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Characterization of the bovine ε gene

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SUMMARY

Immunoglobulin E is quantitatively a minor immunoglobulin class in serum, but nevertheless the major class of antibody mediating type I hypersensitivity reactions and hence, type I allergic phenomena. The bovine ε gene is one of the as yet uncharacterized mammalian immunoglobulin genes. We have therefore cloned and determined the cDNA sequence and genomic organization of the gene. It contains four constant region domain-encoding exons (CH1 to CH4) with a high homology to sheep Cε (87%) and to a lower degree to dog (62%), horse (58%), chimpanzee, orangutan, human (55%), mouse (52%) and rat (52%) Cε genes. Southern blot analysis of bovine genomic DNA, revealed the existence of a single Cε gene with a presence of allelic restriction fragment length polymorphism (RFLP).

INTRODUCTION

Immunoglobulins are divided into five major classes, IgM, IgG, IgA, IgD and IgE, a classification which is based on antigenic differences in the heavy chain constant regions as defined by serology. IgE is an immunoglobulin class that is normally present in only trace amounts in serum and constitutes a monomer with a higher molecular weight than other immunoglobulins (196 000 MW). This is because of an additional domain in the heavy chain, which is thought to be of importance for binding to high affinity Fc receptors on mast cells.[1] The main physiological function of IgE-mediated responses is the defence against parasite infections[2,3] and high serum levels are seen in certain parasitic infections, particularly roundworms.[4] The killing mechanism is mediated via cells such as mast cells and basophils.[5]

The presence of IgE may not only be advantageous as it may also give rise to type I hypersensitivity reactions such as conjunctivitis, rhinitis and asthma.[6–8] Thus, it is important to be able to measure the level of IgE responses in animals. Diagnostic tests for IgE responses require anti-IgE reagents as well as purified IgE. As the level of IgE in the sera of all mammals is very low, even during parasitic infections and allergic disorders,[9] it is difficult to purify IgE using traditional protein purification methods.[10] Thus, the availibility of sequence data on IgE permits the making of IgE specific peptides, which can be used for production of anti-bovine IgE.

Human,[11] mouse,[12] rat,[13,14] dog[15] sheep,[16] chimpanzee, orangutan[17] and horse[18] Cε genes have previously been characterized whereas the Cε gene of most other species, including the cow, has as yet not been characterized. We have therefore subcloned, sequenced and characterized both the cDNA and genomic DNA sequences of the bovine Cε gene.

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MATERIALS AND METHODS

The recombinant phage EMBL4 containing the bovine Cε gene (phage clone 24), has previously been identified and restriction mapped and was a kind gift from Dr Kathrine Knight.[19] Digestion of this clone with restriction enzymes *Eco*RI and *Bam*HI (Boehringer Mannheim Biochemicals, Mannheim, Germany) gave rise to the whole part of the Cε gene in a 5 kb fragment. This fragment was subcloned into pBLUSCRIPT KS (±), transferred into the *E. coli* strain JM109 (heat shock) and sequenced by both the Taq Dye Deoxy Terminator cycle sequencing and the nucleotide chain termination method using the fmol™ DNA sequencing system (Promega, Madison, WI). The primers for sequencing were the vector primers T7 and T3.

Reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA from bovine peripheral blood lymphocytes (Swedish red and white strain) was purified using the TRIZOL™ reagent (gibco BRL, Life Technologies, Gaithersburg, MD). First-strand cDNA synthesis was performed using a cDNA synthesis kit according to instructions from the manufacturer (Pharmacia, Uppsala, Sweden). For cDNA synthesis, 5 μg of total RNA was used.

Part of the cDNA of Cε gene was amplified by PCR using sense primer 1 (bovine consensus sense V-region, deduced from previously published VDJ sequences)[20] and antisense primer 3 (bovine consensus antisense CH3 region). The exon–intron boundaries beween the CH3 and CH4 was amplified by PCR using the sense primer 2 (bovine consensus sense CH3 region) and antisense primer 4 (bovine consensus antisense CH4 region). The primers 2, 3 and 4 were designed from CH3

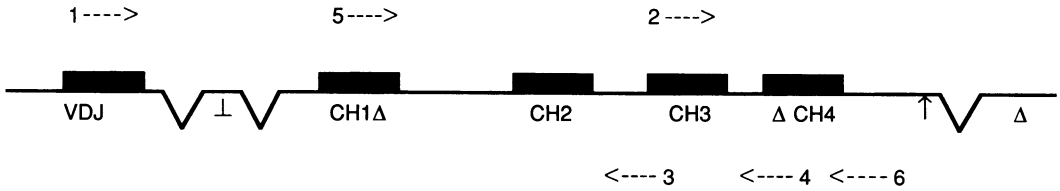

Figure 1. Schematic maps for the coding region of the 5·0 kb *Eco*RI and *Bam*HI fragment (containing the bovine Cε-gene). (→) shows sense primers and (←) shows antisense primers. (△) denotes digestion site for *Pst*I, (⊥) *Bam*HI and (↑) *Eco*RI. The shown distance between *Bam*HI-site and the first exon of the Cε is about 2·0 kb and the distance in the figure is not in accordance with the real distance of that.

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gagtagaagtgagaagctgttggctgagggtccctgtgggtagaggtgagaacagctgctcactggagaggtccctggaagggtcagaggtgagaaaagg   100
acccagaagaaaccatctatccctcaaggctggagcagagccccagtgttccctgagccagaccaccccacctcagctccacagcccaccccaggtgagc   200
agtcagcctggcacatatgggtgagagtcctgagaccagcgctgaccctggctGTCTCCTCAGCCTCCATCCAGGCTCCATCCATCTACCCCTTGAGGCT   300
                                                      V  S  S  A  S  I  Q  A  P  S  I  Y  P  L  R  L

CTGTTGCACAGAAGAAGCCCGTGTAAGGCTGGGCTGCCTGGTCAAGGACTACTTGCCAGGGTCAGTGACTGTGACCTGGGACACAGTGCCCCTGGATGGA   400
 C  C  T  E  E  A  R  V  R  L  G  C  L  V  K  D  Y  L  P  G  S  V  T  V  T  W  D  T  V  P  L  D  G

AGCACCTTGACCTTCCCTAGCATCCAAATGGCGAGCTCCAGTCTCTACATTACCACCAGCCAGCTGACCATCTCGGGCGAGCAGTCCAAGGAGTTCACCT   500
S  T  L  T  F  P  S  I  Q  M  A  S  S  S  L  Y  I  T  T  S  Q  L  T  I  S  G  E  Q  S  K  E  F  T  C

GCAGAGTTTTACACCCTGAGACAATCCAATTGAATAAGACATCAACTGgt gagccagggttggggtagctgggagtaggggggcgggccagccaggctgga   600
 R  V  L  H  P  E  T  I  Q  L  N  K  T  S  T

ggaacagggaggagaggcttggggtctccaggagcccagccccatgcctgtcacctctgccccc agAGTGTGTCAAGAACTTCAGCGACCCCTCAGTGAA   700
                                                                  C  V  K  N  F  S  D  P  S  V  K

GCTCTTCTTCTCCTCATGCAACCCCAATGGCGACACCCAGACCACCATCCACCTCCTGTGCCGGATCTCCGCATACACCCCGGGAAAAATCAAGGTCACC   800
 L  F  F  S  S  C  N  P  N  G  D  T  Q  T  T  I  H  L  L  C  R  I  S  A  Y  T  P  G  K  I  K  V  T

TGGCTGGTGGATGGGCTCCAGTCCGAAGAGCTGTATGCACGATCCGGCCCTGAGATACAGGAGGGCAATCTGACCTCCACCTACAGTGAAGTCAACATCA   900
W  L  V  D  G  L  Q  S  E  E  L  Y  A  R  S  G  P  E  I  Q  E  G  N  L  T  S  T  Y  S  E  V  N  I  T

CACAGGGCCAGTGGGTGTCCGAGAAAACCTACACCTGCCGGGTCAACTATTACGGCTACAACTTCGAAAGCCACGCCCACAGGTGCACAGgt acagccct   1000
 Q  G  Q  W  V  S  E  K  T  Y  T  C  R  V  N  Y  Y  G  Y  N  F  E  S  H  A  H  R  C  T  A

cacctacgccaacactcagacaccaggggctcagggaggagggcggccagcctcacacaggcccctctcgcacac agCCGAGTCCGAGCCCCGCGTGAGC   1100
                                                                  E  S  E  P  R  V  S

GCCTACCTGAGCCCACCCACCCCCCTCGAGCTGTACGTCAACAAGTCACCCAAGATCACCTGCCTGGTTGTGGACCTGGCCAACGAGAAGAATTTGAGCC   1200
A  Y  L  S  P  P  T  P  L  E  L  Y  V  N  K  S  P  K  I  T  C  L  V  V  D  L  A  N  E  K  N  L  S  L

TGACCTGGTCGAGGGCAAACGGGAAGCCTGTGCACGCAGGCCCCCCTGAGATCAAGCGTCAGTTCAACGGGACGGTCACCGTCACGTCCACCCTGCCGGT   1300
 T  W  S  R  A  N  G  K  P  V  H  A  G  P  P  E  I  K  R  Q  F  N  G  T  V  T  V  T  S  T  L  P  V

GGACGTCACTGACTGGGTAGAAGGCGAGACCTACTACTGCAAAGTGAGCCACAGGGACCTGCCCACAGACATCCAGCGATCCATCTCCAAGGATGTTGgt   1400
 D  V  T  D  W  V  E  G  E  T  Y  Y  C  K  V  S  H  R  D  L  P  T  D  I  Q  R  S  I  S  K  D  V  G

gagcggcaggctgaggggaggtaggcgggcctcctgagagaagcctgactaaccacacacctgtctgcag GCAAGCGTCTGGCCCCCAAGGACTACGTGT   1500
                                                                  K  R  L  A  P  K  D  Y  V  F

TCTTGGCAGACGGAAAGGAGCTGGAGAACGAGGAGGAGCTCACCCTCACCTGCATGATCCAGAACTTCTTCCCCAGGGACATCTTTGTGCGGTGGCTGCA   1600
 L  A  D  G  K  E  L  E  N  E  E  E  L  T  L  T  C  M  I  Q  N  F  F  P  R  D  I  F  V  R  W  L  H

TAACAAGGAACTGATGGGGGCAGACCAGCACACCACCACACAGCCCCACAGGGACGACAACAACACCCCAGCCTTCTTCGCCTACAGCCGCCTGGCGGTC   1700
 N  K  E  L  M  G  A  D  Q  H  T  T  T  Q  P  H  R  D  D  N  N  T  P  A  F  F  A  Y  S  R  L  A  V

CCTAGGGCCAACTGGAAGAGGGGCGATGAGTTCACCTCCCAAGTGATCCACGAGGCACTGCCCAGGACTAGGACCCTGGAGAAATCGGTGTTCATTAACA   1800
P  R  A  N  W  K  R  G  D  E  F  T  S  Q  V  I  H  E  A  L  P  R  T  R  T  L  E  K  S  V  F  I  N  S

GCGGTAAATAAcgcccagcctcccgcctgcccctctccaggg   1842
 G  K  *
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Figure 2. Complete nucleotide sequence and deduced amino acid sequence of the bovine epsilon heavy chain constant region. The inferred amino acid sequence (single letter code) is indicated under the first base of each codon, and stop codons are indicated by an asterisk. The carbohydrate attachment sites are labelled by three bold and underlined letters. The splice sites, inferred by comparison with the cDNA sequence are indicated by small letters. The position of splice sites are underlined.

Table 1. Nucleotide sequences of primers. 1, bovine consensus sense variable region. 2, bovine consensus sense CH3 region. 3, bovine consensus antisense CH3 region. 4, bovine consensus antisense CH4 region. 5, bovine consensus sense CH1 region. 6, bovine consensus antisense down stream of Cε-gene

Primers	Nucleotides
1	5′-GAC CCT CTC CCT CAC CTG CAC GGT-3
2	5′-CCT GCC CAC AGA CAT CCA-3′
3	5′-CTT CCC GTT TGC CCT CGA-3′
4	5′-CAC TTG GGA GGT GAA CTC ATC GCC-3′
5	5′-CCT CCA CAC AGA CCC ATC C-3′
6	5′-GGG CTC TGA GCA GGC ACA GTT TAT T-3′

and CH4 of the bovine Cε gene based on our sequence data from genomic DNA. These PCR products were subcloned into the pGEM-T vectors (Promega), transferred into the *E. coli* strain JM109 and sequenced. Nucleotide sequences of the primers are shown in Table 1 and position of them on the Cε gene are shown in Fig. 1.

Southern blot

Southern blot analysis was performed after digestion of 10 different bovine genomic DNA samples (Swedish red and white strain) with the restriction enzymes *Eco*RI, *Hind*III and *Pst*I, respectively. The digested samples were separated in 0·8% agarose gels and transferred to Hybond membranes (Amersham, Slough, UK). Hybridizations were carried out as previously described.[21] The probe was the PCR product, obtained from amplification of Cε phage (clone 24)[19] with the sense primer 5 (start point of the CH1 of bovine Cε gene) and antisense primer 4 (last intron following CH4 of bovine Cε gene). The PCR product was about 1·5 kb. The approximate positions of above mentioned primers are shown in Fig. 1 and the sequences in Table 1.

Phylogenetic analysis

To construct the phylogenetic tree, we used the secretory form of the complete amino acid sequences of IgE heavy chain constant region of eight species (for name and accession number see Fig. 3). Calculation of relatedness data was done by a computer program, Megalian (DNA Star Inc., Madison, WI).

RESULTS

We first cloned secretory form of the bovine Cε cDNA and determined its nucleotide sequence (Fig. 2). To determine the exon–intron boundaries, the genomic DNA of the bovine Cε was then sequenced. The gene contains four constant region domain-encoding exons (CH1 to CH4) as in other species and acceptor–donator splicing sites were normal. The comparison of nucleotide sequences of the bovine Cε cDNA with its counterpart in other mammals revealed that the bovine Cε has a high similarity to sheep Cε (87%) and to a lower degree to dog (62%), mouse (58%), rat (58%), orangutan (56%), chimpanzee (55%), human (55%) and horse (52%) Cε (Fig. 3). Deduced amino acid sequences of the bovine Cε revealed eight carbohydrate attachment sites (Asn-X-Thr/Ser)[22] (Fig. 2).

Southern blot analysis of 10 different samples of bovine genomic DNA revealed one or two bands of 3·5, 4·0 and 4·5 kb in *Eco*RI and 5·8 and 6·3 kb in *Bam*HI digested samples (Fig. 4a,b). Representative results are shown in the figures. Four samples of *Eco*RI and five samples of *Bam*HI digested DNA showed only one band, indicating a single gene and the remaining samples showed two bands which can be due to allelic restriction polymorphism. Southern blot analysis of *Pst*I digested DNA from the above mentioned samples, using the same probe revealed three to five bands. Two constant bands 1·1 and 3·7 kb appeared in all samples. These correspond to restriction sites found in our sequence as depicted in Fig. 1with a 1·1 kb (CH1–CH4) band and a 3·7 kb band derived from

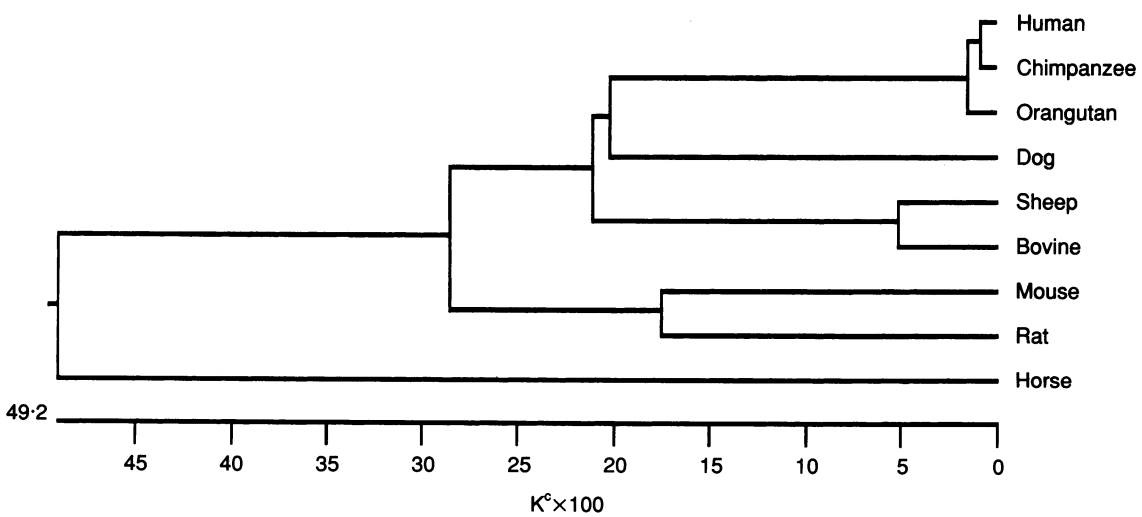

Figure 3. Cε gene phylogeny in mammalians. Homology searching, based on the complete amino acid sequences of secretory form of the IgE heavy chain constant region of eight species. The name and accession number of these data are: bovine (U63640, this paper), sheep (M84356), dog (L36872), horse (U15150), chimpanzee (M15399), orangutan (M15399), human (K01241), mouse (M22934) and rat Cε (J00744, J00742, J00743, K00390). The scale represents the number of amino acid substitutions per 100 sites (k^c value × 100).

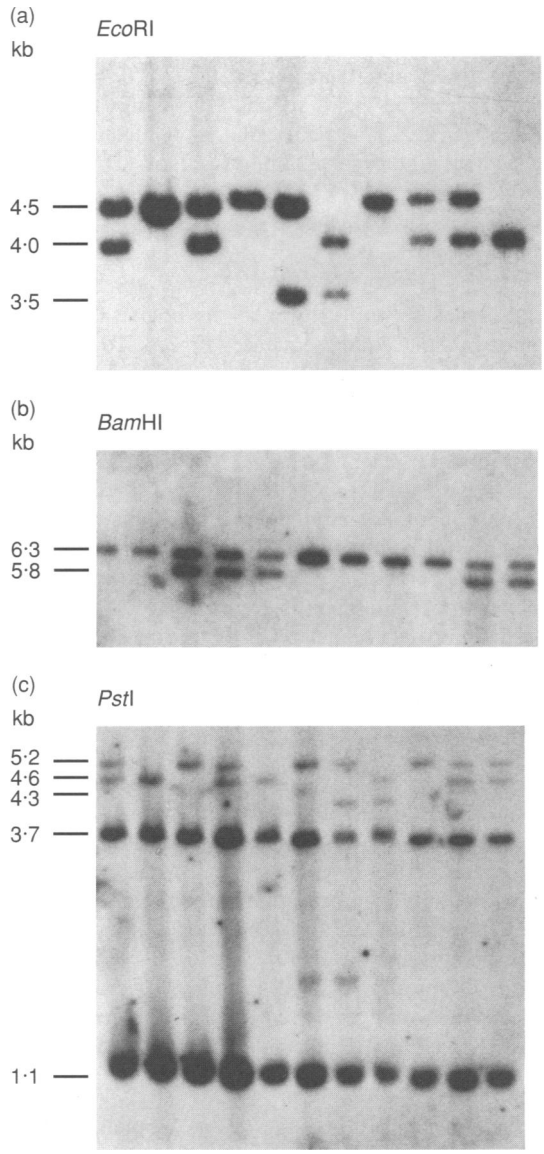

Figure 4. Southern blot analysis of 10 to 11 different samples of bovine genomic DNA; (a) digested with *Eco*RI giving bands of 4·4, 4·0, 3·5 kb; (b) digested with *Bam*HI giving bands of 6·3, 5·8 kb; (c) digested with *Pst*I giving bands of 5·2, 4·6, 4·3, 3·7 and 1·1 kb. All samples were hybridized with a genomic bovine Cε gene.

the 3′ portion of the gene (based on digestion of the Cε gene containing phage) (data not shown). One to three additional weak bands in various sizes can due to restriction fragment length polymorphism (RFLP) of the *Pst*I site on 5′ portion of the Cε (Fig. 4c). The approximate position of *Pst*I sites on the Cε is shown in Fig. 1.

DISCUSSION

Schistosomiasis is a serious veterinary problem in the world. A report from Sudan[23] reports a prevalence of nearly 90% in 18-month-old cattle and more than half of the cattle in Zambia are infected.[24] As IgE is essential in elimination of schistosomes by mediating binding of effector cells to the parasite, knowledge about IgE is important in veterinary immunology and immunopathology.

Evolutionary aspects of mammalian development is based on comparison of amino acid or nuclotide sequences from different species.[25–27] We tried to determine the phylogeny of the bovine Cε gene by comparing its deduced amino acid with its counterpart in other mammalian species. It revealed a marked homology to sheep Cε genes as do other bovine immunoglobulin genes such as Cγ,[28–30] Cα[31] and Cμ (personal communication), suggesting a close relationship.

One feature of IgE in all species is the high level of glycosylation.[22] An analysis of the bovine IgE amino acid sequences revealed eight potential glycosylation sites (Asn-X-Thr/Ser), which is less than that of mouse[12] and sheep[16] but more than human[32] and horse IgE.[18] The differences of glycosylation level, between different mammalians raises a question of its effect on the biological properties of IgE.

Cystein and tryptophan residues are highly conserved in immunoglobulin domains among different species[22] and the position of these two amino acids is also well conserved in bovine IgE but the numbers of both cysteine and tryptophan residues are less than that of human, chimpanzee, orangutan and sheep IgE, which again might have bearing on its biological effects.

The mammalian immunoglobulin heavy chain constant region (CH) gene cluster has evolved through multiple gene duplications, resulting in an increasing number of immunoglobulins genes. During the evolution of the Cε gene, two or more non-allelic Cε have been found in man, chimpanzee and gorilla,[33–35] while other mammalian species such as orangutan, gibbons, old world monkeys[36] and sheep[37] have only one ε-gene. A previous report suggested, based on Southern blot analysis of Swiss Brown cow germ-line DNA with *Eco*RI or *Bam*HI, that there may be two Cε genes in the bovine genome.[19] However, concidering our results from Southern blot analysis, this seems unlikely as it is very rare that two genes would show exactly the same size in digestion with two different restriction enzymes and thus coincide on same band. We therefore interpret our data to suggest that there is only one Cε gene in the bovine genome and that the existence of more than one band in some of the samples is caused by allelic RFLP.

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The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence (NCBI) and have been assigned, the accession numbers U63640 and 1575497.

REFERENCES

1. Helm B., Marsh P., Vercelli D., Padlan E., Gould H. & Geha R. (1988) The mast cell binding site on human immunoglobulin E. *Nature* **331,** 180.
2. Capron M., Capron A., Joseph M. & Verwaerde C. (1983) IgE receptors on phagocytic cells and immune response to schistosome infection. *Monogr Allergy* **18,** 33.

3. JARRETT E.E. & MILLER H.R. (1982) Production and activities of IgE in helminth infection. *Progr Allergy* **31,** 178.
4. CAPRON A., DESSAINT J.P., CAPRON M., JOSEPH M., AMEISEN J.C. & TONNEL A.B. (1986) From parasites to allergy: a second receptor for IgE. *Immunol Today* **7,** 15.
5. CAPRON A. & DESSAINT J. (1985) Effector and regulatory mechanisms in immunity to schistosomes: a heuristic view. *Annu Rev Immunol* **3,** 455.
6. ISHIZAKA K., ISHIZAKA T. & HORNBROOK M.M. (1966) Physicochemical properties of reaginic antibody. V. Correlation of reaginic activity with gamma-E-globulin antibody. *J Immunol* **97,** 840.
7. BENJAMIN E. & LESKOWITZ S. (1991) Antibody-mediated type I anaphylactic reactions. In: *Immunology* (eds. E. Benjamin & S. Leskowitz), p. 243, Wiley-Liss, New York.
8. MARTI E., GERBERT H., ESSICH G., OULEHLA J. & LAZARY S. (1991) On the genetic basis of equine allergic diseases. I. Chronic hypersensitivity bronchitis. *Equ Vet J* **23,** 457.
9. ISHIZAKA T. & ISHIZAKA K. (1975) Biology of immunoglobulin E. Molecular basis of reaginic hypersensivity. *Progr Allergy* **19,** 60.
10. SUTER M. & FEY H. (1981) Isolation and characterization of equine IgE. *Z für Veterinarmedicin* **28,** 414.
11. SENO M., KUROKAWA T., ONO Y. *et al.* (1983) Molecular cloning and nucleotid sequencing of human immunoglobulinepsilon chain cDNA. *Nucl Acids Res* **11,** 719.
12. LIU F.T., ALBRANDT K., SUTCLIFFE J.G. & KATZ D.H. (1982) Cloning and nucleotide sequence of mouse nucleotide sequences of immunoglobulins – epsilon chain cDNA. *Proc Nat Acad Sci USA* **79,** 7852.
13. KINDSVOGEL W.R., REDDY E.P., MOORE J.M. & FAUST C.H. (1982) A cloned cDNA probe for rat immunoglobulin epsilon heavy chain: construction, identification and DNA sequence. *DNA* **1,** 335.
14. HELLMAN L., PETTERSSON U. & BENNICH H. (1982) Characterization and molecular cloning of the mRNA for the heavy (epsilon) chain of rat immunoglobulin E. *Proc Nat Acad Sci USA* **79,** 1264.
15. PATEL M., SELINGER D., MARK G.E., HICKEY G.J. & HOLLIS G.F. (1995) Sequence of the dog immunoglobulin alpha and epsilon constant region genes. *Immunogenetics* **41,** 282.
16. ENGWERDA C.R., SANDEMAN R.A., STUART S.J. & SANDEMAN R.M. (1992) Isolation and sequences of sheep immunogobulinE heavy chain complementary DNA. *Vet Immunol Immunopathol* **34,** 115.
17. SAKOYAMA Y., HONG K.J., BYUN S.M. *et al.* (1987) Nucleotide sequences of immunoglobulins – epsilon genes of chimpanzee and orangutan: DNA molcular clock and hominoid evolution. *Proc Nat Acad Sci USA* **84,** 1080.
18. NAVARRO P., BARBIS D.P., ANTCZAK D. & BUTLER J.E. (1995) The complete cDNA and deduced amino acid sequence of equine IgE. *Molec Immunol* **32,** 1.
19. KNIGHT K.L., SUTER M. & BECKER R.S. (1988) Genetic engineering of bovine Ig. Construction and characterization of hapten-binding bovine/murine chimeric IgE IgA, IgG1, IgG2 and IgG3 molecules. *J Immunol* **140,** 3654.
20. ARMOUR K.L., TEMPEST P.R., FAWCETT P.H. *et al.* (1994) Sequence of heavy and light chain variable regions from four bovine immunoglobulins. *Molec Immunol* **31,** 1369.
21. OLSSON P.G., RABBANI H., HAMMARSTRÖM L. & SMITH C.I.E. (1993) Novel human immunoglobulin heavy chain constant region gene deletion haplotypes characterized by pulsed-field electrophoresis. *Clin Exp Immunol* **94,** 84.
22. LIU F.T. (1986) Gene expression and structure immunoglobulin epsilon chains. *Crit Rev Immunol* **6,** 47.
23. MAJID A., MARSHALL T.D.C., HUSSEIN M. *et al.* (1980) Observations on cattle schistosomiasis in the Sudan. I Epiziootiologic observation in the White Nile Province. *Am J Trop Med Hyg* **29,** 435.
24. DE BONT J., VERCRUYSSE J., SOUTHGATE V.R., ROLLINSON D. & KAUKAS A. (1994) Cattle schistosomiasis in Zambia. *J Helminthol* **68,** 295.
25. ANDREWS P. & CRONIN J.E. (1982) The relationships of *Sivapithecus* and *Ramapithecus* and the evolution of the orangutan. *Nature* **297,** 541.
28. CLARKSON C.A., BEALE D., COADWELL J.W. & SYMONS D.B. (1993) Sequences of ovine Ig gamma 2 constant region heavy chain cDNA and molecular modelling of ruminant IgG isotypes. *Molec Immunol* **30,** 1195.
29. DUFOUR V., MALINGE S. & NAU F. (1996) The sheep Ig variable region repertoire consists of a single VH family. *J Immunol* **156,** 2163.
30. RABBANI H., BROWN W.R., BUTLER J.E. & HAMMARSTRÖM L. (1997) Genetic polymorphism of the IgGHG3 gene in cattle. *Immunogenetics* **46,** 326.
31. BROWN R.W., RABBANI H., BUTLER J.E. & HAMMARSTRÖM L. (1997) Characterisation of the bovine Cα gene. *Immunology* **91,** 1.
32. MAX E.E., BATTEY J., NEY R., KIRSCH I.R. & LEDER P. (1982) Duplication and deletion in the human immunoglobulin epsilon genes. *Cell* **29,** 691.
33. FLANAGAN J.G. & RABBITTS T.H. (1982) The sequences of a human immunoglobulin epsilon heavy chain constant region gene and evidence for three non-alleic genes. *EMBO J* **1,** 655.
34. KAWAMURA S., OMOTO K. & UEDA S. (1989) Nucleotid sequences of the gorilla immunoglobulin alpha 1 gene. *Nucl Acids Res* **17,** 6732.
35. UEDA S., MATSUDA F. & HONJO T. (1988) Multiple recombinational events in primate immunoglobulin epsilon and alpha genes suggest closer relationship of humans to cheempanzee than to gorillas. *J Molec Evol* **27,** 77.
36. UEDA S., TAKENAKA O. & HONJO T. (1985) A truncated immunoglobulin epsilon pesedogene is found in gorilla and man but not in chimpanzee. *Proc Nat Acad Sci USA* **82,** 3712.
37. CLARKE R.A. & BEH K.J. (1993) Organisation of the ovine immunoglobulin C-epsilon gene locus: evidence for a deletion 5′ of the gene. *Vet Immunol Immunopathol* **39,** 381.