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ENZYME VARIANTS OF EIMERIA PARASITIZING THE DOMESTIC FOWL AND POSSIBILITIES OF SPECIES DIAGNOSTICS

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Abstract. Electrophoretic variation of the enzymes lactate dehydrogenase (LDH) and glucosephosphate isomerase (GPI) of *Eimeria* parasitizing the domestic fowl in Czechoslovakia is summarized and the differentiation of species of poultry coccidia is discussed. A new method for evaluation of zymograms of coccidial enzymes is presented. This method enables the results of different experiments to be compared by calculating standardized rates of mobility of each enzyme band relative to the positions of reference variants coded LDH-8 or GPI-9.

During 1987 and 1989 a study on the distribution of different species of coccidia on 29 poultry farms was carried out in Czechoslovakia (Kučera 1990 a, b). The identity of different coccidia species was determined using both classic diagnostic methods (Long and Reid 1982) and enzyme analysis (Shirley and Rollinson 1979, Shirley 1986, 1989). In this paper, a description of the enzyme variants present in zymograms prepared during the above work is presented together with description of an improved method of zymogram evaluation based on methods used previously for other protozoans, e.g. for *Acanthamoeba* (Ward 1985) and *Leishmania* (Le Blancq et al. 1986).

MATERIALS AND METHODS

1. Parasites. Most isolates originate from the study carried out in Czechoslovakia in 1987—1989 on the distribution of individual *Eimeria* species in domestic fowl (Kučera 1990b, partly Kučera 1990a).
Laboratory strains are listed in Table 1. Coccidia were propagated in three weeks old Hybro cockerels kept in isolators and some strains were passaged in chicken embryos.
2. Enzyme electrophoresis. The preparation of oocysts, electrophoresis in thin-layer starch gels and detection of lactate dehydrogenase (LDH) and glucosephosphate isomerase (GPI) were as described previously (Kučera 1989a, 1990a). A strain of *E. tenella* characterized by LDH-8 and GPI-9 (Table 1) was run in each experiment as a reference sample. Additionally other strains of other species (Table 1) were sometimes run as reference samples.
3. Retrospective evaluation of zymograms. Zymograms, i.e. glass plates with starch gels dried after electrophoresis and visualization of enzymes (Kučera 1989b), prepared during the study by Kučera (1990a, b) were used for the retrospective evaluation. The distance from the origin to the centre of each individual enzyme band was measured.
A standardized rate of mobility of an enzyme was determined from the measured distance using a formula:

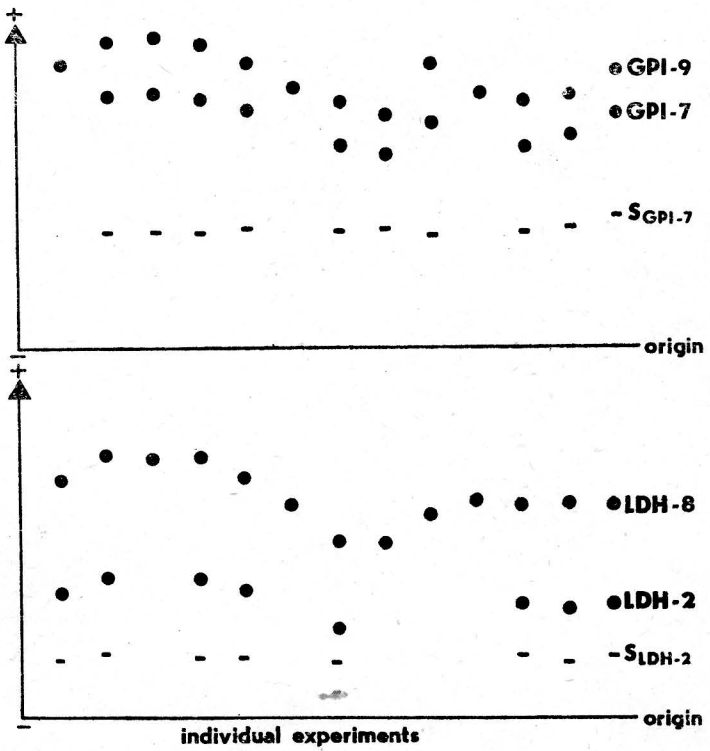
$$S_x = S_r \cdot A_x/A_r,$$

where A_x = actual distance migrated, A_r = actual distance migrated by reference variant LDH-8 or GPI-9 and S_r = constant, which was defined according to the mean values of A_r in our experiments as $S_{r\,LDH-8} = 3.62$ cm and $S_{r\,GPI-9} = 2.51$ cm.

Table 1. Survey of laboratory strains of *Eimeria* and enzyme variants found

Eimeria sp.	Strain	Enzyme variants[a]	Comment
E. praecox	Trnová	LDH-5, GPI-5, GPI-H	Isolated from one oocyst from isolate No. 1 (Table 1)
E. mitis	Bylany KE	LDH-1, GPI-C	Passaged in chicken embryos
E. acervulina	Chotouň	LDH-2, LDH-B, GPI-7	
E. tenella	Jena	LDH-8, GPI-9	
E. tenella	Chotouň	LDH-8, GPI-9	
E. tenella	Ch E-A	LDH-8, GPI-9	Passaged in chicken embryos
E. tenella	NEC (Houghton?)	LDH-8, GPI-1	Originated from a contaminated strain of *E. necatrix* kindly presented by Dr. Chapman from Houghton.
E. brunetti	Chrudim	LDH-4, GPI-6	Isolated from one oocyst from isolate No. 9 (Table 2)
E. maxima	Jesenice	LDH-3, GPI-4	

[a]Enzyme variants numbered in accordance with Shirley and Rollinson (1979) and Kučera (1990a).



RESULTS

Although the same conditions of electrophoresis were used in each experiment there were considerable run-to-run differences in the mobilities of all variants including reference variants LDH-8 and GPI-9 (Fig. 1). Direct comparison of zymograms was not possible. However, the ratio of mobility of an enzyme variant to that of a reference variant was constant (compare the ratio of the positions of LDH-2 or GPI-7 to the positions of LDH-8 or GPI-9 in Fig. 1).

Calculated standardized rates of mobility S_x of individual enzyme variants showed only little variation (see e.g. S_{LDH-2} and S_{GPI-7} in Fig. 1), so that they could have been used for comparison of different experiments.

Mean values of S_x of the different enzyme variants are presented in Table 3 and in comparison with other up to now described enzyme variants are depicted in Fig. 2.

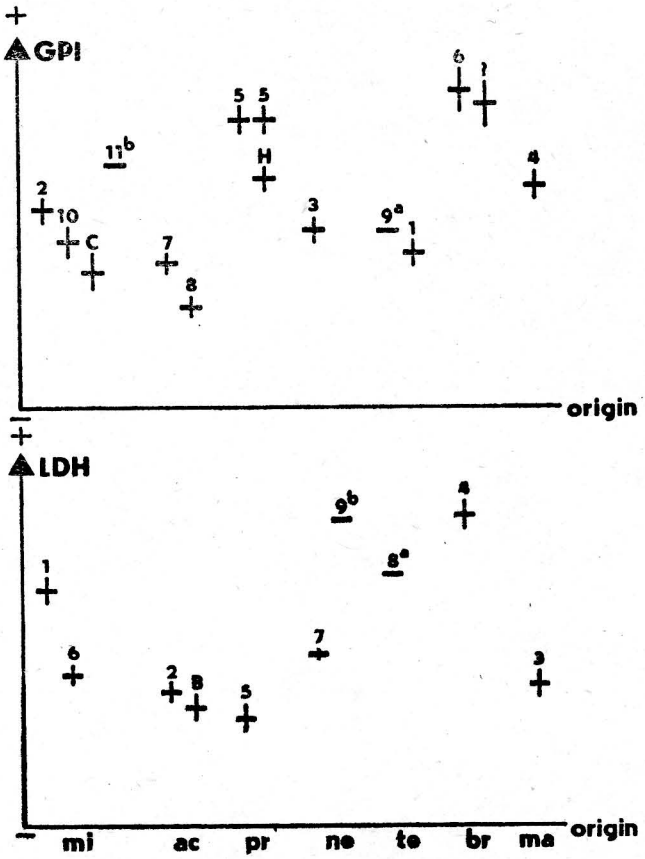


Fig. 2. Comparison of mean S_x of individual enzyme variants. See also Table 3 and Materials and Methods.

◀ **Fig. 1.** Comparison of some experiments, in which large differences in mobilities of reference LDH-8 and GPI-9 (black circles) were encountered. S_{LDH-2} and S_{GPI-7} show the position of the standardized rate of mobility of LDH-2 and GPI-7, respectively, reduced by 1.

Table 2. Survey of enzyme variants found in *Eimeria* of the domestic fowl in Czechoslovakia

Locality[a]	Enzyme variants[b] found in *Eimeria*[c]													
	pra		mit		ace		ten		bru		max		nec	
	LDH	GPI	LDH	GPI	LDH	GPI	LDH	GPI	LDH	GPI	LDH	GPI	LDH	GPI
1. Trnová	5	5 H			2	7								
2. Pičín 1					2	7	8	9			3	4		
3. Pičín 2	5	5 H	6	10	2	7	8	9			3	4		
4. Meely			1	2	2	7					3	4		
5. Písek			1	10			8	9			ND[d]			
6. Bálkova Lhota	5	5 H			2 B	7	8	9						
7. Sedlec	5	5 H	1	10	2	7	8	9	4	6	3	4		
8. Jeníčkova Lhota			1 6	2	2	7	8	9						
9. Chrudim					2	7			4	6				
10. Čimelice 1			1	10	2	8	8	9	4	6	3	4		
11. Čimelice 2			1	2	2	8	8	9						
12. Mirotice			6	10	2	7 8								
13. Hradiště			6	10	2	7 8								
14. Jinošov			1 6	2	2	7	8	9	4	6				
15. Písek			1 6	10	2	7 8	8	9					7[e]	
16. Lišno	5	5			2 B	7	8	9						
19. Police/M							8	9						
20. Skalsko			1	2	2	7					3	4		
21. Řepníky	5	5	1	2	2	7					ND[d]			
22. Chotouň	5	5 H	1	2 10					4	6				
23. Luže	5	5 H	1	10	B	7					ND[d]			
24. Velká			6	10	B	7 8								
25. Markovice					B	7	8	9	4	6				
26. Kunovice	5	5 H	1	2 10	2	7							7	3
27. Selice			1	2	2	7	8	9			3	4		
28. Bojanovice			1	2 10	2	7 8	8	9						
29. Bratřínov	5	5	1	2 10	2	7 8								
No. isolates	10		19		24		15		6		7		2	

[a] Locality numbers correspond with those in Kučera (1990a, b).
[b] Enzyme variants numbered in accordance with Shirley and Rollinson (1979) and Kučera (1990a).
[c] pra = *Eimeria praecox*, mit = *E. mitis*, ace = *E. acervulina*, ten = *E. tenella*, bru = *E. brunetti*, max = *E. maxima*, nec = *E. necatrix*.
[d] No living parasites suitable for enzyme analysis isolated.
[e] GPI not seen due to small number of oocysts in the sample — pure culture of *E. necatrix* was not isolated.

Table 3. Mean S_x of individual enzyme variants[a]

Eimeria sp.	LDH			GPI		
	variant	n	S_x[b]	variant	n	S_x[b]
E. mitis	LDH-1	41	3.40 ± 0.19	GPI-2	21	2.86 ± 0.19
	LDH-6	15	2.18 ± 0.14	GPI-10	20	2.42 ± 0.24
				GPI-C	4	1.97 ± 0.25
				GPI-11		(3.5)[c]
E. acervulina	LDH-2	59	1.93 ± 0.16	GPI-7	60	2.09 ± 0.15
	LDH-B	20	1.70 ± 0.18	GPI-8	16	1.46 ± 0.15
E. praecox	LDH-5	33	1.55 ± 0.20	GPI-5	33	4.15 ± 0.2
				GPI-H	22	3.30 ± 0.17
E. necatrix	LDH-7	3	2.46 ± 0.06	GPI-3	2	2.55 ± 0.18
	LDH-9		(4.4)[d]			
E. tenella	LDH-8		3.62[e]	GPI-1	5	2.22 ± 0.19
				GPI-9		2.54[e]
E. brunetti	LDH-4	15	4.46 ± 0.21	GPI-6	12	4.56 ± 0.31
				GPI-?[f]	4	4.36 ± 0.33
E. maxima	LDH-3	13	2.02 ± 0.18	GPI-4	17	3.18 ± 0.19

[a] Thin-layer starch gel electrophoresis, Tris/citric acid buffer system pH 6.7 (see Kučera 1989b for more details).
[b] Arithmetic mean ± standard deviation multiplied by 2 (interval showing the range of high and low values).
[c] Recounted from Shirley et al. (1983), Pl. 3, Fig. D.
[d] Recounted from Shirley (1985), Fig. 3c.
[f] Constant defined for the reference strain of *E. tenella*. See Materials and Methods.
[e] Subband of GPI-6. See the Discussion.

Table 2 shows a survey of enzyme variants found in coccidia isolated in Czechoslovakia. LDH-5 and GPI-5 accompanied in seven samples with the subband GPI-H (see Kučera 1990a) were the only variants found in 10 isolates of *E. praecox*. Of 19 isolates of *E. mitis*, LDH-1 was found in 15 (79 %), LDH-6, in seven (37 %), GPI-10, in 14 (74 %) and GPI-2, in ten (53 %). In 24 isolates of *E. acervulina* LDH-2 was found in 21 (88 %), a slightly slower variant LDH-B (see Kučera 1990a) in five (21 %), GPI-7 in 22 (92 %), and GPI-8 in eight (33 %). The only variants found in 15 isolates of *E. tenella* were LDH-8 and GPI-9, in *E. brunetti* (6 isolates) LDH-4 and GPI-6, in *E. maxima* (7 isolates) LDH-3 and GPI-4, and in 2 isolates of *E. necatrix* LDH-7 and GPI-3, respectively.

DISCUSSION

Individual species and some strains of coccidia may be differentiated from each other by comparison of their electrophoretic mobility profiles with those of reference laboratory strains (Rollinson 1975, Shirley 1975, 1986, 1989, Shirley and Rollinson 1979, Chapman 1982, Kučera 1990a). Because seven species of *Eimeria* infect the domestic fowl and some species possess more than one variant of some enzymes (Shirley 1986), all known reference samples theoretically shall be necessary for investigation of an unknown sample. The method presented here using the

standardized rate of mobility, S_x, is more convenient because it requires only one reference sample on each zymogram. Comparison of calculated S_x with the tabulated values (Table 3, Fig. 2) enables, in most cases, direct determination of an enzyme variant and consequently the identity of the parasite species. The method also enables comparison of results from different experiments.

Some enzyme variants overlap in their S_x values (Table 3 and Fig. 2). The interval between high and low values reflects the statistical variability of S_x between different experiments. On each particular zymogram, enzyme variants, whose mean S_x values are sufficiently distinct from each other, will be depicted as clearly distinct bands. However, mean S_x values for some variants are very close, e.g. LDH-3 and LDH-2; LDH-9 and LDH-4; GPI-C and GPI-1 and GPI-7; GPI-7 or GPI-10; GPI-3 and GPI-4, respectively. Common forms of GPI for *E. tenella* (now coded as GPI-1) and *E. acervulina* (GPI-7) and LDH for *E. tenella* (LDH-8) and *E. mitis* (syn. *mivati*) (LDH-7) have been already reported by Shirley (1975). Nakamura et al. (1986) also noticed that GPI in *E. mitis* (probably GPI-C) and *E. acervulina* (probably GPI-7), as well as GPI in *E. necatrix* (GPI-3) and *E. tenella* (GPI-9) showed the same mobilities during electrophoresis in starch gel. These "identical" variants can be distinguished according to Shirley (pers. com.) and Shirley and Rollinson (1979) by modifications to the buffer systems used during electrophoresis, namely by changing the pH.

Our results thus confirm the former opinions (Shirley and Rollinson 1979, Kučera 1989b) that the enzyme analysis cannot be the only method for species differentiation and that it must be combined with other diagnostic characteristics such as morphology of oocysts, approximate prepatent period and pathomorphological findings on the gut of experimentally infected chickens.

The retrospective study of zymograms enabled to revise the previous survey of enzyme variants found in Czechoslovakia (Kučera 1990a). The presumed new variant of *E. acervulina* coded LDH-C (Kučera 1990a) proved to be LDH-6 of *E. mitis*. The variant from *E. mitis* coded GPI-C must be regarded as valid, because the laboratory strain *E. mitis*-Bylany-ChE repeatedly passaged in chicken embryos is also characterized by LDH-1 and GPI-C (Table 1). GPI-C is, however, difficult to detect in mixture samples of *E. mitis* and *E. acervulina*, because the very common variant *E. acervulina* GPI-7 has almost the same mobility.

It would be, therefore, desirable to verify the presence of GPI-C in purified cultures of *E. mitis*. A distinctly different subband slower than GPI-6 occurred in two samples of *E. brunetti* (No. 7 and 8) in some experiments. Because this subband was not found in some other experiments with the same cultures, it was probably an artefact. Repeated experiments also proved, that the presumed new variant *E. maxima* LDH-H (Kučera 1990a) was in fact LDH-3.

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D. W. T. Crompton, M. C. Nesheim, Z. S. Pawlowski (Eds): Ascariasis and its prevention and control. *Taylor and Francis, London, New York and Philadelphia 1989, 406 pp. Price 35.00 GBP.*

This publication is based on the conference, organized by the World Health Organization in Penang, Malaysia, in February 1988. The first editor works at the Department of Zoology, University of Glasgow, while the second one is from the Division of Nutritional Sciences, Cornell University in Ithaca/NY/. The third editor is from the Clinic of Parasitic and Tropical Diseases of the Medical School in Poznań and is known for his activities in the Parasitic Disease Programme of the WHO in Geneva. The conference, promoted by ICI Pharmaceuticals, followed one held in Canada in 1984. Its results were published under the title "Ascariasis and its Public Health Significance" by the same editors in the same publishing house in 1985 (for review see Folia Parasitologica 34: 232, 1987). The present publication is a team effort of 22 contributors, five being from Great Britain, others from the U.S.A., the Philippines, Switzerland, Poland and further from Brazil, Tanzania, Burma, Malaysia and Pakistan. In the welcoming address Dr. H. Nakajima, the WHO Director for Western Pacific, emphasized ascariosis as a very important helminthosis in this region, especially with regard to those rural and semi-urban areas with unsatisfactory environmental hygiene. High rates of infection occur here in connection with nutritional inbalance, intestinal disorders and other complications. Heavy infection in children causes retardation of growth and development. Control measures against ascariosis are to be aimed at the development of national control programmes within the framework of primary health care, education and training of health personnel and resource allocation. Opening articles emphasize the high global prevalence of ascariosis, affecting 22 % of the world's population,