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SEROLOGICAL SCREENING FOR INFECTIOUS CATTLE DISEASES. II.ASSOCIATION BETWEEN PREVALENCE AND LEVEL OF ELISA RESPONSE

TRIAGEM SOROLÓGICA DE DOENÇAS INFECCIOSAS. II.ASSOCIAÇÃO ENTRE PREVALÊNCIA E NÍVEIS DE POSITIVIDADE AO ELISA

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SUMMARY

A herd of cattle (Holstein-Zebu crosses) was screened every two months by ELISA during a period of two years for IgG antibodies against 19 infectious disease agents. Two hundred and ninety five sera were collected from 157 young animals (0-4 months of age), 1037 sera from 292 developing animals (4-36 months of age) and 1468 sera from 259 producing animals (> 36 months of age). The results indicate that the difference in ELISA between positive and negative tests is associated with the overall prevalence of positive tests. When the prevalence of positive tests is low the difference between positive and negative tests is greater than when the prevalence is intermediate or high. This means that ELISA, presumably other serological tests for IgG antibodies, is more reliable at low disease (antibody) prevalence. This will tend to offset the declining predictive value of positive tests at low prevalence and may contribute to the successful use of serological tests in disease eradication.

Key words: serological screening, cattle diseases, ELISA.

RESUMO

Um rebanho bovino (cruza Holandês-Zebu) foi monitorado durante um período de dois anos pelo teste imuno-enzimático (ELISA) para detectar anticorpos contra 19 agentes infecciosos. Duzentos e noventa e cinco soros foram coletados de 157 animais jovens (0-4 meses de idade), 1037 soros de 292 animais em crescimento (4-36 meses de idade) e 1468 soros de 259 animais em produção (>36 meses de idade). Os resultados indicam que a diferença entre resultados positivos e negativos ao ELISA está associada com a prevalência geral de testes positivos. Quando esta prevalência for baixa a diferença entre resultados positivos e negativos é maior do que quando esta for intermediária ou alta. Isto significa que o ELISA é mais confiável quando em prevalências baixas. Isto tenderá a compensar diminuindo a probabilidade de valores positivos em prevalências baixas podendo contribuir no uso da sorologia em programas de erradicação.

Palavras-chave: triagem sorológica, doença de bovinos, ELISA.

INTRODUCTION

Serological testing for circulating antibodies is convenient for detection of infectious diseases. The reliability of testing of is customarily expressed in terms of sensitivity and specifity of the test and as the derived predictive values, which depend on the prevalence of the infection. Specifity is a relative term since it depends on cross reactions with other infectious agents the prevalence of which may vary geographic location and age and species of the host. Sensitivity depends on the preparation of the antigen used and may vary according to the degree of seroresponse of the host.

The present study deals with observations which indicate that a low prevalence of an infection in a population is associated with a more reliable discrimination between positive and negative tests.

MATERIAL AND METHODS

The study was conducted in a cattle herd at the center for Research, Teaching and Extension in Tropical Livestock (Centro de Investigación, Enseñanza y

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Extensión en Ganadería Tropical-CIEEGT) located in the North-central part of the state of Veracruz, Mexico. Blood samples were collected bimonthly during 1988 and 1989 and the sera subjected to ELISA with 19 antigens prepared from infectious agents as described by BARAJAS-ROJAS et al (1993).

Two hundred and ninety five serum samples were collected from 157 young animals (< 4 months of age), 1037 samples were collected from 292 developing animals (4-36 months of age) and samples from 259 producing animals (> 36 months of age).

RESULTS

The mean percent ELISA and standard deviations for positive tests (tests above the cut off point) and negative tests (tests below the cut off point) are shown in Table I. Plots of mean of percent ELISA an standard deviation for positive and negative tests against the prevalence of positive tests are shown in Figure 1, 2 and 3. These plots suggest that as the prevalence decreases the difference between the mean percent ELISA of positive and negative tests increases; this applies to all 3 age groups. Plots of logarithms of percent prevalence against difference between positive and negative ELISA (Figure 4, 5 and 6) illustrates the association more clearly and indicates that the association is best for the producing animals and least clear for the most heterogeneous age group, the developing animals.

Table I Mean percent ELISA and standard deviation of positive and negative tests in cattle from the tropics of Mexico over a two year period (1988-1989)

AGENT		PRODUCING POSITIVES	ANIMALS NEGATIVES	DEVELOPING POSITIVES	ANIMALS NEGATIVES	YOUNG * POSITIVES	ANIMALS NEGATIVES
CF	MEAN	65.78	35.52	64.22	29.98	69.67	25.22
	SD	15.38	13.18	16.53	10.77	9.52	9.58
LH	MEAN	68.47	30.25	74.68	27.07	64.28	20.43
	SD	17.47	11.63	19.97	14.02	10.51	13.14
BTV	MEAN	79.55	41.23	88.38	37.73	73.45	37.93
	SD	24.52	6.58	26.44	12.42	17.75	8.13
MB	MEAN	73.83	36.66	67.83	33.15	59.83	23.25
	SD	43.15	10.39	15.53	12.47	7.05	15.09
AM	MEAN	70.53	34.29	78.10	37.83	73.36	27.26
	SD	17.06	10.43	20.41	9.67	20.88	9.28
CB	MEAN	65.66	32.28	64.46	30.88	61.86	24.84
	SD	14.97	7.34	13.42	9.61	11.13	10.29
TG	MEAN	70.54	32.85	68.57	29.37	67.73	27.23
	SD	16.71	11.26	15.22	11.54	14.55	13.95
ST	MEAN	69.06	35.05	66.57	30.42	60.44	23.55
	SD	19.46	9.16	14.35	11.44	9.81	11.01
CL	MEAN	63.14	34.08	71.04	31.3	59.67	20.91
	SD	15.21	9.51	21.19	10.51	12.06	9.86
PM	MEAN	74.77	31.64	65.32	27.74	70.41	24.78
	SD	23.76	11.27	13.08	12.17	14.07	10.61
SD	MEAN	63.91	34.95	62.77	29.47	60.73	16.61
	SD	15.38	10.45	10.94	12.31	9.32	12.06
BRSV	MEAN	73.13	32.28	70.19	36.32	63.14	28.76
	SD	18.23	12.83	20.55	19.81	17.99	11.53
BVD	MEAN	78.83	23.56	63.73	19.09	67.20	18.25
	SD	28.75	9.02	10.96	12.86	11.16	11.63
BA	MEAN	0	10.14	0	9.29	0	7.97
	SD	0	7.81	0	5.73	0	6.29
RV	MEAN	66.88	34.98	67.36	33.05	62.33	31.53
	SD	14.45	11.51	21.04	10.97	13.58	9.78
IBR	MEAN	74.00	18.54	0	13.77	52.67	11.61
	SD	26.78	12.07	0	10.84	8.33	10.06
PI3	MEAN	70.80	33.75	65.73	23.47	57.72	25.44
	SD	19.97	27.53	15.94	12.86	7.01	12.65
HS	MEAN	69.42	28.79	73.78	27.42	57.33	15.44
	SD	28.24	11.08	20.19	11.83	7.99	7.24
LM	MEAN	69.25	30.16	66.32	28.89	62.55	27.29
	SD	19.93	11.02	16.63	12.32	12.92	12.55

* Young animals were tested only for four months

CF= Campylobacter fetus
LH= Leptospira interrogans serovar hardjo
BTV= Bluetongue virus
MB= Mycoplasma bovis
AM= Anaplasma marginale
CB= Coxiella burnetii
TG= Toxoplasma gondii
ST= Salmonella typhimurium
CL= Chlamydia psittaci-trachomatis
PM= Pasteurella multocida
SD= Salmonella dublin
BRSV=Bovine Respiratory Syncytial Virus
BVD= Bovine Viral Diarrhea
BA= Brucella abortus
RV= Rotavirus
IBR= Infectious Bovine Rhinotracheitis
PI3= Parainfluenza 3
HS= Haemophilus somnus
LM= Listeria monocytogenes

DISCUSSION

The results presented here may not be surprising since a high prevalence may mean a high endemic level of infection with seroconversion and a waning of antibodies going on at the same time with the result that a considerable number of animals have ELISA values close to the cut-off point. The fluctuations in ELISA values related to season and state of pregnancy (BARAJAS-ROJAS et al, 1993) show that many infections (seroresponses) have such a dynamic behavior. A low prevalence on the other hand may represent a situation where diseases transmission (infections) is not very active. Most animals will then have reduced antibody levels and the ELISA positive animals may represent chronic or sporadic infection. It may be argued that the pattern found in this study is due to a higher sensitivity of the antigens associated with high prevalence with the result that many tests will cluster close to the ELISA cut-off point, assuming that percent ELISA will rarely exceed 100. However this is not a likely explanation; furthermore there is no apparent association between the prevalence of positive tests and the recorded maximum percent ELISA (not shown).

It may also be argued that repeated testing of the same animal (maximum 5.7 tests per producing animals) may cause distortion. However the number of repeated tests is small compared to the total number of tests and the absence of complete "independence" of test will hardly cause any bias; furthermore all we deal with in serological testing is a population of test results and dynamics of many diseases suggest that result from an animal at one point in time may be quite independent of result obtained at a different time. This is indicated in the report by BARAJAS-ROJAS et al (1993) and will be discussed again in a subsequent article.

According to conventional wisdom, which assumes that test specifity is independent of the prevalence the condition tested for, the predictive value of positive tests wanes as the prevalence

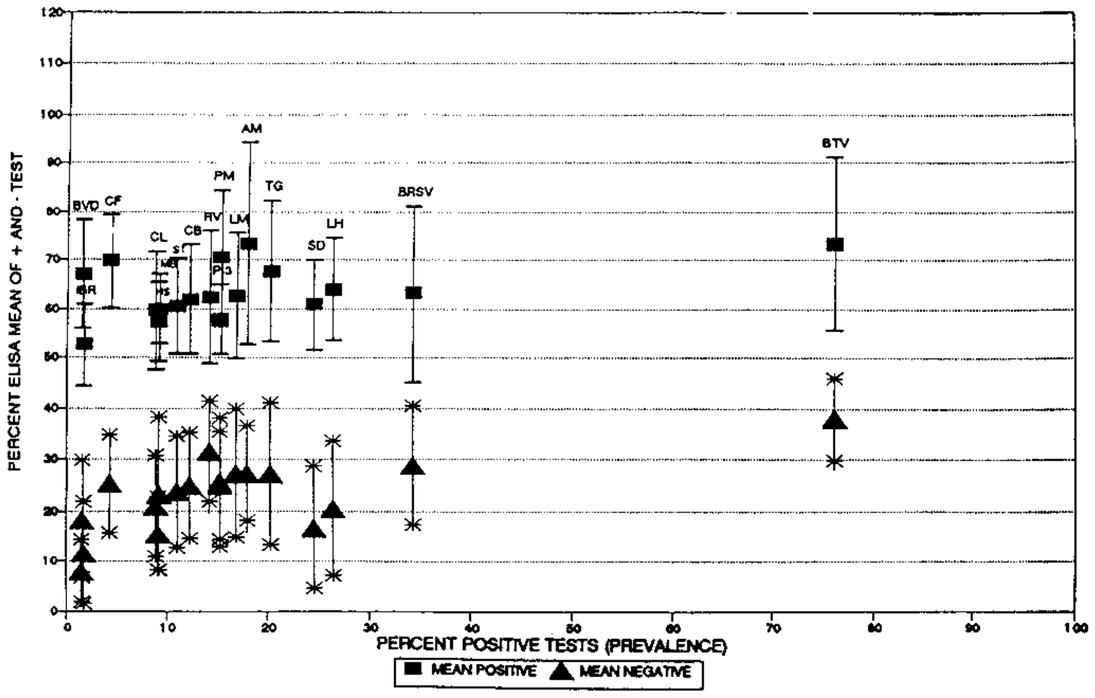

Fig. 1. Mean percent ELISA and standard deviation of positive and negative tests on young cattle versus prevalence of positive tests. Tropics of Mexico; 1988, 1989.

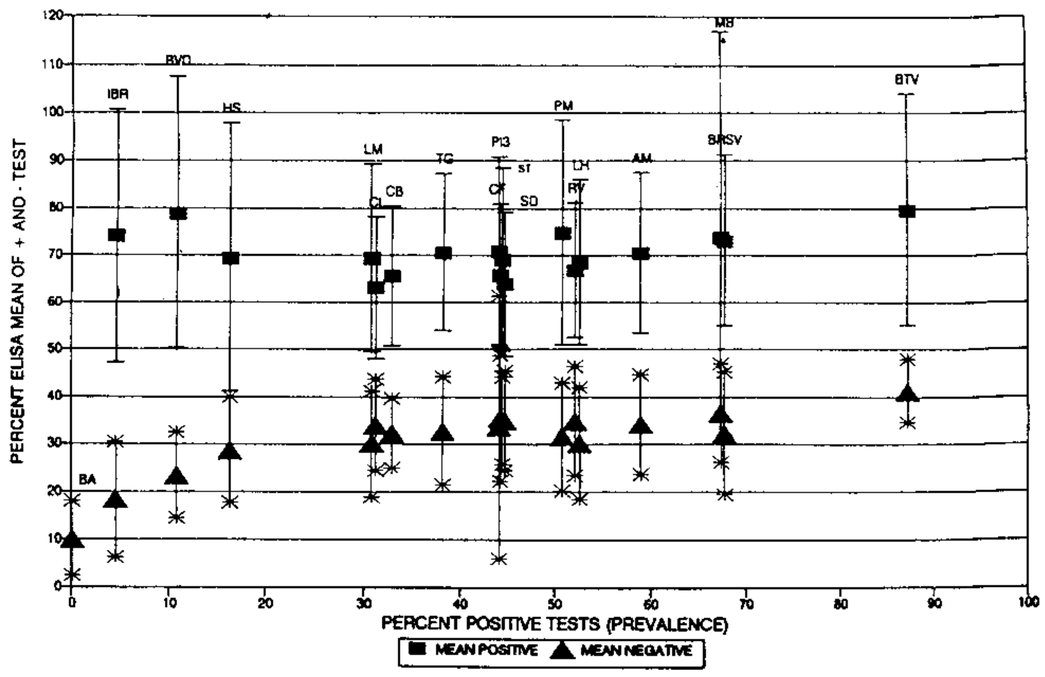

Fig. 3. Mean percent ELISA and standard deviation of positive and negative tests on producing animals versus prevalence of positive tests. Tropics of Mexico; 1988, 1989.

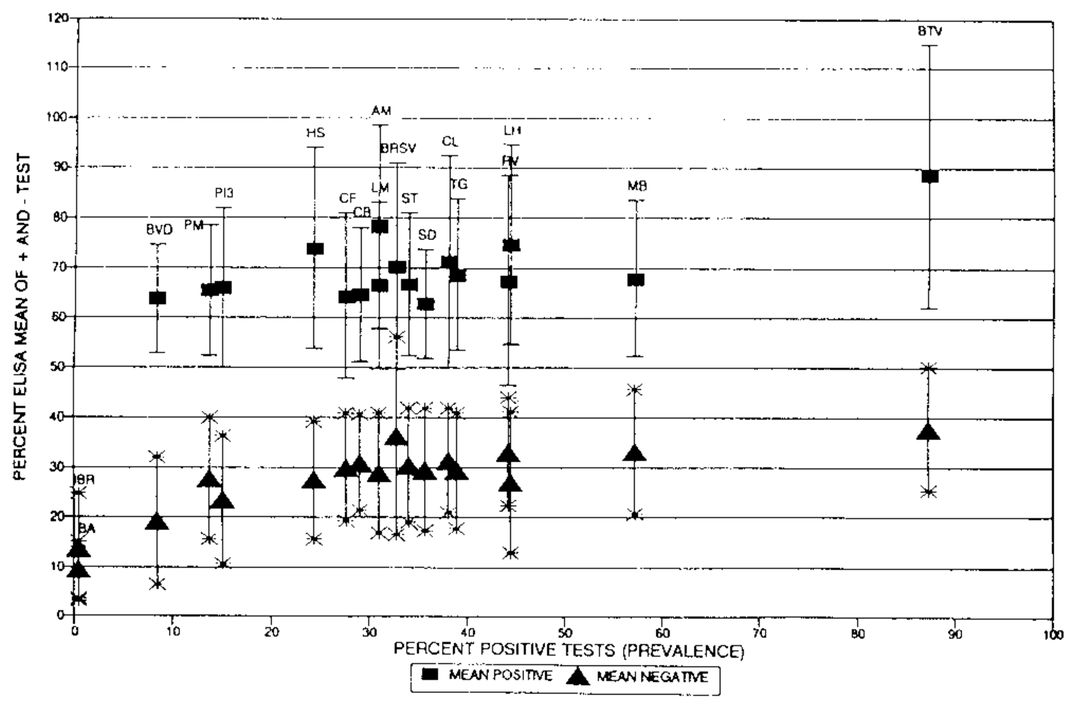

Fig. 2. Mean percent ELISA and standard deviation of positive and negative tests on developing animals versus prevalence of positive tests. Tropics of Mexico; 1988, 1989.

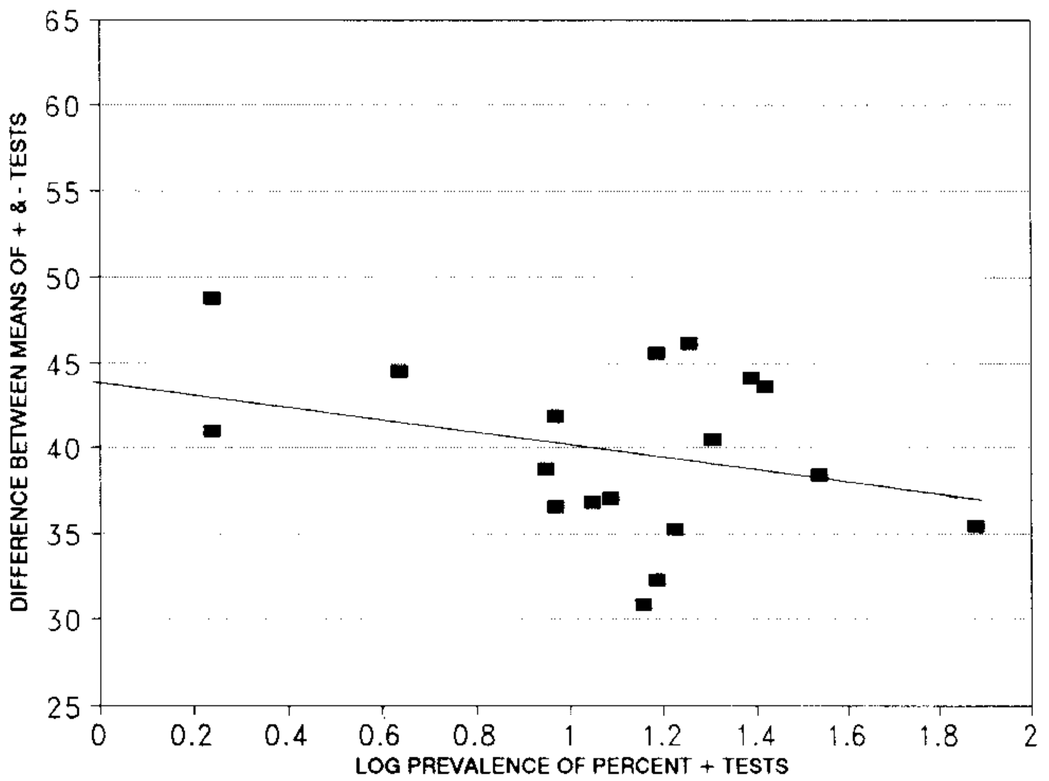

Fig. 4. Regression of difference between mean percent ELISA of positive and negative tests on the logarithm of percent positive test. Young animals. Tropics of Mexico; 1988, 1989. $\hat{y} = 44.49 - 4.23x$; $R^2=0.12$

approaches zero. Taken to its logical consequence this means that it would be next to impossible to base eradication of a disease on serological testing. Practical experience show otherwise (e.g. eradication of brucellosis, bovine leukemia, infectious bovine rhinotracheitis and pseudorabies) and part of the explanation may be the widening gap between positive and negative tests as prevalence decreases.

The evidence presented here together with the known facts that test results may vary with geographic location as well as age, species and breed of animals make the conventional concepts of sensivity and specifity almost meaningless. At the very least estimates of sensivity and specifity should be based on specification of host species and age as well as on geographic locality and prevalence of the condition tested for.

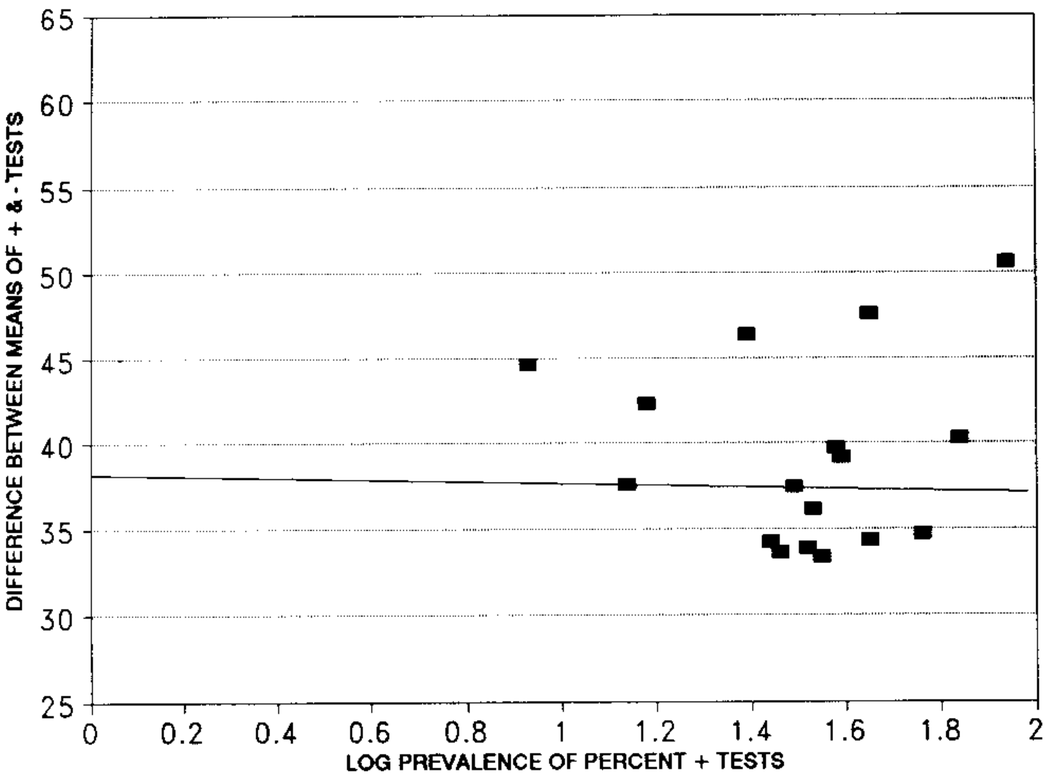

Fig. 5. Regression of difference between mean percent ELISA of positive and negative tests on the logarithm of percent positive test. Developing animals. Tropics of Mexico; 1988, 1989. $\hat{y}$ = 38.33 + .56x; R^2=0.0007

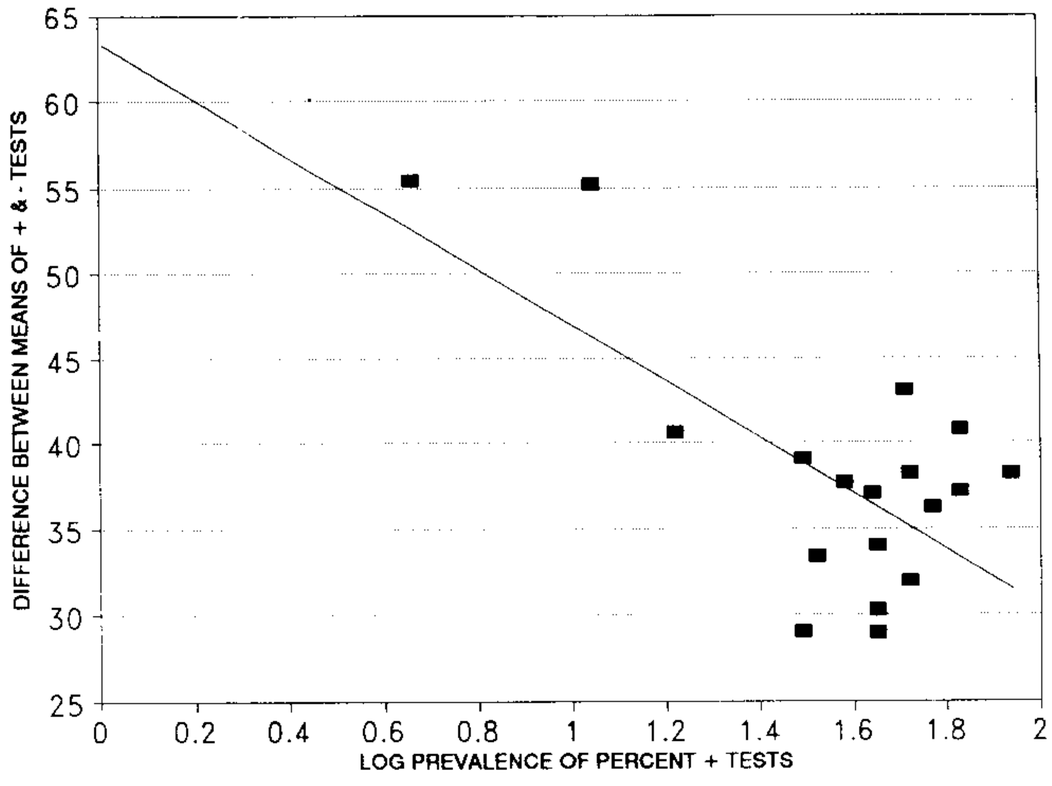

Fig. 6. Regression of difference between mean percent ELISA of positive and negative tests on the logarithm of percent positive test. Producing animals. Tropics of Mexico; 1988, 1989. $\hat{y}$ = 63.48 - 16.22x; R^2=0.46

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REFERENCES

BARAJAS-ROJAS, J.A., RIEMANN, H.P., FRANTI, F.E. Application of enzyme linked immunosorbent assay (ELISA) for epidemiological studies of diseases of livestock in the tropics of Mexico. **Rev Sci Tech Off Int Epiz,** v. 12, n. 1, 1993. In Press.