DISCONTOOLS

Mycoplasma bovis
Summary

Introduction

1. This note provides a brief summary of the Disease and Product analysis prepared by a DISCONTOOLS group of experts on *Mycoplasma bovis (M. bovis*). They reviewed the current knowledge on the disease, considered the existing disease control tools, identified current gaps in the availability and quality of the control tools and finally determined the research necessary to develop new or improved tools. Full details are available on the web site at http://www.discontools.eu/.

Disease profile

2. *M. bovis* associated infections are a major economic constraint on intensive cattle production (both beef -particularly in feed lots- and milk –high yielding herd-) worldwide. The clinical signs are variable, with three dominant ones: mastitis, bovine respiratory disease (BRD) and arthritis. Some signs can evolve from acute to chronic, with an important impact on animal welfare. Infected cattle can become asymptomatic carriers and may shed the organism through nasal discharges or in milk for months to years. *M. bovis* infections are not zoonotic but other animals may become infected, often as asymptomatic carriers. *M. bovis* associated clinical signs are not specific, hence a laboratory differential diagnostics is necessary. The exact role of *M. bovis* amongst other infectious agents still needs to be clarified.

Risk

3. *M. bovis* can spread very rapidly once introduced into a herd. Spread to new herds is usually due to the movement of asymptomatic carriers being purchased and introduced into a clean herd or through semen. The primary routes of infection can vary but are usually close contact (like direct nose-to-nose transmission), via aerosols, by the ingestion of infected milk, by semen, or by contact with contaminated surfaces like shared feeding systems or milking parlour for mastitis. It is suspected that stress of cattle through adverse weather (climatic changes), overcrowding, poor feed quality, introduction of new animals, and transportation might trigger an outbreak. *M. bovis* survival in the herd environment is poorly documented as a route of re-infection. Common-sense biosecurity measures are often effective in controlling the disease despite some remaining lack of knowledge in transmission routes, infectious doses and protective immunity.

Diagnostics

4. Direct diagnosis is most often ensured by molecular methods such as rtPCR with several kits available on the market (either as stand-alone tests targeting only *M. bovis* or “screening” tests targeting several agents potentially involved in BRD and mastitis including *M. bovis*). Culture-based identification tests (like MALDI-TOF) should be encouraged as they go through strains isolation that are then available for antibiotic (AB) susceptibility testing (AST). Some techniques (PCR DGGE, MALDI-TOF…) allow a differential diagnosis from other mycoplasma species that can also be present in clinical specimens (*M. bovirhinis*, *M. dispar*, *M. arginini*, etc.). Intermittent shedding of organisms and the inhibitors present in milk may reduce the effectiveness of some tests.
5. The main indirect test in use is ELISA, with several commercial kits currently available although with variable performance. Most of them are poorly evaluated for use on milk (individual or tank).
6. There is a need for comparing the performance (sensitivity and specificity) and costs of the different diagnostics method through interlaboratory trials (only a few have been conducted so far). Pen side tests performable in the field and at local point-of-care centres would be welcome. Similarly, more rapid (less than one hour) and cost-effective tests are still required, such as LAMP, other isothermal PCRs, latex agglutination tests or lateral flow immunoassay devices. Accessibility to standardised growth medium and controlled selectivity would be welcome. Methods for diagnosis should include routine-friendly AST in real time.
7. Genomic data have largely increased recently and are helpful to monitor epidemiological spread of *M. bovis*. Subtyping isolates might be pertinent in certain epidemiological situations.

Vaccines.

8. Two vaccines, of the bacterin-type, are licensed in the USA and commercially available. Both showed moderate to low efficacy in reducing lung lesions but neither protected against *M. bovis* upper-respiratory tract colonisation and otitis morbidity. Recent trials in the UK showed a potential reduction in post-weaning mortality and AB usage after vaccination. A new live attenuated (temperature sensitive) vaccine has been temporarily licensed for use in France. Its main target is BRD with some promising results during an experimental challenge.
9. Autogenous vaccines are produced and used in different countries. Most often they give the impression of positive results, but no real structured assessments have been conducted. Effects seem to be limited without additional improvements in herd management / housing.
10. Research projects to develop vaccines are numerous but the ideal vaccine, *i.e.* i) safe, ii) effective against all disease manifestations, iii) usable at all stages of animal production, iv) active against all *M. bovis* variations, v) stable, vi) single shot, vii) providing long-term effective protective immunity and viii) usable in all countries has not yet been found. There is still a long way to go in understanding the immune response against mycoplasmas and the different steps in disease development. The contribution of the immune response to the development of chronic lesions indicates caution in the use of vaccines as some vaccines may result in exacerbation of disease. Other concerns relate to the variability of the surface proteins expressed by *M. bovis* and the possible requirement to use multiple strains in a vaccine. Adjuvant selection could be critical in stimulating a protective immune response.

Pharmaceuticals

11. *M. bovis,* like all mycoplasmas, is wall-less and is consequently insensitive to ABs targeting the cell wall, such as the commonly used beta-lactams. A range of ABs, with a potential efficacy, has a marketing authorization worldwide and is currently used against *M. bovis* (except for some molecules of critical importance, like fluoroquinolones). However, there is a poor response to treatments in both BRD and mastitis and high levels of resistance in vitro have been described worldwide for almost all families. Target modification by chromosomal mutations is the most commonly described mechanism of resistance in mycoplasmas but other might exist.
12. Currently, animal AB use is decreasing in Europe and the USA and development of new ABs is not seen as socially responsible and hence not a priority for many industries. Effectiveness of probiotics, medicinal plants and new cocktails of ABs should be assessed.

Knowledge

13. Increased knowledge on the pathophysiology of *M. bovis* infections, including host invasion mechanisms and its predilection for specific body sites is required and could hint towards new therapeutic developments. Experimental models for reproducing the disease, and mimicking the interaction with the host immune system are required.
14. Possible differences in route of transmission, infectious dose per route, host susceptibility, age, breed etc. also require investigation.
15. The role of some predicted virulence factors is still to be ascertained *in vivo*.
16. There is a need for interlaboratory trials to validate diagnostics methods including AST and for clinical interpretative criteria of AST results. Recommendations for “targeted” sampling (time, volume, frequency, …) are needed to maximise detection in different matrices (bulk tank milk, semen, etc.).

Conclusions

17. *M. bovis* infections have a significant negative economic impact on cattle rearing worldwide.
18. Main problems are: i) no effective vaccines available, ii) insidious infection not always easily diagnosed, iii) difficulty to eliminate the disease from a herd, iv) difficulty to assess the contribution of *M. bovis* in the BRD complex when a number of other pathogens are also involved and finally v) development of resistance against most of the ABs currently in use.
19. Currently the most widely used preventive measure is AB therapy with poor success, but the alternative test and slaughter is a crude and less economical strategy to help control this disease. The disease in its chronic form has also important implications for animal welfare (raising awareness).
20. More research is needed to inform and harmonise regulatory control measures (e.g. trade of semen, diagnostic procedures).