Detection of bacterial DNA in synovial fluid in dogs with arthritis – a comparison between culture and 16S rRNA gene sequencing

NoVOS 2017

Alexandra Vilén
Leg vet, Specialistkompetens i Hunden och Kattens Sjukdomar, under utbildning till specialist i kirurgi (Steg II)
Introduction

- Septic bacterial arthritis (SA) serious condition requiring prompt diagnosis & treatment
  - Monoarticular pain & lameness
  - Swelling & increased heat
  - Lethargy & pyrexia
  - Onset of symptoms usually fast, but in case of a chronic or low grade SA an insidious presentation possible
  - Septic polyarthritis uncommon but do occur
    - e.g. dxx Immune mediated polyarthritis (IMPA)

*(Bennett 1988, Caywood 1977)*
Bacteria on synovial fluid cytology confirms SA

- Bacteria only found on cytology in 16-54% samples (dogs with SA)
  
  (Marchevsky1999, Clements 2005)

- In humans with SA 19-27%
  
  (Souza Miyahara 2014, Kim 2010)
Positive bacterial culture confirms SA

- Susceptability test available
- Takes 2-3 days
- Culture usually reported positive in only 50%
  

- Diagnosis through a combination of clinical and laboratory findings and good response to joint flush and antibiotic therapy
PCR 16s rRNA analyse

- Diagnostic tool in humans with SA
- Faster (hours) & a more sensitive method
- Several critical steps (DNA extraction and PCR amplification)
  - False positive
  - False negative

(Kim 2010, Bonilla 2011, Mariani 1995)
PCR in animals with SA

Equine study, 57 synovial fluid samples.

• Bacterial culture
  – Agar plate medium – sensitivity 37.8%
  – Blood culture medium - sensitivity 77.6%

• 16S PCR - sensitivity 89.5%

(Pille 2007)
Hypothesis

That PCR would be a more sensitive method to detect bacteria in SF from dogs with SA than conventional culture.
Material and methods

Dogs treated at Evidensia Small Animal Referral Hospital Helsingborg, 2010-2013

Inclusion criteria:

- Clinical symptoms of SA (10 dogs included)
- Clinical symptoms of osteoarthritis (9 dogs included) = Control group 1
- Clinical symptoms of immune mediated polyarthritis (IMPA, 9 dogs included) = Control group 2

and

- A minimum of 0.8 ml synovial fluid collected from at least one joint + in the IMPA group an additional minimum of 0.25 ml from at least one other affected joint to confirm the diagnosis of IMPA (≥ 2 joints)
Material and Methods

- Cytology (EDTA tube)
- Bacterial culture
  - Pediatric blood culture medium (incubation in 37°C for 24 h before inoculating on a blood agar plate)
- 16s rRNA PCR
  - SF immediately transferred to a sterile container, which was placed in –20°C within 30 minutes. Stored 1-6 months
  - Klinisk Mikrobiologi in Lund (Human clinical laboratory)
Results

<table>
<thead>
<tr>
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<th>Sensitivity (95 % CI)</th>
<th>Specificity (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriological culture</td>
<td>70 (35 - 93)</td>
<td>100 (85 - 100)</td>
</tr>
<tr>
<td>PCR</td>
<td>20 (3 - 56)</td>
<td>100 (85 - 100)</td>
</tr>
<tr>
<td>Bacteriological culture + PCR</td>
<td>70 (35 - 93)</td>
<td>100 (85 - 100)</td>
</tr>
</tbody>
</table>

Confidence interval are exact based on the binomial distribution

18 dogs with SA (19 joints) bacterial culture sensitivity 63.2% versus 16S PCR 73.7%

(Scharf 2015)
• Sample preparation
  • Critical steps (DNA extraction and PCR amplification)
  • Low sample volume
  • PCR inhibitors in SF
    (Mariani 1995)
• Leucocyte DNA compete with bacterial DNA
  (Jordan 2005, Trampuz 2003, Mariani 1995)
• Careful validation of the methods is
  (Marin 2012)
Sample handling and storage

- Freezing of DNA considered standard
- DNA stability and load influenced by freezing?
- Aquous solution influenced by freezing (clinical samples versus frozen DNA extract)

(Podivinsky 2009, Carlsen 2010, Coreless 2000)
Limitations

• Low sample size

• Many pitfalls in DNA analyse

• Clear diagnostic criteria of SA lacking
  – Promising results on lactate as a diagnostic tool to be used in future studies

(Proot 2015)
Conclusion

16S rRNA PCR in dogs with suspected septic arthritis much less sensitive than we expected (sensitivity of 20%)

• Reason for this unclear but sample storage might have influenced?

• Bacterial culture sensitivity 70%
  • Inoculation in pediatric blood culture medium and incubation 24 h superior to direct swab on agar plate

• More clinical prospective studies needed
• Validated guidelines for optimal handling of clinical samples prior to analyse
Thank you for the attention!

Acknowledgements

• Staff at Evidensia Small Animal Refferral Hospital
• Henriette Ström
• Thure and Karin Forsbergs Foundation
• Michael Forsgrens Foundation
• Stiftelsen Svensk Djursjukvård
REFERENCES