Epidemiol. Infect. (2002), **128**, 333–336. © 2002 Cambridge University Press
DOI: 10.1017/S0950268801006732 Printed in the United Kingdom

IS*200* fingerprinting of *Salmonella enterica* serotype Abortusovis strains isolated in Iran

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(*Accepted 19 November 2001*)

SUMMARY

Salmonella enterica serovar Abortusovis is one of the most common pathogens responsible for abortion in sheep. In Iran, the spread of Abortusovis is highly dependent on the nomadic life style. In this study we performed IS*200* fingerprinting to identify the clonal lines circulating in Iran. All the isolates contained 4 or 5 copies of the transposon and could be classified in 4 genotypes. A single genotype was highly prevalent and very likely it has circulated in Iran since 1970. All the isolates showed a high degree of relatedness.

In Iran, nomadism has considerable historical, political and economical importance. This life style is based on sheep and goat husbandry. Abortion in sheep, caused by bacterial pathogens, drastically affects the nomads' economy. Among these pathogens, *S. enterica* sv. Abortusovis and *Brucella* species are the most common agents that have been isolated in Iran (Asia) [1–3]. In the last few years in Chaharmahal-Bakhtiary province, located in the western area of Iran, a modification in the top rank of bacteria causing abortion in sheep flocks has been observed. Specifically, the frequency of *Brucella* sp. isolation from cases of infectious abortion has dropped from 13·8% in 1993 to 9% in 2000, whereas the cases of abortion caused by serovar Abortusovis did not vary significantly during the same years (from 1·8% in 1993 to 1·5% in 2000) (Nikbakht, personal communication).

Abortusovis is a host-restricted pathogen which induces abortion in sheep and goats, generally during the first pregnancy. Lambs delivered by infected ewes usually are weak, suffer bacteriaemia, enteritis, pneumonia and generally die during the first week of age [4, 5]. Remarkably, serovar Abortusovis does not cause any sign of sickness in non pregnant ewes and in rams. Thus, serovar Abortusovis infection in sheep differs from infection caused by other *Salmonella* serovars (i.e. *S. enterica* svs Dublin and Typhimurium), that are characterized by enteritis, metritis, anorexia in adult animals, and might lead to ewes death [4, 6–8].

In endemic areas, abortion can affect up to 50% of the ewes in an infected flock causing heaving economic losses in regions with a sheep-based economy [4].

In countries with a nomadic life style, like Iran, the spread of infection is not limited within a flock or a province, but may disseminate rapidly nationwide. It is noteworthy that flocks that wander generally have insufficient health management and may become more susceptible to the infection.

In order to limit economic losses caused by sheep abortion in Iran, epidemiological surveys are necessary to monitor the spread of the infection. In addition to biochemical and serological analysis required to identify the causative agents of infections, molecular methods to identify the genotypes responsible for abortion in a defined geographic area should be applied.

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Fig. 1. Map of Iran with details of Iranian Provinces.

Schiaffino et al. demonstrated that IS*200* fingerprinting is a useful tool to discriminate between clonal lines of serovar Abortusovis [9]. Application of IS*200* for epidemiological purposes benefits from two characteristics: (i) presence of several copies of the IS*200* element in Abortusovis, and (ii) rare transposition frequency [7, 10, 11]. Southern blot hybridization with an IS*200* derived probe was previously carried out in serovar Abortusovis [9]. An IS*200* band of ~ 9 kb was found in all the isolates tested from different geographic areas, and it was described as serovar-specific band. According to these results, a pair of primers that specifically amplified IS*200* in serovar Abortusovis was designed [12].

In this paper we applied IS*200* fingerprinting to 39 serovar Abortusovis strains isolated in Iran. To the best of our knowledge, this is the first report of molecular characterization of serovar Abortusovis from this country.

The serovar Abortusovis strains under study were isolated in three different provinces of Iran (see map in Fig. 1); 34 strains were isolated from Chaharmahal-Bakhtiary in 1999–2000, 3 from Esfahan and 2 from Varamin, the latter collected during the 1970s. Serovar Abortusovis SS44 was used as reference strain [9]. All Iranian samples were obtained from stomach of aborted fetuses. After enrichment in selenite broth and culture on selective media (MacConkey or Salmonella Shigella agar), presumptive salmonella colonies were purified. Biochemical tests were performed with ID 32 E system (Bio Merieux, Marcy l'Etoile, France). Serotyping was achieved by standard

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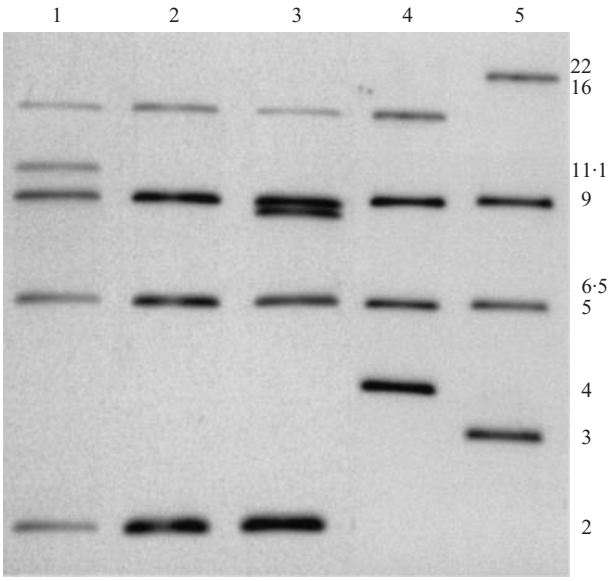

Fig. 2. Distribution of insertion element IS*200* in *S. abortusovis* isolated in Iran. Lane 1: strain CH1494, profile A; Lane 2: strain CH1493, profile B; Lane 3: strain CH1498, profile C; Lane 4: strain VAR1268, profile D; Lane 5: SS44 (Sardinian strain).

slide agglutination using specific antisera (Difco). Antigenic profiles were, as expected, O4, 12:c: 1,6 for all the isolates.

To isolate genomic DNA, phenol extraction was carried out according to Ausubel et al. [13]. DNA was then digested with the restriction enzyme *Pst*I (Amersham) which does not cut within IS*200* [10]. Separation of fragments was carried out by agarose gel electrophoresis (0·8%). Southern hybridization was performed as described by Schiaffino et al. [9].

The discrimination index (DI) was calculated according to the formula of Hunter and Gaston [14]. This value represents the probability that two unrelated strains sampled from a population will fall into different groups using the above method.

Analysis of restriction fragments obtained with *Pst*I digestion revealed the presence of 4 or 5 copies of IS*200* in all the Iranian strains. The IS*200* elements were located in restriction fragments with size about 2–16 kb (Fig. 2).

As previously recorded for serovar Abortusovis strains of different geographic origin, we could identify the serovar-specific band of about 9 kb in all Iranian strains [9]. The strains were also further confirmed using PCR, as described before by Beuzon et al. [12]. All the Iranian strains gave rise to an amplicon of 900 bp, as expected (data not shown).

Based on IS*200* profile, the Iranian isolates were classified in four genotypes: A, B, C, D (Table 1).

Table 1. *IS*200 *profiles of* Salmonella *Abortusovis strains isolated in Iran*

Strains no.	Pattern	Number of IS*200* copies
CH1493	B	4
CH1494	A	5
CH1495	B	4
CH1496	B	4
CH1497	B	4
CH1498	C	5
CH1499	C	5
CH1500	B	4
CH1501	B	4
CH1502	A	5
CH1503	B	4
CH1504	C	5
CH1505	B	4
CH1506	C	5
CH1507	C	5
CH1508	C	5
CH1509	B	4
CH1510	B	4
CH1511	B	4
CH1512	B	4
CH1513	C	5
CH1514	B	4
CH1515	C	5
CH1516	B	4
CH1517	B	4
CH1518	B	4
CH1519	A	5
CH1520	B	4
CH1521	B	4
CH1522	B	4
CH1523	B	4
CH1524	B	4
CH1525	B	4
CH1526	B	4
ES1260	B	4
ES1262	B	4
ES1269	B	4
VAR1268	D	4
VAR1527	D	4

Three bands of ~5 kb, ~9 kb and ~16 kb were found in to all Abortusovis isolates. Twenty-three out of 34 strains isolated from the Chaharmahal-Bakhtiary province showed an IS*200* fingerprinting characterized by four bands (profile B). The same profile was observed among the three isolates from Esfahan province. These data are not surprising since Chaharmahal-Bakhtiary and Esfahan provinces share a common border (see map). The other isolates from Chaharmahal-Bakhtiary province showed a similar IS*200* pattern with, in addition, a ~11 kb band

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(profile A, 3 strains) or a ~ 8·2 kb band (profile C, 8 strains).

Two isolates from the village of Varamin, Tehran province, showed a genotype that possessed the three bands common to all Iranian isolates (~ 5 kb, ~ 9 kb and ~ 16 kb) and a fourth band of ~ 4 kb (profile D).

IS*200* fingerprinting analysis of all Iranian strains showed a DI of 0·52.

Fingerprinting based on IS*200* demonstrated that serovar of Abortusovis strains isolated from unrelated geographic areas are highly polymorphic [9]. Also, serovar Abortusovis strains isolated within the same geographic area could be divided into different clonal lines.

In addition to the ~ 9 kb serovar-specific band, all epidemic strains isolated in Iran shared two bands of ~ 5 kb and ~ 16 kb. The band of ~ 5 kb, but not that of ~ 16 kb, was also detected in Abortusovis strains isolated in Sardinia (Italy), the United Kingdom and Russia [9]. Therefore, the presence of the ~ 16 kb band appears to be restricted only to Iranian strains.

Most of these strains (26 out of 38) belonged to profile B, that was highly prevalent. Interestingly, we found this genotype both in strains from Chaharmahal-Bakhtiary province isolated in 1999–2000 and in strains from Esfahan province isolated in 1970. These results strongly suggest that this genotype has circulated in Iran for at least 30 years. Furthermore, it is tempting to speculate that the profile B might represent an ancestral clone from which the other genotypes have risen. This hypothesis is supported by the observation that all the other genotypes collected in the western area of Iran shared the four copies pattern of profile B, plus an additional band.

Profile D was found only in strains isolated in 1970 from Varamin, Tehran province, in the Northern part of Iran. It is characterized by 4 bands, 3 of which are common to those of profile B.

Our results confirm that fingerprinting based on IS*200* hybridization pattern is a suitable marker to describe the genotypes responsible of abortion in a defined area.

The clonal lines we have found in all Iranian strains showed a high degree of relatedness, as described before for Sardinian strains [9]. Variation in one area might be due to flocks' movements inside and outside the provinces.

IS*200* fingerprinting of Iranian strains demonstrated a high resolution and good discrimination power (0·52) [14] and, as regard to other results, we confirmed this procedure to be valid as a typing method for serovar Abortusovis strains.

ACKNOWLEDGEMENTS

We are grateful to Simin Aghili who kindly provided our isolates from Chaharmahal-Bakhtiary.

REFERENCES

1. Tadjbakhche H, Desliens M, Hedjazi M. Bacteriological study of enzootic abortion on sheep due to *Salmonella abortusovis* is Iran. Rev Med Vet 1971; **122**: 621–7.
2. Tadjebakhche H, Gillet RY. Epidemiology of a new outbreak of abortion due to *Salmonella abortusovis* infection in sheep at Varamin, Tehran Province. Bull Soc Sciences Vet Med Comparee Lyon 1973; **75**: 241–6.
3. Tadjebakhche H, Gatel A. Serological survey of antibodies to *Salmonella abortusovis* in domestic animals in Iran. Recueil de Médecine Véterinaire 1972; **148**: 1027–30.
4. Jack E J. *Salmonella abortusovis*: an atypical Salmonella. Vet Rec 1968; **82**: 558–61.
5. Pardon P, Sanchis R, Marly F, Lantier F, Pepin M, Popoff M. Salmonellose ovine due à *Salmonella abortusovis*. Ann Rech Vet 1988; **19**: 221–35.
6. Rubino S, Rizzu P, Erre G, et al. Mechanism of pathogenicity in *Salmonella abortusovis*. NATO ASI series. Series A, Life Sciences (USA) 1993; **245**: 141–7.
7. Rubino S, Leori G, Rizzu P, et al. *Salmonella abortusovis* mutants unable to adhere to epithelial cells and with reduced virulence in mice. Infect Immun 1993; **61**: 1786–92.
8. Uzzau S, Brown DJ, Wallis T, et al. Host adapted serotypes of *Salmonella enterica*. Epidemiol Infect 2000; **125**: 229–55.
9. Schiaffino A, Beuzon CR, Uzzau S, et al. Strain typing with IS*200* fingerprints in *Salmonella abortusovis*. Appl Environ Microbiol 1996; **62**: 2375–80.
10. Gibert I, Barbe J, Casadesus J. Distribution of insertion sequence IS*200* in *salmonella* and *shigella*. J Gen Microbiol 1990; **136**: 2555–60.
11. Rubino S, Muresu E, Solinas M, et al. IS*200* fingerprint of *Salmonella enterica* serotype Typhimurium human strains isolated in Sardinia. Epidemiol Infect 1998; **120**: 215–22.
12. Beuzon CR, Schiaffino A, Leori G, Cappuccinelli P, Rubino S, Casadesus J. Identification of *Salmonella abortusovis* by PCR amplification of a serovar-specific IS*200* element. Appl Environ Microbiol 1997; **63**: 2082–5.
13. Ausubel FM; Brent R, Kingston, RE, et al., eds. Current protocols in molecular biology. New York: Green Publishing Associates & Wiley Interscience, 1987.
14. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's Index of Diversity. J Clin Microbiol 1988; **26**: 2465–6.