Mycobacterium Tuberculosis Complex

Subjects: Respiratory System | Veterinary Sciences | Microbiology
Contributor: Mark Chambers

The Mycobacterium tuberculosis complex (MTBC) is a group of bacteria that cause tuberculosis (TB) in diverse hosts, including captive and free-ranging wildlife species. There is significant research interest in developing immunodiagnostic tests for TB that are both rapid and reliable, to underpin disease surveillance and control.

Keywords: MTBC ; Mycobacterium bovis ; immunological ; diagnostic ; tuberculosis

1. Introduction

The *Mycobacterium tuberculosis* complex (MTBC) is a group of genetically similar bacteria that cause the disease tuberculosis (TB) in a range of hosts [1]. The MTBC comprises the major pathogenic mycobacteria species *M. tuberculosis, M. bovis, M. africanum*, *M. canettii, M. microti*, *M. caprae, M. pinnipedii*, *M. mungi*, *M. suricattae*, and *M. orygis* [2]. Cattle are considered the primary host of *M. bovis*; however, infection is not limited to livestock but also affects humans and many other free-ranging and captive wildlife species [3]. Notably, the European badger (*Meles meles*) in the United Kingdom, Brushtail possum (*Trichosurus vulpecula*) in New Zealand, and White-tailed deer (*Odocoileus virginianus*) in the United States are all species implicated in transmission of *M. bovis* to livestock [3]. As reviewed by Miller and Olea-Popelka, different control strategies for TB are implemented in different countries based on the level of disease transmission and prevalence within that country, and considering which species are infected or at risk of infection [4]. Common control strategies include surveillance, culling of reservoirs and infected animals, increased biosecurity and vaccination underpinned by diagnostic testing [5].

Zoonotic transmission of TB may be more likely in zoos due to the close contact of staff with animals, as well as the potential for transmission of infection from human to animal, although this is very rare [6]. In addition, zoos may find it problematic to maintain biodiversity and conserve valuable or endangered species and to exchange genetic resources and animals with one another where TB outbreaks occur, as reviewed by Lécu and Ball [7]. Accurate diagnosis of TB in captive wildlife is therefore important but challenging, given the diversity of species susceptible to the MTBC.

Conventional diagnostic tests for TB are considered the gold-standard and comprise of bacterial culture, histopathology, and post-mortem examination [8]. Often, these conventional tests are used in combination with one another or are used as a confirmatory test for newer immunological diagnostics, as discussed by Salfinger and Pfyffer [9]. Culture and post-mortem examinations are relatively expensive tests that require laboratory facilities for isolation and identification of mycobacteria. Culture is the primary gold-standard test for TB. However, as mycobacteria are slow growing, culture can be protracted as well as being liable to cross-contamination with other environmental bacteria [10]. Culture also varies in sensitivity depending upon the type of sample used [11].

Immunological diagnostics based on the humoral immune response rely on the detection of antibodies specific to MTBC antigens. Whilst easy to perform, they may be a poor indicator of TB infection because antibody titers tend to increase as disease progresses [12]. Hence, the humoral response is less reliable for the detection of asymptomatic cases or cases early on in infection but can be used to monitor the progress of infection, as reviewed in Pollock et al., 2001 [13]. Efforts to increase the sensitivity of antibody-based tests have included the use of multiple antigens, most recently, the P22 complex, made up of 118 different antigenic targets, including MPB83, MPB70 and ESAT-6 [14].

In contrast, the cell-mediated immune (CMI) response is characterized by the production of cytokines, such as IFN-γ released by stimulated lymphocytes. As discussed in the Pollock et al. review, relative to antibody production, the CMI response generally occurs earlier after infection and is considered to play a major role in controlling TB [13]. The intradermal delayed-type hypersensitivity tuberculin skin test (TST) involves intradermal injection of tuberculin, a complex mix of antigens derived from *M. bovis*-purified protein derivative (PPD) and measurement of swelling at the injection site usually 72 hrs later [15]. The TST is generally unreliable in most non-bovine species, such as European badgers, producing a weak response, which can be altered by the stress of capture [15][16]. In addition, the TST is often considered

impractical for free-ranging wildlife because of the need to capture and retain the animal to read the test, as discussed and reviewed by De Lisle et al. [15].

Indicators often used to measure diagnostic test performance include, but are not limited to, sensitivity and specificity, predictive values, likelihood ratios, and receiver operating characteristic curve (ROC curve) analysis. Another, but less used, method is the diagnostic odds ratio (DOR). The DOR is a single indicator of test performance, being a ratio of the odds of a positive result in a diseased individual relative to the odds of a positive result in a non-diseased individual [17]. DOR can range from 0 to infinity, but a value of 1 demonstrates that the test has no discrimination between an individual with and without disease. The higher the DOR value, the better able a test is in discriminating infected from non-infected individuals [17].

2. Diagnosing Tuberculosis in Captive and Free-Ranging Non-Bovid Species

This entry was intended as a review of the tests available for diagnosing TB in non-bovid species, focusing on immunological tests and highlighting any advances from previous reviews undertaken in 2009 and 2012 [18][19]. Common indicators of diagnostic performance include sensitivity, specificity, PPV, and NPV; however, these factors are insufficient to demonstrate diagnostic performance alone [17]. Sensitivity and specificity indicators are based on a proportion of results showing positive or negative results among diseased or healthy individuals and do not consider cut off values [17]. NPV and PPV are generally not good indicators of diagnostic performance per se as they are dependent on the prevalence of infection and therefore assess diagnostic performance in a context-dependent situation [17][20]. For this study, DOR was chosen as the primary method of evaluating diagnostic performance because it serves as a single measure of test performance independent of disease prevalence [17], making comparisons across studies more straightforward. This is the first study to use DOR to assess diagnostic test performance in animals, although it has been used to assess the performance of TB tests in humans, e.g., [21].

Wild boar, badger, and deer were the most common species used in studies, with 27.5% of studies carried out in suid species (pigs, wild boar, and warthogs). Wild boar, badger and white-tailed deer are all significant maintenance hosts of TB in different countries [3]. Wild boars have been documented across Europe showing marked increase in numbers [22]. Throughout Europe, wild boar are showing higher levels of transmission of TB, without the requirement of livestock to maintain infection in the ecosystem, as reviewed in Gortázar et al., 2012 [23]. This has an impact on the population of wild boar itself but also increases the chance of transmitting the disease to other wildlife [24]. The increased awareness of wild boar as an important vector of animal TB is reflected the increase in the number of papers reporting the use of immunodiagnostics for suids, 14 papers in this report in comparison to only three papers covering a similar span of time in the last review [19]. The performance of diagnostic tests was reported in two new species since the previous reviews: meerkat and African wild dog, both being the focus of one study each. TB in meerkats is similar to that in other mammalian species [25], and their study has shed light on the behaviors and social interactions that may affect transmission of TB within social mammal species [26]. African Wild Dogs are classed as a threatened species that are currently under high pressure of infection which may impact their long-term survival and conservation [27]. A study looked at 21 packs of wild dog in Kruger National Park, where TB is endemic in African buffaloes and found using an IGRA that 20/21 of the packs studied had been sensitized to *M. bovis*, showing an 83% prevalence of infection [27]. Despite these results, the species is currently considered stable but highlights the potential threat that could occur with changes in biological and environmental factors such as habitat availability and reproductive rates [27].

Antigenic targets identified frequently were ESAT-6, CFP10, MPB83 and MPB70. Recombinant proteins like CFP10/ESAT-6 have demonstrated high sensitivity and specificity for TB detection in people in comparison to conventional CMI diagnostics like the TST [28]. CFP10 and ESAT-6 may also be the target of strong antibody-positive responses when included in serology tests for both elephants and wild boar [29][30] but show poor diagnostic performance in badgers, with no significant increase in antibody response associated with disease progression [31]. Therefore, the diagnostic performance of CFP10 and ESAT-6 antigens cannot be generalized across species, as is the case with many antigenic targets, but does demonstrate potential for accurate detection of TB in certain species. Individually, MPB83 induces high antibody responses across a range of species including cattle, badger, deer, wild boar, and primates [32][33][34][35]. P22 was described in 2017 [14], and therefore, was not reviewed previously. P22 complex is a mix of 118 different proteins, some of the most abundant being MPB70, MPB83, and ESAT-6 [14]. P22 complex was reported to have reduced cross-reactivity with *Mycobacterium avium*, having greater sensitivity than other antigenic targets, like bPPD, [14] in different species, including llamas, cattle, goats, pigs, and sheep [36][37]. In our review, although MPB83 and P22 appeared most frequently as antigenic targets in the top-ranking tests according to sensitivity, specificity, or DOR, they did not appear any more frequently than would be expected by chance, their appearance among the best performing tests more

likely indicating how commonly these antigens are used. Nonetheless, both antigens gave good performance in a variety of test platforms against a range of non-bovid species. P22 as an antigenic target gave sensitivity and specificity values of 70.1–96.7% and 75.0–100.0%, respectively, across studies in wild boar, pig, deer, and badgers. Interestingly, the inclusion of multiple antigens usually increases the likelihood of FP occurring, but this was not seen with P22, despite it being a complex of 118 different antigens. When a P22-based ELISA was compared to the diagnostic performance of MPB83 as a target, it produced similar diagnostic results; however, when used in parallel, sensitivity was increased [38]; some infected animals were only detectable using MPB83 antigen, whilst others were only detectable using the P22 complex [38]. This was surprising since MPB83 is an abundant component of P22. Consequently, when used in parallel, a greater range of animal species were detected. More research is required using field samples to compare and validate the potential of P22 across a wider array of species to confirm the findings above.

Serological diagnostics were more common than CMI tests, with more serological tests appearing in the top ten. Generally, CMI tests are considered to give high sensitivity; however, this was not seen in this review as CMI tests did not appear among the tests with the highest DOR values. In general, the CMI tests were not carried out in suid species but instead in lions and deer, and this could explain the cause of their lower apparent performance, particularly as the high performing tests were carried out in suid species. Suid species are noted to have a detectable humoral response soon after *M. bovis* exposure which is maintained with disease progression, allowing for rapid detection [30][39]. Moreover, as reviewed by Berger, in most species, the humoral antibody response is dependent upon the cell-mediated response initiating a T helper cell response to activate macrophages and other essential cytokines for antibody activation [40]. However, it has been suggested that suid species have a dichotomy between the humoral and CMI response, meaning that a strong humoral response can occur independently of a cell-mediated response [41][42][43].

Despite a test having a high accuracy, it did not always correlate with high diagnostic performance, based on DOR. For example, TB ELISA-VK [44], t-bPPD [44], and bPPD2 [44] were all ranked among the top ten performing tests according to DOR but did not appear in the top ten for either sensitivity or specificity. Conversely, the Ingezim TB-CROM [32], Indirect PPD ELISA [45], and TB ELISA-VK [45] appeared in either or both top ten for specificity and sensitivity but not DOR. We reason that DOR is a better metric for assessing the performance of a diagnostic test since sensitivity and specificity (as pooled or indiviudal indicators) do not represent discriminatory performance, since a high sensitivity can be accompanied by a low specificity, as shown particularly for the TB ELISA-VK [45]. In contrast, DOR is a combination of both sensitivity and specificity, increasing when they become near perfect.

3. Conclusions

In conclusion, a variety of diagnostic tests are now available for an array of wildlife species, with increasing variety of species being studied. The focus of this review was on diagnostic tests that detect or measure the host immune response to infection. It was evident that serological tests are surpassing tests like the TST and even other CMI-based tests, such as IGRA for diagnostic performance. Obtaining proof of high accuracy in tests is still an issue, restricting validation of many tests. We used DOR to evaluate diagnostic performance, which to the best of our knowledge has not been used previously for assessing TB diagnostic tests in animals. P22 complex was identified as a promising, new antigenic target, which alongside MPB83 demonstrated potential for use as an accurate seroantigenic target. We believe these conclusions to be consistent with the evidence and arguments presented.

References

1. Gagneux, S. Host-pathogen coevolution in human tuberculosis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2012, 367, 850–859.
2. Brites, D.; Loiseau, C.; Menardo, F.; Borrell, S.; Boniotti, M.B.; Warren, R.; Dippenaar, A.; Parsons, S.D.C.; Beisel, C.; Behr, M.A.; et al. A new phylogenetic framework for the animal-adapted Mycobacterium tuberculosis complex. Front. Microbiol. 2018, 9, 2820.
3. Palmer, M.V. Mycobacterium bovis: Characteristics of wildlife reservoir hosts. Transbound. Emerg. Dis. 2013, 60, 1–13.
4. Miller, M.; Olea-Popelka, F. One health in the shrinking world: Experiences with tuberculosis at the human-livestock-wildlife interface. Comp. Immunol. Microbiol. Infect. Dis. 2013, 36, 263–268.
5. Fitzgerald, S.D.; Kaneene, J.B. Wildlife reservoirs of bovine tuberculosis worldwide: Hosts, pathology, surveillance, and control. Vet. Pathol. 2013, 50, 488–499.

6. Dalovisio, J.R.; Stetter, M.; Mikota-Wells, S. Rhinoceros' rhinorrhea: Cause of an outbreak of infection due to airborne Mycobacterium bovis in zookeepers. Clin. Infect. Dis. 1992, 15, 598–600.
7. Lécu, A.; Ball, R. Mycobacterial infections in zoo animals: Relevance, diagnosis and management. Int. Zoo Yearb. 2011, 45, 183–202.
8. Silva, D.A.V.D.; Siconelli, M.J.L.; Bürger, K.P.; Keid, L.B. Comparison between tests for tuberculosis diagnosis in slaughtered bovines. Arq. Inst. Biológico 2018, 85, 85.
9. Salfinger, M.; Pfyffer, G.E. The new diagnostic mycobacteriology laboratory. Eur. J. Clin. Microbiol. Infect. Dis. 1994, 13, 961–979.
10. Pfyffer, G.E. Mycobacterium: General characteristics, laboratory detection, and staining procedures. In Manual of Clinical Microbiology, 11th ed.; Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S., Warnock, D.W., Eds.; ASM Press: Washington, DC, USA, 2015; Volume 1, pp. 536–569.
11. Hines, N.; Payeur, J.B.; Hoffman, L.J. Comparison of the recovery of Mycobacterium bovis isolates using the BACTEC MGIT 960 system, BACTEC 460 system, and Middlebrook 7H10 and 7H11 solid media. J. Vet. Diag. Investig. 2006, 18, 243–250.
12. Welsh, M.D.; Cunningham, R.T.; Corbett, D.M.; Girvin, R.M.; McNair, J.; Skuce, R.A.; Bryson, D.G.; Pollock, J.M. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology 2005, 114, 101–111.
13. Pollock, J.M.; McNair, J.; Welsh, M.D.; Girvin, R.M.; Kennedy, H.E.; Mackie, D.P.; Neill, S.D. Immune responses in bovine tuberculosis. Tuberculosis 2001, 81, 103–107.
14. Infantes-Lorenzo, J.A.; Moreno, I.; Risalde, M.L.A.; Roy, A.; Villar, M.; Romero, B.; Ibarrola, N.; de la Fuente, J.; Puentes, E.; de Juan, L.; et al. Proteomic characterisation of bovine and avian purified protein derivatives and identification of specific antigens for serodiagnosis of bovine tuberculosis. Clin. Proteom. 2017, 14, 36.
15. de Lisle, G.W.; Bengis, R.G.; Schmitt, S.M.; O'Brien, D.J. Tuberculosis in free-ranging wildlife: Detection, diagnosis and management. Rev. Sci. Tech. 2002, 21, 317–334.
16. Higgins, D.A. The skin inflammatory response of the badger (Meles meles). Br. J. Exp. Pathol. 1985, 66, 643–653.
17. Glas, A.S.; Lijmer, J.G.; Prins, M.H.; Bonsel, G.J.; Bossuyt, P.M. The diagnostic odds ratio: A single indicator of test performance. J. Clin. Epidemiol. 2003, 56, 1129–1135.
18. Chambers, M.A. Review of the diagnosis and study of tuberculosis in non-bovine wildlife species using immunological methods. Transbound. Emerg. Dis. 2009, 56, 215–227.
19. Chambers, M.A. Review of the diagnosis of tuberculosis in non-bovid wildlife species using immunological methods—An update of published work since 2009. Transbound. Emerg. Dis. 2013, 60, 14–27.
20. Altman, D.G.; Bland, J.M. Diagnostic tests 2: Predictive values. BMJ 1994, 309, 102.
21. Greco, S.; Girardi, E.; Navarra, A.; Saltini, C. Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. Thorax 2006, 61, 783–790.
22. Massei, G.; Kindberg, J.; Licoppe, A.; Gacic, D.; Sprem, N.; Kamler, J.; Baubet, E.; Hohmann, U.; Monaco, A.; Ozolins, J.; et al. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest. Manag. Sci. 2015, 71, 492–500.
23. Gortázar, C.; Delahay, R.J.; McDonald, R.A.; Boadella, M.; Wilson, G.J.; Gavier-Widen, D.; Acevedo, P. The status of tuberculosis in European wild mammals. Mammal. Rev. 2012, 42, 193–206.
24. Barasona, J.A.; Gortazar, C.; de la Fuente, J.; Vicente, J. Host richness increases tuberculosis disease risk in game-managed areas. Microorganisms 2019, 7, 182.
25. Drewe, J.A.; Foote, A.K.; Sutcliffe, R.L.; Pearce, G.P. Pathology of Mycobacterium bovis infection in wild meerkats (Suricata suricatta). J. Comp. Pathol. 2009, 140, 12–24.
26. Drewe, J.A. Who infects whom? Social networks and tuberculosis transmission in wild meerkats. Proc. Biol. Sci. 2010, 277, 633–642.
27. Higgitt, R.L.; Louis van Schalkwyk, O.; de Klerk-Lorist, L.M.; Buss, P.E.; Caldwell, P.; Rossouw, L.; Manamela, T.; Hausler, G.A.; Hewlett, J.; Mitchell, E.P.; et al. Mycobacterium bovis infection in african wild dogs, kruger national park, south africa. Emerg. Infect. Dis. 2019, 25, 1425–1427.
28. Hemmati, M.; Seghatoleslam, A.; Rasti, M.; Ebadat, S.; Mosavari, N.; Habibagahi, M.; Taheri, M.; Sardarian, A.R.; Mostafavi-Pour, Z. Expression and purification of recombinant Mycobacterium tuberculosis (TB) antigens, ESAT-6, CFP-10 and ESAT- 6/CFP-10 and their diagnosis potential for detection of TB patients. Iran. Red Crescent Med. J. 2011, 13, 556–563.

29. Kerr, T.J.; de Waal, C.R.; Buss, P.E.; Hofmeyr, J.; Lyashchenko, K.P.; Miller, M.A. Seroprevalence of Mycobacterium tuberculosis complex in free-ranging african elephants (Loxodonta africana) in kruger national park, south africa. J. Wildl. Dis. 2019, 55, 923–927.
30. Miller, M.A.; Gortazar, C.; Roos, E.O.; Risalde, M.A.; Johnathan-Lee, A.; Sridhara, A.A.; Lyashchenko, K.P. Serological reactivity to MPB83 and CFP10/ESAT-6 antigens in three suid hosts of Mycobacterium bovis infection. Vet. Microbiol. 2019, 235, 285–288.
31. Ashford, R.T.; Anderson, P.; Waring, L.; Dave, D.; Smith, F.; Delahay, R.J.; Gormley, E.; Chambers, M.A.; Sawyer, J.; Lesellier, S. Evaluation of the dual path platform (DPP) VetTB assay for the detection of Mycobacterium bovis infection in badgers. Prev. Vet. Med. 2020, 180, 105005.
32. Fresco-Taboada, A.; Risalde, M.A.; Gortazar, C.; Tapia, I.; Gonzalez, I.; Venteo, A.; Sanz, A.; Rueda, P. A lateral flow assay for the rapid diagnosis of Mycobacterium bovis infection in wild boar. Transbound. Emerg. Dis. 2019, 66, 2175–2179.
33. Lyashchenko, K.P.; Greenwald, R.; Esfandiari, J.; Greenwald, D.; Nacy, C.A.; Gibson, S.; Didier, P.J.; Washington, M.; Szczerba, P.; Motzel, S.; et al. PrimaTB STAT-PAK assay, a novel, rapid lateral-flow test for tuberculosis in nonhuman primates. Clin. Vaccine Immunol. 2007, 14, 1158–1164.
34. Waters, W.R.; Palmer, M.V.; Bannantine, J.P.; Whipple, D.L.; Greenwald, R.; Esfandiari, J.; Andersen, P.; McNair, J.; Pollock, J.M.; Lyashchenko, K.P. Antigen recognition by serum antibodies in white-tailed deer (Odocoileus virginianus) experimentally infected with Mycobacterium bovis. Clin. Diagn. Lab. Immunol. 2004, 11, 849–855.
35. Waters, W.R.; Palmer, M.V.; Thacker, T.C.; Bannantine, J.P.; Vordermeier, H.M.; Hewinson, R.G.; Greenwald, R.; Esfandiari, J.; McNair, J.; Pollock, J.M.; et al. Early antibody responses to experimental Mycobacterium bovis infection of cattle. Clin. Vaccine Immunol. 2006, 13, 648–654.
36. Infantes-Lorenzo, J.A.; Moreno, I.; Roy, A.; Risalde, M.A.; Balseiro, A.; de Juan, L.; Romero, B.; Bezos, J.; Puentes, E.; Akerstedt, J.; et al. Specificity of serological test for detection of tuberculosis in cattle, goats, sheep and pigs under different epidemiological situations. BMC Vet. Res. 2019, 15, 70.
37. Infantes-Lorenzo, J.A.; Whitehead, C.E.; Moreno, I.; Bezos, J.; Roy, A.; Dominguez, L.; Dominguez, M.; Salguero, F.J. Development and evaluation of a serological assay for the diagnosis of tuberculosis in alpacas and llamas. Front. Vet. Sci. 2018, 5, 189.
38. Arrieta-Villegas, C.; Infantes-Lorenzo, J.A.; Bezos, J.; Grasa, M.; Vidal, E.; Mercader, I.; Singh, M.; Domingo, M.; de Juan, L.; Perez de Val, B. Evaluation of P22 antigenic complex for the immuno-diagnosis of tuberculosis in BCG vaccinated and unvaccinated goats. Front. Vet. Sci. 2020, 7, 374.
39. Barasona, J.A.; Barroso-Arevalo, S.; Rivera, B.; Gortazar, C.; Sanchez-Vizcaino, J.M. Detection of Antibodies against Mycobacterium bovis in Oral Fluid from Eurasian Wild Boar. Pathogens 2020, 9, 242.
40. Berger, A. Th1 and Th2 responses: What are they? BMJ 2000, 321, 424.
41. Boadella, M.; Lyashchenko, K.; Greenwald, R.; Esfandiari, J.; Jaroso, R.; Carta, T.; Garrido, J.M.; Vicente, J.; de la Fuente, J.; Gortazar, C. Serologic tests for detecting antibodies against Mycobacterium bovis and Mycobacterium avium subspecies paratuberculosis in Eurasian wild boar (Sus scrofa scrofa). J. Vet. Diagn. Investig. 2011, 23, 77–83.
42. De Bruin, M.G.; De Visser, Y.E.; Kimman, T.G.; Bianchi, A.T. Time course of the porcine cellular and humoral immune responses in vivo against pseudorabies virus after inoculation and challenge: Significance of in vitro antigenic restimulation. Vet. Immunol. Immunopathol. 1998, 65, 75–87.
43. Kimman, T.G.; De Bruin, T.M.; Voermans, J.J.; Peeters, B.P.; Bianchi, A.T. Development and antigen specificity of the lymphoproliferation responses of pigs to pseudorabies virus: Dichotomy between secondary B- and T-cell responses. Immunology 1995, 86, 372–378.
44. Cardoso-Toset, F.; Luque, I.; Carrasco, L.; Jurado-Martos, F.; Risalde, M.A.; Venteo, A.; Infantes-Lorenzo, J.A.; Bezos, J.; Rueda, P.; Tapia, I.; et al. Evaluation of five serologic assays for bovine tuberculosis surveillance in domestic free-range pigs from southern Spain. Prev. Vet. Med. 2017, 137, 101–104.
45. Roos, E.O.; Buss, P.; de Klerk-Lorist, L.M.; Hewlett, J.; Hausler, G.A.; Rossouw, L.; McCall, A.J.; Cooper, D.; van Helden, P.D.; Parsons, S.D.C.; et al. Test performance of three serological assays for the detection of Mycobacterium bovis infection in common warthogs (Phacochoerus africanus). Vet. Immunol. Immunopathol. 2016, 182, 79–84.