Tuberculosis in Camelids

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TUBERCULOSIS IN CAMELIDS

- Diagnosis in New and Old World Camelids (NWC/OWC)
  - NWC – EU, South America, US
  - OWC – UAE, Africa, Pakistan, Kazakhstan, US
- Isolation of *M. bovis, M. tuberculosis & M. microti* from Mycobacterium Tuberculosis Complex (MTBC)
  - *M. avium, M. kansasii* - environmental
- Typing of *M. bovis* found in camelids reflects locally predominant genotypes in cattle and wildlife (Rhodes 2014)
- Outbreaks rare in natural habitat
CLINICAL SIGNS

- Changes in behavior
- Anorexia and cachexia
- Respiratory distress, coughing
- None
- Sudden death or euthanasia for deteriorating body condition

Photograph courtesy Kerstin Joensson, AP
TRANSMISSION ROUTES

- Respiratory
- Oral
- Other routes rare
  - Milk
  - Vertical
PATHOLOGY

- Respiratory tract - lungs and associated lymph nodes
- GI tract
- Liver
- Mammary gland (Richey et al. 2011)
- Small multifocal white-yellowish caseous nodules
- Large abscesses
- Miliary

Photograph courtesy James Barnett, AHVLA
DIAGNOSIS

- **Post-mortem:**
  - Compatible lesions found at necropsy/slaughter (usually first indication of issue in herd)
  - Histopathology – Acid-Fast Bacilli
  - Culture – *M. microti* difficult to isolate

- **Ante-mortem:**
  - Cellular
    - Skin testing
      - For movement purposes
      - Required for herd test after confirmed positive at necropsy
    - Interferon-gamma (IFN-γ)
  - Humoral (Serology)
SKIN TESTING

- **Single Intradermal Test (SIT)**
  - Purified Protein Derivative Bovis (PPDB) – 0.1ml injected into shaved area in axilla & read 72 hours post injection
    - Increase in skin thickness over pre-injection reading measured with calipers: <2.0mm increase = negative, 2-4 mm increase = suspect, >4.0mm = positive
  - 1º test in US

- **Single Comparative Intradermal Test (SCIT)**
  - PPDB & Purified Protein Derivative Avium (PPDA) – 0.1mL injected into shaved area in axilla & read 72 hours post injection
    - Increase in skin thickness over pre-injection reading measured with calipers: negative bovine reaction, or a positive or inconclusive bovine reaction ≤ a positive or inconclusive avian reaction and the absence of clinical signs in both cases = negative, a positive or inconclusive bovine reaction which is from 1 - 4 mm > the avian reaction, & the absence of clinical signs = inconclusive; bovine reaction which is more than 4 mm > the avian reaction, or the presence of clinical signs = positive
  - 2º test in US, 1º test in EU
SKIN TESTING

- Lacks sensitivity and specificity in naturally infected animals (Dean et al. 2009)
- SCIT used more to minimize false positives due to environmental mycobacteria (Alvarez et al. 2011)
- Several different injection sites used to enhance sensitivity – neck (pre-scapular), axillary, tail
- Variation in reading times used to evaluate skin fold increase (Bezos et al. 2013, Wernery et al. 2007)
NWC
- SCIT may still miss *M. microti* infection – no specific intradermal tests using *M. microti* antigens (Alvarez et al. 2011)

OWC
- Limited numbers of OWC studies
- Lesions found at slaughter revealed environmental mycobacteria (Mamo et al. 2011)
- SIT/SIDT 55.55 Se 93.33 Sp (Narnaware et al. 2015)

Photo courtesy Alex Turner, CDA
Camelidae (Camel, Llama, Alpaca, & Guanaco) – All species of camelidae may be tested with a tuberculin skin test in the hairless area behind the elbow, although it should be noted that the sensitivity and specificity of this test in these species is markedly lower than the skin tests for cattle and bison. Therefore, it is essential that a clinical diagnosis of TB be given some consideration even in the face of a negative tuberculin test. PPD Bovis is used at 0.1 ml dose, with observation and palpation for swelling at 48, 72, and 96 hours.
2.2 Tuberculosis

Negative results must be obtained on two (2) tuberculosis intradermal tests, using the post-axillary injection site, at least 90 days apart, with the second being performed no more than 30 days prior to export.

Testing procedures must be administered by a veterinarian competent in the specified procedure in the exported species.

The tuberculosis test to be conducted is the intradermal test with a dose rate of 0.1 ml of bovine PPD tuberculin (or product of equivalent potency approved by CFIA) injected at the post-axillary site, the injection site identified with a permanent ink marker, and the thickness of the skin recorded with calipers. The skin thickness will be measured seventy-two (72) hours post injection.

A responder is any animal in which there is an increase in the thickness of the skin greater than 1.5 mm at the site of injection in response to the initial injection of tuberculin.

Any responder animal to either the intradermal test is to be removed from the group of animals intended for export, and the entire testing protocol needs to begin again for the remainder of the group. A minimum of 90 days is always required between any intradermal test.

The results of all the tuberculin tests (including the dates of test readings) must be shown on the required health certificate for the animal to be imported.
Interferon-gamma (IFN-γ)

- Developed for use in South American Camelids (SAC) that would not be dependent on completion of prior skin test (Rhodes et al. 2012)
- \textit{In vitro} stimulation of blood cells
- Responses on whole heparinized blood too low but significant responses seen when peripheral blood mononuclear cells (PMBCs) separated out and stimulated with PPDB, PPDA, ESAT6 – CFP10 (EC) peptide cocktail
- 80-98% Sp, 15-80% Se
- Detects other MTBC mycobacteria
SEROLOGY

- Infected animals can be detected before onset of clinical signs (Lyashchenko et al. 2007)
  - 2 animals seroreactive 1-2 years before clinical signs
- Used alone or in combination with skin testing – increased sensitivity (amnestic response) (Stevens et al. 1998) (Bezos et al. 2013)
SEROLOGY

- **MAPIA – Multiantigen print immunoassay (Chembio)**
  - Uses range of *M. bovis* specific antigens including MPB70 & MPB83 printed on nitrocellulose membrane incubated with serum samples in laboratory

- **Lateral flow based rapid test (RT) - VetTB Stat-Pak (Chembio)**
  - No longer in production, replaced by DPP
  - Uses *M. tuberculosis* antigens - MPB83, ESAT-6 and CFP10

- **Dual Path Platform (DPP) VetTB assay (Chembio)**
  - Licensed in US for cervids and elephants, animal-side test with results available in 15 minutes
  - Uses *M. bovis* recombinant MPB83 protein and CFP10/ESAT-6 fusion
  - Read by a DPP optical reader which measures reflectance

- **ELISA**
  - IDEXX ELISA (IDEXX Laboratories) - plates pre-coated with MPB83 and MPB70 antigens
  - Enferplex antibody ELISA (Enfer Scientific) – includes seven antigens PPDB, SAT6, CFP10, Rv3616c, MPB83, MPB70 and MPB70 peptide
EVALUATION OF STAT-PAK AND DPP IN SAC - (LYASHCHENKO ET AL. 2011)

- 156 alpacas and 175 llamas from GB, Switzerland and US
- Confirmed TB group (Culture +, and/or typical gross lesions or clinical signs)
  - 35 alpacas/17 llamas
    - 34 alpacas/10 llamas with M. Bovis (GB); 1 alpaca/7 llama with M. microti (Switzerland)
  - All except 2 M. bovis llamas negative on skin test
- TB free group
  - Negative controls including 96 alpacas/122 llamas known to be TB free (Switzerland, US)
RESULTS – (LYASHCHENKO ET AL. 2011)

- TB confirmed (M. bovis & microti)
  - Alpacas – 25/35 (71% Se) reacted to Stat-pak, 26/35 (74%) reacted to DPP
  - Llamas – 13/17 (77% Se) for both Stat-pak and DPP
  - In parallel: Alpacas – 31/35 (89% Se) Llamas 15/17 (88% Se)
  - In series: Alpacas 57% Se; Llamas 65% Se

- TB free
  - Alpacas - 98% Sp for both Stat-pak and DPP
  - Llamas – 94% Sp Stat-pak, 98% Sp DPP,
  - In parallel: Alpacas - 97% Sp; Llamas 93% Sp
  - In series: Alpacas and Llamas 100% Sp

- Parallel testing in alpacas 95% accuracy vs. 90% accuracy Stat-pak/ 92% DPP alone
- 96% accuracy for llamas with DPP alone
EVALUATION OF GAMMA INTERFERON AND ANTIBODY TUBERCULOSIS TESTS IN ALPACAS - (RHODES ET AL. 2012)

- Research conducted by Animal Health and Veterinary Laboratories Agency funded by industry
- Analysis of IFN-γ & 4 serological tests (STAT-PAK, DPP, IDEXX ELISA & multiplex ELISA)
- All tests conducted on 48 diseased (typical gross visible lesions (VL) consistent with mycobacteria) alpacas from 10 infected GB herds, 257 TB-free alpacas from 17 GB herds, and 49 serum samples from the US
- IFN-γ assay: PPDB & PPDA used to generate comparative PPD response; ESAT6/CFP10 peptides used for increased specificity for bovine TB
- Data analyzed to suggest test combinations
RESULTS - (RHODES ET AL. 2012)

- Camelid IFN-γ detects MTBC (M. microti) – not specific
- Serology - ~97% (Sp) & 60-70% (Se)
- Combination of IFN-γ with Stat—pak and DPP or IDEXX in parallel provided greatest Se (100%)
- Combination of tests in serial interpretation – 99.7% (Sp), 56% (Se)
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<tr>
<th>Test</th>
<th>n/total</th>
<th>% Sensitivity</th>
<th>95% CI</th>
<th>n/total</th>
<th>% Specificity</th>
<th>95% CI</th>
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<tr>
<td>STAT-PAK</td>
<td>35/52</td>
<td>67.3</td>
<td>54.5-80.8</td>
<td>8/306</td>
<td>97.4</td>
<td>95.6-99.2</td>
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<td>DPP</td>
<td>30/52</td>
<td>57.7</td>
<td>44.3-71.1</td>
<td>10/306</td>
<td>96.7</td>
<td>94.1-98.4</td>
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<td>IDEXX</td>
<td>36/52</td>
<td>69.2</td>
<td>56.7-81.7</td>
<td>8/306</td>
<td>97.4</td>
<td>95.6-99.2</td>
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<td>ENFERplex</td>
<td>32/48</td>
<td>66.7</td>
<td>53.4-80.0</td>
<td>8/257</td>
<td>96.9</td>
<td>94.8-99.0</td>
</tr>
</tbody>
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ASSESSMENT OF PCR TESTING OF NASAL SWABS AND FECES FOR DETECTION OF M. BOVIS IN SAC – (CRAWSHAW ET AL. 2014)

- 44 Nasal swabs & fecal samples collected from 63 SAC carcasses from culture confirmed M. bovis infected herds
- Carcasses received a pathology score on PM. Values assigned according to tissues affected & extent of lesions (min score 0, max score 12); carcasses reflected full range of pathology scores, including 8 SACs with no gross lesions
- Samples were tested for IS1081 PCR and RD4 Real Time PCR (to detect M. bovis specifically)
- 23/44 positive on either test (64% Se),
  - Nasal swabs 14/44, Fecal swabs 21/44 positive to both IS1081 & RD4 PCR confirming M. bovis
- Culture performed on 14/44 (5 negative on culture & PCR)
  - 9 positive on culture, fecal PCR detected 4 & the nasal PCR detected 5 (a combination of the PCRs did not detect 3)
CAMELID TUBERCULOSIS IN THE UK

- Legislation introduced in 2006 required veterinarians to notify veterinary authorities of all tuberculous lesions in carcasses of livestock and pets
  - Movement restrictions pending culture results
  - SCIT repeated at 90 day intervals
- UK Voluntary camelid TB scheme (Hayton et al. 2014)
  - Defra, AHPA, Industry, British Veterinary Camelid Society, SureTest, Enfer Scientific
  - Camelids skin tested with SCIT 10-30 days before blood testing
  - Enferplex test – 2 antigen level: 66.7 (Se) 97 (Sp); 4 antigen level 100 (Sp)
  - Annual herd surveillance, pre-movement and export testing
TUBERCULOSIS IN OWC - (WERNERY ET AL. 2007)

- Outbreak in UAE Dromedary racing herd Diagnosed on PM as M. bovis (antelope)
- SIT and SCIT used to determine optimal test sites and reading times
- Serum samples collected for Stat-pak and MAPIA 3 times within 6 months (2mo prior to skin test, immediately before skin test, 6wk post skin test)
- Serum samples from camels assayed for M. paratuberculosis using IDEXX-ELISA modified for camelids
TUBERCULOSIS IN OWC - RESULTS

- 2 camels consistently positive to Stat-pak and MAPIA, positive on SCIT confirmed on PM
- Strongest Stat-pak & MAPIA responses in index case, moderate in number 1&2
- Best responses for both skin tests – axillary read at 5 days
- All 3 positives detected on Stat-pak /MAPIA, no false positives
- Presence of false positive SCIT responses in camelids may be due to environmental mycobacteria
CONCLUSIONS

- Serology tests show promise for ante mortem detection of TB in Camelids
- Current protocols for skin testing are unlikely to detect infection in herds
  - More data needed on appropriate protocol for skin testing in Camelids
- More research needed on test performance in naturally infected and non-infected camelids with known infection status
- Additional research needed in OWC
- True prevalence of TB in camelids is unknown due to lack of surveillance and substandard tests
REFERENCES


QUESTIONS?

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Photograph courtesy Tim Fox, VS