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## Occurrence of caprine leucocyte antigens (CLA) in Creole goats susceptible/resistant to heartwater

RUFF (G.), MAILLARD (J.C.), CAMUS (E.), DEPRES (E.), MATHERON (G.). Antigènes leucocytaires caprins (ALC) chez les chèvres Créole sensibles ou résistantes à la cowdriose. *Revue Élev. Méd. vét. Pays trop.*, 1993, 46 (1-2) : 205-207

Certaines lignées de chèvres ont montré une prédisposition génétique à manifester des symptômes cliniques de la cowdriose. Afin d'élucider une implication possible du complexe majeur d'histocompatibilité (CMH) dans la pathogénie de la cowdriose, les antigènes ALC de classe I, codés par le CMH caprin, de plus de 100 chèvres Créole, ont été typés sérologiquement. Le CMH est un système génétique qui influence des processus immunologiques différents, c'est-à-dire sur la réponse immunitaire individuelle. À l'aide de nos allosérums ont été détectés 13 allèles de ALC différents qui se retrouvent également dans les races suisses, deux antigènes non-CMH et un nouveau groupe défini par la combinaison de deux antigènes. Les fréquences alléliques des antigènes ALC détectés étaient différentes entre les groupes résistant et sensible. Il reste à éclaircir si les différences représentent des effets régionaux de population, ou si elles indiquent une pression de sélection par l'agent pathogène. Des tests additionnels sont en cours sur des chèvres résistantes ou sensibles originaires d'un même environnement, ainsi que sur des animaux issus de croisements entre caprins résistants et sensibles.

*Mots clés* : Caprin - Chèvre Créole - Cowdriose - Antigène - Complexe majeur d'histocompatibilité - Allèle - Sérologie - Immunité.

### INTRODUCTION

A genetic predisposition to the manifestation of heartwater disease (cowdriosis) in Creole goats could be demonstrated (6). The caprine major histocompatibility complex (MHC), representing a genetic marker system of individual immunocompetence, was studied regarding its involvement in the pathogenesis of heartwater disease.

The MHC codes for its gene products, the so-called caprine leucocyte (CLA) antigens. They can be differentiated into various classes according to their structure and function. In the goat, class I antigens are expressed on all nucleated cells, class II antigens are primarily expressed on B-cells and on activated T-cells.

The caprine MHC has been characterized by several techniques. The earliest reports described serological techniques (9, 10, 14), later biochemical methods were applied (3, 4) and in the most recent studies molecular genetic analysis was carried out (12, 13). Up to the present, a total of 27 class I antigens belonging to one locus and one class I antigen belonging to another locus could be detected by serological means. Eighteen of these gene products could also be confirmed using biochemical detection, whereas one antigen could be split. A total of seven class II antigens could be characterized by the serological and biochemical techniques. The application of DNA analysis resulted in 22 different nucleic acid sequences of the class II (DRB) type. Whether they all code for different gene products remains to be clarified. Three non-MHC antigens have also been described (9).

The authors applied serological characterization of MHC class I products in the present study. Using this technique a high degree of polymorphism can be rapidly detected causing relatively little expenses. Furthermore, class I and class II antigens are closely linked and are thus inherited on the same chromosome. In this way it is possible to conduct linkage studies within family material using the class I antigens as markers in various projects.

### MATERIAL AND METHODS

A total of 112 Creole goats belonging to CIRAD-EMVT and INRA have been serologically typed for their CLA class I antigens. Thirty-nine goats were classified as resistant type after challenge and 73 others, including animals native from the Les Saintes archipelago, were presumed to be of the susceptible type before being challenged to heartwater (6).

CLA alloantiserum production, cell isolation and CLA-typing procedures have been carried out as described in detail elsewhere (1, 11).

For the statistical evaluation typing results have been computed. Gene frequencies and relative risks have been calculated using Chi-square analysis with Yate's correction where necessary (5, 7).

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## RESULTS AND CONCLUSIONS

Of the already known caprine class I antigens, 13 could be detected in the Creole goats. In addition, a new combination of two reagents could be found, characterizing a local breed-specific antigen (Gu1). Furthermore, two of the three non-MHC antigens were present in the Creole goats.

The gene frequency of all the determined CLA class I antigens was estimated for the presumed susceptible and the resistant group. For the antigens with a statistically different gene frequency for the two groups, the relative risk and the probability value was calculated (table 1). Four antigens showing significantly different P-values ( $P < 0.05$ ) could be detected. The non-MHC antigen CLY 1.1 (9) and the CLA antigen Be13 occurred in an increased frequency in the resistant group. The CLA antigens Be4 and Be9 were more frequent in the susceptible group.

These differences might represent pathogen-induced selection pressure, but they might also be due to frequency differences found at the population level. In order to clarify this hypothesis, further investigations are necessary. Genetic comparison between these two populations of different geographical origin, using other genetic marker systems is presently under investigation (PEPIN, thesis in preparation). Furthermore a crossbreeding programme has been initiated in order to study the inheritance of the different CLA class I marker antigens in the offspring of resistant/susceptible parents. A final number of over 200 offspring is expected and will be challenged with heartwater before May 1994. CLA-typing and challenge testing of these animals will enable a better understanding of the underlying disease mechanisms and the genes linked to susceptibility/resistance in the pathogenesis of heartwater disease.

If the serologically detected MHC antigens will not provide sufficient information about disease association, more refined techniques for analysis of MHC or other genetic marker systems have to be considered. Today, an increasing number of molecular genetic techniques such as RFLP, PCR, sequencing, oligotyping (12) and various DNA probes including microsatellites (8) have already been developed for small ruminants. These sophisticated methods represent powerful tools in association studies with various traits in sheep and goats.

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## REFERENCES

1. BÖYUM (A.) Separation of leucocytes from blood and bonemarrow. *Scand. J. Clin. Lab. Invest.*, 1986, **21** (Suppl. 197) : 31-50.
2. CAMERON (P.U.), TABARIAS (H.A.), PULENDRAN (B.), ROBINSON (W.), DAWKINS (R.L.). Conservation of the central MHC genome : PFGE mapping and RFLP analysis of complement, HSP70, and TNF genes in the goat. *Immunogenetics*, 1990, **31** : 253-264.
3. JOOSTEN (I.), RUFF (G.), SANDERS (M.F.), LAZARY (S.), HENSEN (E.J.). Biochemical and serological typing of caprine class I and II products. *Anim. Genet.*, 1991, **22** (Suppl. 1) : 48.
4. JOOSTEN (I.), RUFF (G.), SANDERS (M.F.), HENSEN (E.J.). Use of isoelectric focusing to define MHC class I polymorphism in goats. *Anim. Genet.* (in press).
5. MANTEL (N.), HAENSZEL (W.). Statistical aspects of the analysis of data from retrospective studies of diseases. *J. Nat. Cancer Inst.*, 1959, **22** : 719-748.

TABLE 1 Gene frequencies, relative risk and P-values of CLA antigens in Creole goats, resistant (R) or susceptible (S) to heartwater (cowdriosis).

	Specificity	Sera	Gene frequencies			Probabilities		
			R N = 39	S N = 73	$\chi^2$	P (11.d.)	R.R.	Sign.
R	CLY panleuc.	94 N	0.168	0.028	13.28	< 0.001	0.13	+++
	Be 13	154 N	0.108	0.028	4.54	< 0.035	0.22	++
	Be 1	332 K	0.108	0.042	3.51	< 0.065	0.35	+
S	Be 4	Boby	0.08	0.250	9.18	< 0.01	4.29	+++
	Be 9	Kaffea	0	0.078	4.93	< 0.03	-	++
	Be 20-D3	Venus	0.013	0.086	3.51	< 0.065	7.47	+
	Be 22	1 N	0.013	0.086	3.51	< 0.065	-	+

6. MATHERON (G.), BARRÉ (N.), CAMUS (E.), GOGUE (J.). Genetic resistance of Guadeloupe native goats to heartwater. *Onderstepoort J. vet. Res.*, 1987, **54** : 337-340.

7. MATTIUZ (P.L.), IHDE (D.), PIAZZA (A.), CEPPELLINI (R.), BODMER (W.). New approaches to the population genetic and segregation analysis of the HLA system. In : TERAZAKI (P.I.), Ed. *Histocomp. Testing*. Copenhagen, Munksgaard, 1970. P. 193-205.

8. MOORE (S.S.), BARENDSE (W.), BERGER (K.T.), ARMITAGE (S.M.), HETZEL (J.). Bovine and ovine DNA microsatellites from the EMBL and GENBANK data bases. *Anim. Genet.*, 1992, **23** : 463-468.

9. NESSE (L.L.), RUFF (G.). A comparison of lymphocyte antigen specificities in Norwegian and Swiss goats. *Anim. Genet.*, 1989, **20** : 71-77.

10. RUFF (G.) Investigations on the caprine leucocyte antigen (CLA) system. Thesis no. 8468, ETH Zurich, 1987. 223 p.

11. RUFF (G.), LAZARY (S.). Evidence for linkage between the caprine leucocyte antigen (CLA) system and susceptibility to CAE virus-induced arthritis in goats. *Immunogenetics*, 1988, **28** : 303-309.

12. SCHWAIGER (F.W.), RUFF (G.), WEYERS (E.), LAZARY (S.), EPPLEN (J.T.). Polymorphism of the caprine and ovine MHC class II DRB genes detected by oligonucleotide typing. *Anim. Genet.*, 1992, **23** (Suppl. 1) : 43.

13. SCHWAIGER (F.W.), BUITKAMP (J.), RUFF (G.), WEYERS (E.), EPPLEN (J.T.). Highly efficient oligonucleotide typing of artiodactyl MHC genes on the basis of intronic hypervariable simple repeated DNA sequences. *Molecular Ecol.* (accepted).

14. VAN DAM (R.), VAN WERKHOFEN (C.B.), VAN DER DONK (J.A.), GOUDSWAARD (J.). Histocompatibility in ruminants. The production and evaluation of alloantibodies for CLA-typing in goats. *J. Immunogenet.*, 1976, **3** : 237-244.

**RUFF (G.), MAILLARD (J.C.), CAMUS (E.), DEPRES (E.), MATHERON (G.).** Occurrence of caprine leucocyte antigens (CLA) in Creole goats susceptible/resistant to heartwater. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 205-207

A genetic predisposition to the manifestation of disease symptoms has been demonstrated for heartwater in certain goat lines. In order to clarify a possible involvement of the major histocompatibility complex (MHC) in the pathogenesis of heartwater, over 100 Creole goats have been typed serologically for their CLA class I antigens coded by the caprine MHC. The MHC is a genetic system that influences different immunological processes, i.e. the individual immune response. With our alloantisera we were able to detect 13 different CLA alleles also present in the Swiss breeds, two non-MHC antigens, a new cluster defined by the splitting of two antigens and several specific reaction patterns of single reagents. The allele frequencies of the detected CLA antigens differed for the resistant and susceptible groups examined. Whether the differences represent regional sire effects or indicate pathogen-induced selection pressure remains to be clarified. Additional testing of resistant/susceptible goats originating from common environment as well as of specially crossbred (resistant x susceptible) animals are underway.

*Key words* : Goat - Creole goat - Heartwater - Antigen - Major histocompatibility complex - Allele - Serology - Protection.

**RUFF (G.), MAILLARD (J.C.), CAMUS (E.), DEPRES (E.), MATHERON (G.).** Aparición de antígenos leucocitarios caprinos (CLA) en cabras Criolla susceptibles o resistentes a la coudriosis. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 205-207

En ciertas líneas caprinas se ha demostrado la predisposición genética a la manifestación de los síntomas de la coudriosis. Con el fin de aclarar la posible acción del complejo mayor de histocompatibilidad (MHC) en la patogénesis de la coudriosis, se tipificaron serológicamente alrededor de 100 cabras Criolla para el antígeno de CLA clase I, codificado con el MHC caprino. El MHC es un sistema genético que influye sobre procesos inmunológicos diferentes, como por ejemplo la respuesta inmune individual. Gracias a nuestros anti-sueros de misma especie, se pudieron identificar 13 alelos diferentes de CLA, también presentes en razas suizas, dos antígenos no MHC, un nuevo grupo definido mediante la separación de dos antígenos y varios patrones específicos de reacción de agentes individuales. Las frecuencias alélicas de la detección de antígenos de CLA difieren para los grupos resistentes y susceptibles examinados. No es claro si las diferencias representan efectos reguladores regionales o indican una presión selectiva patógeno-inducida. Se llevaron a cabo otros exámenes de caprinos resistentes o susceptibles provenientes de un medio ambiente común, así como de una raza producto de un cruce específico (resistente x susceptible).

*Palabras claves* : Caprino - Cabra Criolla - Coudriosis - Antígeno - Complejo mayor de histocompatibilidad - Alelo - Serología - Inmunidad.