

Farmer's lung precipitins in Doubs (a department of France): prevalence and diagnostic value

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In a French region where farmer's lung (FL) is common, we determined the prevalence of FL precipitins in dairy farmers and analyzed the relation between the presence of FL precipitins and the clinical probability of the disease. All the exposed dairy farmers of both sexes ($n = 2555$) from five districts of the Doubs department were asked to respond to a medical and professional questionnaire. A total of 1763 (69%) farmers agreed to participate. Precipitins tests were conducted in 551 (31%) farmers who showed any respiratory symptom and in a random sample of 100 asymptomatic farmers. Serum for each farmer was analyzed by both double diffusion and immunoelectrophoresis against *Micropolyspora faeni* (MF) and extracts of moldy hay (HE) from Doubs. The 651 farmers were then divided into four groups (G 1–4) with a decreasing probability of FL (G1: typical FL symptoms; G4: asymptomatic farmers). The estimated prevalence of precipitins in the whole population was as follows: 1) by double diffusion, against HE: 83%, against MF: 27%; 2) by immunoelectrophoresis, against HE: 26%, against MF: 19%. There was a close "linear" relation between the prevalence of precipitins detected by immunoelectrophoresis against HE and the symptoms: 51% in G1, 36% in G2, 29% in G3, and 13% in G4. Precipitins detected by immunoelectrophoresis were also related to exposure and geography (more immunization in tableland area than in plain or mountain area). Presence of precipitins detected by double diffusion was not related to symptoms, exposure, or geography. This study shows that the prevalence of precipitins is high in the exposed dairy farmers of Doubs, and suggests that immunoelectrophoresis (with the antigens used) is a more effective method for the diagnosis of FL than double diffusion.

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Farmer's lung (FL) is an extrinsic allergic alveolitis caused by chronic inhalation of the microorganisms, essentially thermophilic actinomycetes, found in moldy vegetable material. Its prevalence, in exposed subjects in the Northern Hemisphere, is variable, ranging from 0.5% (11) to more than 5% (10). These differences are due to occupational or climatic factors, but also to variations in the diagnostic criteria adopted in epidemiologic studies.

The diagnosis is based upon clinical, radiologic, respiratory function, and immunologic (presence of serum precipitins and lymphocytic alveolitis by bronchoalveolar lavage) indicators. However, radiologic and respiratory function abnormalities are often transitory and may be absent (2, 7, 11). It is difficult to include bronchoalveolar lavage in epidemiologic studies, and this indicator also lacks specificity (20).

This is why the presence of precipitins, the detection of which is easy, often plays an important diagnostic role, particularly in epidemiologic studies. Their presence is indicative of immunization caused by chronic exposure to FL antigens, but the links between immunization and disease are loose. According to different studies, the sensitivity of serologic tests is between 12 and 100% (12, 15), and their specificity is between 66 and 100% (13, 18).

The Doubs department of northeastern France is a semimountainous, cold, damp, fodder-producing region in which FL is common (6). The aims of the study were to determine the prevalence of precipitins in dairy farmers in Doubs and to analyze the relationship between the presence of precipitins and the clinical probability of FL.

Material and methods

The study was conducted in cooperation with the Doubs Mutualité Sociale Agricole (Agricultural Health Insurance Scheme), the medical unit of which organizes 5-yearly physical examinations for all farmers in the department. It involved dairy farmers from five districts of the Doubs department representative of its geography.

All practicing dairy farmers of both sexes, aged 16–65 years inclusive, were requested to fill out a questionnaire and give a blood sample. Questionnaires were sent by mail and collected about 10 days later during physical examinations which took place near the homes of the farmers concerned. Questionnaires were checked immediately and completed, if necessary, by the same investigator throughout the study. All subjects filling out the questionnaire provided a blood sample for the detection of precipitins. The questionnaire included personal identification and demographic information, respiratory past history, an inventory of recent respiratory symptoms, smoking habits, and occupational information (size of farm, size of cattle herd, method of storing fodder, organization of farm work). Questions on respiratory symptoms were derived from the questionnaire of the American Thoracic Society (8). Additional questions about farmer's lung symptoms were asked. Those intended to detect typical semi-delayed symptoms (see "major criterion") were as follows:

- when handling stored plant material such as fodder, straw, grain, do you (or did you) experience one or more of the following symptoms: sneeze; cough; shortness of breath; dyspnea; fever; other, specify ...?
- do (or did) these symptoms begin while you are (were) handling *or* after handling the material: during or after handling? how long after?
- what is (are) the material(s) responsible for these symptoms: fodder; straw; grain; other, specify...?
- if you experience(d) such symptoms, when did they begin? do (did) they happen every year? at what season? how many episodes per year?

Of the 2555 practicing farmers in the five districts studied, 1763 participated fully in the study (questionnaire and blood sample), i.e., a response rate of 69%. This group of 1763 subjects was representative of the farming population of the five districts in age, sex, and occupational characteristics of their farms ("Mutualité Sociale Agricole" and "Direction Départementale de l'Agriculture" of Doubs, Besançon).

Study population

Sera were studied for the detection of precipitins in all farmers with at least one respiratory symp-

tom, i.e., 551 subjects (31.2%) and in a sample of 100 subjects representative of asymptomatic farmers. These 100 subjects were extracted from the 1212 asymptomatic subjects after stratification according to age, sex, smoking habits, exposure, and the geographic location of the farmhouse based upon three different relief zones (plain, tableland, mountain).

The 651 practicing farmers (551 symptomatic and 100 asymptomatic farmers) were then divided into four groups with a decreasing probability of having FL, according to definitions based upon the following major and minor diagnostic criteria.

Major criterion

The major criterion was acute, recent (past two winters), and recurrent (at least two episodes per winter) episodes of cough, dyspnea, fever, or any combination of these three symptoms, of delayed onset (4–8 h) after handling barn-stored fodder during the winter.

Minor criteria

Minor criteria were as follows:

- shortness of breath or respiratory discomfort when mold present in fodder
- dyspnea present exclusively or worsening during period of indoor housing of cattle
- unexplained winter weight loss
- prolonged influenza-like episodes (2 weeks or more) during period of indoor housing of cattle.

Definition of patient groups according to symptoms

Group 1. Probable farmer's lung. This group included farmers fulfilling the major criterion and at least two minor criteria.

Group 2. Possible farmer's lung. This group included subjects who had respiratory symptoms after handling barn-stored fodder, minor criteria, or both, but who did not show the full definition criteria of Group 1.

Group 3. Nonspecific respiratory symptoms. This group included farmers who had respiratory symptoms not linked to antigen exposure: cough, expectoration, chronic bronchitis, or chronic dyspnea.

Group 4. Asymptomatic subjects.

Estimation of antigen exposure

All study subjects were exposed in that they were practicing farmers using fodder, but the degree of

exposure was different, and two groups were defined as follows.

Mild exposure ($n = 95$). The first group comprised farmers no longer having cattle or not participating regularly in their care (less than twice a week).

Heavy exposure ($n = 548$). The second group comprised farmers with cattle and regularly involved in their care.

The other exposure factors studied were the number of cattle, the method of storing fodder (middle-density bales, round bales, loose hay), the method of drying fodder (natural drying or artificial barn drying), and whether or not the farmer's living quarters were separate from actual barn buildings.

Precipitin tests

Each serum was tested by methods adapted from Ouchterlony's double diffusion (17) and Grabar & Williams' immunoelectrophoresis (9) for four antigens: *Micropolyspora faeni*, the strain of which was provided by the Mycological Reference Laboratory (London, UK), and three extracts of hay from Doubs: one extract from the plain, one from the tableland, and one from the mountain. Antigenic extracts were prepared in four stages as follows.

Preparation of raw extract. This included a stage of defatting by exposure to ether solution for 24 h, then extraction in Coca fluid (Cl Na_4 , CO_3HNa 2.75 g, phenol 4 g, water 1000 g for 8 days, and, finally, filtration to eliminate high-mol.-mass proteins (Seitk membrane).

Purification of raw extract. The extract was purified by overnight dialysis in a dialysis tube consisting of a semipermeable cellulose membrane.

Freeze-drying. A PP3-Serial type freeze-dryer was used.

Standardization. This consisted of confirmation of the precipitating capacity of the extracts prepared by reaction of these extracts with the serum of a strongly immunized subject suffering from FL.

The two methods of detection of precipitins used were as follows.

Immunodiffusion. In this double-diffusion system, antibody precipitins and soluble antigens are allowed to diffuse toward each other from wells cut in agar, and a line of precipitation develops where the two compounds are at optimal concentration. Molten agar solution (15 ml) on a 11×5 -cm glass slide was used. Wells were cut in the agar; serum wells should

be 12.5 mm in diameter and antigen wells 4 mm in diameter. The serum and antigens wells were separated by 6 mm edge to edge. The center well was filled with serum and the peripheral wells with antigenic extracts with a micropipette (0.25 ml for serum and 0.02 ml for antigenic extracts). Slides were positioned in a moist chamber and incubated at 4°C for 5 days, but they were examined daily for precipitin lines (viewed by transmitted light). The slides were washed in changes of 9% saline containing 1 mg/ml sodium azide for at least 5 days to remove unprecipitated protein, and, finally, they were washed in distilled water. The slides were dried by covering the gel surface with a Whatman no. 1 filter and being left at room temperature, and were then stained by immersion in amido black solution for 10 min. The slides were then decolorized in several changes of the above solvent and air-dried.

Immunoelectrophoresis. Thirty-five milliliters of 1.2% agar (in half-strength 0.1 M Barbitone acetate buffer, pH 8.6) on a 20.5×7.5 -cm glass slide was used. Wells were cut in agar layer; the wells should be 2 mm in diameter and should be separated by 12.5 mm edge to edge. The wells were filled with antigens. Slides were positioned in an electrophoresis tank filled with the same buffer (at full strength), and then connected to the buffer by wicks of filter paper. Electrodes were connected to a DC power supply, and a constant current of 25–30 mA for the whole slide for 90 min was used. Slides were removed from the tank, and channels 50 mm long and 1.5 mm wide were cut. The channels were filled with 250 μl of serum and incubated for 48 h at 4°C in a moist chamber. Slides were washed in changes of 9% saline containing 1.0 mg/ml sodium azide for at least 48 h, and then washed in distilled water for 2 h to remove the sodium chloride. Slides were dried by covering the gel surface with Whatman no. 1 filter paper, and then stained, decolorized, and dried as described in the immunodiffusion method.

These methods with these antigens were tested in a group of 20 nonfarmer subjects (13 men) living in rural zones, but not exposed to farming antigens for at least 5 years. No subject had precipitins against *M. faeni*; four had precipitins against hay extracts; one detected by both immunoelectrophoresis (one arc) and double diffusion (two arcs), and three by double diffusion (one arc).

Antigen preparation and the detection of precipitins took place in the Clermont-Ferrand immunology laboratory (J. M. Aiache).

Data analysis

Serologic results were analyzed according to respiratory symptoms, antigen exposure, and the charac-

teristics of the study population (age, sex, smoking habits, geography). Serologic results were expressed both qualitatively (presence or absence of precipitation arcs) and quantitatively (number of precipitation arcs).

Quantitative variables were expressed as the mean ± standard deviation (SD), or as the mean ± standard error (SEM). Qualitative variables were compared by the chi-square test, and quantitative variables by nonparametric analysis of variance (Kruskal-Wallis test). Cochran’s trend test was used to seek a linear relationship between serologic results and the probability of having FL. A polychotomic logistic regression model was used to analyze the relationship between serologic results and the clinical probability of having FL, after adjustment for age, sex, smoking, geography, and exposure.

Results

Description of study population

The four groups of subjects are described in Table 1. Because of their respiratory symptoms, one patient in group 1 and six in group 2 had decreased their exposure during recent months and no longer tended cattle.

Serologic results

The estimated prevalence of immunization in the 1763 farmers studied was as follows:

- As detected by double diffusion, 83.5% of farmers had precipitins regardless of the antigen, 82.6% had precipitins directed against hay extracts, and 27.1% had precipitins directed against *M. faeni*.
- As detected by immunoelectrophoresis, 26.2% of farmers had precipitins regardless of the antigen, 19% had precipitins directed against hay extracts, and 10.4% had precipitins directed against *M. faeni*.

Table 2 shows the prevalence of precipitins detected by the two methods in relation to sex, age, smoking, geography, and exposure. The presence of precipitins was linked neither to sex nor smoking habits. There was a statistically significant influence of age only for *M. faeni* detected by double diffusion. The prevalence of precipitins was higher in farmers living in tablelands. Finally, the presence of precipitins was linked to the degree of exposure (heavy or mild), particularly for *M. faeni* detected by immunoelectrophoresis.

Neither methods of storing and drying fodder nor

Table 1. Description of studied population

	Group 1 n = 35	Group 2 n = 140	Group 3 n = 376	Group 4 n = 100	P
Sex					
Men	28 (80%)	86 (61.4%)	220 (58.5%)	53 (53%)	<0.05
Women	7 (20%)	54 (38.6%)	156 (41.5%)	47 (47%)	
Age (years)					
< 30	0	20 (14.3%)	20 (5.3%)	11 (11%)	<0.01
30–39	3 (8.6%)	15 (10.7%)	58 (15.4%)	10 (10%)	
40–49	9 (25.7%)	28 (20%)	62 (16.5%)	24 (24%)	
50–59	19 (54.3%)	52 (37.1%)	138 (36.7%)	37 (37%)	
≥ 60	4 (11.4%)	25 (17.9%)	98 (26.1%)	18 (18%)	
Mean age (±SD)	51.1 (±8.5)	47.8 (±12.5)	50.6 (±11.8)	48.7 (±11.5)	NS
Geography					
Plain	1 (2.9%)	21 (15%)	51 (13.6)	21 (21%)	<0.05
Tableland	29 (82.8%)	104 (74.3%)	292 (77.6%)	61 (61%)	
Mountain	5 (14.3%)	15 (10.7%)	33 (8.8%)	18 (18%)	
Smoking		n=137	n=365	n=94	
Nonsmokers	22 (62.8%)	98 (71.6%)	218 (59.7%)	67 (71.3%)	NS
Ex-smokers	5 (14.3%)	25 (18.2%)	96 (26.3%)	16 (17%)	
Smokers	8 (22.9%)	14 (10.2%)	51 (14%)	11 (11.7%)	
Pack-years* (mean±SD)	20.1 (±12.7)	18.6 (±15.3)	18.4 (±14.6)	17.9 (±11.1)	NS
Exposure		n=139	n=371	n=98	
Mild	1 (2.9%)	15 (10.8%)	63 (17%)	16 (16.3%)	NS
Heavy	34 (97.1%)	124 (89.2%)	308 (83%)	82 (83.7%)	

* Pack-years in smokers and ex-smokers.

** Chi-square test for qualitative variables; nonparametric analysis of variance (Kruskal-Wallis) for quantitative variables.

Table 2. Prevalence (%) of positive precipitin reactions related to sex, age, smoking habits, geography, and degree of exposure

	Double diffusion		Immunoelectrophoresis	
	HE	MF	HE	MF
<i>Sex</i>				
Men (n=387)	83.7	24.8	28.4	13.2
Women (n=264)	83.7	26.5	30.3	8.3
<i>P*</i>	NS	NS	NS	NS
<i>Age</i>				
< 30 (n=51)	84.3	13.7	17.6	2
30-39 (n=86)	82.6	24.4	27.9	9.3
40-49 (n=123)	84.6	34.1	27.6	16.3
50-59 (n=246)	86.2	28.9	32.9	11.8
≥ 60 (n=145)	79.3	17.2	29	10.3
<i>P*</i>	NS	<0.01	NS	NS
<i>Smoking</i>				
Nonsmokers (n=405)	84.7	26.2	29.6	12.1
Ex-smokers (n=84)	89.3	27.4	31	9.5
Smokers (n=142)	79.6	24.6	28.9	11.3
<i>P*</i>	NS	NS	NS	NS
<i>Geography</i>				
Plain (n=94)	89.4	14.9	17	0
Tableland (n=486)	82.5	27.6	32.7	13.1
Mountain (n=71)	84.5	25.3	21.1	8.4
<i>P*</i>	NS	<0.05	<0.01	<0.001
<i>Exposure</i>				
Mild (n=95)	77.9	14.7	18.9	1.1
Heavy (n=548)	84.6	27.4	32.2	12.8
<i>P*</i>	NS	<0.05	<0.05	<0.01

* Chi-square test; HE: hay extracts; MF: *M. faeni*.

the size of cattle herds had any influence on the presence of precipitins.

Relation between symptoms and precipitins

Of asymptomatic farmers, 83% had precipitins by detected double diffusion and 22% by immunoelectrophoresis (Table 3). As detected by double diffusion, almost all patients who had precipitins directed against *M. faeni* also had them directed against hay extracts, unlike immunoelectrophoresis.

Table 3. Relation between prevalence (%) of precipitin-positive reactions and clinical probability of farmer's lung

	Group 1 n=35	Group 2 n=140	Group 3 n=376	Group 4 n=100	<i>P*</i>	<i>P**</i>
<i>Double diffusion</i>						
Hay extracts (HE)	82.8	91.4	81.4	82	NS	NS
<i>M. faeni</i> (MF)	28.6	28.6	23.4	28	NS	NS
HE or MF	82.8	91.4	82.2	83	NS	NS
<i>Immunoelectrophoresis</i>						
Hay extracts (HE)	51.4	35.7	29	13	<0.0001	<0.0001
<i>M. faeni</i> (MF)	25.7	11.4	10.1	10	<0.05	<0.05
HE or MF	60	39.3	31.9	22	<0.001	<0.001

* Chi-square test; ** linear trend test (Cochran).

Table 4. Clinical probability of farmer's lung (expressed by odds ratio and confidence interval at 95%) related to precipitin-positive reactions by immunoelectrophoresis, adjusted for sex, age, smoking habits, geography, and exposure (polychotomic logistic regression model)

	Group 1 n=35	Group 2 n=140	Group 3 n=376	Group 4 n=100	<i>P**</i>
<i>Precipitin positive</i>					
Hay extracts (HE)	5.8 (2.3-15)	3.6 (1.8-7.4)	2.6 (1.3-5)	1*	<0.001
<i>M. faeni</i> (MF)	1.6 (0.6-4.7)	0.8 (0.3-2)	0.8 (0.4-1.8)	1*	NS
HE or MF	4 (1.7-9.4)	2.1 (1.1-3.8)	1.4 (0.8-2.5)	1*	<0.005

* Risk threshold = 1.

** Chi-square test.

There was no relation between the presence of precipitins detected by double diffusion and symptoms, regardless of the detection antigen. In contrast, there was a strong correlation between the presence of precipitins detected by immunoelectrophoresis, notably against hay extracts, and the clinical probability of having FL: 51.4% of patients in group 1 had precipitins against hay extracts, 35.7% of patients in group 2, 29% in group 3, and 13% in group 4 ($P<0.0001$). The prevalence of precipitins increased proportionally and linearly with the clinical probability of having FL (Table 3).

In Table 4, this relation between symptoms and immunoelectrophoresis precipitin results is expressed after adjustment for age, sex, geography, and exposure, by a polychotomic logistic regression model. It was statistically significant only for the presence of precipitins directed against hay extracts ($P<0.001$). This indicates that farmers, when they had precipitins, had a 5.8 times greater risk of belonging to group 1, a 3.6 times greater risk of belonging to group 2, and a 2.6 times greater risk of belonging to group 3, than of belonging to group 4.

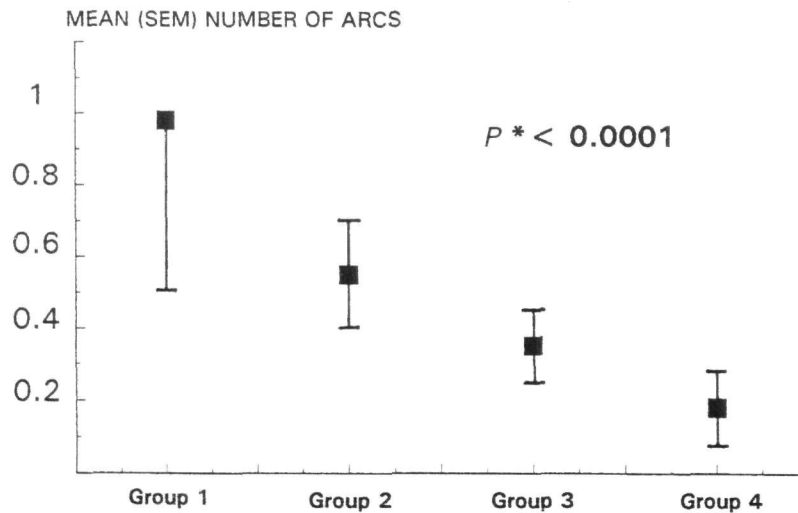


Fig. 1. Relation between number of precipitation arcs against hay extracts (immunoelectrophoresis method) and probability of farmer's lung (* Kruskal-Wallis test).

Moreover, there was a strong relation between the number of precipitation arcs against hay extracts and the symptoms confirmed by immunoelectrophoresis (Fig. 1), but not double diffusion.

Discussion

This study showed the existence of a strong relation between the clinical probability of having FL and the presence of precipitins against hay extracts, as detected by immunoelectrophoresis. It also showed that the presence of precipitins was linked to the degree of exposure, notably when they were detected by immunoelectrophoresis.

The validity of this study is based upon the definition of the four groups of farmers. The patients of group 1 were patients who had typical symptoms of FL, but the diagnosis could not be confirmed without further special investigations. The existence of semidelated respiratory symptoms may also be indicative of organic dust toxic syndrome. This syndrome is probably due to the massive inhalation of endotoxins from Gram-negative bacteria. In a previous study in the same region, we found that there were insignificant numbers of Gram-negative bacteria, a source of endotoxins (3). Furthermore, respiratory challenge tests with liquid extracts of moldy hay in a group of Doubs farmers with semidelated respiratory symptoms showed results suggestive of FL, and not of organic dust toxic syndrome (5).

It is probable that most patients in group 1 really had FL. However, it is possible that there were also patients with FL in group 2 and in group 3, which included farmers with respiratory symptoms of a nonspecific nature. In contrast, the patients of group 4 had no respiratory symptoms, and it is reasonable to assume that none of them had FL. We thus feel that, even though the definition of groups was based only upon clinical indicators, the probability of having FL decreased from group 1 to group 4.

The results of the present study showed a close link between the presence of precipitins, detected by immunoelectrophoresis, against hay extracts and symptoms. There was even a linear, "dose-effect" type relation between the presence of these precipitins and the clinical probability of having FL, after adjustment for confusion factors. This relation did not exist for precipitins against *M. faeni*. The microbiologic study cited above showed that there were essentially actinomycetes of the genus *Streptomyces* in Doubs dairy farms. It is possible that *M. faeni* is not the major FL antigen in our region. We chose these antigens (*M. faeni* and hay extracts) to detect precipitins in the study, because they have been used in routine diagnosis work for more than 10 years in the region.

Twenty-two percent of subjects in group 4 had precipitins detected by immunoelectrophoresis. This confirms the lack of specificity of these serologic methods, in agreement with other studies (13, 19, 23).

The presence of precipitins detected by double diffusion was not correlated with symptoms: more than 80% of farmers were positive by double diffusion regardless of symptoms. Double diffusion is often considered to be a more sensitive method than immunoelectrophoresis. In this study, almost all farmers who had arcs by immunoelectrophoresis also had them by double diffusion. All the farmers were chronically exposed to moldy hay. Precipitins against moldy hay extracts detected by double diffusion probably reflected this chronic exposure. This method is probably not effective to distinguish exposure from disease. Moreover, unexposed subjects may also have precipitins detected by this method (4/20 subjects; see Material and methods section).

The presence of precipitins was linked to geography. Immunization was greater in farmers living in tablelands. We had already noted, in earlier studies (4, 6), that the incidence of respiratory symptoms was higher in tablelands. This is probably due to climatic factors: annual rainfall and rainfall during haymaking are higher in tablelands than in the plain; and humidity there is certainly greater than in the mountains because of less sun. As a result, it is probable that barn-stored fodder in the tableland regions is particularly rich in molds and thermophilic actinomycetes. This influence of climate on the presence of precipitins has already been reported in other studies (16, 21), as has the link between the incidence of farmer's lung and rainfall during haymaking (10, 22).

Finally, the presence of precipitins was linked to exposure. The essential factor determining the presence of precipitins was the fact of dealing with the fodder provisions of cattle. This agrees with earlier studies (1, 11, 14). In contrast, the organization of the farm, the methods of storage and drying of fodder, and herd size had no influence on immunization. This confirms the results of studies in Wisconsin (11) and Canada (1).

In conclusion, this study suggests that immunoelectrophoresis is a more effective method for the diagnosis of FL than double diffusion. There is a fairly strong link between the presence of precipitins (and also the number of arcs of precipitation) detected by immunoelectrophoresis and FL, but the specificity of the method is weak. The detection of precipitins by double diffusion, with the antigens used, was not found to be of any value in the diagnosis of the disease.

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