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QTL detection and candidate gene study for traits related to food quality and safety in sheep (meat and dairy) production within the framework of a European research contract called "genesheepsafety"

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SUMMARY – The "genesheepsafety" project addresses the safety and quality of food in the sheep production chain using genetics. It concerns research partners from France, UK, Italy and Spain involved in a European contract (QLK5-2000-00656) of the 5th Framework Programme. The project started in February 2001 and will end in July 2004. The project will share genetic resource populations (backcross between Sarda and Lacaune breeds in Italy, divergent Lacaune lines in France, purebred families in Churra in Spain, purebred families in Basco-Bearnaise, Lacaune and Manech breeds in France, backcross between a lean and a fat line of Blackface in UK) and will also share the genotyping of the animals using microsatellite markers. The traits measured, related to food quality and safety, include CLA (conjugated linoleic acid) of milk, mastitis resistance and milkability, nematode parasite resistance, nasal botfly resistance and out of season breeding. It is planned to study both candidate genes or functional genomics for those traits, and QTL analysis. Outputs include knowledge on food and quality traits, QTL and candidate genes, in order to implement breeding strategies on those traits.

Key words: Food safety, sheep products, genetics, QTL, candidate genes, dairy and meat sheep.

RESUME – *"Détection de QTL et étude de gènes candidats pour des caractères liés à la qualité et la sécurité alimentaires en production d'ovins (viande et lait) dans le cadre d'un contrat européen de recherche intitulé "genesheepsafety"". Le projet d'acronyme "genesheepsafety" concerne la maîtrise de la sécurité et la qualité de la chaîne alimentaire ovine en utilisant la génétique. Les partenaires de ce contrat de recherche (QLK5-2000-00656) dans le cadre du 5ème PCRDT viennent de France, Angleterre, Italie et Espagne. Ce projet a débuté en février 2001 et il se terminera en juillet 2004. Le projet va partager diverses populations ressources (croisement en retour entre les races Sarde et Lacaune en Italie, lignées divergentes Lacaune en France, familles de race pure Churra en Espagne, familles de race pure Basco-Béarnaise, Lacaune ou Manech en France, croisement en retour entre une lignée maigre et une lignée grasse en race Blackface en Angleterre) et va partager aussi le génotypage des animaux à partir de marqueurs microsatellites. Les caractères mesurés, en relation avec la qualité et sécurité alimentaire, incluent : ACL (acide conjugué linoléique) du lait, la résistance aux mammites et la facilité de traite mécanique, la résistance aux nématodes et aux oestres, l'aptitude au désaisonnement. Il est prévu de mettre en oeuvre à la fois une approche gène candidat ou génomique fonctionnelle, et la détection de QTL. Les retombées incluent des connaissances sur des caractères de sécurité et qualité des produits, sur des QTL et gènes candidats, dans le but de mettre en place des stratégies de sélection de ces caractères.*

Mots-clés : *Sécurité alimentaire, produits ovins, génétique, QTL, gène candidat, ovins lait et viande.*

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Introduction

In the EU, the consumer demand for safety and "healthy" food products is increasing in such a way that the reduction of contaminants and zoonoses in the chain food became a major goal for the production systems. Under these conditions, our project which deals with sheep products aims to use genetics to improve the quality and safety of the sheep chain. This new goal can be achieved by producing products more protective for the human health and by reducing the use of antibiotic treatments or drugs. It means that breeding programmes in Europe must in the future concentrate more: (i) on modification of product composition related to protective factors for human health; and (ii) on selection of animals which are more resistant to diseases, in situations where a genetic strategy is sound and likely to be successful.

Objectives of the project

Production context

Sheep production is mainly located in less favoured rural regions in Europe, and contributes largely to the maintenance of activities and populations in these areas. This industry includes dairy sheep in Mediterranean countries and meat sheep throughout Europe. *Dairy sheep* systems have a dual purpose, with income from meat (milk-fed lambs) and milk, the milk being processed into high quality cheese, generally PDO cheese as laid down in Community legislation. Due to evolution of the CAP and consumers demand for typical PDO products, and for more quality and safety, Mediterranean dairy sheep have to be improved for production trait (milk yield) and milk composition quality (fat and protein) to maintain competitiveness of this production, but also for a number of new traits for which the economic importance has increased rapidly in the last years. These new traits are related to the reduction of production costs and the increase of the quality and safety of the products, as: (i) milkability or longevity of the animals; and (ii) resistance to mastitis or parasitic diseases. *Meat sheep* are found in many agricultural areas of Europe, but especially in the less favoured hill areas. The industry is reliant almost entirely in one product, meat, which is facing challenges from a decreased market share compared to other meats, especially white meat, and also cheap imports from outside of Europe. The challenge facing the meat sheep sector is to cost-effectively produce a product which is attractive to the consumer, i.e. high quality and perceived to be safe. For this purpose, the meat sheep breeding programmes of Europe must in the future concentrate on: (i) animal health issues, to reduce risks of food contamination and zoonoses, and to reduce the reliance on chemicals; and (ii) direct product quality, i.e. carcass composition and meat quality.

Scientific context

Genetic maps of livestock based on molecular genetic markers provide new tools for the detection and mapping of genes of economic importance in farm animals. This provides new opportunities to select for the necessary product quality and safety traits which are often difficult to measure. To face the genetic challenge described above, a series of regional projects have started in the European Union to explore the ovine genome and detect Quantitative Traits Loci (QTL). But most of these projects are too small to give strong and conclusive answers to the questions being addressed, or lack a critical mass of researchers in all the multidisciplinary knowledge needed: under these conditions, production traits (i.e. milk yield and milk composition for dairy sheep, or growth and carcass traits for meat sheep) are more extensively and generally studied in these existing projects. *Thus new traits related to the quality and safety of the sheep products will be addressed in the framework of the European project QLK5-2000-00656*.

Description of the project

Therefore, our European project aims to combine several regional programmes of QTL detection in dairy or meat sheep to investigate new traits of which the economic importance will increase in the future, since they are directly related on the one hand to the optimum use of inputs and farming systems protective of the environment, on the other hand to the quality and safety of the sheep products (Barillet, 1999). It also builds a critical mass of researchers addressing these issues.

Six participants are involved in this project which started in February 2001 and will end in July 2004: (i) INRA (participant 1 – P1) in France; ENVT (participant 2 – P2) in France; (iii) Roslin Institute (participant 3 – P3) in UK; (iv) University of Leon (participant 4 – P4) in Spain; (v) IZCS (participant 5 – P5) in Italy; and (vi) University of Glasgow (participant 6 – P6) in UK.

Summary of the project (Table 1)

To maintain and share between participants resource populations of sheep

The shared informative populations will be made, on the one hand of differing breeds or lines and their crosses, on the other hand of collection of large sized purebred families.

(i) The first crosses correspond to Backcross (BC) ewes between an Italian (Sarda) and a French (Lacaune) dairy sheep breed (S × L BC), and they are bred in Italy (Sardinia) by P5 on an experimental farm.

(ii) The second crosses will be backcross lambs between a lean and a fat line of Blackface (Bl) meat sheep (Bl BC), bred on an experimental farm in Scotland by P3.

(iii) The third differing lines corresponds to dairy Lacaune sheep divergent lines (L divergent lines) for milk production on an experimental farm of P1.

(iv) The purebred families will be collected on-farm and AI centres from the dairy Churra breed (C families) in Spain by P4, dairy Basco-Bearnaise, Lacaune and Manech breeds (B, L or M families) in France by P1, taking advantage of the existence of on-farm breeding programmes based on artificial insemination rams.

Sharing the populations means that the participants will organize cooperative research to compare the results of the different populations combined with the exchange of biological material (blood, milk, DNA...) from these populations to each participant according to his competence and tasks to achieve. This is particularly important in the measurements of new traits as described later.

To share genotyping of the animals of the resource populations of sheep

The resource populations will be genotyped using 3 panels of microsatellite markers provided for two of them by the participants P1 and P4. Each panel will be composed of up to 150 loci, to enable us to scan all the genome of S × L BC by P5 (and P1), the genome of Bl BC by P3, the genome of C families by P4. On the other hand, P1 will perform a partial genotyping of B, L or M families.

To measure traits related to the quality and safety food

(i) Conjugated Linoleic Acid (CLA) content of sheep milk by P5: in S × L BC sheep measurement of CLA of milk in the middle of the second lactation.

(ii) Mastitis resistance: Somatic Cell Counts (SCC), cases of clinical mastitis, udder score, digital pictures and milkability (milk flow rate) will be recorded in the S × L BC by P5 and L divergent lines by P1; (SCC) and udder score in the C families by P4 and (SCC) in the B, L or M families (population) by P1.

(iii) Out of season lambing: spontaneous ovarian activity recorded in S × L BC before mating in spring by twice progesterone assay by P1.

(iv) Nematode and Oestrus ovis resistance: for nematodes, faecal eggs counts will be measured on Bl lambs by P3 and P6, S × L BC by P5 and P1 and C ewes by P4.

(v) Oestrus ovis resistance determined in the S × L BC by IgA and IgG in sera and milk by P1.

Table 1. Summary of the genesheepsafety project

Population	Backcross between Sarda and Lacaune breeds	Backcross between a lean and a fat line of Blackface	Divergent lines in Lacaune breed	Churra families	Basco-Bearnaise, Lacaune and Manech families
Bred (in)	Experimental farm	Experimental farm	Experimental farm	Private farms	Private farms
Genotyping	Genome scan	Genome scan	Genomic approach	Genome scan	Partial genotyping
Measurement of production traits	Yes (milk production)	Yes (growth rate and carcass traits)	Yes (milk production)	Yes (milk production)	Yes (milk production)
Traits related to quality and safety food	CLA in milk				
	Mastitis resistance, udder score and milkability		Mastitis resistance, udder score and milkability	Mastitis resistance and udder score	Mastitis resistance and (udder score)
	Out of season breeding				
	Nematode parasite resistance	Nematode parasite resistance		Nematode parasite resistance	
	Oestrus parasite resistance				
Candidate genes or functional genomics		MHC and nematode parasite resistance			
	Melatonin receptor and out-of-season breeding 4 candidate genes and CLA				
			Functional genomics and mastitis resistance-milkability		
Detection of QTL	Yes	Yes	No	Yes	No
Verification of QTL					Yes
Candidate genes/functional genomics	Yes	Yes	Yes	No	No

To study candidate genes or functional genomics

Utilising previous knowledge, a candidate genes approach will be implemented.

(i) By P6 and P3 for nematodes parasites and Major Histocompatibility Complex (MHC) in Bl BC.

(ii) By P1 for out of season breeding and melatonin receptor in S × L BC.

(iii) And CLA by P1 using the S × L BC for a candidate genes (lipoprotein lipase, fatty acid synthase, acetyl-CoA carboxylase, and stearoyl-CoA desaturase) approach based on the quantification of their mRNA.

On the other hand, a functional genomic approach will be carried out by P1 for mastitis resistance and milkability from the L divergent lines.

To carry out biological analysis

The previous steps of the project will allow the participants to analyse the data for QTL or candidate genes detection using state-of-art techniques. Then they will compare the results in different environments × populations for the same trait to get more powerful results (meta-analysis). Finally they will be able to study the possible genetic relationships between these new traits and the production traits.

Conclusion

The project started in February 2001 and will end in July 2004. The first QTL detections are expected in 2002. The first publications are presented during this meeting of the FAO/CIHEAM subnetwork on Sheep and Goat genetic resources held in May 2002 in Sardinia (Carta *et al.*, this volume; Marie-Etancelin *et al.*, this volume) or will be presented in August 2002 at the 7th World Congress on Genetics Applied to Livestock Production in France (Carta *et al.*, 2002; El Zarei *et al.*, 2002; Schibler *et al.*, 2002).

Benefits from the project will be to integrate molecular information about genetic variability into marker assisted selection in sheep breeding programmes in the future. It should be pointed out that in the participating countries the breeding infrastructure exists to enable marker assisted selection to be successfully applied to the sheep populations.

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