PARASITIC PROTOZOANS IN LIVESTOCK AND PETS IN ESTONIA. REVIEW

Brian Lassen, Heli Talvik
Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia,
Tel. (+372) 5288411, e-mail: brian.lassen@gmail.com

Summary. Valuable scientific knowledge on parasitic protozoans in Estonian livestock and companion animals is currently disappearing and has been unavailable to the international scientific community due to location or language barriers. Extracts of relevant publications from MEDLINE, AGRIS, the library of the Estonian University of Life Sciences, and largely personal collections, in English, Estonian and Russian, have been summarized for this review. Prevalence studies of *Eimeria*, *Sarcocystis*, *Babesia,* and *Neospora* were discovered in cattle, together with smaller investigations of *Cryptosporidium*. Pigs and sheep have been studied for coccidia, while chicken were only examined for *Eimeria*, and included the possible discovery of the species of *E. sporadica*. Cattle and chicken were subjects of drug trials in attempts of controlling *Sarcocystis* and *Eimeria*. Primary hosts such as dogs have been studied for *Sarcocystis*, *Cystoisospora* and *Toxoplasma*, while cats have only been in focus for the latter parasite. Though human and animal infections indicate that several of the parasites are currently well established, and some increasing, the previous investigations do not indicate any serious actions taken to limit the parasites.

Key words: *Sarcocystis, Eimeria, Cryptosporidium, Isospora, Toxoplasma*, Estonia.

PARAZITINIŲ PIRMUONIŲ PAPLITIMAS TARP ESTIJOS GYVŪNŲ. LITERATŪROS APŽVALGA

Brian Lassen, Heli Talvik
Estijos gyvybės mokslų universitetas, Kreutzwaldi 62, 51014 Tartu, Estija
tel. (+372) 528 84 11, el. paštas: brian.lassen@gmail.com

Santrauka. Praeito amžiaus antrojoje pusėje dėl politinės situacijos Estijoje mokslinės publikacijos tarptautiniuose žurnaluose apie parazitinių pirmuonių paplitimą tarp žemės ūkio ir naminių gyvūnų tapo negalimos ir buvo skelbiamos estų bei rusų kalbomis tarybiniuose žurnaluose**.** Taigi nusprendėme pasinaudoti interneto šaltiniais – MEDLINE, AGRIS, taip pat Estijos gyvybės mokslų universiteto bibliotekoje esančiais fondais bei asmeninėmis bibliotekomis ir paruošti šią literatūros apžvalgą. Buvo tirtas tarp Estijos galvijų parazitinių pirmuonių *Eimeria*, *Sarcocystis*, *Babesia ir Neospora,* iš dalies – *Cryptosporidium* paplitimas. Kokcidijų paplitimas buvo tirtas tarp kiaulių ir avių, o eimerijų – tarp viščiukų, įskaitant galimai naują rūšį, pavadintą *E. sporadica*. Be to, buvo atlikti bandymai tiriant vaistų poveikį galvijus ir vištas gydant nuo sarkocistų ir eimerijų. *Sarcocystis*, *Cystoisospora* ir *Toxoplasma* paplitimas buvo tirtas tarp šunų ir tik pastaruoju metu – tarp kačių. Mūsų atlikta analizė parodė, kad kai kurių žmonių ir gyvūnų užkrečiamųjų ligų vis daugėja. Prieita prie išvados, kad anksčiau atlikti moksliniai tyrimai nepadėjo Estijoje sumažinti pavojingų pirmuonių sukeliamų parazitinių ligų paplitimo.

Raktažodžiai: *Sarcocystis, Eimeria, Cryptosporidium, Isospora, Toxoplasma*, Estija.

Introduction. Protozoan parasites span seveal thousand species and are known to cause production losses in animal husbandry (Daugschies and Najdrowski, 2005; Chi *et al*., 2002; Wyss *et al.,* 2000). Estonian meat and dairy products represent 48% of the total food production in the country, making these parasites of economic importance in livestock industry (Ministry of Agriculture, 2007). Care and expenditure on pets, including parasite control, have increased tremendously over the past decade in Estonia. The impacts of parasitological infections on health and productivity of livestock animals and possible zoonotic rolesare in lack of accessible documentation and contemporary studies.

From the mid to the end of the last century, Estonian veterinary scientists were hindered in accessing and publishing in international journals. As a result, the research papers were most often submitted to periodicals in Estonian and Russian. The language barriers and isolated publication channels have kept a long period of research obscured to the international community. The original work of Estonian veterinarians is disappearing together with the original authors who often are in possession of the last articles accessible.

Inquires in MEDLINE, AGRIS, and library database of the Estonian University of Life Sciences (former Estonian Agricultural University and erstwhile Estonian Agricultural Academy) did not reveal many hits on Estonian authors in parasitology or keywords related to parasitic protozoans in Estonia. Relevant literature was however discovered in the library of the university by manual search for specific titles, not listed in the database. Far better results were achieved by using the network of local scientists. Individual archives and personal knowledge on the publications turned to be the most effective method and gave the largest number of relevant references. The accumulated and summarized material is presented in this review arranged by parasite species.

BABESIA spp.

The epidemiology, mortality, and treatment of *Babesia divergens* (syn. *B. bovis*) was studied in 1966-1968 by

Jaan Praks (1969). His investigations included both, experiments, observations and the use of submitted data from the national Registry of the Estonian Soviet Socialist Republic (ESSR). By studying the parasites location in 2000 erythrocytes per blood smear using the Romanovski-Gimza staining method Praks identified 23 out of 162 farms positive for *B. divergens*. The farms were located in 6 of 15 National districts. By pooling the retrospective data from the years 1963-1968, with Praks own prevalence studies, the number of districts with reported cases of clinical Babesiosis has raised up to 13 during the same period. According to the National Registry data from 1960-1968 a total of 16 133 cases of babesiosis had been reported, with a 4.89% mortality rate. On national level, 0.36% of all cattle were estimated to have had the clinical disease, with case reports peaking in June, while mortality was highest in October and November. Only South Eastern Estonia was free from clinical outbreaks of Babesiosis in this particular period.

To test the efficiency of Haemosporidin, Acaprin, and Azidin on in cattle, a trial was performed using a herd of 93 cattle infected with *B. divergens* and 372 controls (Praks, 1969). A group of infected cattle was, further split into three subgroups and given single treatment with Haemosporidin, Acaprin, and Azidin, at the dose rate of 0.5 mg/kg, 1.0 mg/kg and 3.5 mg/kg of body weight, respectively. Azidin was reported as being the most efficient in the control of clinical Babesiosis caused by *Babesia divergens*.

The annual variations in the recorded cases between 1960-1968 of bovine Babesiosis were observed by Praks (1969), but without finding tendencies of an increase or decrease. Interestingly, recent reports on tick transmitted human infections show a dramatic increase in tick-born encephalitis and Lyme borreliosis in Baltic residents and especially in the border country Latvia (Randolph, 2008; Bormane *et al*., 2004). As it was postulated, the peak in human infection presumably was influenced by the socio-economic upheaval following the collapse of the Soviet system in East European countries including Estonia (Sumilo *et al.,* 2008).

Human babesiosis has been diagnosed in Europe since 1957 (Gorenflot *et al.*, 1998), and is currently under further investigation in Estonia (Estonian Research Portal, current ETF project 6938, www.etis.ee).

CRYPTOSPORIDIUM

Cattle

First description of *Cryptosporidium* in bovines was a case study of a farm experiencing acute gastro-enteritis and mortalities of calves (Talvik and Daughschies, 2004). Bloody stool was observed in two of the stables housing 2-4 month and 4-6 month old animals respectively. Faecal examinations detected *Cryptosporidium spp*., *E. zuernii,* and *E. ellipsoidalis* oocysts in the younger animals, and additionally *E. bovis* and *E. pellita* in the older ones. Rota virus tests were negative, and the likely main pathogenic cause of the problem may have been *Eimeria*, perhaps as a result of compromised immunity due to low quality feed. Ongoing investigations back up the first findings in that *Eimeria* and *Cryptosporidium* are often found together, and are found in almost all farms (Lassen *et al.,* 2008). However, the investigated farms rarely indicate having detected parasitic diseases previously, and almost none coccidia, but do observe diarrhoea problems in calves periodically. Only very recently samples from diarrhoea cases have been sent to diagnostics for coccidia, but at a modest pace and mostly driven by initiatives of medical companies. Coccidian samples investigated indicate that they do cause problems in Estonia, but require higher awareness before cattle health will benefit from the existing knowledge of their presence and the resulting production losses. Occasional infections of veterinarians and scientists Estonian cattle farms occurs questing. The zoonotic aspects of this parasite however has caught even less attention than the bovine situation, and it is likely that every person handling the animals will be infected at one point.

Pigs

Cryptosporidium has been noted in general parasitological investigations (Järvis and Mägi, 2008), and to be more frequent in organic farming than industrial enterprises (Lassen *et al.,* 2007). The outspread and impact of this protozoan on productivity of pigs is not investigated in more detail.

EIMERIA

Cattle

The first investigations of bovine eimeriosis were carried out on selected farms by Parre and Praks who were the first to determine *Eimeria* species and their sporulation time in Estonia (Parre and Praks, 1974; Praks and Parre, 1973). Samples collected in 1971 from clinically sick calves in two farms in Pärnumaa (n=23) and Raplamaa (n=32) listed five species of bovine *Eimeria* which were identified by sporulated morphology: *E. auburnensis* (7% and 3 %)*, E. bovis* (26% and 71%)*, E. ellipsoidalis* (15% and 10 %)*, E. subspherica* (11% and 4 %)*,* and *E. zuernii* (41% and 12 %). Calves found with five (70% and 82%) or four species (17% and 9%) at the same time were most common. Only 9% in both farms shed three species. Double infections were only seen in 4% of the farms in Pärnumaa, and single infections were not observed. All the infected calves were found with both of the most pathogenic species: *E. zuernii* and *E. bovis*. The sporulation periods, defined as the time for half of the oocysts in 2% potassium dichromate solution to sporulate, were recorded to take 2-3 days for all species, with the exception of 5 days for *E. subspherica.* In conclusion, these presumably first publications on *Eimeria* in Estonia emphasize that similar situations in other native farms may be expected and that more than the identified species may be present. However, the third investigation by Praks and Parre (1974), to elucidate the spread of *Eimeria* on Estonian farms, confirmed the same 5 species found in 1971 in three farms without clinical cases of eimeriosis and good hygiene standards. A small deviation was the absence of *E. subspherica* in one farm. The study stratified the sampling on 3-4 month old calves, 1-1.5 year old heifers, and cows. Heifers and calves had *Eimeria* oocysts in 87% of samples, while 27% of the cows were positive. The authors gave a conclusive warning to less ideal farms when reporting the pathogenic *E. bovis* and *E. zuernii* were found in all age groups in all farms.

A full national investigation of the parasite was undertaken mare than 10 years later by Karis (1987). In total 3,372 faecal samples from all counties were collected from 5 different age classes. All farms investigated had animals shedding oocysts, and the sample prevalence was calculated to 35.3%. The age distribution of positive samples was: <1 month, 10.1%; 1-2 months, 42.7% ; 2-4 months, 58.3%; 4-6 months, 29.5%; and >1 year, 12.0 %. The species identified in the samples were identical to those found by Parre and Paraks: *E. ellipsoidalis* (49.8%), *E. bovis* (30,8%), *E. auburnensis* (11.6%), *E. zuernii* (6.5%), *E. subspherica* (1.3%). The difference to the previous findings was that neither *E. bovis* nor *E. zuernii* dominated as was previously observed, what would be expected from the two most pathogenic species. It is curious why in three reports the same five species were mentioned, as if only these were present at that time. Karis carried out an extensive experiment with the prophylaxis treatments using available drugs and concluded Rigecoccin-25 the best option. Infection studies were also included observing the contents of calf blood and weight gain when infected with *Eimeria*. Calves infected with 400 000 oocysts (90% *E. bovis*, 9% *E. zuernii*, and 1% other) required 2-3 times the feed to gain a kilo over 28 days compared to controls. Simultaneously, digestibility of food components was reduced by 4.2-5.6%. Leukocytes and urea increased, as potassium, calcium, and phosphate decreased in calves when fed a dose of 300 000 oocysts. The same animals were diagnosed with metabolic acidosis 21 days post infection. The infection doses in these trials were quite high compared to other experiments investigating distortion of weight gains by doses of 100 000-250 000 *E. bovis* oocysts (Mundt *et al*., 2003; Heath *et al.,* 1997). The effect measured by Karis, 1987 is comparable to the study when 250 000 of *E. bovis* oocysts were fed to the calves with 40-50% reduction in feed uptake after 4 weeks (Sartin *et al.,* 2000). However, dose dependence of oocysts on weight loss and feed uptake is not well documented for cattle. The method of analysis in Estonian parasitology labs traditionally was non-quantitative simple flotation, and probably for this reason none of the studies reported infection intensities. It is therefore difficult to estimate the extent of eimeriosis in Estonian farms previously. The current situation is still much the same as previously reported (personal communication). More species have established in the herds and larger portions of older animals have become infected regardless of dramatic modernisation in production forms since 2000 onwards (Veermäe *et al.,* 2001). Eimeriosis is currently a problem in Estonian cattle production, and though the most thoroughly investigated of the protozoan diseases, has yet to succeed in catching the attention of the animal keepers.

Pigs

Post mortem investigation of 1 172 pigs between 1968 and 1977, diagnosed *E. deblicki* in 27 of the cases (Ridala *et al.,* 1981). Gastro-enteritis accompanied by pneumonia from side infections was the most common cause of the death. Opportunistic pathogens were speculated to be results of reduced immunity in animals fed poor quality food. More recent studies report prevalence from different production systems (Järvis and Mägi, 2008; Lassen *et al.,* 2007). Low numbers of animals with *Eimeria* were found in industrial farms (<10%), while conventional and small farms had infection levels at 58% and 44%. Pigs on ecological and wild boar farms had almost all *Eimeria* oocysts, which can be associated with the increased risk of animals having access to pasture continuously or at periods (Roepstorff *et al.,* 1998). In total 9.9% of the piglets and 22.3% of sows and fatteners shed the oocysts placing the Estonian prevalences for these age groups above the Nordic prevalence for older animals, and lower for piglets (Roepstorff *et al.,* 1998). No oocysts of *Isospora spp.* were found in the study, which may be due to the limited sensitivity of the McMaster method used to detect the transparent oocysts. The parasite can be found in most farms since Laaneoja (2004) previously identified *Isospora spp.* on 12 out of 14 farms from 2-3 old week piglets. Järvis and Mägi have reported infection intensities between 220-1 000 oocysts per gram (opg) faeces, but in routine diagnostics up to 100 000 opg can be observed (data not shown). So far *E. porci, E. polita, E. suis, E. neodebliecki, E. scrofae*, and *E. guevarai* have been morphologically identified. Research of coccidia in Estonian pig production is ongoing, but awareness towards the parasites is modest. Organic farming in Estonia has increased more than 13-fold since 1999, including pig production (Ministry of Agriculture, 2007). The parasite burden in these groups is normally higher, due to outdoor access and restrictions on treatment, and parasites are likely to be of increasing importance in the future (Eijck and Borgsteede, 2005).

Sheep

A seven year study of older sheep from 8 farms detected *Eimeria spp.* oocysts in 25% of the breeding ewes grazing with lambs (Kaarma and Mägi, 2002). Half of the young grazing lambs, ageing between 1-4 months, shed oocysts. Out of 1950 lambs in this study up to the age of 7 months, 5% developed clinical signs of eimeriosis. Though sheep and goats are not strongly represented in Estonian livestock they are susceptible to coccidia much more likely than cattle (Foreyt, 1990).

Poultry

During the 1948-1950ies, Estonian chicken farms experienced dramatic 50-80% mortality rates caused by *E. tenella* (Plaan, 1951). Iodine, milk, phenothiazine, and DDT (Dichloro-Diphenyl-Trichloroethane) failed in controlling the outbreaks, but sulphonamide trails showed good results. Cage bottoms made of net wiring proved most efficient as a method of reducing the spread of *Eimeria* in the long term. Commonly *E. maxima, E. mitis, E. acervulina*, and *E. necatrix* were discovered. Plaan described an oocyst found in a 5 week old chicken that did not exactly fit any existing morphologies. He named the new species *E. sporadica*. A summary of his description include an egg-shaped (22.1 x 22.2 μm) oocyst with polar granule and sporulation within 48 hours. It was found in the middle part of the intestine where it caused haemorrhagic inflammation and bleeding. *Eimeria sporadica* has not since been identified elsewhere to our knowledge, and the farm on which it was detected has long since been destroyed. Plaans description of *E. sporadica* also lies within the definition of *E. necatrix* (Levine, 1970), and probably needs to be rediscovered to be internationally accepted as a separate species.

In the 70'is Parre and colleagues picked up on *Eimeria* in poultry and in particular the immunological aspects. Chicken experiments with *E. tenella* infections had an outcome of lessened mitotic activity and increased numbers of degenerative cell form in the thymus (Parre *et al.,* 1977). Later immunization trials of 11 day old chick embryos were attempted by injecting *E. tenella* sporozioites included adding specific immune sera and immunoglobulins from an immunized chicken (Parre and Suigusaar 1977). The chorioallantoic membranes of the embryos that received antibodies from a donor chicken had less colonies of sporozoite lesions and additionally produced antibodies against the parasite later in life. In another vaccine trial Parre and Olkonen (1977) administered single low doses of sporulated *E. tenella, E. acervulina*, and *E. brunetti* oocysts to 10 day old chicks with a 2 week interval. Two weeks after the last dose the chicken were challenged with either $1x10^6$ *E. tenella,* $5x10^6$ *E. acervulin*a, or $2x10^6$ *E. brunetti* oocysts. The best vaccination properties were recorded for both chicken kept in battery cages and on deep litter when fed a combination of 1 000 *E. tenella,* 5 000 *E. acervulina*, and 3 000 *E. brunetti* sporozoites. The effect could be further enhanced when administering 0.0125% Zoalene to the feed. Judging from these publications, these trials were among the first ones and similar trails continue today, Parre and his colleagues were well up in the time regarding vaccine trials (Weber and Evans, 2003). Treatment options are now available to the farmer to control this problematic pathogen in chicken, but we have no idea of the extent of *Eimeria* in Estonian poultry and its mortality today.

NEOSPORA

Cattle

Bulk milk antibodies against *Neospora caninum* were first investigated in a pilot study of 65 random Estonian herds in 2007 using the SVANOVIR@Neospora-ab ELISA kits (Lassen et al., 2008). The result of 26% positive farms encouraged an expanded investigation of 320 additional herds in 2008 and establishing a national prevalence around 16%. Blood samples from 59 and 24 cows in two of the bulk milk positive farms were tested with the same ELISA brand and 33% and 19% respectively of the animals had antibodies against *Neospora*. The Estonian herd prevalence is basically identical to recent Latvian studies that report 15.5% (Ehivalde et al., 2007), hinting common epidemiological factors for this parasite. Abortion causes in Estonian cattle lack good documentation, and *Neospora* has not previously been taken into account. Dogs, the primary host, have not yet been investigated but they are very common in farms and often have access to the interior.

SARCOCYSTIOSIS

Cattle

A national investigation of *Sarcocystis* in cattle meat samples collected from slaughter houses can be credited to the work of Miller (1994). Samples of the right atrium and ventricle or apex of the heart were collected from nearly 3 000 cows and heifers from 13 counties and examined microscopically by the compression method. *Sarcocystosis* cysts were found in 57.5% of the cows with a mean infection intensity (II) of 66,7 sarcocysts per gram muscle tissue (SGM), and mostly found in the East Viirumaa region (83,6%). Heifers had a slightly lower prevalence of 49.0%, a mean II of 61.7 SGM, and high infection levels in West Virumaa (75.3%), Tartumaa (76.6%), Valgamaa (63.4%), and Pärnumaa (65.7%). Morphological differentiation of 24 cow samples identified *Sarcocystis cruzi* as the species found in the samples. Refinement of the diagnostic method by examining 21 sites of the slaughtered animals resulted in the cardiac apex, cervical part of the oesophagus, and the apex of the heart as the most suitable sample locations. Again Millers research provides the only source of this potentially zoonotic disease in the secondary host. Data on the primary host, the dog, have been acquired by Talvik (1998) through routine sample investigations of almost a thousand faecal samples. Oocysts were identified as *Sarcocystis spp*. in 0.09% cases and *Cystoisospora spp.* in 1.8%. The samples investigated were not classified by location, so it is not clear which proportion of dogs came from rural areas where they are more likely to have been in contact with cattle.

TOXOPLASMOSIS

Cats

Testing of Toltrazuril against shedding of *Toxoplasma gondii* oocysts in cat faeces, were run by Annus and others in 1997. In a case-control trial both groups were fed 10 grams of mouse meat containing *Toxoplasma* cysts per kilo bodyweight. One group was additionally fed Toltrazuril, and did not shed oocysts in the faeces. Ten weeks post infection both groups were challenged with infected mouse meat, and neither produced oocysts. Two new groups fed $2.5x10^6$ sporulated *T. gondii* oocysts, and one of the groups were treated with the drug resulting in no oocyst shedding and seroconversion of 7 out of 12 cats. Annus concluded a reduction in oocyst production due to the drug, regardless of the infection routes, without affecting the immunity against reinfection. Though the experimental animals were German SPF cats, this work is of interest since it is the only scientific animal study on this zoonotic parasite. Still sero-prevalence of the population of Tartu, Estonia, reach 54,9% - a significantly higher number compared to cities in neighbouring countries like Uppsala, Sweden (23.0%) and Reykjavik, Finland (9.8%) (Birgisdóttir *et al*., 2006). Though a study like this strongly indicates a high transmission rate between cats and humans, regardless of strong winters that can destroy the oocysts, we know preciously little about *T. gondii* in Estonian cats. A recent report on human zoonoses examined blood samples from five zoo animals, two dogs, and three cats (The Veterinary and Food Board, 2007). Two zoo animals and a dog were tested positive. The same publication list the first human cases of toxoplasmosis from 1997-2007 ranging between 0.1-1.2% per 100 000 persons.

Discussion and Conclusion

National investigations of several of the parasitic protozoans were carried out in the past but they were mainly focused on cattle and outdated. Though it may seem unlikely that the pathogens would remain unchanged in a country that has spent large sums on modernisation, both from national and international funds, it may be the case to some extent. The situation of *Eimeria* in cattle has not changed dramatically over the last 20 years. Perhaps because these pathogens, together with other parasites, are not communicated or considered of so high relevance they have been reduced to shadow existences of other problematic pathogens. The past studies may still carry some relevance to the situation today, and certainly fill some information into a previous blank page.

Cattle have received most attention in regard to protozoans, and some husbandry animals are totally or partly unexplored (Table 1). Horses, sheep, goats, and chicken deserve a closer look regarding most of the parasites. More importantly, the primary hosts of many of the diseases in question, the cat and dog, have been overlooked. They are of particular interest since they are vectors of animal and human infections not only for protozoan, but also other parasitic zoonoses. In Estonia, both pets and stray cats and dogs have easy access to many farms, and in rural areas they can also get in contact with sylvan reservoirs for many diseases.

Table 1. **Documented protozoan parasites in Estonian livestock and pets**

Protozoa	Hosts								
	Cattle	Horse	Swine	Sheep	Goat	Chicken	Cat[1]	Dog	References
Babesia	x								Praks 1969
Cryptosporidium	x		x						Talvik and Daugschies 2004, Järvis and Mägi 2008, Lassen *et al.* 2007
Cystoisospora	x		x	x		x		x	Talvik 1998
Eimeria									Plaan 1951, Praks and Parre 1973, Parre and Praks 1974, Praks and Parre 1974, Parre *et al*. 1977, Parre and Olkonen 1977, Parre and Suigusaar 1977, Karis 1987, Kaarma and Mägi 2002
Isospora			x						Laaneoja *et al.* 2004
Neospora	x								Lassen *et al.* 2008
Sarcocystis	x							x	Miller 1994, Talvik 1998
Toxoplasma							x		The Veterinary and Food Board 2007

[1] Zoo felids

This historical review left us with an impression how important the communication to farmers and veterinarians is for the veterinary research. The experience from communication with the groups involved in animal care shows that they are unaware of the existing knowledge, and find alternative information elsewhere. A study based on these motivations should be worth while for any scientists desiring to improve conditions with informed strategies. Decades of research and vast resources can be spent on accumulating data in periodicals and dissertations like the ones presented here. But if we do not succeed in passing on the knowledge, we might be better off conserving our energy.

Acknowledgements

We would like to thank all the scientists who helped us in recovering the many articles mentioned in this review: Toivo Järvis, Jaan Praks, Erika Mägi, Eino Krall, and Illa Miller. In addition, we would like to thank Saulius Petkevičius for translation of abstract into Lithuanian language.

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Received 16 January 2009
Accepted 19 June 2009