

## Evaluation of Faecal Shedding of Acid-fast *Mycobacterium avium* subsp. *paratuberculosis* (map) in both Intradermal Johnin Test- and Serologically (Elisa) Map-positive Cattle

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### ABSTRACT

Paratuberculosis is a debilitating disease of livestock caused by *Mycobacterium avium* (MAP). As a result of economic impact caused, disease is now one of the most important problems of dairy industry. Detection of animals shedding MAP organism plays a crucial role in herd eradication of the disease. Aim of the presented study was to evaluate relationship between MAP ELISA results, Intradermal Johnin test and fecal MAP shedding ratio. 40 ELISA and Johnin test positive animals were selected for the study. 25 of 40 animals (62.5%) were shedding MAP organisms with their feces. Results of the present study indicate that combination ELISA and Johnin test results is a useful tool for prediction of animals shedding MAP.

**Key Words:** Paratuberculosis, Cattle, Faecal shedding, ELISA, Ziehl-Nielsen, Johnin test

## İntradermal Johnin Test ve Serolojik (ELISA) olarak *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Pozitif Olan Sığırlarda Dışkı ile Asit-Dirençli MAP Saçılımının Değerlendirilmesi

### ÖZET

Paratüberküloz çiftlik hayvanlarında, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) tarafından meydana getirilen kronik bir hastalıktır. Hastalık meydana getirdiği ekonomik zararlar dolayısı ile günümüzde süt sığırı endüstrisinin en önemli problemleri arasında yer almaktadır. Hastalığın sürüden eradikasyonunda, MAP saçıyan hayvanların belirlenmesi büyük önem taşımaktadır. Sunulan çalışmanın amacı, MAP ELISA sonuçları, deri içi Johnin testi ve MAP saçılım oranı arasındaki ilişkinin araştırılmasıdır. Çalışmanın canlı hayvan materyalini 40 adet ELISA ve Johnin pozitif inek oluşturmaktadır. 40 inekten 25 adedinin (%62.5), dışkı ile MAP saçtığı belirlenmiştir. Sunulan çalışmanın sonuçları ELISA ve Johnin testinin kombine şekilde kullanılmasının, dışkı ile MAP saçıyan hayvanların belirlenmesinde kullanılabilecek bir araç olduğunu göstermektedir.

**Anahtar Kelimeler:** Paratüberküloz, Sığır, Dışkı Saçılımı, ELISA, Ziehl-Nielsen, Johnin test

### INTRODUCTION

Paratuberculosis is a debilitating disease of livestock caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease has an important economical impact on the dairy industry because of the losses caused by a decreased culling weight, a lowered milk yield and pregnancy rates and treatment costs. (Murray and Smith 2002, Radostits 2004).

Testing cattle with clinical signs of paratuberculosis is an important step of the surveillance for paratuberculosis (Collins et al. 2006, Martin 2008). The clinical presumptive diagnosis of paratuberculosis can be confirmed by demonstrating the presence of MAP in the faeces, by the evaluation of a cell-mediated immune response or by the detection of antibodies against MAP (Weber et al. 2009). Methods to demonstrate the presence of MAP include faecal culture, the microscopic examination of Ziehl-Neelsen (ZN)-stained faecal smears for the presence of clumps of acid-fast MAP organisms (ZN-test), and polymerase chain reaction (PCR) assays (National Research Council of the National Academies, 2003). Methods to identify antibodies against MAP include the complement fixation test and enzyme-linked immunosorbent assay (ELISA) (Kalis et al. 2002).

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A well-known cell-mediated immune (CMI) test for mycobacterial infections is the intradermal skin test, which measures a delayed type of hypersensitivity to mycobacterial antigens. The swelling formed within two to three days after the injection of a mycobacterial antigen preparation known as PPD (purified protein derivative) is measured using a set of callipers to determine the increase of the skin thickness (Kalis et al. 2003).

The purpose of this study is to determine the faecal shedding ratio of acid-fast MAP organisms in both MAP ELISA and intradermal Johnin test-positive cattle. Our goal was to evaluate the usefulness of the combination of a MAP ELISA and intradermal Johnin test for eradication and control programs of paratuberculosis in dairy cattle.

## MATERIALS AND METHODS

Forty intradermal Johnin test and ELISA positive for MAP Holstein-Friesian dairy cattle, aged from 3 to 8 years, were evaluated for paratuberculosis by the microscopic examination of their faeces for acid-fast organisms using Ziehl–Neelsen staining. The serological evaluation was performed using an ELISA (Para-TB-Ab ELISA, Svanovir; Svanova Biotech AB Inc., Sweden), according to the manufacturer protocol. For the ELISA, the samples were added to the wells of microplates coated with lipoarabinomannan derived from MAP. The results were measured using a Biotek ELx800 reader at 450 nm. The percent positivity (PP) values were calculated for the samples using the following formula:

$$\text{PP} = \frac{\text{mean optical density (OD) value of sample}}{\text{mean OD value of positive control}} \times 100$$

The serum samples with PP values of 53 or more were evaluated as positive, values of 37 to 57 were evaluated as doubtful, and values of 31 or less were evaluated as negative.

At the time of the serum and faeces collection for the serologic and faecal examination, the intradermal Johnin test (Central Veterinary Control and Research Institute, Etlik, Ankara) was performed by injecting 0.2 ml of Johnin's PPD fraction intradermally in the cervical area of the cattle. The results were evaluated 48 hours after injection. An increase of the skin fold thickness equal and/or greater than 3 mm was considered to be a positive result. The absence of a reaction was classified as negative, and an oedematous reaction was categorised as suspect.

For the study, cattle that were MAP positive by both the intradermal Johnin test and ELISA 40 were selected. Faeces from all of the cows were examined for parasites before and after the study to eliminate the possible indirect effect of intestinal parasitism on the immune response to MAP. All of the samples examined were negative for parasites.

The faecal samples of 40 MAP ELISA and intradermal Johnin test-positive cattle were collected by the rectal route and the faecal smears were stained with Ziehl–Nielsen stain for the identification of acid-fast microorganisms. All of the slides were examined under 1000x magnification, and the observations were recorded according to Acid Fast Bacilli (AFB) scoring criteria, as follows (Fujiki 2001). no acid-fast bacilli in at least 100 microscopic fields was scored as negative (-); one to nine acid-fast bacilli in 100 microscopic fields was scored as doubtful (?); and 10 to 99 acid-fast bacilli in 100 microscopic fields was scored as positive (+).

## RESULTS

The ZN-test results of the 40 samples that were both MAP sero-positive and intradermal Johnin test (JT)-positive were retrieved (Table 1). As shown in Table 1, 25 (62.5%) of the 40 cases produced both positive JT-ELISA and ZN-test results, whereas 15 (37.5%) of the 40 cases exhibited positive JT-ELISA and negative ZN-test results. To support culling decisions in cases of suspected of paratuberculosis, a fast confirmation of the clinical presumptive diagnosis is preferred. Faecal culture is often regarded as a gold standard but takes at least several weeks before a test result is obtained, depending on the culture method used (Weber et al. 2009). Therefore, in

the field, cattle suspected of paratuberculosis are often tested by practical, cheaper and faster methods, such as the intradermal Johnin test, ZN-test on faecal samples and serum-ELISA; thus methods forementioned were used in this study.

**Table 1.** Results of intradermal Johnin test, ELISA and microscopic examination of Ziehl–Neelsen stained faecal smears of 40 cattle per age group.

Age	Test results		
	JT*-ELISA + ZN** +	JT-ELISA + ZN-	JT-ELISA + ZN ?
< 4	4	-	-
≥4	21	15	-
Total	25	15	-

\* Intradermal Johnin Test, \*\* Ziehl-Neelsen staining

## DISCUSSION

The skin test takes advantage of the development of a delayed-type hypersensitivity (DTH) reaction to the intradermal injection of a mycobacterial extract, PPD. Intradermal skin testing has been and continues to be commonly used for the diagnosis of bovine paratuberculosis (Rideout et al. 2003). In our study, we aimed to evaluate the faecal shedding ratio of acid-fast MAP organisms in cattle that were positive by both the MAP ELISA and intradermal Johnin tests. In the present study, 25 of the 40 (62,5%) MAP ELISA and intradermal Johnin test-positive cattle shed acid-fast organisms, as based on the results of faecal smear staining. The results of our study indicate that the combination of the MAP ELISA and Johnin test are useful indicators for the detection of MAP-shedding animals in a herd and thus these test could be used in combination in control and eradication programs against MAP. Therefore, in ZN test-positive cattle showing or not showing the clinical signs of the disease, it is extremely important to eliminate positive and especially shedder animals from the herd.

## REFERENCES

- Collins M.T, Gardner I.A, Garry F.B, Roussel A.J and Wells S.J (2006). Consensus recommendations on diagnostic testing for the detection of paratuberculosis in cattle in the United States. *J. Am. Vet. Med. Assoc.*, 229: 1912–1919.
- Fujiki A (2001). Direct smear examination. In: *TB Bacteriology Examination to Stop TB*. The Research Institute of Tuberculosis, ed. Fujiki A, Japan International Cooperation Agency JINNOU Co. pp 7
- Kalis C.H, Barkema H.W, Hesselink J.W, Van C. Maanen and Collins M.T ( 2002). Evaluation of two absorbed enzyme-linked immunosorbent assays and a complement fixation test as replacements for fecal culture in the detection of cows shedding *Mycobacterium avium* subspecies paratuberculosis. *J Vet Diagn Invest.*, 14: 219-24.
- Kalis C.H.J, Collins M.T, Hesselink J.W and Barkema H.W (2003). Specificity of two tests for the early diagnosis of bovine paratuberculosis based on cell-mediated immunity: the Johnin skin test and the gamma interferon assay. *Veterinary Microbiology.*, 97:73-86.
- Martin P.A.J (2008). Current value of historical and ongoing surveillance for disease freedom: surveillance for bovine Johne's disease in Western Australia. *Prev. Vet. Med.*, 84: 291–309.
- Murray M.J. and Smith B.P (2002). Diseases of the alimentary tract. In: *Large Animal Internal Medicine*, 3<sup>rd</sup> edn. Smith BP, ed. London: Mosby, pp 779–783.
- National Research Council of the National Academies (2003). *Diagnosis and Control of Johne's Disease*, National Academies of Sciences, Washington, DC, pp 229.
- Radostits O.M, Gray C.C, Blood D.C and Khinchcliff K.E (2004). Paratuberculosis (Johne's disease). In *Veterinary Medicine: A Textbook of the Disease of Cows, Sheep, Pigs, Goats and Horses*. 9<sup>th</sup> edn. W.B. Saunders, pp 920-934.
- Rideout B.A, Brown S.T, Davis W.C, Gay J.M, Giannella R.A, Hines M.E, Hueston W.D and Hutchinson L.J (2003). *Diagnosis and Control of Johne's Disease*. National Academies Press, Washington, D.C., USA, pp 45-65.
- Weber M.F, Verhoeff J, G.Van Schaik and C. Van Maanen (2009). Evaluation of Ziehl–Neelsen stained faecal smear and ELISA as tools for surveillance of clinical paratuberculosis in cattle in the Netherlands. *Preventive Veterinary Medicine.*, 92: 256–266.