Research Paper

Isolation, Molecular Identification and Genomic Pattern of *Mycobacterium Bovis* Isolates Collected from Tuberculin-positive Cattle in Infected Farms of Shiraz, Iran

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Mycobacterium bovis, Tuberculination, PGRS-RFLP, PVUII, Strain

ABSTRACT

Background *Mycobacterium bovis* is the main cause of tuberculosis in cattle. The most commonly used method to identify bovis-infected cattle is tuberculin test.

Objective The present study aimed to investigate the population structure of *Mycobacterium bovis* in infected cattle farms of Shiraz City in Iran.

Methods In this descriptive cross-sectional study, 50 pathological samples from tuberculin-positive cattle that were collected from two abattoirs were cultured on glycerinated and pyruvated Lowenstein-Jensen media. Genomic material from culture-positive slopes was extracted and used in polymerase chain reaction (PCR)-16S rRNA, PCR-IS6110, and PCR--regions of difference (RD) typing. All the *M. bovis* isolates were then digested by PvuII restriction enzyme and genotyped by polymorphic guanine/cytosine-rich repetitive sequences (PGRS)-restriction fragment length polymorphism (RFLP) technique.

Findings In bacterial culture, 13 (26%) of samples had living mycobacteria where PCR test results revealed their identity as *Mycobacterium bovis*. Genotype profiling by RFLP-PGRS method displayed two patterns with 10 isolates shared a single profile identical to that of *M. bovis* bacillus calmette-guerin (BCG) strain (1173 P2) and three isolated with a different genotype.

Conclusion Higher prevalence of BCG-like *M. bovis* (as a typical characteristic of Iranian *M. bovis* population) in cattle farms of Shiraz City was expected. This may indicate the local evolution of new *M. bovis* strains in the region or the infiltration of such strains through cattle farming activities.

Extended Abstract

1. Introduction

Bovine tuberculosis has been known for a long time as the most important zoonotic disease and a mater of concern in the dairy industry worldwide [1]. The *mycobacterium tuberculosis* complex (MTC) consists of a number of species and subspecies that can cause tuberculosis in humans or animals [3, 4]. One of the globally important members of this complex is *Mycobacterium bovis* (*M. bovis*) which is the leading cause of tuberculosis in cattle [7]. Tuberculin test is the most commonly used method for detection of *M. bovis* in cattle [8]. Restriction fragment length polymorphism (RFLP) method using IS6110, direct repeat

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(DR) and Polymorphic Guanine/cytosine-rich Repetitive Sequences (PGRS) markers have been among the earliest strain typing systems used for genotyping of the *M. bovis* [12]. The present study aimed to improve the genomic knowledge of the population structure of *M. bovis* isolates collected from tuberculin-positive cattle in infected cattle farms of Shiraz, Iran.

2. Materials and Methods

In this descriptive cross-sectional study, conducted within 13 months (from January 2016 to February 2017), pathological samples were collected from 50 slaughtered tuberculin-positive cattle belonged to 6 cattle farms located in Shiraz, Iran which had been sent to two abattoirs. The frozen samples were then transferred to Bovine Tuberculosis Reference Laboratoriy of Razi Vaccine and Serum Research Institute in Karaj, Iran. Using sterile scissors, scalpels and forceps, the samples with tuberculosis lesions were dissected. The small cut tissue pieces were then homogenized using sterile sea sand, mortar and pestle. Ten ml of NaOH (4%), according to the Petroff's decontamination method, was added to the homogenates with continuous but gentle stiring of the pestle content using the mortar for 20 minutes to ensure the completion of decontamination process. After 20 minutes of settling, 5 mL of supernatant was transferred to a Falcon tube and centrifuged at 5,000 x g for 15 minutes. The supernatant was removed and the centrifuge deposit was used to inoculate traditional (glycerinated) and pyruvated (0.2% sodium pyruvate) Lowenstein-Jensen media [19, 20]. The genomic contents of the culture-positive slopes were extracted using the Van Soolingen method [1] and subjected to polymerase chain reaction (PCR)-16SrRNA, PCR-IS6110, and the quad PCR-regions of difference (RD) (RD1, RD4, RD9 and RD12) protocols [21-23]. The detected MTC isolates were further digested by PvuII restriction enzyme and the strain was typed by PGRS-RFLP technique. The obtained patterns were evaluated by the Gel-Pro Analyzer [24].

3. Results

Of 50 study samples, 13 (26%) had mycobacterial growth-positive cuture. The PCR-16SrRNA and PCR-IS6110 results (Figure 1) confirmed their identity as MTC bacteria. No other MTC members execpt *M. bovis* was detected in PCR-RD typing between the isolates (Figure 2). All isolates were typeable by RFLP strain typing method. The genotype profiling of *M. bovis* isolates resulted in detection of two patterns among which 10 isolates (76.92%) shared a single profile identical to that of *M. bovis* Bacillus Calmette-Guerin (BCG) strain (1173 P2; Figure 3A), while the remaining three isolates (23.08%) displayed a different genotype (Figure 3B). Although it has been previously reported in Iran (Urmia City), but this type seems to be new in Shiraz City.

4. Discussion

Our results showed a relatively high prealence (77%) of the BCG-like *M. bovis* isolates in cattle farms of Shiraz city

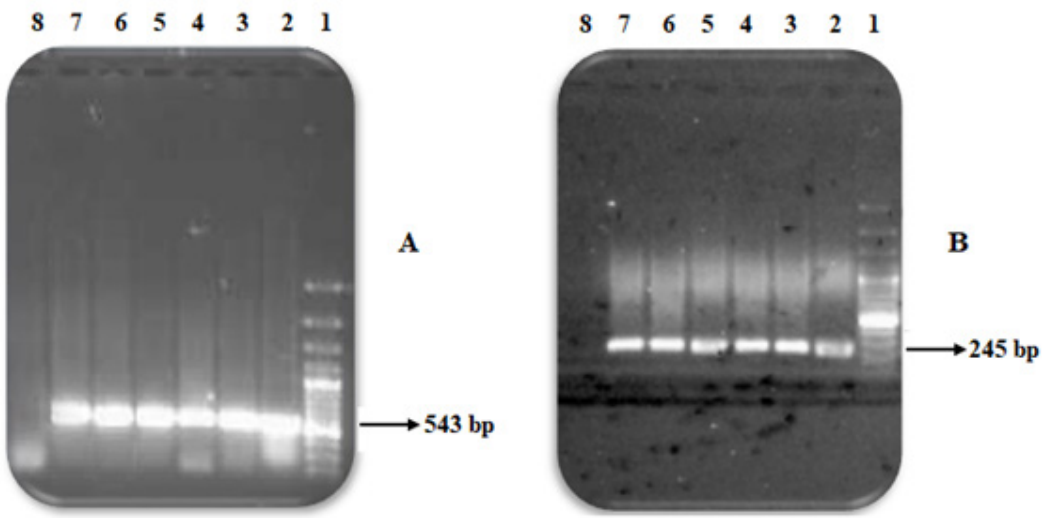

Figure 1. Electrophoresis

A. PCR-16S rRNA; and B. PCR-IS6110 products

Lane 1: Marker 100 bp; lanes 2 to 6: Positive mycobacterial samples; lane 7: BCG (1173 P73) as positive control; lane 8: Negative control.

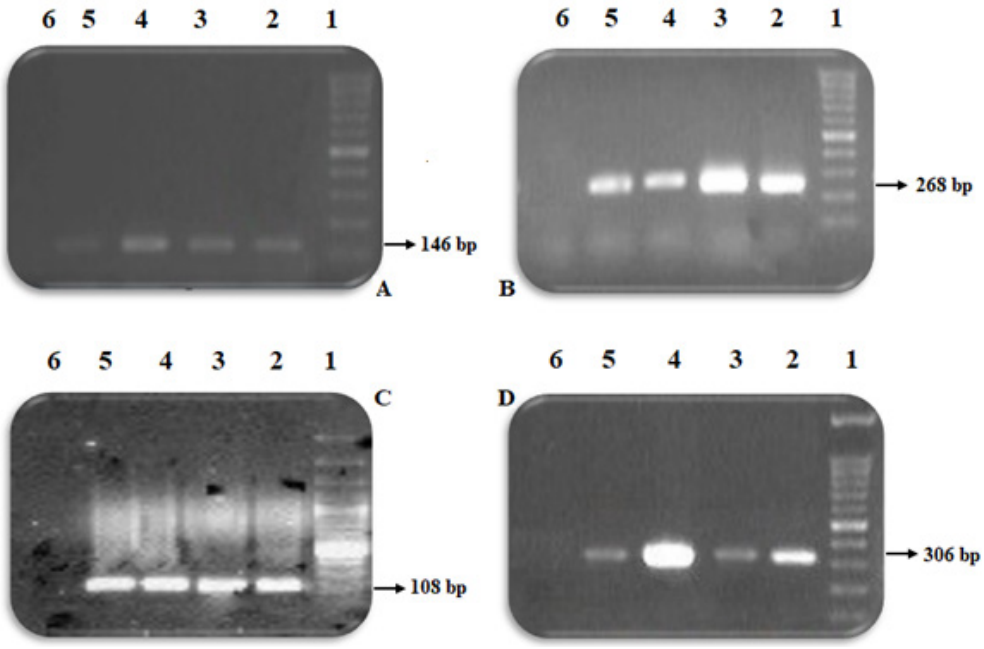

Figure 2. Electrophoresis

A. PCR-RD1; B. PCR-RD4; C. PCR-RD9; and D. PCR-RD12 products
Lane 1: 100 bp marker; lane 2: (A) M. bovis strain C and (B, C, D) M. bovis strain AN5 as a positive control; lanes 3 to 5: Positive samples; lane 6: Negative control.

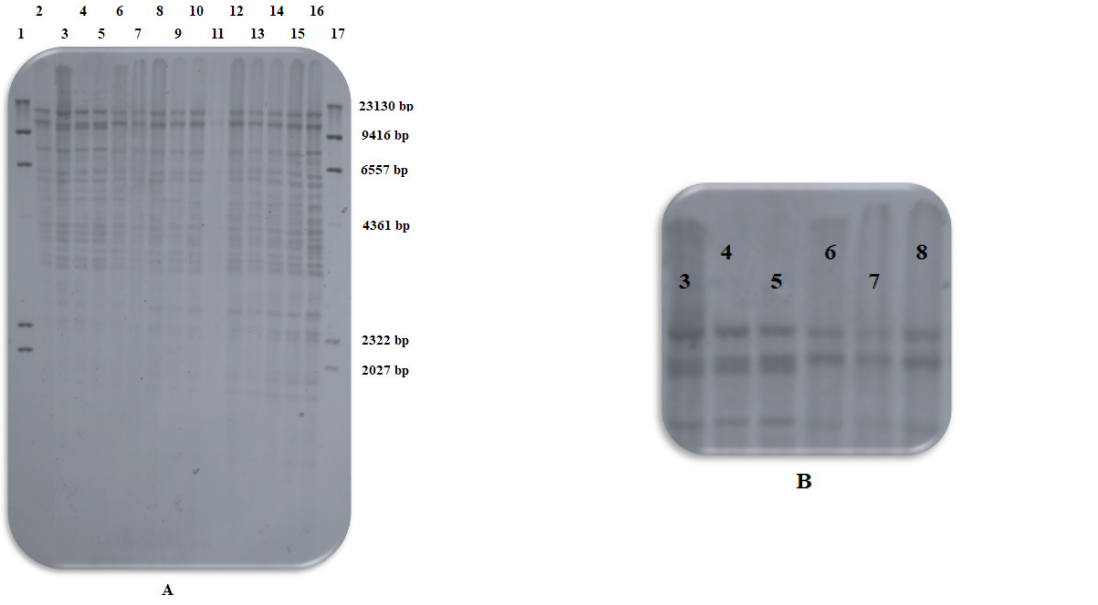

Figure 3. RFLP patterns of PvuII-digested DNA from M. bovis isolates hybridized with PGRS probe

A: Lane 1, DNA molecular weight marker II; lane 2, BCG (1173 P2) as positive control; lanes 3 to 15, *M. bovis* isolates; lane 16, *M. bovis* (AN5 strain) as positive control; lane 17, DNA molecular weight marker II. B: Lanes 13 to 15, strains different from BCG strain; lanes 10 to 12, BCG like strain.

which is consisting with previous studies in Iran [9, 17, 30]. Finding homogeneous population of *M. bovis* isolates in Shiraz and in other cities of Iran at a larger scale indicates the local evolution of new *M. bovis* strains in the region or the entery of such strains through livestock production, especifically cattle farming.

Ethical Considerations

Compliance with ethical guidelines

The present study used the biological samples and tissues prepared from abattoirs by the inspectors of the Veterinary Department in Shiraz. No any experiment was performed on living animals.

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Authors' contributions

Conceptualization: Nader Mosavari, Masoud Haghkhah, Keyvan Tadayon; Field and laboratory experiments: Hossein Ghaderi; Data analysis, draft preparation: Nader Mosavari, Keyvan Tadayon, Hossein Ghaderi; Editing and review: Keyvan Tadayon.

Conflicts of interest

The authors declared no conflict of interest.

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