240 - $5,152 = $15,088
  and 1,012 cat care days
What we used to do

• Exam and Wood’s lamp exam at intake

• Isolated and treated all “high-risk” suspects and exposed cats while waiting for culture results

• Cultured, lime dipped (usually once) and monitored “low-risk” suspects
What we do now

- WL for all, stronger focus on lesion checks
- Positive lesion check, positive Wood’s lamp – consider positive, isolate
- Positive lesion check, negative Wood’s lamp – PCR and lime dip
  - Medical observation until PCR result for most
  - Isolate/quarantine if very suspicious
How is this working for us?

• Very well! E.g. groups of cats from an institutional hoarder with known dermatophytosis
  • Only a few cats per transfer of 20-40 cats have required isolation and treatment; the rest are moved ahead quickly

• The number of cats being isolated for dermatophytosis in our shelter has dropped dramatically
Summary: IDEXX® PCR

- Excellent method to rapidly rule out dermatophytosis and for initial diagnosis
- False positives outweighed by rapid results for true negatives
- Culture remains the method of choice to determine mycological cure
- Ct values can help in decision-making but there is no reliable cut-off during treatment
  - Ct value $\geq 35.7$ at intake – in individual cases, may suggest a false-positive PCR