Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: http://www.tandfonline.com/loi/taar20

Preliminary association analysis of microsatellites and Mycobacterium avium subspecies paratuberculosis infection in the native Garfagnina goats

Francesca Cecchi, Claudia Russo, Filippo Fratini, Barbara Turchi, Giovanna Preziuso & Carlo Cantile

JOURNAL OF APPLIED ANIMAL RESEARCH, 2018
VOL. 46, NO. 1, 879–882
https://doi.org/10.1080/09712119.2017.1417129

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

OPEN ACCESS Check for updates

Preliminary association analysis of microsatellites and *Mycobacterium avium* subspecies paratuberculosis infection in the native Garfagnina goats

Francesca Cecchi, Claudia Russo, Filippo Fratini, Barbara Turchi, Giovanna Preziuso and Carlo Cantile

Department of Veterinary Science, University of Pisa, Pisa, Italy

ABSTRACT
Paratuberculosis disease occurs with high frequency in many places of the world and it is a chronic infection of ruminants, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The objective of this study was to investigate the association between MAP-resistant or MAP-susceptible goats and short tandem repeats (STR) markers. Blood samples were collected from 48 adult goats (27 positive and 21 negative) of the Italian native Garfagnina goat breed from a single flock that had experienced annual mortalities due to MAP infection. Diagnosis was achieved by serological tests and by post-mortem examination of affected animals. To investigate possible genetic influences on susceptibility or resistance of goats to MAP disease, 12 STR markers were used. For each marker, allele and genotypes frequencies between MAP-positive and MAP-negative groups of animals were compared using the chi-square test and Fisher's exact tests. In this study, two microsatellite loci SRCRSP05 and ETH10 were associated with the disease playing an interesting role in the susceptibility or resistance to the disease. Although the present study should be considered preliminary, our results reveal for the first time that two microsatellite loci were associated with the development of lesions due to MAP in the Garfagnina goat breed.

ARTICLE HISTORY
Received 18 July 2017
Accepted 10 December 2017

KEYWORDS
Mycobacterium avium; *paratuberculosis*; microsatellite; disease susceptibility

1. Introduction

Paratuberculosis, or Johne's disease, is an intestinal chronic granulomatous infection of ruminants, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease causes persistent diarrhoea, progressive weight loss, debilitation, anaemia, and eventually death (Minozzi et al. 2010). Paratuberculosis is one of the most important infectious diseases causing damage to livestock, especially because it is hardly recognized and diagnosed by farmers and veterinarians. In goats, clinical signs of paratuberculosis are not characteristic and it is difficult to early identify the disease, although weight loss could be an efficient indicator of the progression of the disease (Munjal et al. 2005). Economic losses experienced by farmers (Dennis et al. 2011), the possible role of MAP in Crohn's disease of humans (Sechi LA et al. 2005) and avoiding unnecessary suffering of the animals are the major reasons for interventions against MAP. Although no prevalence data are available, the disease is thought to be widely distributed in Italy. Many studies have investigated the presence of paratuberculosis in Italian sheep population (Nebbia et al. 2006; Attili et al. 2011; Galiero et al. 2015; Galiero et al., 2016), whereas, to our knowledge, studies conducted on Italian goat population are limited (Nebbia et al. 2006; Galiero et al. 2017). As reported by Moioli et al. (2016), there are no effective treatments against MAP. Control programmes are very complex and expensive for farmers, and also vaccination did not prove to be sufficiently protective (Reddacliff et al. 2006). However, although MAP is widespread in the environment, the low rate of infected individuals, even within the same flock, suggested the presence of genetic factors that could influence individual resistance against the disease. The genetic susceptibility to MAP infection in domestic ruminants has been investigated using quantitative and/or molecular genetics. Resistance to MAP infection has been found to be heritable (Koets et al. 2000; Mortensen et al. 2004; Gonda et al. 2006; Settles et al. 2009), with heritability estimates ranging from 0.06 to 0.102 in cattle and from 0.01 to 0.15 in different goat breeds (Singh et al. 1990). Despite low heritability estimates, all studies confirm genetic influence on paratuberculosis susceptibility.

Attempts to locate loci associated with resistance to paratuberculosis have been made. Gonda et al. (2007) found evidence for a quantitative trait locus on Bos taurus chromosome 20 (BTA20) associated with paratuberculosis susceptibility. Hinger et al. (2007) investigated the association of eight microsatellites with paratuberculosis in German Holstein cows. However, none showed any significant associations. On the contrary, Reddacliff et al. (2005) found an association of one microsatellite allele in SLC11A1 (formerly NRAMP1) with MAP resistance in sheep. Garfagnina is an Italian native goat breed registered on the Tuscan regional repertory of genetic resources at risk of extinction with about 745 animals belonging to 17 flocks. The origin of this population is still uncertain, even if it seems to derive from crossings between native goats from Alpine Arc and from the Tuscan-Emilian Apennines; local breeders refer that the population was reared for generations for its milk and meat production.

CONTACT Francesca Cecchi francesca.cecchi@unipi.it

In a previous paper (Cecchi et al. 2017b), a genome-wide scan was performed on the individual marker genotypes on the same goats' population and nine significant markers located within, or nearby to annotated genes, were highlighted. Two genes found encoded or are linked to protein kinases that are among the most important enzymes involved in the immune response to Johne's disease and four genes are involved in the functions of the Golgi complex. Given the importance of MAP as a pathogen of animals, and as a potential risk factor for important human diseases, the objective of this study was to deepen the association studies in this native Italian goat population using short tandem repeats markers.

2. Materials and methods

All animal procedures used in this study were in agreement with the ethical and animal welfare concerns of the Committee on the Ethics of Animal Experiments of Minimally Invasive Surgery Centre and fully complied with recommendations outlined by the Italian laws.

2.1. Diagnostic assessment and selection of animals for genotyping

The study was performed in a Garfagnina dairy goat breed flock consisting of 269 females and 20 males. Age ranged from 2 to 9 years. All animals were registered in the herdbook, but genealogical information was not available. The flock was located in the Garfagnina district (Media Valle del Serchio, Lucca, Central Italy) and a semi-extensive farming system was practised. The goats grazed during the morning (feed supplements were given mainly over the winter) and were housed overnight, when they received an integration of forage and feed. Flock management was of a family farm type. Milking was practised twice a day using a trolley milking machine and the milk was stored in refrigerated tanks. Affected animals showed weight loss and decrease in milk production. Approximately one year before the trial, all the goats of the flock have been subjected to serological screening by agar gel immunodiffusion test, and all the suspicious of infection goats were identified. These goats and the healthy goats were subjected to further and periodical serological analysis. In particular, serum samples were analysed by ELISA ID screen® paratuberculosis indirect screening test (ID.VET, Montpellier, France) and the positive samples were subsequently tested with ELISA ID screen® paratuberculosis indirect confirmation test (ID.VET, Montpellier, France) (Galiero et al. 2017).

Through this test, 27 MAP-positive goats have been identified; and therefore, the trial has begun. Twenty-one serologically negative goats were considered as the control group. Diagnosis was also achieved by post-mortem examination of affected animals (Cecchi et al. 2017b).

2.2. Genotyping

Blood samples from the 48 goats of the two groups were collected. Whole blood was collected in Vacutainer tubes with K-EDTA as an anticoagulant and stored at −20°C until genomic DNA was extracted using a Qiagen QIAamp DNA blood mini/midi kit (Qiagen, San Diego, CA, USA).

Twelve microsatellites (MAF65, SRCRSP5, INRA023, MCM527, CSRD247, SRCRSP23 OarFCB20, TGLA53, INRA005, INRA063, ETH10 and ILSTS87), located in 12 chromosomes and amplified in one multiplex PCR reactions, were investigated. Detailed information of these markers is reported in Table 1.

2.3. Statistical analysis

For each marker, the following parameters were computed using the Molkin v2.0 program (Gutièrrez et al. 2005): number of alleles, effective allele size, observed heterozygosity and polymorphism information content (PIC). Allelic and genotype frequencies were estimated by direct counting. To investigate possible genetic influences on susceptibility or resistance of goats to MAP, for each marker, alleles and genotypes frequencies between MAP-positive animals and the control group (MAP-negative) were compared using the chi-square test and Fisher's exact tests.

3. Results and discussion

The results of the microsatellite analysis in terms of number of alleles observed, alleles size, PIC and observed heterozygosity of the analysed Garfagnina goat population are summarized in Table 1. In total, 71 alleles were observed for the 12 microsatellite loci analysed (Cecchi et al. 2017a). Table 2 reports the number of alleles and genotypes for each marker in the two populations and the number of alleles and genotypes common to the two groups. All 12 microsatellite markers resulted to be polymorphic in the whole sample and in each group (Table 2).

Table 1. Locus, dye, range, number of alleles, effective allele size (EfAlSize), observed heterozygosity (Ho) and PIC for the 12 microsatellite loci.

Locus	Dye	Range	Chromosome	No. of alleles	Ef. al. size	Ho	PIC (%)
SRCRSP23	6-FAM	69–111	unknown	9	5.35	0.831	79.31
OarFCB20	PET	86–118	2	4	2.57	0.611	56.30
MAF065	VIC	115–151	15	9	5.87	0.803	80.97
ILSTS87	6-FAM	137–151	28	7	5.38	0.814	78.77
INRA005	NED	110–126	12	5	2.49	0.599	53.34
TGLA53	PET	130–160	16	5	3.14	0.681	61.89
McM527	NED	162–178	5	6	3.58	0.721	67.30
SRCRSP05	VIC	153–181	21	6	4.17	0.760	72.51
INRA063	6-FAM	169–179	18	4	1.93	0.482	40.72
INRA023	VIC	190–220	3	7	2.91	0.656	61.98
ETH10	NED	198–286	5	3	2.53	0.605	53.60
CSRD247	6-FAM	211–263	14	6	3.62	0.724	67.31

Table 2. Number of alleles and number of genotypes for each marker in the MAP-positive and control (negative) groups.

	No. of alleles			No. of genotypes		
	Positive	Negative	Shared alleles	Positive	Negative	Shared genotype
Marker						
SRCRSP23	8	9	7	17	15	5
OarFCB20	4	4	4	6	7	5
MAF065	9	9	9	16	16	9
ILSTS87	7	5	5	15	10	7
INRA005	4	5	4	7	6	3
TGLA53	5	4	4	7	6	6
McM527	5	5	4	10	9	7
SRCRSP05	6	6	6	13	11	6
INRA063	4	2	2	6	3	3
INRA023	7	5	5	10	7	4
ETH10	3	3	3	6	5	5
CSRD247	5	6	5	9	8	6

A total of 67 alleles were found in MAP-positive group and 63 in the control group, with the number of alleles (Na) ranging from 3 to 9 (mean value 5.6 ± 1.83) and from 2 to 9 (mean value 5.2 ± 2.09), respectively in MAP-positive and control groups (Table 2). The most polymorphic loci were: MAF065 (9 alleles in both groups) and SRCRSP23 (8 alleles in MAP-positive group and 9 alleles in the control group) (Table 2); the less polymorphic loci were: INRA063 (4 alleles in MAP-positive group and 2 alleles in the control group) and ETH10 (3 alleles in both groups).

Although a comparison with other breeds can be biased due to the different marker sets used by different authors, it may be noted how the mean number of alleles per locus was slightly lower than that reported by Ramamoorthi et al. (2009) on the Barbari goats, and by Sechi T et al. (2005) on three Sardinian goat populations, but similar to what observed in Orobica and Girgentana goats by Negrini et al. (2012). These latter breeds exhibited small genetic variability and therefore evidence of a recent bottleneck. However, our animals were derived from a single flock.

The PIC per locus showed only one marker with values under the 50% and an average value of 64.5% (±12.30). The PIC estimated in the present study is comparable with that obtained in other goat breeds, such as Saanen (Negrini et al. 2012). The PIC was originally introduced by Botstein et al. (1980) and it refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency and has been proved to be a general measure of how informative a marker is; the higher is the PIC value the more informative a marker is. In the present study, MAF065 and SCRRSP23 and ILSTS87 microsatellites appeared as the most informative, whereas INRA63 was the less informative.

Ten of the twelve markers considered in this research were used also by Sechi et al. (2005) for the study of the genetic variability in Maltese, Sardo autochthonous goats and their mixed blood population, and by Negrini et al. (2012) who analysed the genetic structure of eight Italian goat breeds (Camosciata delle Alpi, Valodostana, Bionda dell'Adamello, Orobica, Grigia Molisana, Girgentana, Argentata dell'Etna and Sarda).

In this study, differences in alleles and genotypes frequencies between the animals belonging to the MAP-positive and control groups were highlighted. In fact, a total of 12 group-specific alleles was observed (8 in MAP-positive group and 4 in control group), but all at a frequency lower than 10%. Table 3 reports the percentage of each of the most frequent alleles (>15%) for each marker in the MAP-positive group and the control group.

Association studies showed a likely association of polymorphism at microsatellite loci SRCRSP05 and ETH10.

The control group showed a higher frequency of allele 163 bp of SRCRSP05 locus (0.40 vs. 0.10 of MAP-positive group; $P < .05$); regarding ETH10, the same group showed a higher frequency of allele 205 bp (0.62 vs. 0.30 of MAP-positive group; $P < .05$) and a less frequency of allele 203 bp (0.10 vs. 0.36 of MAP-positive group).

Table 3. Percentage of each of the most frequent alleles (>15%) for each marker in MAP-positive and control (negative) groups.

Group	*n*	Allele frequencies							
		OarFCB20			ILSTS87				
		93	97		137	139	141	143	145
Positive	54	0.15	0.61		0.15	0.17	0.19	0.22	0.17
Negative	42	0.33	0.43		0.19	0.14	0.36	0.10	0.21
			INRA005			MAF065			
		115	117	121	121	131	133		
Positive	54	0.50	0.31	0.15	0.17	0.30	0.28		
Negative	42	0.60	0.31	0.02	0.12	0.26	0.14		
			McM527			TGLA53			
		152	162	164	134	136	146		
Positive	54	0.26	0.22	0.39	0.37	0.39	0.17		
Negative	42	0.21	0.31	0.38	0.36	0.36	0.26		
			INRA063			SRCRSP05			
		173	175		163	173	179		
Positive	54	0.22	0.69		0.10	0.39	0.22		
Negative	42	0.38	0.62		0.40	0.31	0.12		
			ETH10			INRA023			
		203	205	207	197	211	215		
Positive	54	0.36	0.30	0.34	0.11	0.52	0.19		
Negative	42	0.10	0.62	0.29	0.19	0.55	0.19		
			CSRD247			SRCRSP23			
		228	230	240	79	91	105		
Positive	54	0.31	0.28	0.28	0.31	0.11	0.20		
Negative	42	0.29	0.24	0.40	0.38	0.17	0.05		

The frequencies of all specific genotypes of each group were quite low. However, three genotypes of three different markers, with high frequency in both groups, showed statistically significant different frequencies. The MAP-positive group presented a higher frequency of genotype 173179 of SRCRSP05 marker (39.63% vs. 4.76%; $P < .01$), a higher frequency of genotype 175175 of INRA63 marker (48.15% vs. 28.09%) and a higher frequency of genotype 203205 of ETH10 marker (25.96% vs. 12.28%; $P < .05$). Conversely, the control group presented a higher frequency of genotype 205205 of ETH10 marker (47.61% vs. 18.52%).

Genotype 173/179 of SRCRSP05 was associated with the disease ($P < .05$). Marker ETH10 plays an interesting role in the susceptibility or resistance to the disease: important are the frequencies of both alleles and of genotypes 203/205 (susceptible) and 205/205 (resistant). Association studies were previously reported only for two infectious diseases of goats, namely Heartwater disease (Cowdriosis) and Nematode-resistance (Obexer-Ruff et al. 2003).

4. Conclusions

Although the present study should be considered preliminary since the analyses were performed on a limited number of animals, our results reveal for the first time that two microsatellite loci (SRCRSP05 and ETH10) were weakly associated with the development of lesions due to MAP in the native Garfagnina goat breed confirming that some of the genes involved in MAP infection are related to the Golgi apparatus.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by PRA2016 (Prof. Carlo Cantile); Fondi Ateneo (Dr Francesca Cecchi); University of Pisa.

References

Attili AR, Ngu NV, Preziuso S, Pacifici L, Domesi A, Cuteri V. 2011. Ovine Paratuberculosis: a seroprevalence study in dairy Glocks eared in the Marche Region, Italy. Vet Med Int. Article ID 782875.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 32:314–331.

Cecchi F, Russo C, Cantile C, Preziuso G. 2017a. Preliminary genetic variability analysis of the native Garfagnina goats based on microsatellite polymorphism. Large Anim Rev. 23:191–193.

Cecchi F, Russo C, Iamartino D, Galiero A, Turchi B, Fratini F, Degl'Innocenti S, Mazza R, Biffani S, Preziuso G, Cantile C. 2017b. Identification of candidate genes for paratuberculosis resistance in the native Italian Garfagnina goat breed. Trop Anim Health Prod. 49(6):1135–1142.

Dennis MM, Reddacliff LA, Whittington RJ. 2011. Longitudinal study of clinicopathological features of Johne's disease in sheep naturally exposed to *Mycobacterium avium* subspecies Paratuberculosis. Vet Pathol. 48:565–575.

Galiero A, Fratini F, Mataragka A, Turchi B, Nuvoloni R, Ikonomopoulos J, Cerri D. 2016. Detection of *Mycobacterium avium* subsp. paratuberculosis in cheeses from small ruminants in Tuscany. Int J Food Microbiol. 217:195–199.

Galiero A, Fratini F, Turchi B, Colombani G, Nuvoloni R, Cerri D. 2015. Detection of *Mycobacterium avium* subsp. paratuberculosis in a sheep flock in Tuscany. Trop Anim Health Prod. 47:1567–1571.

Galiero A, Turchi B, Pedonese F, Nuvoloni R, Cantile C, Colombani G, Forzan M, Cerri D, Fratini F. 2017. Serological, culture and molecular survey of *Mycobacterium avium* paratuberculosis in a goat flock in Tuscany. Folia Microbiol. doi:10.1007/s12223-017-0518-7.

Gonda MG, Chang YM, Shook GE, Collins MT, Kirkpatrick BW. 2006. Genetic variation of *Mycobacterium avium* ssp. paratuberculosis infection in US Holsteins. J Dairy Sci. 89:1804–1812.

Gonda MG, Kirkpatrick BW, Shook GE, Collins MT. 2007. Identification of a QTL on BTA20 affecting susceptibility to *Mycobacterium avium* ssp. Paratuberculosis infection in U.S. Holsteins. Anim Genet. 38:389–396.

Gutièrrez JP, Royo LJ, Alvarez I, Goyache F. 2005. Molkin v2.0: A computer program for genetic analysis of populations using molecular coancestry information. J Hered. 96:718–721.

Hinger M, Brandt H, Horner S, Erhardt G. 2007. Association analysis of microsatellites and *Mycobacterium avium* subspecies paratuberculosis antibody response in German holsteins. J Dairy Sci. 90:1957–1961.

Koets AP, Adugna G, Janss LL, van Weering HJ, Kalis CH, Wentink GH, Rutten VP, Schukken YH. 2000. Genetic variation of susceptibility to *Mycobacterium avium* subsp. Paratuberculosis infection in dairy cattle. J Dairy Sci. 83:2702–2708.

Minozzi GL, Buggiotti L, Stella A, Strozzi F, Luini M, Williams JL. 2010. Genetic loci involved in antibody response to *Mycobacterium avium* ssp. Paratuberculosis in cattle. PLoS ONE. 5:e11117.

Moioli B., D'Andrea S., De Grossi L., Sezzi E., De Sanctis B., Catillo G., Steri R., Valentini A., Pilla F. 2016. Genomic scan for identifying candidate genes for paratuberculosis resistance in sheep. Anim Prod Sci. 56:1046–1055.

Mortensen H, Nielsen SS, Berg P. 2004. Genetic variation and heritability of the antibody response to *Mycobacterium avium* subspecies paratuberculosis in Danish Holstein cows. J Dairy Sci. 87:2108–2113.

Munjal SK, Tripathi BN, Paliwal OP. 2005. Progressive immunopathological changes during early stages of experimental infections of goats with *Mycobacterium avium* subspecies paratuberculosis. Vet Pathol. 42:427–436.

Nebbia P, Robino P, Zoppi S, De Meneghi D. 2006. Detection and excretion pattern of *Mycobacterium avium* subspecies paratuberculosis in milk of asymptomatic sheep and goats by nested-PCR. Small Rumin Res. 66:116–120.

Negrini R, D'Andrea MS, Crepaldi P, Colli L, Nicoloso L, Guastella AM, Sechi T, Bordonaro S, Ajmone-Marsan P, Pilla F, the Econogene Consortium. 2012. Effect of microsatellite outliers on the genetic structure of eight Italian goat breeds. Small Rumin Res. 103:99–107.

Obexer-Ruff G, Sattler U, Martinez D, Maillard JC, Chartier C, Saitbekova N, Glowatzki ML, Gaillard C. 2003. Association studies using random and "candidate" microsatellite loci in two infectious goat diseases. Genet Sel Evol. 35:S113–S119.

Ramamoorthi J, Thilagam K, Sivaselvam SN, Karthickeyan SMK. 2009. Genetic characterization of Barbari goats using microsatellite markers. J Vet Sci. 10:73–76.

Reddacliff L, Beh K, McGregor H, Whittington R. 2005. A preliminary study of possible genetic influences on the susceptibility of sheep to Johne's disease. Aust Vet J. 83:435–441.

Reddacliff L, Eppleston J, Windsor P, Whittington R, Jones S. 2006. Efficacy of a killed vaccine for the control of paratuberculosis in Australian sheep flocks. Vet Microbiol. 115:77–90.

Sechi LA, Scanu AM, Molicotti P, Cannas S, Mura M, Dettori G, Fadda G, Zanetti S. 2005a. Detection and isolation of *Mycobacterium avium* subspecies paratuberculosis from intestinal mucosal biopsies of patients with and without Chron's disease in Sardinia. Am J Gasteroenterol. 100:1529–1536.

Sechi T, Usai MG, Casu S, Carta A. 2005. Genetic diversity of Sardinian goat population based on microsatellites. Ital J Anim Sci. 4:58–60.

Settles M, Zanella R, McKay SD, Schnabel RD, Taylor JF, Whitlock R, Schukken Y, Van Kessel JS, Smith JM, Neibergs H. 2009. Whole genome association analysis identifies loci associated with *Mycobacterium avium* subsp. paratuberculosis infection status in US holstein cattle. Anim Genet. 40:655–662.

Singh N, Kala SN, Vihan VS, Singh VS. 1990. Genetic study on susceptibility to Johne's disease in goats. Indian J Anim Sci. 60:1163–1165.