International Journal of Mycobacteriology xxx (2016) xxx–xxx

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Comparative evaluation of diagnostic tests for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in the tissues of sheep affected with distinct pathology of paratuberculosis

Ganesh G. Sonawane [a,*], *Bhupendra N. Tripathi* [b]

[a] *Animal Health Division, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India*
[b] *ICAR-National Research Centre on Equines, Hisar, Haryana, India*

ARTICLE INFO

Article history:
Received 4 September 2016
Accepted 14 September 2016
Available online xxxx

Keywords:
Diagnostic evaluation
Mycobacterium avium subsp. *paratuberculosis*
Pathology
Sheep

ABSTRACT

Aims and objective: Paratuberculosis or Johne's disease is a chronic infectious granulomatous enteritis, mainly of cattle, sheep, goats, and other domestic and wild animals caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Currently, MAP has been recognized as an important animal pathogen with significant zoonotic and public health concerns. The early detection of infected animals using suitable diagnostic methods helps in developing control and preventive strategies for the herd. Therefore, the present study was aimed to determine the comparative efficacy of certain diagnostic methods used in the identification and confirmation of MAP in the ovine tissues with distinct pathology of paratuberculosis.
Methods: The ileum and mesenteric lymph node (MLN) tissues were collected from 38 sheep infected with paratuberculosis from organized farms of Rajasthan. These animals were further classified as paucibacillary (PB; $n = 15$) or multibacillary (MB; $n = 23$) on the basis of histopathological findings and mycobacterial loads. The ileum and MLN tissues of these animals were subjected to IS900 and 251 gene polymerase chain reaction (PCR) and bacterial culture. The tissue sections from MB, PB, and uninfected control sheep groups were stained using indirect immunoperoxidase technique (IPT) and Ziehl–Neelsen (ZN) method.
Results: On bacterial culture examination of the ileum and MLN tissues using Herrold's egg yolk medium, MAP was isolated in 14 (60.9%) MB and 5 (33.3%) PB sheep. Of 38 sheep, IS900 PCR detected 21 (55.2%) positive for MAP, of which 19 (82.6%) were MB and 2 (13.3%) were PB sheep. Similarly, 251 gene PCR detected 25 (65.7%) sheep positive for MAP infection, of which 21 (91.3%) were MB and 4 (27%) were PB sheep. Thus, 251 gene PCR was found superior to IS900 PCR in the detection of MAP from the tissues.

In PB sheep, IPT and ZN tests were positive in 87.5% and 50% of ileum sections and 70% and 37.5% in MLN sections, respectively. In MB sheep, IPT and ZN tests detected all animals as positive for MAP organisms or antigen and had equal sensitivity in the detection of MAP. The overall sensitivity of IPT was found superior (95%) to ZN staining (80%) in the demonstration of acid-fast bacteria or its antigen in the tissues.

* Corresponding author at: Animal Health Division, ICAR-Central Sheep and Wool Research Institute, Avikanagar, Dist. Tonk 304501, Rajasthan, India.
E-mail address: sganesh413@gmail.com (G.G. Sonawane).
Peer review under responsibility of Asian African Society for Mycobacteriology.
http://dx.doi.org/10.1016/j.ijmyco.2016.09.005

Conclusion: The sensitivity of all the tests in the detection of MAP was lower in PB sheep than in MB sheep. Bacterial culture detected MAP in only 50% of sheep and was found less sensitive than other tests used in the present study. Comparing the overall sensitivity of both the PCR assays, 251 gene PCR was found superior to IS900 gene PCR. The sensitivity of IPT was found superior (95%) to the ZN staining (80%) in the demonstration of acid-fast bacteria in the tissues. In the present study, IPT was found superior in the detection of MAP in PB and MB form of ovine paratuberculosis. This test can be used in the confirmation of post mortem diagnosis and research of paratuberculosis along with histopathology. However, 251 gene PCR assay was found easier to perform than IPT and could be used as paratuberculosis screening test in endemic sheep farms for blood and fecal samples.

Conflicts of interest

The authors have no conflicts of interest to declare.

Please cite this article in press as: GG Sonawane, B.N. Tripathi Comparative evaluation of diagnostic tests for the detection of Mycobacterium avium subsp. paratuberculosis in the tissues of sheep affected with distinct pathology of paratuberculosis. Int. J. Mycobacteriol. (2016), http://dx.doi.org/10.1016/j.ijmyco.2016.09.005