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from each group were inoculated once with a double dose, for safety testing. Blood samples were drawn before first administration, before the booster and every two months after, up to 8 months since the first administration. The immune responses were evaluated by the microscopic agglutination lyses reaction, using six *Leptospira* serovars: *pomona, icterohaemorrhagiae, canicola, hardjo, grippotyphosa and australis*, as required by NSVFSA regulations.

Results and Conclusion .The analytical interpretation of the results shows that the vaccine elicits a protective immune response starting at 21 days after the second administration, with antibodies against all five serovars, and lasting over a period of 6 months. No adverse reactions were recorded while administering a double dose.

The vaccine complies with the tests for identity, sterility, safety, inactivation and potency. Upon completion of all stability tests, Romvac Company intends to submit applications for manufacturing and marketing authorizations.

STUDIES ON CYTOTOXICITY AND ANTIBACTERIAL EFFECT OF ARTEMISININ

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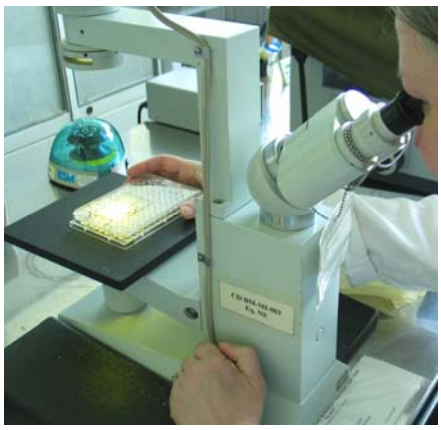
Introduction: Artemisinin, an extract of sesquiterpene lactone endoperoxide obtained from *Artemisia annua*, is routinely used in the treatment of malaria and various forms of human cancer. In order to extend its therapeutic range, studies were done on Artemisinin cytotoxicity on chicken embryo fibroblasts (CEF) in parallel with tests on Vero cells, and the effect assessment on *Salmonella* spp strains of avian origin.

Material and methods: The cytotoxicity tests were carried out on monolayers of chicken embryo fibroblasts (20,000 and 10,000 cells / ml), and Vero cells (10,000 cells / ml). Artemisinin (dissolved in DMSO, DMF respectively, mixed with PBS or MEM) was placed in contact with the cells for 15 minutes and 1 hour, in concentrations of 177.10, 17.71, 8.85 and 4.43 nM. Evaluation of cytotoxic effect was performed by MTT assay, reading at 540 nm at intervals of 15 minutes, 1, 2 and 21 hours post-treatment. The antibacterial effect was tested by Kirby - Bauer disk diffusion assay, according to SFM, on four strains of Salmonella enteritidis, two of S. typhimurium and one strain of S. gallinarum of avian origin, in concentrations of 80 and 160 ug / disk.

Results and Conclusions: On CEF, there were noticed differences due to the solvents used, for the same amount of Artemisinin and a cytotoxic effect was recorded at the concentration of 177.10 nM. The cytotoxic effect was recorded on Vero cells including concentration of 4.43 nM, regardless of the organic solvent used. In disk diffusion antibiogram the two concentrations had no anti-Salmonella effect (inhibition diameters were of 6-9 mm).

STUDIES ON CYTOTOXICITY AND ANTIBACTERIAL EFFECT OF ARTEMISININ*

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Introduction: Artemisinin, an extract of sesquiterpene lactone endoperoxide obtained from *Artemisia annua*, is routinely used in the treatment of malaria and various forms of human cancer. In order to extend its therapeutic range, studies were done on Artemisinin cytotoxicity on chicken embryo fibroblasts (CEF) in parallel with tests on Vero cells, and the effect assessment on *Salmonella* spp strains of avian origin.

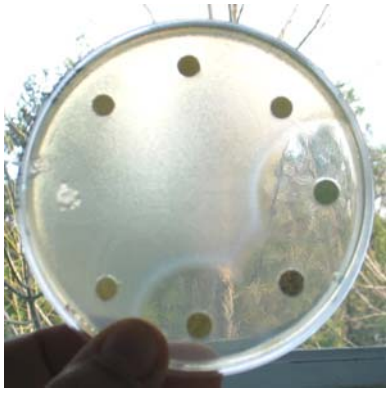


Material and methods: *Cytotoxicity tests*: Monolayers of CEF (chicken embryo fibroblasts, 20,000 and 10,000 cells / ml), and Vero cells (10,000 cells / ml). Artemisinin (dissolved in DMSO, DMF respectively, mixed with PBS or MEM), in concentrations of 177.10, 17.71, 8.85 and 4.43 nM, placed for 15 minutes and 1 hour. MTT assay: reading at 540 nm at intervals of 15 minutes, 1, 2 and 21 hours post-treatment; interpretation: the average of absorbance of the control (no chemical) regarded as 100% and the percentage of cell growth in each well was calculated (% of control).

	Artemisinin : DMSO				Artemisinin : DMF				Control CEF
	177.09 nM	17.70 nM	8.85 M	4.42 M	177.09 nM	17.70 nM	8.85nM	4.42 nM	
15'	101.00	118.40	111.99	128.21	105.49	116.96	140.86	134.24	100
1h	176.98	175.51	144.97	141.63	116.85	137.08	127.40	131.29	100
2h	135.65	174.51	104.10	93.58	106.51	112.30	116.76	110.52	100

	Artemisinin : DMSO				Artemisinin : DMF				Control Vero
	177.09 nM	17.70 nM	8.85 nM	4.42 nM	177.09 nM	17.70 nM	8.85 nM	4.42 nM	
15'	73.88	86.46	95.23	103.90	77.29	118.30	133.20	104.02	100
1h	95.63	93.89	97.72	86.00	99.15	142.11	131.76	120.14	100
2h	80.21	111.60	85.54	129.41	118.75	109.37	105.38	120.27	100
20h	71.15	86.74	64.10	78.04	69.20	73.19	108.88	84.23	100

Anti-bacterial effect: Kirby - Bauer disk diffusion assay (FSM protocol). 4 *Salmonella enteritidis*, 2 *S. typhimurium*, 1 *S. gallinarum* strains of avian origin. Artemisinin in concentrations of 80 and 160 ug / disk.



Results and conclusions: On CEF, there were noticed differences due to the solvents used, for the same amount of Artemisinin and a cytotoxic effect was recorded at the concentration of 177.10 nM. The cytotoxic effect was recorded on Vero cells including concentration of 4.43 nM, regardless of the organic solvent used. In disk diffusion antibiogram the two concentrations had no anti-*Salmonella* effect (inhibition diameters were of 6-9 mm).

Crt. no.	Bacterial strain	AMOXICILLIN 25ug	GENTAMICIN 10ug	ENROFLOXACIN 5ug	ARTEMISININ:DMSO		ARTEMISININ:DMF	
					160ug	80ug	160ug	80ug
1.	S.typhimurium 179	30	19	27	6	6	6	6
2.	S.enteritidis 2488	32	25	27-31	6	6-7	6	6
3.	S. typhimurium 504	32	21	28-33	6-8	6	6	6
4.	S.enteritidis 13a	32	21	24-28	6-8	6-7	6	6
5.	S.gallinarum. 91	39	28	32	6	6-9	6	6
6.	S.enteritidis. 288	6.5	21-28	24-30	6-8	6	6	6
7.	S.enteritidis. 290	28-32	20	25-29	6	6	6	6

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