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Study of human allergic milk whey protein from different mammalian species using computational method

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Abstract:
Nowadays, safety and quality assessment of food used for human consumption have to consider by its possible contribution to the maintenance or improvement of the consumer's health. Milk is an important food with many nutrients. Cow milk is an important source of energy, protein, vitamins and minerals for the growing child as well as adults. But, numerous cow milk proteins have been implicated in allergic responses and most of these have been shown to contain multiple allergic epitopes. The present study disclosed best alternatives to cow milk, which are not allergic and as good as cow milk in nutritional value. The *in silico* analysis of casein (*alpha* s1, *alpha* s2, *beta* and *kappa*) and *beta*-lactoglobulin, unveils that sheep milk is a more suitable alternate to cow milk for allergic infants and buffalo milk for allergic adult humans.

Keywords: Casein, *beta*-lactoglobulin, Multiple Sequence Alignment, Neighbour joining method

Background:
Milk is a white liquid produced by the mammary glands of mammals and is an important food with many nutrients. The cow (Bos taurus), domestic goat (Capra hircus), Domestic sheep (ovis aries) and Water buffalo (Bubalus bubalis) plays important role as milk producers **[1].** The best nutritional option for newborn infants is mother's milk; however some infants may not be exclusively breast fed during the first month of life. In that case, another substitute must be provided for cow milk. This substitution results in an allergic disease known as cow milk protein allergy (CMPA) in 2-6% of children **[2].** Nowadays, most common alternatives are soy and extensively hydrolyzed formulae. However, there is evidence that 10-20% of children allergic to cow milk do not tolerate soy derivatives **[3-5],** and some cases of high immunological reaction to extensively hydrolyzed formulae have been reported **[6-8].** Allergies to cow milk are often broadly classified into immunoglobulin E (IgE)-mediated allergy and non-IgE-mediated allergy **[9].** Cow's milk contain more than 25 different proteins, but only the whey proteins *alpha*-lactalbumin, *beta*-lactoglobulin, BSA and lactoferrin as well as 4 caseins, have been identified as allergens **[10].** The casein fraction is composed of alpha s1, alpha s2, beta and kappa-casein, of which alpha-s1 casein seems to be a major allergen according to IgE and T-cell recognition data **[11-14].**

In developed countries there is increasing interest in goat milk and its derivatives, the quality of which is considered of special importance in the light of current tendencies favoring healthy eating. In particular, the composition of goat milk is said to have certain advantages over that of cow milk, and thus former is preferable for some consumers **[15].** In one case Scientific and clinical studies also suggest that infants and children who are sensitive to cow milk based product often thrieve better when goat milk based product is substituted **[16].** In order french extensive clinical studies with children allergic to cow milk, the treatment with goat milk produced positive results in 93% of the children and was recommended as a valuable aid in child nutrition because of less allergenicity and better digestibility than cow milk **[17].** But in some cases goat milk also proved to be allergenic and intolerant for infants **[18].** In some childrens

and adult humans,water buffalo milk show more tolerance than cow milk. Repeat skin testing was performed at age 2 years of children and continued to be positive for cow milk but children found to be negative for skin prick tested to water buffalo milk based yogurt **[19].** On these bases, selection of suitable milk for consumption is an important target for nutritionist and pediatricians. Therefore, the present study leads to evaluate the suitability of buffalo and sheep milk for nutrition to the people and children allergic to cow milk by study of amino acid composition, secondary structure, CLUSTALW2 analysis and phylogenetic relationship of Cow, Goat, Sheep and Buffalo milk whey proteins.

Methodology:

Dataset

Search for some milk whey protein was made in GenBank and EMBL databases. Obtained protein sequences filtration was done to remove any redundant sequences from the population and cross-checked by UniProtKB data base. The sequences are available at NCBI (http://www.ncbi.nlm.nih.gov/protein) with accession no. as given in **Table 1 (see supplementary material).** The dataset consists of casein protein (α-s1, α-s2, β and κ) and β-lactoglobulin.

Sequence analysis

The ProtParam tool of ExPASy (http://www.expasy.ch/) was used to analyze amino acid and atomic composition **[20].** Further isoelectric point, Extinction coefficient and hydropathicitywas observed and compared between four species.Secondary structure predictions was done using SABLE (http://www.sable.cchmc.org) of obtained sequence in dataset.

Multiple Sequence Alignment

CLUSTALW2 at European Bioinformatics Institute (www.ebi.ac.uk/tools/msa/clustalw2/) was used for protein multiple sequence alignment (MSA) using standard parameters.

Phylogeny tree construction

The Phylip tool (http://bioweb.pasteur fr/phylogeny/intro.en.html) was used to construct phylogenetic trees from aligned sequences. Computation of distance was done by program Protdist, which constructs distance matrices by a process called "Bootstrapping". A rooted tree was plotted using NJ plot software package **[21]** and distance matrix was calculated between each selected species **[22].**

Discussion:

Sequence analysis

Number of amino acid residue in selected milk whey protein in each species given in **Table 2 (see supplementary material).** These proteins also share considerable similarity in their percentage content of amino acids as given in **Table 3-7 (see supplementary material).** There was considerable similarity in predicted protein secondary structure of *Capra hircus* and *Ovis aries* as shown in **Figure 1 (A-T).** At the same time there were some similarities as well as some differences in predicted protein secondary structure of *Bos Taurus* and *Bubalus bubalis* as shown in **Figure 1(A-T).**

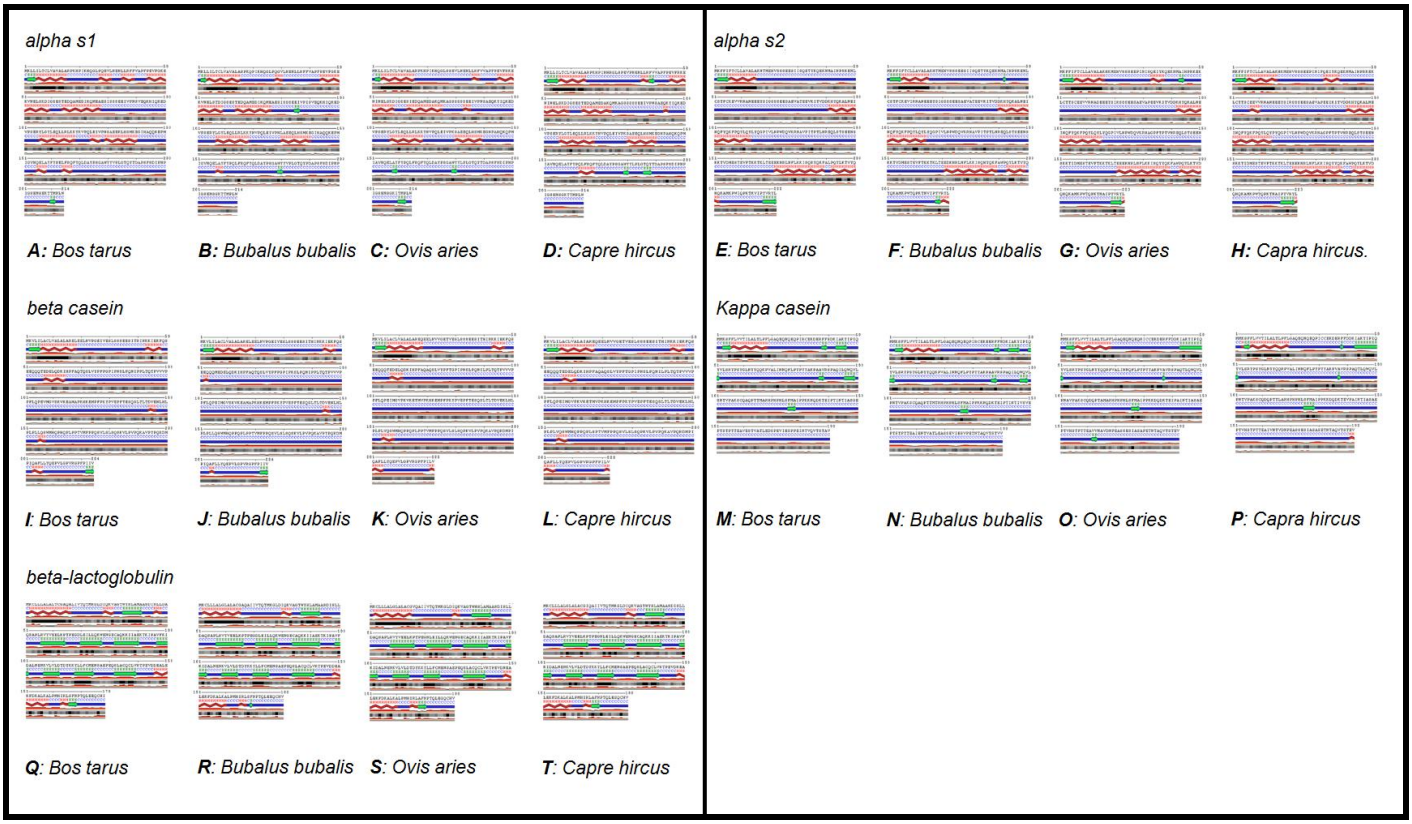

Figure 1: Predicted secondary structure in different species.

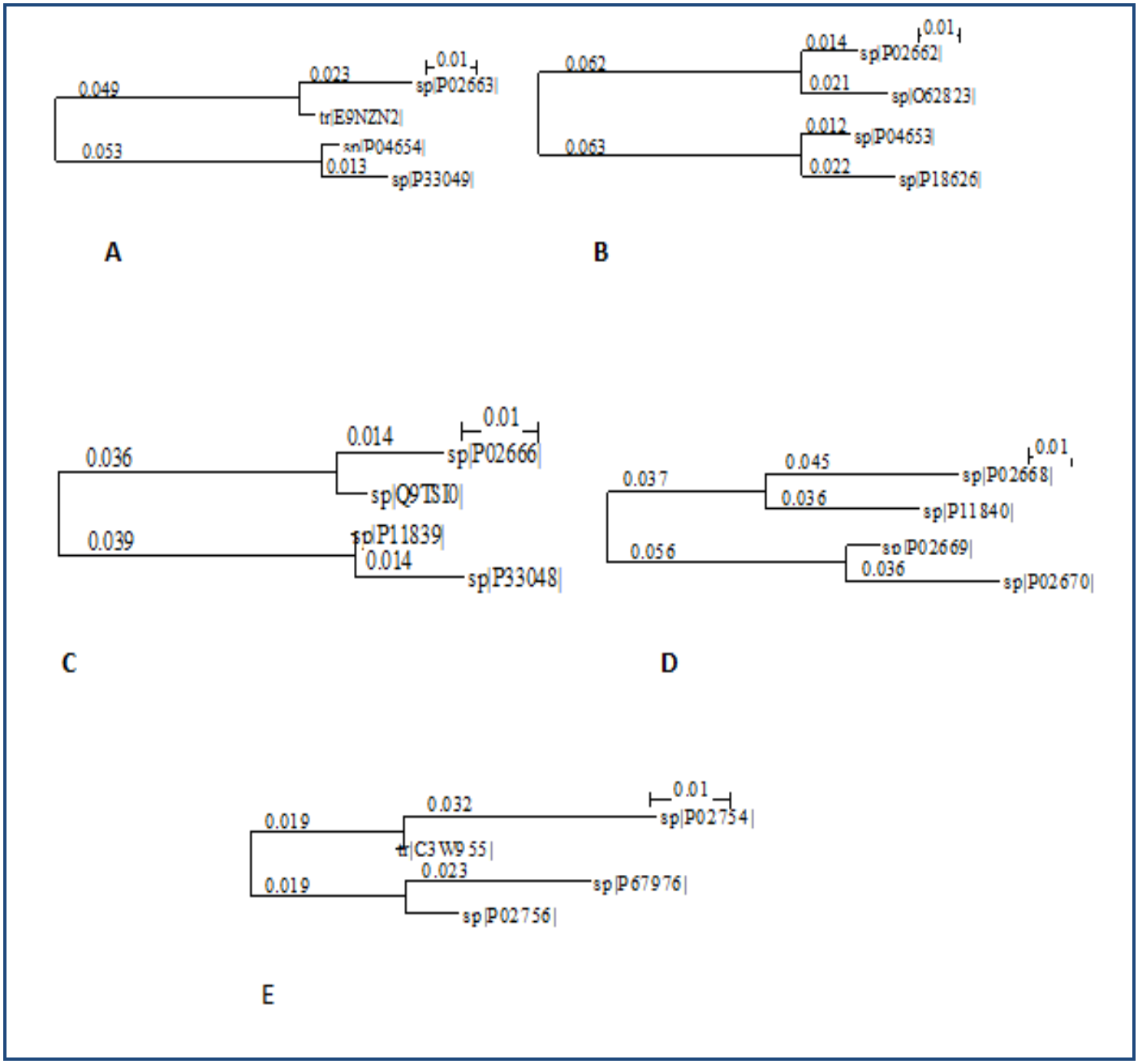

Figure 2: A) casein *alpha* sl**; B)** casein *alpha* s2**; C)** casein *beta***; D)** casein *kappa***; E)** *beta*-lactoglobulin

Multiple sequence Alignment

The MSA of protein sequences obtained from CLUSTALW2 including the family of candidate protein sequences, show the maximum pairwise similarity in beta-casein group (95%) between goat and sheep. On the other hand minimum pair wise similarity shown in kappa casein (85%) between cow - goat and cow- sheep. The most common and generally more accessible approach to protein function prediction is 'inheritance through homology'- that is, the knowledge that proteins with similar sequences frequently carry out same function **[23].** For a majority of proteins it is already posible to predict their approximate function with resonable accuracy based on their evolutionary relationship or sequence similarity to proteins with known functions **[24-26].** Since the whey protein from goat and sheep share a great similarity this supports functional similarity between goat and sheep milk. At the same time buffalo shows great similarity with cow at sequence level but some dissimilarities at secondary structure level makes it less allergenic. The reason behind this may be absence of allergenic epitopes but it should be analyzed further *in vitro*.

Phylogenetic tree construction

The result of phylogenetic tree analysis shown that for casein and beta-lactoglobulin each subgroup is divided in to two sub groups containing two species (*Bos Taurus, Bubalus bubalis*) in one group and two species (*Capra hircus, Ovis aries*) in another group is shown in **Figure 2 (A-E)**. Distance matrix for each protein groups in between four different species was calculated as given in **Table 8-12 (see Supplementary material).** Calculated distance matrix evaluates close relationship between goat-sheep and cow-buffalo.

Conclusion:
In case of cow milk allergic infants, since milk whey proteins of sheep and goat are almost identical , hence sheep milk is suitable and acceptable substitute of goat milk. In cow milk allergic childrens and adult humans buffalo milk is a good substitute of cow milk without any change in nutritional quality.

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Supplementary material:

Table 1: Selected proteins for comparison analysis

Milk whey protein	*Bos Taurus*	*Capra hircus*	*Ovis aries*	*Bubalus bubalis*
	Genbank Accession no.			
α-s1 casein	P02662	P18626	P04653	O62823
α-s2 casein	P02663	P33049	P04654	E9NZN2
β casein	P02666	P33048	P11839	Q9TSI0
K casein	P02668	P02670	P02669	P11840
β -lactoglobulin	P02754	P02756	P67976	C3W955

Table 2: No of amino acid in selected proteins

Milk whey protein	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
	No. of amino acid			
α-s1 casein	214	214	214	214
α-s2 casein	222	223	223	222
β casein	224	222	222	224
K casein	190	192	192	190
β -lactoglobulin	178	180	180	180

Table 3 & 4: Amino acid composition of casein alpha s1 and s2 protein

Alpha s1	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***	**Alpha s2**	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
Ala	5.0%	5.4%	5.4%	5.0%	Ala	5.6%	7.0%	7.0%	5.6%
Arg	2.7%	3.1%	3.1%	2.3%	Arg	2.8%	3.3%	2.8%	2.3%
Asn	6.3%	5.8%	5.4%	5.9%	Asn	3.7%	5.1%	4.7%	3.7%
Asp	1.8%	2.2%	2.7%	2.3%	Asp	3.3%	3.3%	3.3%	3.3%
Cys	1.4%	1.3%	1.3%	1.4%	Cys	0.5%	0.5	0.5%	0.5%
Gln	7.2%	7.2%	7.2%	7.2%	Gln	6.5%	6.5%	7.0%	7.9%
Glu	10.8%	11.2%	11.2%	10.4%	Glu	11.7%	9.3%	9.3%	10.3%
Gly	0.9%	0.9%	0.9%	0.9%	Gly	4.2%	4.2%	4.2%	5.1%
His	1.4%	2.2%	2.2%	2.3%	His	2.3%	1.9%	1.9%	1.9%
Ile	5.4%	5.8%	5.4%	5.4%	Ile	5.6%	4.7%	5.6%	6.1%
Leu	7.2%	5.8%	6.3%	6.8%	Leu	10.3%	10.3%	10.7%	10.7%
Lys	11.3%	11.2%	11.2%	10.8%	Lys	7.0%	6.5%	7.0%	6.1%
Met	2.3%	2.2%	2.2%	2.3%	Met	2.8%	2.8%	2.8%	2.8%
Phe	4.1%	4.5%	4.0%	4.1%	Phe	3.7%	3.3%	3.3%	3.7%
Pro	4.5%	5.8%	5.4%	4.5%	Pro	7.9%	8.9%	7.9%	8.4%
Ser	7.7%	6.3%	6.7%	7.7%	Ser	7.5%	8.4%	8.9%	7.0%
Thr	7.2%	6.7%	6.7%	7.7%	Thr	2.8%	2.8%	1.9%	2.8%
Trp	0.9%	1.3%	1.3%	1.4%	Trp	0.9%	0.9%	0.9%	0.9%
Tyr	5.4%	5.4%	5.4%	5.9%	Tyr	4.7%	5.1%	5.1%	4.7%
Val	6.8%	5.4%	5.8%	6.3%	Val	6.1%	5.1%	5.1%	6.1%

Table 5 & 6: Amino acid composition of casein beta and kappa protein

Beta-lactoglobulin	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
Ala	10.7%	11.1%	11.1%	11.1%
Arg	1.7%	1.7%	1.7%	1.7%
Asn	2.8%	3.3%	3.9%	2.8%
Asp	5.6%	4.4%	4.4%	5.6%
Cys	3.9%	3.9%	3.9%	3.9%
Gln	5.6%	5.6%	5.6%	5.6%
Glu	9.0%	8.3%	8.3%	8.9%
Gly	2.8%	3.9%	3.9%	3.3%
His	1.1%	1.1%	1.7%	1.1%
Ile	5.6%	6.1%	5.6%	5.6%
Leu	15.2%	15.0%	15.0%	15.0%
Lys	9.0%	9.4%	8.9%	8.9%
Met	2.8%	2.8%	2.8%	2.8%

Phe	2.2%	2.2%	2.2%	2.2%
Pro	4.5%	4.4%	4.4%	4.4%
Ser	3.9%	3.3%	3.3%	3.9%
Thr	5.1%	4.4%	4.4%	4.4%
Trp	1.1%	1.1%	1.1%	1.1%
Tyr	2.2%	2.2%	1.7%	2.2%
Val	5.1%	5.6%	6.1%	5.6%

Table 7: Amino acid composition of casein Beta-lactoglobulin protein

Beta-actoglobulin	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
Ala	10.7%	11.1%	11.1%	11.1%
Arg	1.7%	1.7%	1.7%	1.7%
Asn	2.8%	3.3%	3.9%	2.8%
Asp	5.6%	4.4%	4.4%	5.6%
Cys	3.9%	3.9%	3.9%	3.9%
Gln	5.6%	5.6%	5.6%	5.6%
Glu	9.0%	8.3%	8.3%	8.9%
Gly	2.8%	3.9%	3.9%	3.3%
His	1.1%	1.1%	1.7%	1.1%
Ile	5.6%	6.1%	5.6%	5.6%
Leu	15.2%	15.0%	15.0%	15.0%
Lys	9.0%	9.4%	8.9%	8.9%
Met	2.8%	2.8%	2.8%	2.8%
Phe	2.2%	2.2%	2.2%	2.2%
Pro	4.5%	4.4%	4.4%	4.4%
Ser	3.9%	3.3%	3.3%	3.9%
Thr	5.1%	4.4%	4.4%	4.4%
Trp	1.1%	1.1%	1.1%	1.1%
Tyr	2.2%	2.2%	1.7%	2.2%
Val	5.1%	5.6%	6.1%	5.6%

Table 8: Distance matrix of four species in casein alpha s1

Casein alpha s1	*Bos Taurus*	*Capra hircus*	*Ovis aries*	*Bubalus bubalis*
Bos Taurus	0	0.161	0.151	0.035
Capra hircus	0.161	0	0.034	0.168
Ovis aries	0.151	0.034	0	0.158
Bubalus bubalis	0.035	0.168	0.158	0

Table 9: Distance matrix of four species in casein alpha s2

Casein alpha s2	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
Bos Taurus	0	0.138	0.125	0.023
Capra hircus	0.138	0	0.013	0.115
Ovis aries	0.125	0.013	0	0.102
Bubalus bubalis	0.023	0.115	0.102	0

Table 10: Distance matrix of four species in casein Casein Beta Casein Kappa

Casein Beta	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
Bos Taurus	0	0.103	0.089	0.014
Capra hircus	0.103	0	0.014	0.089
Ovis aries	0.089	0.014	0	0.075
Bubalus bubalis	0.014	0.089	0.075	0

Table 11: Distance matrix of four species in casein Casein Kappa

Casein Kappa	***Bos Taurus***	***Capra hircus***	***Ovis aries***	***Bubalus bubalis***
Bos Taurus	0	0.174	0.138	0.081
Capra hircus	0.174	0	0.036	0.165
Ovis aries	0.138	0.036	0	0.129
Bubalus bubalis	0.081	0.165	0.129	0

Table 12: Distance matrix of four species in casein Casein Beta- lactoglobulin

Casein Beta- lactoglobulin	*Bos Taurus*	*Capra hircus*	*Ovis aries*	*Bubalus bubalis*
Bos Taurus	0	0.070	0.093	0.032
Capra hircus	0.070	0	0.023	0.038
Ovis aries	0.093	0.023	0	0.061
Bubalus bubalis	0.032	0.038	0.061	0