Tuberculosis in camelids – present situation and tests

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ABSTRACT

TB in camelids has historically been swept under the rug as it was deemed of little significance. Our understanding of the disease has increased over the years and it is becoming more apparent these species are far more susceptible than previously assumed.

As more animals are lost due to TB, it is time to reassess their role in bTB epidemiology, address the virtually unchecked zoonotic potential that camelids pose, as well as the possible implications for animal welfare. This article reports on clinical manifestations, the potential different mycobacteria involved and available options for detection with their potential limitations.

Historically, camelids were seen as mere spillover, dead-end hosts posing a negligible risk in the TB epidemiology. This, combined with the legal wasteland when it comes to these animals and the lack of mandatory registration, has meant eradication efforts have almost completely passed them by.

Our understanding of the disease in camelids has evolved in recent years, with more evidence hinting towards transfer within the herd. This capacity to function as an amplification host and reservoir (de la Rua-Domenech, 2006; Twomey et al, 2009) implicates a potential for a far more active role in the epidemiology of TB than was previously assumed.

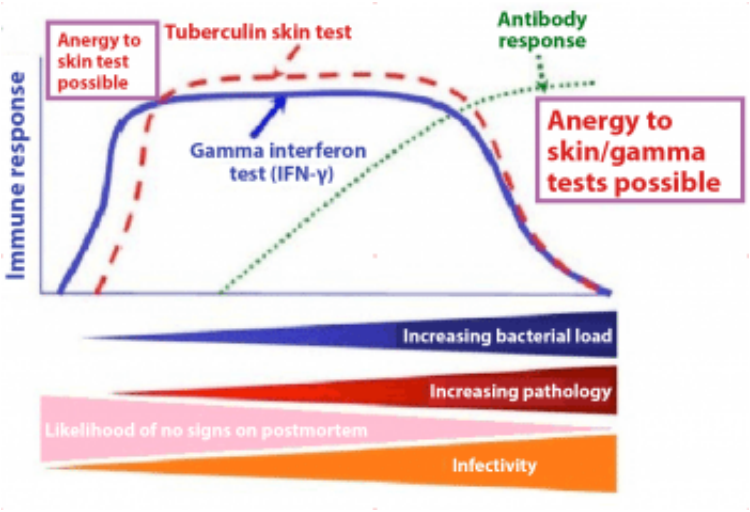

Figure 1. Tuberculosis progression in bovines.

The zoonotic potential from TB in camelids has also become increasingly apparent, primarily posing a risk to vets (de la Rua-Domenech, 2006; Posthaus et al, 2011; Twomey et al, 2010a; Veen et al, 1991) and owners (de la Rua-Domenech, 2006), but also potentially to the public.

Although only a very limited number of camelids enter the food chain, it is the tendency to have close interactions with live animals that poses a risk of direct transfer to humans. More lamoids are kept as novelties in petting zoos, open farm settings and trekking or even therapeutic animals. This, combined with popular public shows, means plenty of potential exists for zoonotic spread to people, or to infect other livestock.

Unfortunately, about half of the registered camelid herds are located within highly endemic TB areas (British Alpaca Society; BAS/Defra data), with the potential for spread from local cattle and wildlife reservoirs. Despite this, breeders have generally been very reluctant to actively address the risks faced by the industry from TB.

Animals continue to move virtually unchecked between herds and any addition or movement (Twomey et al, 2009) poses a potential biosecurity nightmare. In 2013 alone, 200 registered animals were lost to TB and, so far, 94 herds have suffered a confirmed breakdown (BAS). No mandatory testing interval exists, despite a poll launched by Defra in 2014, nor are there any requirements for pre-movement or post-movement testing in the UK.

However, the number of camelids tested that are co-located or in close vicinity to a breakdown in a different species has risen, as well as trace back and forward animals, and TB suspect animals found by vets.

Voluntary testing options can be granted by the APHA, but uptake has been hesitant by the majority of the camelid community, despite a BAS-sponsored trial to assess the available

serological-based tests in 2012 (Rhodes and Vordermeier, 2012) and a serological test that became available in 2014 (Hayton et al, 2014). Consequently, many herd breakdowns are still tentatively diagnosed on gross postmortem findings (Alvarez et al, 2012), after which final culture confirmation can cost precious time.

This lack of screening invariably means the disease has time to get well established before animals are, in fact, diagnosed as suffering from TB. This delay will likely expose and claim a far larger proportion of the herd than would have been necessary.

It has been shown in cattle an inverse relationship exists between the cellular and humoral-based tests – the latter being better suited to finding advanced disease stages (Ritacco et al, 1991; **Figure 1**).

It is especially costly to miss animals late in the disease process, since these are far more likely to be shedding large numbers of TB bacilli. Therefore, it makes sense to emphasise on humoral tests for voluntary screens.

Symptoms

Panel 1. Clinical tuberculosis signs reported in camelids.

Associated with lung pathology[1]

- Exercise intolerance
- Chronic persistent coughing and other respiratory signs

Non-specific symptoms

- Sudden death
- Bruxism
- Anorexia, weight loss and emaciation[2]
- Weakness and lethargy
- Agitation
- Discharging skin lesions
- Superficial lymph node swelling

[1]On postmortem exams, lung involvement can be very extensive, despite an apparent lack of clinical manifestation. [2]Routinely, animals are reported to be in good body condition score. Crawshaw et al, 2013; Garcia-Bocanegra et al, 2010; Lyashchenko et al, 2007; Ryan et al, 2009; A Twomey et al, 2010; Twomey et al, 2012.

The disease process in camelids is poorly understood so far; sparse documentation is available that often deals with limited numbers.

Experimental infection shows a rapid onset of disease and death, although this is likely to be dose-dependant and spoligotype-dependent. Interestingly, no respiratory distress was apparent before acute death, despite extensive lung pathology on postmortem (Stevens et al, 1998).

TB often seems to have a predilection for affecting the lung tissue in various animals, giving rise to respiratory signs. It is, however, likely the camelid physiological adaptations to cope with life in low oxygen environments enables them to remain often symptomless.

A list of reported clinical manifestations in “naturally” infected herds can be found in **Panel 1**. As is apparent, a wide range of often vague clinical manifestations and symptoms exists, probably depending on the affected body system. The apparent lack of a pathognomonic symptom – or, indeed, a typical disease progression – hinders diagnosis on symptoms alone (Wernery and Kinne, 2012).

Many non-specific symptoms – such as wasting, acute death, anorexia and lethargy – are common reasons to submit samples or to refer animals, but these are often not caused by TB (Zanolari et al, 2009). Common symptoms, such as ill thrift and weight loss, are more often caused by parasitism and Johne’s disease than TB (Twomey et al, 2014). These vague clinical symptoms complicate diagnosis based on clinical signs alone.

Host immunity and TB bacterial interactions have been extensively documented and are shown to be very complex in humans. The disease in bovines seems to mimic this (Neill et al, 2001; Pollock et al, 2001; Waters et al, 2010). It is, therefore, not unlikely these interactions will prove to be equally complex in camelids, further necessitating a multi-level approach for successful diagnosis, which goes well beyond basing a plausible diagnosis on clinical symptoms alone.

Finding granulomatous lesions on postmortem has long been perceived as almost synonymous to TB infection, but this does not rule out other infectious agents, which can present with similar lesions. Coccidioidomycosis or other non-TB bacteria, such as *Mycobacterium avium* subspecies *avium* and *avium paratuberculosis* (Johne’s disease), can also cause granulomatous lesions in several organs, including the lung (Crawshaw et al, 2013; Fowler and Bravo, 2010).

This necessitates culture to be definitive, which can take an extended period of time. As can be derived from **Figure 1**, these lesions need time to develop. Early microscopic lesions or atypical affected locations can also easily be missed on postmortem.

Epidemiology in camelids

Infection is often caused by the same spoligotypes abundant in the local cattle and wildlife reservoirs, implicating a local acquisition source (Connolly et al, 2008; Crawshaw et al, 2013; Twomey et al, 2007; Twomey et al, 2012). Various wildlife reservoirs have been extensively researched to determine their possible role in bovine cases of TB (Delahay et al, 2002; Delahay et al, 2007; Hardstaff et al, 2014; Scantlebury et al, 2004; Ward et al, 2009) and the situation in cattle can locally be highly endemic (Defra, 2014).

Mycobacteria are shown to be able to survive for extended periods of time, making indirect infection through fields plausible, as well as a possible reason for recrudescence within herds (Ghodbane et al, 2014; O'Reilly and Daborn, 1995). Although most domesticated mammals are very resilient against TB infection (Broughan et al, 2013a), camelids seem to be highly susceptible in the UK setting (Pesciaroli et al, 2014). Infection can also be actively brought in by adding a carrier camelid into the herd (Twomey et al, 2009).

After initial infection, most of the spread seems horizontal between animals, although a degree of vertical spread could be possible (Richey et al, 2011; Zanolari et al, 2009), as is also seen in cattle. Infectious agents can be droplets over short distances, all excrements and open skin lesions. All these present a possible zoonotic risk as well.

Different mycobacteria in camelids

Table 1. Different mycobacteria isolated from camelids

Mycobacteria strain	Complex	"Natural host"	Endemic in UK?	Zoonotic potential	Infects camelids?
Mycobacterium tuberculosis Fowler and Bates, 2010; Penha et al, 2011	TB complex	Humans	Yes, but most cases are carriers entering the UK from endemic countries	Yes	Possible, but very rare
Mycobacterium bovis O'Reilly and Daborn, 1995	TB complex	Bovines, amplification in badgers, deer, goats and camelids	Yes, most common cause of bTB in wild and domesticated ruminants	Yes	Yes
Mycobacterium microti Roring et al, 2000; Hamilton et al, 2001; Cavanaugh et al, 2004; Zanolari et al, 2009; Hermans et al, 2006	TB complex	Rodents	Yes	Yes	Yes
Mycobacterium ulcerans O'Brien et al, 2013	TB complex	Ubiquitous	Not yet reported, reported in camelids in Australia	Yes, causes Buruli ulcer	Yes
Mycobacterium caprae Aranaz et al, 2003; Prodinger et al, 2014; Rodriguez et al, 2011	TB complex	Goats	Not yet reported, prolific in goats in Spain, also diagnosed in Germany	Yes	Yes
Mycobacterium kansasii Bauer et al, 2009; Johnson et al, 1993	Atypical non-TB complex mycobacteria	Ubiquitous	Yes	Yes	Possible, but very rare
Mycobacterium avium subspecies *avium* Lara et al, 2003	Avium (non-lung/ intracellulare) complex	Birds	Yes	Yes	Possible, but very rare. Can clinically mimic Johne's disease
Mycobacterium avium subspecies *paratuberculosis* Aldrup et al, 1994; Crawshaw et al, 2013; Richey et al, 1995	Avium (non-lung/ intracellulare) complex	Ruminants	Yes	Disputed, possible link with Crohn's disease	Yes, causes Johne's disease, mimicking clinical TB

Table 1. Different mycobacteria isolated from camelids.

Various mycobacteria have been implicated to cause disease in camelids, and an overview of these can be found in **Table 1**. The majority of camelid cases are either caused by *Mycobacterium bovis* or *Mycobacterium microti*. Other mycobacteria have been reported both in the UK and around the world, possibly complicating future eradication efforts as animals are being transported

internationally.

Testing options

Classical skin test

Historically, the skin test has been used to identify TB-infected camelids. Sensitivity (SN) and specificity (SP) issues have been routinely voiced by vets in practice. Multiple reports exist on the suggested performance of the single intradermal comparative cervical tuberculin (SICCT) test in camelids, but drawing any definitive conclusions is difficult. A lack of a gold standard testing protocol exists. Various reading intervals, injection sites and skin interpretations are described and this, combined with the limited sample size, makes any comparison unfounded (Alvarez et al, 2012).

To further complicate this, relative performance is often measured against animals either showing visible lesions at postmortem or positive on culture and, as such, only represents a limited subset of infected animals. This makes these relative SN/SP of questionable credibility (Bezos et al, 2013; Bezos et al, 2014; Broughan et al, 2013b; Lyashchenko et al, 2011).

Although a consensus is lacking among authors regarding the sensitivity and specificity of employing skin tests in camelids, it is usually regarded as disappointing (Lyashchenko et al, 2011; Twomey et al, 2012; Wernery and Kinne, 2012). This low sensitivity has undoubtedly hindered both the initial disclosure of TB, as well as eradication once infection was found.

Anergy to skin tests has been a long standing complication in cattle and this also seems to exist in camelids, as negative animals on the skin test can have extensive postmortem pathology. Reasons for skin test failure are believed to be partly down to equipment and operator errors, but also due to the different stages in the disease process (de la Rua-Domenech et al, 2006; Lepper et al, 1977; **Figure 1**). Certain animal factors that cause immunosuppression (such as pregnancy, stress, concurrent infection or malnutrition), as well as differences in virulence between strains, are likely contributors (Lepper et al, 1977).

As different mycobacteria species are often very homologous, a risk exists for immunological cross-reactions compromising the test's specificity in practice. Cross-reactions are known to exist between *M bovis* and *Mycobacterium avium*, but also between *M bovis* and other TB complex mycobacteria – and even non-pathological mycobacteria. Possible cross-reactivity to environmental mycobacteria has been known for a long time in cattle (Karlson, 1962) and these cross-reactions are also likely to exist in camelids, as the tuberculin used has the same complex purified protein derivative (PPD) make-up. *M kansasii*, for instance, is not part of the TB complex, but has been shown to experimentally give rise to a limited cross-reaction with PPD in cattle (carries ESAT-6 and CFP-10). Although actual infection and lesions from *M kansasii* can occur in cattle and camelids, these are considered very rare and not classified as TB (Braun et al, 2009;

Waters et al, 2006; Waters et al, 2010).

Ancillary mandatory testing protocols

The emphasis for mandatory testing still lies predominantly on the comparative skin test, modified for use in camelids (90-day intervals, different interpretation/inoculation site), but there has been a push towards complementing these with serological follow-up tests.

The use of pure-specific recombinant protein antigens has shown great potential in cattle to differentiate between different mycobacteria (Amadori et al, 2002). These have also shown potential for their application in camelids (Rhodes and Vordermeier, 2012). Available serology panels are either DPPVetTB+IDEXX ELISA in serial or parallel interpretation, or the Enferplex test.

Voluntary testing

Voluntary tests need to be condoned by the APHA and permission needs to be sought before any type of test can take place. Both skin and serological tests, as mentioned for mandatory tests, can be granted at the owner's expense. The Enferplex test can also be performed without the pre-sampling booster of tuberculin, if desired.

Future alternatives

Many other tests for TB control in cattle have been described. In camelids, one of the most promising is PCR testing.

These have already been trialled successfully on postmortem samples and could become a future option (Crawshaw et al, 2014). Care must be taken on such sensitive tests that other environmental mycobacteria sometimes found (Twomey et al, 2010a) do not give rise to false-positive results.

Some authors have concluded different in vitro tests are able to find different subpopulations of infected animals and, therefore, combining tests might prove useful in eradication (Chambers, 2013; Pesciaroli et al, 2014). Combining different tests can, indeed, be used to sacrifice SP for SN, which is potentially a valuable trade when in a breakdown setting (Rhodes et al, 2012). It has also been shown the anamnestic response can be used to increase serological sensitivity (Waters et al, 2011), although unfounded concerns exist that this might negatively affect specificity by creating more background noise.

Tests using multiple antigen markers seem to be able to find anergic reactors missed by cell-mediated tests (Dean et al, 2009; Whelan et al, 2011; Zanardi et al, 2013).

Figure 1 shows a schematic overview of disease progression during TB infection in cattle, to

illustrate this trade off between cellular immunity-based tests, such as skin and gamma tests, and the serology-based tests.

Table 2. Different available tests for tuberculosis in camelids

Test name	Works by	Sensitivity (SN)/specificity (SP)	Notes
Single intradermal comparative cervical tuberculin[1]	Evoked cell-mediated response causing a delayed type hypersensitivity reaction	Varies greatly between authors, but generally presumed to be low	Purified protein derivative or mixed complex antigens used for the test
Gamma interferon Rhodes et al, 2012.	Cellular-mediated response	Unclear. In the past SP has been likely compromised by microti strains in camelids	
IDEXX ELISA Rhodes and Vordermeier, 2012.	Serological response	SN 69.2% SP 97.4%	Antigen: MPB83/ MPB70 (mix)
DPPVetTB lateral flow Rhodes and Vordermeier, 2012.	Serological response	SN 57.7% SP 96.7%	Antigen: MPB83 CFP10/ESAT 6 (mix)
IDEXX+DPPVetTB Rhodes and Vordermeier, 2012.	Serological response	SN 81.3% SP 95.8% SN 55.8% SP 99.7%	Parallel/ breakdown testing Serial/ voluntary testing
Enferplex Rhodes and Vordermeier, 2012.	Serological response	(Two spot) SN 66.7%, SP 96.9%[2] (Four spot) SN 55.1% SP 99.8%	Antigen: MPB70[2] MPB83 MPB70 ESAT 6 ESAT 10 Alfa-crystalline-2 Rv3618O

[1]Test not applied at cervical region, but at anterior axilla region in the UK.
[2]Statistical package in place.

Table 2. Different available tests for tuberculosis in camelids.

In cattle, gamma-interferon response takes one to five weeks to develop, while skin reactions take between two to six weeks (de la Rua-Domenech et al, 2006). In bovines, TB is a slowly progressing chronic infection that will often remain subclinical for a long time (de la Rua-Domenech et al, 2006). This might be completely different in camelids, but often, the disease does seem to go undetected for a long time. The main reason clinical TB in cattle is a rare find in modern veterinary practice is the stringent testing intervals that catch most cases well before the clinical stages develop.

In camelids, this is, unfortunately, not the case in the UK, and animals are either severely clinically diseased, suggestive of TB – and, therefore, tested by vets in practice – or found to display gross pathology resembling TB on postmortem examinations. Both situations would likely mean the animal is in the end stage of the disease, which means a high likelihood exists that the animal has been shedding large numbers of TB bacilli (**Table 2**).

Enferplex in comparison to skin testing and other blood essays

The Enferplex test employs seven different antigen wells that are not mixed and have individual thresholds. These readings are quantitative, not qualitative as with other serological tests, which leaves potential to change individual cut-off values at the individual antigen level to further

modulate SN/SP.

The seven different antigen markers will both maximise the chance to pick up various disease stages, as antigen expression is stage-dependent. For example, MBP83 and ESAT 6 are classified as early markers, while MPB70 is a late-stage marker.

It might also be possible to differentiate between certain different mycobacteria that can cause TB. Although this functionality is not absolute, *M microti* strains that were ESAT-6 negative have been successfully differentiated from *M bovis* by this (Enferplex data).

Cross reactions due to *M avium* infection or Johne's disease vaccinations, which can be an issue when using cellular immunity-based tests, should not occur with Enferplex. With this test, it is also possible to differentiate between natural *M bovis* infection and animals vaccinated with BCG vaccine in cattle (Whelan et al, 2010). This might become valuable when a vaccine becomes available.

Table 3. Costing for voluntary serological tests

Test	Costs	Interpretation
Enferplex Sure Farm, 2017.	£12 per sample + £30.55 postage (often shared) + vet costs	Two spot, statistical package/retest if necessary, four spot instant positives[1]
IDEXX+DPPVetTB APHA, 2016.	£21.75/test, if testing ≤5 £18.80/test, when testing ≥5 + vet costs	Serial interpretation only

[1]Statistical package in place.

Table 3. Costing for voluntary serological tests.

The test is still improving as more animals get tested – so far about 2,000 camelids have been tested using Enferplex. When only borderline (two spot) results are found, interpretation is based on a statistical package. It calculates the probability that, in the population tested, this constitutes sufficient evidence that infection is indeed present (with a 99.5% level of certainty). If this level cannot be attained, the positives are determined as inconclusive and retested. This reduces the chance of wrongly classifying an animal/herd as infected, without ignoring borderline results, improving the test's specificity (**Table 3**).

Future opportunities

Camelids will often represent a considerable emotional, genetic and financial value to their owners and investing in their health should be a logical step forward. Investing in preventive biosecurity measures (Barrington et al, 2006), employing risk-based trading and voluntary screening methods will minimise uncontrolled spread and ensure a timely diagnosis, quicker resolution and safeguard animal welfare.

A proactive engagement by the industry and vets in practice to address these welfare and zoonotic

issues will also safeguard the favourable public's perception towards these fascinating creatures. Other countries, such as New Zealand, have already launched industry-led voluntary TB surveillance schemes.

Although the available tests are far from perfect, they are well within justifiable ranges for both SN and SP, if compared to bovine testing modalities. In bovines, the SICCT test appears to have a positive predictive value of around 91% (Goodchild et al, 2015) and the sensitivity can be as low as 50% (Downs et al, 2011).

If we put this into perspective, the range in serological-based tests available for camelids are definitely not outperformed by the skin test in cattle. Although sensitivity seems slightly disappointing, it is useful to remember a typical herd-level breakdown will involve about 10% of a herd. If a test with a sensitivity of "only" 70% is used then the chance to miss infection in a herd of 40 is less than 1% on just one single screening.

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