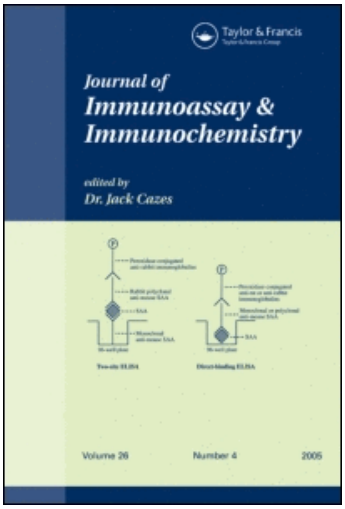

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

IMMUNOGENICITY OF BACILLUS CALMETTE-GUÉRIN (BCG) IN BOVINE NEONATES UNDER TRADITIONAL FARMING IN CENTRAL ETHIOPIA

Tewodros Debebe[a]; Gobena Ameni[a]
[a] Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

Online publication date: 23 March 2010

To cite this Article Debebe, Tewodros and Ameni, Gobena(2010) 'IMMUNOGENICITY OF BACILLUS CALMETTE-GUÉRIN (BCG) IN BOVINE NEONATES UNDER TRADITIONAL FARMING IN CENTRAL ETHIOPIA', Journal of Immunoassay and Immunochemistry, 31: 2, 160 — 168
To link to this Article: DOI: 10.1080/15321811003617479
URL: http://dx.doi.org/10.1080/15321811003617479

Journal of Immunoassay and Immunochemistry, 31:160–168, 2010

ISSN: 1532-1819 print/1532-4230 online
DOI: 10.1080/15321811003617479

Taylor & Francis
Taylor & Francis Group

IMMUNOGENICITY OF BACILLUS CALMETTE-GUÉRIN (BCG) IN BOVINE NEONATES UNDER TRADITIONAL FARMING IN CENTRAL ETHIOPIA

Tewodros Debebe and Gobena Ameni
Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia

□ *Vaccination is an alternative method of controlling bovine tuberculosis (BTB) particularly in developing countries where the test and slaughter control method is not acceptable socially and economically. The objective of this study was to evaluate the immunogenicity of bacillus Calmette-Guérin (BCG) vaccination in bovine neonates. Twelve BTB free bovine neonates (six vaccinated with 0.5 mL of 2.4×10^6 CFU of BCG and six control) with age less than one month were used for this study. Interferon gamma (IFN-γ) and antibody responses to mycobacterial antigens were determined at 0, 1, 3, 7, and 13 weeks of post-vaccination. The mean IFN-γ response to bovine purified protein derivative, PPD in vaccinated group (Mean ± SEM, 0.541 ± 0.216) was greater than the mean IFN-γ response to bovine PPD in non-vaccinated group (Mean ± SEM, 0.253 ± 0.101). Within the vaccinated group, the mean IFN-γ response was greater in cross breed (Mean ± SEM, 0.779 ± 0.458) than in zebu breeds (0.303 ± 0.178). No detectable antibody was observed in both vaccinated and non-vaccinated groups for 13 weeks post vaccination. A sharp rise in IFN-γ response to bovine PPD was observed between at week 3, and then from week 3 to 7 post-vaccination, there was rapid falling of IFN-γ response after which the response remained more or less constant in the consecutive weeks. This preliminary study showed the immunogenicity of BCG in bovine neonates under traditional cattle farming in Ethiopia.*

Keywords BCG, bovine neonates, central Ethiopia, immunogenicity, traditional farming

INTRODUCTION

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis* (*M. bovis*), is a chronic infectious disease of animals characterized by the formation of tubercles in any tissue/organ of the animal.[1] It is widely distributed throughout the world and represents a serious challenge in international trade of animals and their products, causing major economic losses to livestock.[2] More than 50 million cattle are infected with *M. bovis*, resulting in economic losses of approximately $3 billion annually.[3]

Address correspondence to Gobena Ameni, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia. E-mail: gobenachimdi2009@yahoo.co.uk

Apart from being important disease of intensification with a serious effect on animal production, it is an important zoonosis being transmitted to humans by an aerogenous route and/or through consumption of infected milk and other cattle products. In countries where BTB is prevalent and pasteurization is not practiced about 10% to 15% of human tuberculosis (TB) is caused by *M. bovis*.[4]

Many countries have implemented programs to reduce the incidence of TB in cattle, and eradication based on test-and-slaughter-policy.[2] However, in developing countries like Ethiopia, as this is a costly method, alternative means of control such as vaccination need to be implemented. Presently, bacillus Calmette-Guérin (BCG) is the only available vaccine for the control of TB for field use. Different vaccination trials have also been undertaken in cattle in different parts of the world.[5–7] The present study was also undertaken for the generation of additional data on the immunogenicity of BCG in zebu and zebu × Holstein neonates under Ethiopian condition.

EXPERIMENTAL

Study Neonates

Bovine neonates were recruited from Muke-Turi, located in central Ethiopia and known for its livestock production. The neonates were obtained from smallholder farms that were kept on pasture. Twelve, five zebus and seven crosses (zebu × Holstein) were used for the experiment. Six neonates were vaccinated with BCG and six were used as control. The dams of the experimental neonates were negative for BTB on the basis of IFN-γ test 15 days before delivery. Similarly, the neonates were negative for BTB by IFN-γ test prior to vaccination.

BCG Vaccination

The neonates were vaccinated by subcutaneous injection in the side of the neck with 0.5 mL inoculums containing 2.4×10^6 CFU of BCG (Staten Serum Institute, Denmark). The control (non-vaccinated) and vaccinated animals were kept on pasture and sampled at various time points of post vaccination to analyze immune responses.

Blood Collection and Processing

Blood samples were collected from the jugular vein of both vaccinated and non-vaccinated neonates into heparinized vacutainers for IFN-γ test and plain vacutainers for serum extraction at 0, 1, 3, 7, 13 weeks

post-vaccination. Whole blood stimulated with mycobacterial antigens within 4 hours of collection. Briefly, whole-blood cultures were performed in 96-well tissue culture plates. Cultures contained 250 μl heparinized blood and 25 μl of either avian PPD, bovine PPD (Veterinary Laboratories Agency, Weybridge, UK), or saline. After 48 hours incubation at 37°C in a humid 5% CO_2, plasma supernatants were harvested and transferred to a 96-well plate and stored at −20°C until tested by IFN-γ enzyme-linked immunesorbent assay.

Whole-Blood IFN-γ Assay

The BOVIGAM assay[8] was performed according to the manufacturer's instructions (BIOCORE AH, Omaha, NE) with a commercially available kit (BOVIGAM^{TM}, CSL Animal Health). Freeze dried components were reconstituted and green diluents was added to the required wells. Test samples and controls were added into the wells. After 60 minutes incubation at room temperature, the wells were washed with wash buffer and freshly prepared conjugate was added to the wells. Similarly it was incubated for 60 minutes then washed with wash buffer. Following this, freshly prepared enzyme substrate solution was added to the wells then incubated for 30 minutes at room temperature by protecting from direct light. After incubation enzyme stopping solution was added to each well. Finally, within 5 minutes of termination of the reaction the absorbance of each well was measured as optical density (OD) at 450 nm using plate reader (Titertek, Multi scan plus version 1.4, by joint venture company of lab. system and row laboratory, Finland). Accordingly, if response of bovine PPD-response of avian PPD > 0.1, it was considered as positive.[6]

Lateral Flow Assay (Chembio Rapid Test)

Blood samples were collected from both vaccinated and non-vaccinated groups at 0, 1, 3, 7 and 13 weeks post-vaccination for extraction of sera for rapid serological test. A rapid immunochromatographic assay, VetTB STATPAK test, which was developed by Chembio Diagnostic Systems, Inc., Medford, N.Y., to detect antibodies of three isotopes (IgM, IgG, and IgA) against mycobacterial antigens was used for the rapid serological assay. This test was performed following the procedure described by Ref. [9]. Accordingly, 30 microlitres of serum and 3 drops of sample diluents were added sequentially to the sample pad. As the diluted test sample migrates to the conjugate pad, the latex particles conjugated to antigen bind antibody, if present, thus creating a colored immune complex. Driven

by capillary forces, this complex flows laterally across the nitrocellulose membrane impregnated with specific antigen and binds to immobilized antigen, producing a visible blue band in the test area of the device. In the absence of specific antibody, no band is visible in the test window. The liquid continues to migrate along the membrane, producing a similar blue band in the control area of the device, irrespective of the presence of specific antibody in the test sample, demonstrating that the test immunoreagents are functional properly. Test results were read at 20 minutes and interpretation of the result was done on the basis of the intensity of the band in test area. Accordingly, when there was no visible band, weak band, moderate or strong band, the test was considered as negative (0), weak positive (+), moderately positive (++), or strongly positive (+++), respectively.

Statistical Analysis

Data were entered into excel spreadsheet and student *t-test* was used to compare the means IFN-γ responses in vaccinated and non-vaccinated calves.

RESULTS

Interferon-γ Response in Vaccinated and Non-Vaccinated Neonates

Two groups of bovine neonates were included in this study: (i) 6 neonates vaccinated with BCG, and (ii) 6 neonates non-vaccinated and served as control. IFN-γ responses were measured at 0, 1, 3, 7, and 13 weeks after vaccination. Prior to BCG vaccination the intensity of IFN-γ responses to both avian and bovine PPD's was low, and similar in both vaccinated and non-vaccinated calves. Following vaccination intensity of IFN-γ response in BCG-vaccinated was characterized by a sharp increase, attaining its peak at 3 weeks of post-vaccination (Figure 1). However, after the 3rd week of vaccination, there was rapid falling of IFN-γ level, and this sharp falling in the intensities of IFN-γ responses to both avian and bovine PPD's. At week 13 post-vaccination, more or less remained constant.

The means FN-γ responses to avian and bovine PPD's at different weeks in both vaccinated and non-vaccinated neonates are presented in Table 1. The means of IFN-γ responses to both avian and bovine PPD were higher in vaccinated calves than in non-vaccinated controls. But the difference was not statistically significant mainly due the low sample size.

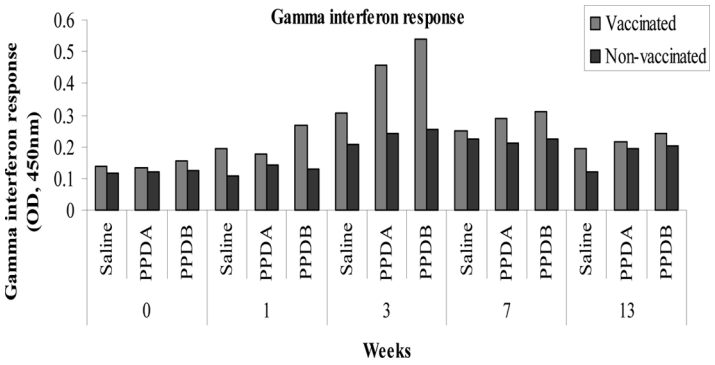

FIGURE 1 Responses of IFN-γ to avian and bovine PPD's in BCG-vaccinated and non-vaccinated bovine neonates in central Ethiopia.

Within the vaccinated group, the means of IFN-γ responses to both PPD's were higher in cross breed than in pure zebu breed (Table 2). The trends of means of the means of IFN-γ responses to both PPD's at different weeks post vaccination in the two breeds of calves is depicted by Figure 2.

TABLE 1 The Means IFN-γ Responses to Avian and Bovine PPD's in BCG-Vaccinated and Non-Vaccinated Bovine Neonates for 13 Weeks in Central Ethiopia

Weeks After Vaccination	Vaccinated Mean ± SEM	Non-Vaccinated Mean ± SEM
Week 0		
Saline	0.138 ± 0.055	0.115 ± 0.046
A-PPD	0.136 ± 0.054	0.122 ± 0.049
B-PPD	0.156 ± 0.063	0.127 ± 0.051
Week 1		
Saline	0.196 ± 0.078	0.106 ± 0.043
A-PPD	0.178 ± 0.071	0.141 ± 0.056
B-PPD	0.269 ± 0.108	0.129 ± 0.052
Week 3		
Saline	0.307 ± 0.123	0.205 ± 0.082
A-PPD	0.459 ± 0.183	0.240 ± 0.096
B-PPD	0.541 ± 0.216	0.253 ± 0.101
Week 7		
Saline	0.252 ± 0.101	0.226 ± 0.090
A-PPD	0.290 ± 0.116	0.212 ± 0.085
B-PPD	0.310 ± 0.124	0.226 ± 0.090
Week 13		
Saline	0.195 ± 0.078	0.122 ± 0.049
A-PPD	0.215 ± 0.086	0.192 ± 0.077
B-PPD	0.243 ± 0.097	0.201 ± 0.081

TABLE 2 The Means IFN-γ Responses to Avian and Bovine PPD's in BCG-Vaccinated Cross Breed and Zebu Calves in Central Ethiopia

Weeks After Vaccination	Zebu Breed Mean ± SEM	Cross Breed Mean ± SEM
Week 0		
Saline	0.125 ± 0.074	0.150 ± 0.088
A-PPD	0.127 ± 0.075	0.144 ± 0.085
B-PPD	0.147 ± 0.086	0.151 ± 0.089
Week 1		
Saline	0.153 ± 0.090	0.146 ± 0.086
A-PPD	0.155 ± 0.091	0.201 ± 0.118
B-PPD	0.166 ± 0.097	0.301 ± 0.177
Week 3		
Saline	0.298 ± 0.175	0.355 ± 0.209
A-PPD	0.381 ± 0.224	0.537 ± 0.316
B-PPD	0.303 ± 0.178	0.779 ± 0.458
Week 7		
Saline	0.217 ± 0.128	0.218 ± 0.128
A-PPD	0.299 ± 0.176	0.236 ± 0.139
B-PPD	0.312 ± 0.183	0.333 ± 0.195
Week 13		
Saline	0.148 ± 0.087	0.156 ± 0.092
A-PPD	0.211 ± 0.124	0.189 ± 0.111
B-PPD	0.238 ± 0.139	0.258 ± 0.152

Measurement of BCG-Specific Immunoglobulin (Ig) Production

The lateral flow assay has recently been developed for the diagnosis of TB, and a cocktail of mycobacterial antigens is used to measure antibody response to mycobacterial infection. In the present study, only one neonate (09) from the control group had showed evidence of antibody production at weeks 0 and 1 post-vaccination (Table 3).

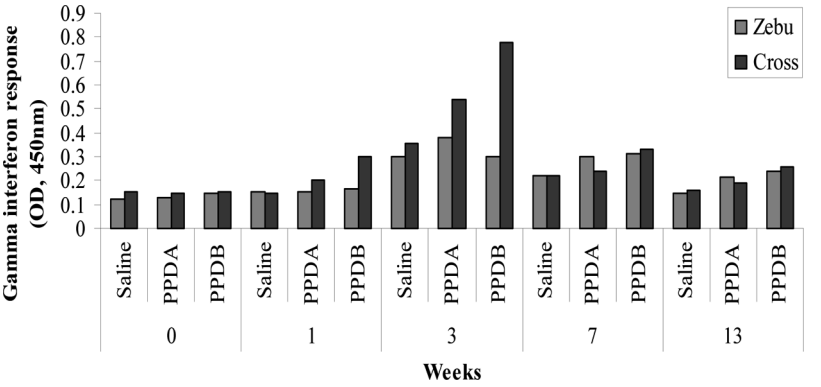

FIGURE 2 Responses of IFN-γ to avian and bovine PPD's in BCG-vaccinated cross breed and zebu calves in central Ethiopia.

TABLE 3 The Magnitude of Antibody in BCG-Vaccinated and Non-Vaccinated Groups at Different Points of Vaccination

ID No.	Group	Weeks After Vaccination				
		Week 0	Week 1	Week 3	Week 7	Week 13
01	Vaccinated	0	0	0	0	0
02	Vaccinated	0	0	0	0	0
03	Vaccinated	0	0	0	0	0
04	Vaccinated	0	0	0	0	0
05	Vaccinated	0	0	0	0	0
06	Vaccinated	0	0	0	0	0
07	Non-vaccinated	0	0	0	0	0
08	Non-vaccinated	0	0	0	0	0
09	Non-vaccinated	+++	++	0	0	0
10	Non-vaccinated	0	0	0	0	0
11	Non-vaccinated	0	0	0	0	0
12	Non-vaccinated	0	0	0	0	0

Negative = (0); Weak positive = (+).
Moderately positive = (++); Strongly positive = (+++).

DISCUSSION

In this study, the level of IFN-γ in the whole blood and the magnitude of antibody in the serum were determined at intervals from week 1 to 13 weeks following vaccination of neonates with BCG. M. avian PPD and M. bovine PPD were used for the stimulation of whole blood. At day 0, IFN-γ responses by neonates from both vaccinates and non-vaccinates to either M. avium or M. bovis PPD were low. Similar findings were reported previously.[10–12] Immediately after vaccination, the IFN-γ concentration in the whole blood started to rise, attaining its peak at three weeks post vaccination. Similar observations were reported earlier by other workers.[5,6,11,12] One reported that calves produced strong IFN-γ response 4 weeks post BCG-vaccination.[5] Similar pattern was observed in this study, and this increased level of IFN-γ in the whole blood may, in part, indicate the proliferation of IFN-γ secreting T cells[13] and correlate with protection.[6,14] In agreement with Ref. [11], in the falling stage, the level of IFN-γ was observed to decrease, first very rapidly, but later slowly. The slow falling in the IFN-γ response might have resulted from the partial control of BCG organisms during the first few weeks following vaccination or due to sequestration of BCG organisms.[5] This phase was followed by a constant stage that shows more or less similar level of IFN-γ release, in which the BCG and the immune component have achieved the equilibrium level.

Interestingly, a high level of IFN-γ release was observed in Cross breed as compared to the Zebu breed in the vaccinated neonates. Before vaccination

(i.e., 0 weeks) more or less similar level of IFN-γ release was observed for both Cross and Zebu breeds. However, after vaccination increased level of IFN-γ release was observed in Cross breeds as compared to Zebu breeds. In agreement with this study,[15] demonstrated higher IFN-γ response in Holstein cattle than in Zebu cattle kept under the same husbandry conditions. The difference in the level of IFN-γ between the two breeds could be due to the difference in the reptoire of the two breeds affecting the recognition of mycobacterial antigens.[15]

In the current study, the difference in the level of IFN-γ release in vaccinated and non-vaccinated (control) groups and between the two breeds (Zebu and Cross) of vaccinated group was statistically not significant ($P > 0.05$). The possible explanation for this insignificancy could be attributed to the small sample size used for this study. Moreover, in agreement with this study many studies demonstrated that efficacy of BCG in cattle and human has not always been positive with a consistently low efficacy or insignificance in many tropical regions of the world where the vaccine is most needed.[16–20]

In this study, both vaccinated and non-vaccinated neonates failed to produce measurable antibody prior and post-vaccination except one neonate from the control group. This could reflect the presence of cross-reactive, maternal antibody acquired through the ingestion of colostrums. The possible reason for the absence of antibody following BCG-vaccination could be due to the initial sensitization cell-mediated immune (CMI) responses with humoral antibody responses developing as bacillary load increases.[21] A similar finding was observed in the study by Ref. [7], vaccinated calves didn't produce measurable antibody in vitro, whereas vaccinated adults developed measurable antibody responses in vitro. The absence of an in vitro antibody response suggests that the young neonates may be limited in its capacity to produce an adult-like antibody response to BCG.[7]

In conclusion, the findings of this study demonstrated that BCG vaccination induced IFN-γ response both in cross and zebus calves. On top of this, this preliminary result suggested that IFN-γ response could be higher in cross breed calves than in pure zebu calves under the same husbandry situation. Therefore, further study using adequate sample size is required to further substantiate this preliminary observation.

ACKNOWLEDGMENT

We acknowledge Dr. Martin Vordermeier for providing us with the reagents.

REFERENCES

1. Radostits, O.M.; Blood, D.C.; Gay, C.C. *Veterinary Medicine. A Textbook Diseases of Cattle, Sheep, Pigs, Goats, and Horses*, 8th ed.; Bailliere Tindall: London, Philadelphia, Sydney, Tokyo, Toronto, 1994; 830–838.
2. Feliciano, M.S.; Ana, M.A.E.; Ruth, M.G.T. A review of M. bovis BCG protection against TB in cattle and other animals species. Prev. Vet. Med. **2003**, *58*, 1–13.
3. Steele, J.H. Regional and country status report. In *Mycobacterium Bovis Infection in Animals and Humans*; Thoen, C. O.; Ed.; Iowa State University Press: Ames, Iowa, 1995; 169–172.
4. Ashford, D.A.; Whitney, E.; Raghunathan, P.; Cosivi, O. Epidemiology of selected mycobacteria that infect humans and other animals. Rev. Sci. Technol., Office International des Epizooties **2001**, *20*, 325–337.
5. Buddle, B.M.; de Lisle, G.W.; Pfeffer, A.; Aldwell, F.E. Immunological responses and protection against Mycobacterium bovis in calves vaccinated with a low dose of BCG. Vacc. **1995**, *13*, 1123–1130.
6. Vordermeier, H.M.; Chambers, M.A.; Cockle, P.J.; Whelan, A.O.; Simmons, J.; Hewinson, R.G. Correlation of ESAT-6-specific gamma interferon with pathology in cattle following Mycobacterium bovis BCG vaccination against experimental bovine tuberculosis. Infect. Immunol. **2002**, *70*, 3026–3032.
7. Nonnecke, B.J.; Waters, W.R.; Foote, M.R.; Palmer, M.V.; Miller, B.L.; Johnson, T.E.; Perry, H.B.; Fowler, M.A. Development of an adult-like cell-mediated immune response in calves after early vaccination with mycobacterium bovis bacillus calmette-guérin. J. Dairy Sci. **2005**, *88*, 195–210.
8. Wood, P.R.; Rothel, J.S. In vitro immunodiagnostic assays for bovine tuberculosis. Vet. Microbiol. **1994**, *40*, 125–135.
9. 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.; Andersen, P.; Lyashchenko, K.P. Early antibody responses to Mycobacterium bovis infection in cattle. Clin. Vacc. Immunol. **2006**, *13*, 648–654.
10. Waters, W.R.; Palmer, M.V.; Whipple, D.L.; Slaughter, R.E.; Jones, S.L. Immune responses of white-tailed deer (odocoileus virginianus) to mycobacterium bovis bcg vaccination. J. Wildlife Dis. **2004**, *40*, 66–78.
11. Ameni, G.; Tibbo, M. Kinetics of Interferon-g (IFN-γ) release in the peripheral blood of calves vaccinated with BCG. J. Immunoass. & Immunochem. **2002**, *23*, 245–253.
12. Buddle, B.M.; Denis, M.; Aldwell, F.E.; Vordermeier, H.M.; Hewinson, R.G.; Wedlock, D.N. Vaccination of cattle with Mycobacterium bovis BCG by a combination of systemic and oral routes, Veterinary Laboratory Agency, Weybridge, Surrey, UK, 2008.
13. Kee, H.N.; Aldwell, F.E.; Wedlock, D.N.; Watson, J.D.; Buddle, B.M. Antigen-induced interferon-g and interleukin-2 responses in cattle innoculated with mycobacterium bovis. Vet. Immunol. Imunopathol. **1997**, *57*, 59–68.
14. Buddle, B.M.; Wedlock, D.N.; Denis, M.; Skinner, M.A. Identification of immune response correlates for protection against bovine tuberculosis. Vet. Immunol. Immunopathol. **2005**, *108*, 45–51.
15. Ameni, G.; Aseffa, A.; Engers, H.; Young, D.; Hewinson, G.; Vordermeier, M. Cattle husbandry in Ethiopia is a predominant factor for affecting the pathology of bovine tuberculosis and gamma interferon responses to mycobacterial antigens. Clin. Vacc. Immunol. **2006**, *13*, 1030–1036.
16. Ten-Dam, H.G. Research on BCG vaccination. Adv. Tuberc. Res. **1984**, *21*, 79–106.
17. Smith, D.W.; Wiegeshaus, E.H.; Edwards, M.L. The protective effects of BCG vaccination against tuberculosis. In *Mycobacterium Tuberculosis*; Bendinelli, M.; Friedman, H.; Eds.; Plenum Publishing Corporation: New York, N.Y., 1988; 341–370.
18. Fine, P.E. The BCG story: lessons from the past and implications for the future. Rev. Infect. Dis. **1989**, *11*, 353–359.
19. Fine, P.E. Variation in protection by BCG: implications of and for heterologous immunity. Lancet **1995**, *346*, 1339–1345.
20. Buddle, B.; Parlane, N.A.; Keen, D.L.; Aldwell, F.E.; Pollock, J.M.; Lightbody, K.; Andersen, P. Differentiation between Mycobacterium bovis BCG-vaccinated and M. bovis-infected cattle using recombinant mycobacterial antigens. Clin. Diagn. Lab. Immunol. **1999**, *61*, 1–5.
21. Pollock, J.M.; Neill, S.D. Mycobacterium bovis infection and tuberculosis in cattle. Vet. J. **2002**, *163*, 115–127.