

Circulating antibodies in human echinococcosis before and after surgical treatment*

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Antibody levels in 89 patients who had been operated on for hydatid disease were studied over a period of 4 years by complement fixation, latex agglutination, bentonite flocculation, and passive haemagglutination tests. The geometric mean titres were much higher in patients with liver echinococcosis than in those whose lungs were affected. In the first month after operation, an increase in antibody level was observed more often in the former than in the latter. The raised level usually persisted for 3-6 months.

In one group of 77 patients, the serological tests either became negative 6 months to 2 years after operation or remained positive during the entire postoperative period. In the other 12 patients, who had recurrent echinococcosis, the tests did not become negative, although there was a reduction in antibody levels during the first and second postoperative years in 6 patients, followed by a rise when the disease recurred. Antibody levels remained high during the entire observation period in the other 6 patients.

The prognosis can be considered favourable in patients with low pre-operative titres, or negative tests, provided the tests remain negative up to the end of the first year after operation or become negative within one and a half years. Where there is only a small reduction in antibody titre, the prognosis may still be favourable if the decrease continues to fall up to the end of the second year. Since a drop in antibody titres occurred in certain cases with recurrent infection, it is advisable to defer an opinion on the prognosis until the end of the second year. If the fall in antibody titres is followed by a steady rise, or if they remain high or show slight fluctuations, a recurrence is almost certain.

The immune response in echinococcosis is the result of permanent diffusion of antigenic substances through the hydatid cyst walls. The antigenic stimulation induces a stable antibody level, but this stimulation is reduced or ceases when certain changes occur in the cyst wall and in the hydatid fluid, or after the surgical removal of the cyst. The antibodies, may persist in low titres for some time, but sooner or later they disappear completely (15).

We have studied the nature of the echinococcus antibodies, their persistence levels, and the dynamics of the immune response during the postoperative period. We have also attempted to determine to what extent the outcome of surgical treatment could be forecast from changes in antibody titres.

* This work was supported by a grant from the World Health Organization.

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MATERIALS AND METHODS

The studies were carried out on 89 patients operated on for echinococcosis. Hydatid cysts were present in the liver in 56 patients, in the lungs in 27 patients, and at other sites in 6 patients. Seventy-five of the patients were operated on for primary and 14 for secondary echinococcosis.

Sera were collected 6-10 times during the first, third, sixth, and twelfth months, as well as during the second, third, and fourth year of the postoperative period, and were stored at -20°C. The patients were examined clinically several times.

The complement fixation (CF) test was performed using a slightly modified version of the method previously described (14). The test volume was set at 0.5 ml (instead of 1 ml) and the reaction was carried out in the cold and read after incubating for 30 min at 37°C. The final reading was made on the next day after storage at 4°C overnight; titres of 1:5 and 4+ were taken as positive.

The latex agglutination (LA) test was performed as described by Szyfres & Kagan (12). Hytex polysty-

Table 1. Comparison of complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF), and passive haemagglutination (PHA) tests in 89 patients with echinococcosis tested before operation

Patients tested	No. of sera tested	CF			LA			BF			PHA		
		No. positive	% positive	GMT ^a	No. positive	% positive	GMT	No. positive	% positive	GMT	No. positive	% positive	GMT
Total hydatid patients	89	81	91	1:28	82	92	1:92	79	89	1:73	82	92	1:8 150
Primary echinococcosis ^b	75	67	89	1:23	68	91	1:72	65	87	1:62	68	91	1:6 120
liver echinococcosis	46	41	89	1:33	42	91	1:120	41	89	1:110	44	96	1:18 000
lung echinococcosis	27	24	89	1:13	24	89	1:30	22	82	1:22	22	82	1:1 010
Secondary echinococcosis	14	14	100	1:60	14	100	1:386	14	100	1:389	14	100	1:43 350

^a Geometric mean titre.

^b Including two patients with hydatid cysts localized elsewhere than in the liver or lung.

rene latex (Hyland Laboratories) was used and a titre of 1:5 was taken as positive.

The bentonite flocculation (BF) test was based on the technique of Bozicevich et al. (2) with slight modifications. The bentonite suspension was centrifuged once at 500 g for 15 min and then at 750 g for a further 15 min. The test was considered positive if the titre was 1:5 or above.

The passive haemagglutination (PHA) test was performed according to the method of Garabedian et al. (6), as modified by Todorov (13). The reaction was considered positive at a minimum titre of 1:200.

Antigens were obtained from fertile hydatid fluid collected from *Echinococcus granulosus* cysts in sheep, and were prepared as described previously (15). The antigens were kept in small quantities at -20°C before use.

RESULTS

Serology of echinococcosis before operation

The four serological tests were found to have almost equal sensitivities in the patients examined, the differences being not statistically significant ($t < 1.96$ Table 1). No differences were found in the percentage of positive tests between patients with liver and lung echinococcosis. However, the geometric mean titres for lung echinococcosis were much lower than those for liver echinococcosis. All patients who had been operated on for secondary echinococcosis gave positive results in all four tests

and had considerably higher geometric mean titres than patients with primary echinococcosis.

Serology of echinococcosis after operation

The patients under study were divided into two groups, one comprising 77 patients in whom an eventual decrease in antibody levels was observed after surgery, the other comprising 12 patients in whom recurrent echinococcosis was confirmed either immunologically or surgically.

First group of cases. Within four years after operation, the serological tests became negative in a significant proportion of patients. The highest percentage of negative results was established with the CF test, followed by the BF, LA, and PHA tests in that order, the differences being not statistically significant. The tests became negative more rapidly and more often in patients with lung echinococcosis than in those with liver echinococcosis. The percentages of patients in whom the serological tests remained positive up to the end of the observation period were:

	CF	LA	BF	PHA
Liver echinococcosis	59	66	72	86
Lung echinococcosis	13	35	14	52
Total	44	56	54	76

During the first months following operation an increase in antibody levels was observed in some

patients, this being more frequent in liver echinococcosis than in lung echinococcosis (Table 2). In other patients the titres increased slightly or remained unaltered, while in a smaller proportion they dropped immediately after operation. The rises in titre were observed as early as the first week after operation, generally reaching a maximum toward the end of the first month, and in some cases even later. The elevated antibodies persisted until the third month in 28% of the patients, up to the sixth month in 53%, and up to 1 year after surgery in 19%; they then began to decrease.

Fig. 1A shows the dynamics of the antibody response during the postoperative period in terms of the geometric mean titres. The titres, increased in all four tests during the first month; after that they began to drop. The reduction in the titres that occurred between the third month and the first year following surgery, was more pronounced in patients with liver echinococcosis (Fig. 1B) than in those with lung echinococcosis (Fig. 1C). Subsequently, the antibody titres followed a similar pattern, although they remained consistently lower in patients with lung echinococcosis.

It was possible to distinguish two types of response: rapid reduction in antibody levels and delayed reduction. The rapid antibody reduction was seen in 51 cases (66%), 28 with liver and 23 with lung echinococcosis. Before operation, 59% of these

cases had low antibody titres and only 27% had high titres. After operation, an increase in antibody titres was observed in 33% of these patients. Cases with one cyst were predominant, 22% of patients having large cysts (over 10 cm in diameter), while 21% had multiple or secondary echinococcosis. In patients with rapid decrease in antibody, the serological tests tended to become negative usually between 6 months and 2 years after operation. This process began as early as the first postoperative month. During the first 3 months after surgery, it was mainly in the patients with lung echinococcosis that the results became negative, while in patients with liver echinococcosis it was not usually until the sixth month that negative results were obtained.

Where the geometric mean titres were of the rapid reduction type, the dynamics was characterized by a steep decrease, which was most pronounced 1–3 months after operation, and by a comparatively low antibody level especially during the last 3 years of the period (Fig. 2A).

The delayed type of antibody reduction was seen in 26 cases (34%), 23 with liver and 3 with lung echinococcosis. Here the antibodies persisted during the entire postoperative period. The serological tests did not become negative. Before the operation all these cases were seropositive, 8% of patients having low titres and 92% high titres. After surgery the antibody titres were increased in 66% of the pa-

Table 2. Changes in titre levels of complement fixation (CF), latex agglutination (LA), bentonite flocculation (BF), and passive hemagglutination (PHA) tests during the first postoperative month

Test and localization	No. of sera tested	Change in titre						
		Increased		Unaltered or slightly increased		Decreased		
		No.	%	No.	%	No.	%	
CF	liver	50	27	54	17	34	6	12
	lung	26	7	27	15	57	4	15
	total	76	34	45	32	42	10	13
LA	liver	50	27	54	17	34	6	12
	lung	26	7	27	14	54	5	19
	total	76	34	45	31	41	11	14
BF	liver	50	25	50	18	36	7	14
	lung	26	6	23	15	57	5	19
	total	76	31	41	33	43	12	16
PHA	liver	50	27	54	17	34	6	12
	lung	26	7	27	14	54	5	19
	total	76	34	45	31	41	11	14

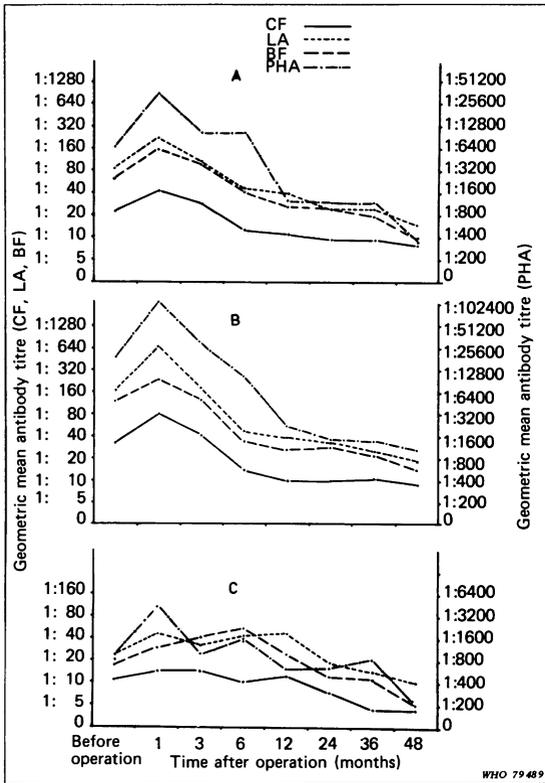


Fig. 1. First group of cases: progressive reduction in geometric mean titres in the CF, LA, BF, and PHA tests in relation to the postoperative period. A: all cases; B: liver infections; C: lung infections.

tients. Large cysts were present in 46% of patients, multiple and secondary echinococcosis in 54%. This type was characterized by very high antibody titres (Fig. 2B), several orders of magnitude higher than those seen in the rapid type of reduction (Fig. 2A).

Second group of cases. This group comprised 12 patients with recurrent hydatid disease. In 7 of them the recurrence was confirmed surgically and in 5 on

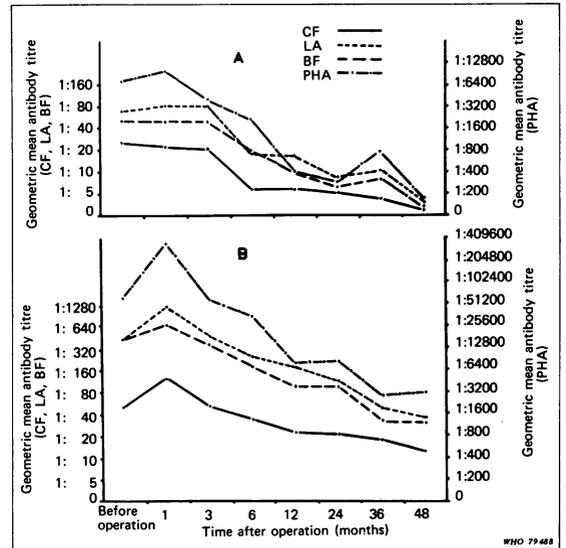


Fig. 2. Postoperative changes in geometric mean titres in the CF, LA, BF, and PHA tests in patients with (A) rapid and (B) delayed type of antibody reduction.

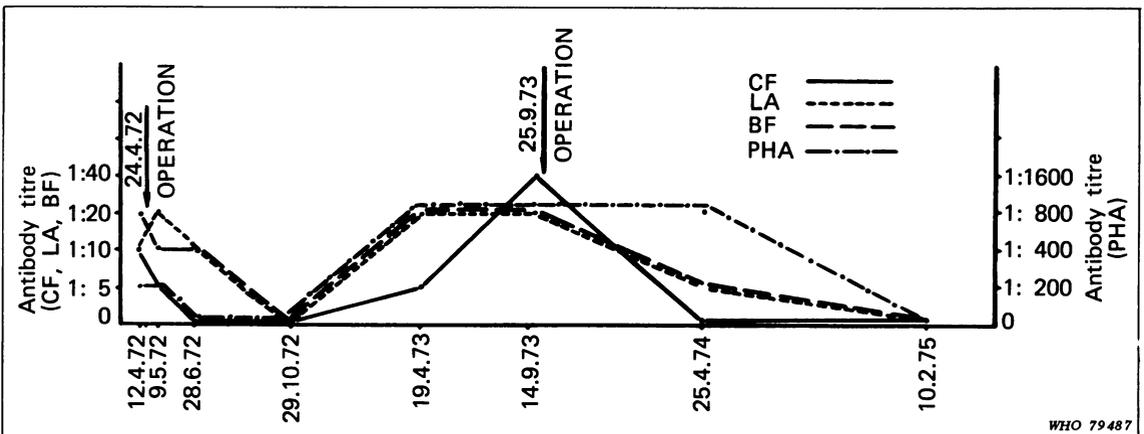


Fig. 3. Antibody response during the postoperative period in a patient with recurrent echinococcosis. All four tests became negative 2-6 months following the

operation. An increase in the titres was observed at the end of the first year after surgery.

the basis of immunological data. These cases had positive serology, the geometric mean titres being rather high: CF test, 1:75; LA test, 1:500; BF test, 1:400; and PHA test, 1:65 000.

In 6 of these cases, the titres dropped postoperatively but in 2 of them they rose again during the first year after surgery and in the other 4 they rose during the second year. In 1 of these patients, operated on for lung echinococcosis, the tests became negative as early as 6 months after operation, increasing again after 1 year but without attaining high values. The persistence of these titres up to the 18 months gave reason to suspect the presence of cysts. This was confirmed by X-ray examination and a second operation was performed (Fig. 3). In another patient operated on for liver echinococcosis, the titres decreased without becoming negative up to 6 months after the operation, but after about 9 months they rose again, reaching the pre-operative level. Hydatid cyst of the spleen was found surgically. In another patient with multiple liver echinococcosis, the titres dropped within 1 year of a second operation being performed. They then began to rise again, attaining high values between 18 and 24 months after operation. This was undoubtedly evidence of a recurrence.

In the remaining 6 cases, the high antibody titres persisted after surgery, though they fluctuated somewhat. In 2 patients with proved multiple echinococ-

cosis of the liver and peritoneum, particularly high levels persisted up to the end of the observation period. In 2 other patients, who had been operated on for lung echinococcosis, the antibody level remained high up to 4 years after surgery. On operation, one of them was found to have 3 cysts in the liver (Fig. 4). In the fifth case, the antibody level showed a slight drop during the first postoperative year, but subsequently remained high for 4 years because of another cyst in the kidney. The sixth case concerned a patient operated on for liver echinococcosis, in whom the titres continued at a high level two years after the last operation.

DISCUSSION

Antibody persistence after operation

There are considerable differences of opinion on the duration of antibody persistence after surgical removal of hydatid cysts. Fairley & Kellaway (5) and Sorice et al. (11) maintain that the CF test remains strongly positive for up to 9-12 months, while Abou-Daoud & Schwabe (1) claim that a positive test can be obtained for as long as 11 years. However, our studies indicate that in cases with lung hydatidosis the CF test could become negative as early as 3 months after operation. By the end of the first year, complement-fixing antibody had disappeared in 30% of cases. Magath (8), Lass et

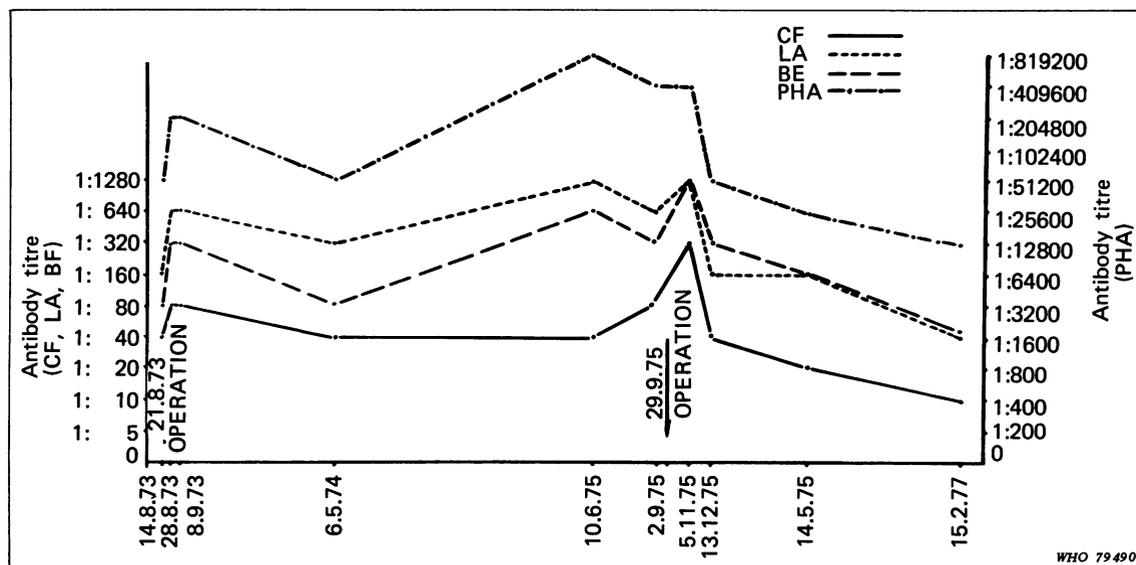


Fig. 4. Antibody response during the postoperative period in a patient with recurrent liver echinococcosis.

All four tests continued to give comparatively high titres until the second operation was performed.

al. (7), and Roy et al. (10) state that the CF test yields negative results 2 months to 1 year after operation. The CF test became negative in the first year after operation in a further 26% of our cases, and the test remained positive in 44% of cases up to the end of the observation period.

Sorice et al. (11) found that the PHA test remained positive for at least two years, after which the titres began to drop. This view, as well as that of Roy et al. (10), who state that the test becomes negative within a year and that of Lass et al. (7) who claim that the test tends to remain positive for at least 11 years, does not apply to all cases. We have shown that the PHA titre began to fall soon after the operation and that by the end of the second year it had become negative in 20% of cases, while in 76% of cases it remained positive up to the end of the observation period.

The results of the present study confirm our previous view (15) and allow some additional conclusions to be drawn regarding factors determining how long echinococcus antibodies persist after operation. The duration of antibody persistence and the antibody level were influenced to a great extent by the organ in which the hydatid cysts were located; according to Yarzabal et al. (16) the condition of the cyst may also be important. With some exceptions, the decrease in antibody response is more pronounced and the serological tests become negative more rapidly in lung than in liver echinococcosis. In cases with high pre-operative serological titres, including those with lung localization, the rate of antibody reduction is delayed. The tests become negative more rapidly in cases with low pre-operative titres. A delayed antibody reduction is observed in patients after operation for recurrences and in those with multiple hydatidosis. Cases with a transitory postoperative increase on titres are characterized by a delayed type of antibody decrease. Obviously, it would not be possible to draw general and definite conclusions concerning the duration and level of antibody persistence after surgery without taking the above-mentioned factors into consideration.

Criteria for forecasting the postoperative course of echinococcosis

Several authors maintain that the immunological data relating to the postoperative course of echinococcosis permit the results of surgical intervention to be evaluated and provide a pointer to the prognosis of the disease. According to Quilici et al. (9), in the case of a favourable prognosis the

serological tests should become negative after 6–12 months. However, our data indicate that this is true for only 10% of patients, using the PHA test, or for 30% of patients, using the CF test. Lass et al. (7) and Coudert et al. (4) consider that successful treatment of echinococcosis is accompanied by a significant and/or a gradual decrease in antibody titres. Zorihina et al. (17) assume that following successful surgery the LA test titre falls within a few months after operation and that the test turns negative after 1–3 years.

Our studies have confirmed these general conclusions and yielded some additional details. According to the data we obtained, a favourable prognosis can be envisaged in the following cases: (1) if the tests are negative before the operation and remain so up to the end of the first year after surgery; (2) if the titres are low before operation, become negative afterwards, and remain negative for one and a half years and (3) if the reduction in the titres is small and the tests do not become negative, but the downward trend continues up to the end of the second year after operation.

According to Sorice et al. (11), in the case of recurrences of echinococcosis, the CF test should remain positive for 2 years after operation. Recurrent hydatid disease is assumed if 6 months (3) or 1 year (11) after surgery the level of antibodies is still high. These views, however, are not applicable to all cases. We have shown that the test may remain positive for more than 2 years and that the titres may be high throughout the first year after surgery in cases with a postoperative increase in antibodies. More plausible is the claim of Lass et al. (7) that "an unchanged and suddenly increased titre would be suggestive of recrudescence". Similarly, Zorihina & Bregadze (18) established that in recurrent echinococcosis LA test titres remain high during the entire observation period.

Our study confirmed in some respects the views of earlier investigators (7, 11, 17, 18). Nevertheless, it seems necessary to define more precisely the immunological criteria indicating the recurrence of echinococcosis. We consider recurrent infection is likely in the following cases: (1) if one and a half years after operation the titres remain high or show only slight fluctuations, without a marked drop and without a transitory rise in titres during the first month after surgery; (2) if there is an increase in the titres during the first postoperative month that might obscure the actual dynamics of the antibody response, and if the titres remain high between the first and the second year, a recurrence would be likely at

the end of the second year, and (3) if the titres drop after operation and then rise steadily the possibility of a recurrence towards the end of the second year after surgery should be considered.

Obviously, the final judgement regarding the outcome of the surgical treatment will depend on

circumstances. For some patients it may be possible to reach a prognosis earlier than for others. In order to arrive at a prognosis, at least 4 serological examinations have to be carried out, namely at 1, 6, 12, and 24 months after surgery.

RÉSUMÉ

LES ANTICORPS CIRCULANTS DANS L'ÉCHINOCOCCOSE HUMAINE AVANT ET APRÈS TRAITEMENT CHIRURGICAL

La dynamique du titre d'anticorps a été étudiée chez 89 malades ayant subi un traitement chirurgical de la maladie hydatique, et soumis pendant 4 ans à des examens sérologiques comportant des épreuves de fixation du complément, d'agglutination au latex, de floculation à la bentonite et d'hémagglutination indirecte.

La sensibilité des diverses épreuves d'immunodiagnostic appliquées avant l'intervention chirurgicale était analogue. La moyenne géométrique des titres était beaucoup plus élevée dans l'échinococcose hépatique que dans l'échinococcose pulmonaire. Le premier mois suivant l'opération, l'augmentation des titres d'anticorps était plus fréquente dans l'échinococcose hépatique que dans l'échinococcose pulmonaire. Ce titre d'anticorps élevé subsistait habituellement pendant 3 à 6 mois. La réduction des titres s'effectue selon une dynamique correspondante: elle est plus forte dans l'échinococcose hépatique, les titres demeurant cependant toujours plus élevés en ce cas.

On observe deux types de diminution du titre d'anticorps: un type rapide et un type retardé. Chez 77 malades, on a observé le type rapide, les épreuves sérologiques devenant négatives entre le sixième mois et la deuxième année suivant l'opération, plus rapidement dans la maladie pulmonaire que dans la maladie hépatique. Le type retardé observé chez 12 malades présentant une échinococcose récurrente, entraînait une diminution du

titre d'anticorps, mais sans négativation des épreuves. Chez 6 de ces malades, on a observé une baisse du titre d'anticorps au cours de la première et de la deuxième année suivant l'opération, puis une nouvelle augmentation due à une récurrence tardive. Les six autres malades ont conservé un titre élevé d'anticorps pendant toute la période d'examen.

La tendance de la dynamique du titre d'anticorps pendant la période post-opératoire permet d'établir le pronostic. Chez les malades présentant avant l'opération une sérologie négative ou un faible titre d'anticorps, le pronostic sera favorable si les résultats négatifs persistent jusqu'à la fin de la première année suivant l'opération, ou si les résultats deviennent négatifs dans les 18 mois suivant l'opération. Si la diminution des titres d'anticorps est plus faible, le pronostic peut être considéré comme favorable si la tendance des titres d'anticorps à la baisse est observée jusqu'à la fin de la seconde année. Toutefois, une baisse des titres d'anticorps a été observée dans certains cas de récurrence. C'est pourquoi le pronostic doit être différé jusqu'à la fin de la deuxième année. Lorsqu'une tendance constante à l'augmentation des titres d'anticorps suit une première diminution, ou lorsque les titres d'anticorps restent élevés ou ne présentent que des fluctuations négligeables, il y a presque inévitablement récurrence.

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