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Serological Investigations of a Bovine Respiratory Disease ("Urner Pneumonie") Resembling Farmer's Lung

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The immunological response of cattle exposed to moldy hay was examined by agar gel diffusion with standard farmer's lung hay antigens. A high incidence of precipitins against *Micropolyspora faeni* (60%) and moldy hay antigen (80%) was detected in exposed but apparently healthy cattle from a region with a high incidence of bovine farmer's lung. In comparison, in the plains, a low incidence area, we found only 1 animal of 164 harboring precipitins against *M. faeni*. We further observed that many animals from exposed populations lost their precipitins during pasturing and regained them during winter housing. Thirty-nine clinical cases of bovine farmer's lung ("Urner Pneumonie") were investigated serologically. Only 49% of these animals showed precipitins against *M. faeni* and 54% showed precipitins against moldy hay antigen. We discuss in this paper the probable causes of this apparent lack of immunological response.

The occurrence of a respiratory disease in Swiss cattle, as a well-defined clinical and histopathological entity, has been recognized for many years (1, 2, 8, 11, 12). Since the disease was first recognized in the valley Schaechental of the Canton Uri, it was named "Urner Pneumonie" (1, 8). Clinical, pathological, and serological features (13) of the bovine disease demonstrate a similarity to the human disease extrinsic allergic alveolitis or farmer's lung. Serum precipitating antibody against antigens of thermophilic *Actinomycetes* occur in both man and cattle, and the pneumonia of each species is thought to be due to interaction of antigen and antibody in the lung parenchyma with production of a hypersensitivity of the Arthus type or type III of Gell and Coombs (4).

Jenkins and Pepys (9), in a serological investigation of "fog fever" in cattle, pointed out that a disease resembling farmer's lung occurs in England. A similar disease has been reported in English calves (14). The recent investigations of Pirie et al. (19) in Scotland leave no doubt about the existence of an allergic pneumonia in cattle which can be called "bovine farmer's lung" in recognition of its clinical and morphological similarity to the human disease.

Antigens reactive with farmer's lung serum are commonly derived from *Micropolyspora faeni*, *Thermoactinomyces vulgaris*, and *Thermomonospora viridis*, commonly isolated from moldy hay (5, 6, 10). The immune response to these antigens apparently follows their inhalation, and the presence of such precipitins in man is generally considered of diagnostic value since they are correlated with disease. The situation in cattle may be different, for recent observations (1, 7) have shown that a high percentage of cattle have serum precipitins without clear correlation with respiratory distress.

In the present paper, we have tried to evaluate in various groups of cattle the diagnostic significance of serum precipitins specific for antigens from *M. faeni*, *T. viridis*, *T. vulgaris*, extract of moldy hay, and *Aspergillus* sp. We examined cattle in the valley of Schaechental suffering from clinical bovine farmer's lung, as well as cattle from the same region without a history of the disease. Serum precipitins were studied during three periods of housing and two periods of pasturing. In comparison, we tested sera of cattle from the plains where bovine farmer's lung was not diagnosed.

MATERIALS AND METHODS

Serum samples. Serum was obtained and was tested unconcentrated as soon as possible or frozen at −20 C until used.

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TABLE 1. *Serological investigation of animals with bovine farmer's lung*

Antigen	1969 (18 cases)	1970 (9 cases)	1971 (12 cases)	Total (39 cases)
Micropolyspora faeni	11[a]	2	6	19 (49%)
Thermoactinomyces vulgaris	0	0	3	3
Thermomonospora viridis	2	0	7	9 (23%)
Aspergillus fumigatus	2	3	2	7
A. niger	1	0	0	1
A. nidulans	0	1	0	1
Extract of hay	7	6	8	21 (54%)

[a] Number of specimens showing lines of precipitation.

Antigens. The following antigen preparations were employed. Moldy hay was defatted with acetone and extracted in Coca's solution for 7 days. The extract was Seitz-filtered, dialyzed against distilled water, and freeze-dried to produce moldy hay extract antigen (18). Glycine extract was prepared from 4-day cultures of *M. faeni* on Nutrient Agar at 55 C (20). A similar glycine extract of *T. viridis* was prepared from 4-day cultures on Brain Heart Infusion Agar at 40 C. *T. vulgaris* glycine extracts were obtained from 10-day cultures on yeast extract-agar at 40 C. *Aspergillus* extracts were prepared from mycelium homogenates of 5-week cultures on Sabouraud Agar.

Agar gel diffusion. Tests were performed in 1% Noble Agar with phosphate buffer (*p*H 7.0) with the standard pattern described by Pepys (15, 17).

Moldy hay and *Aspergillus* antigens were used at a concentration of 30 mg/ml; other antigens, at 10 mg/ml in saline.

Immunoelectrophoresis. The method of Jenkins and Pepys (16) was modified by the use of cellulose acetate sheets in a Beckman Microzone apparatus. Electrophoresis was continued for 25 min with a potential gradient of about 11 v/cm. Antigens were used at a concentration of 90 mg/ml.

RESULTS

Serological pattern of cattle. The immunological response of cattle to farmer's lung hay antigens is similar to that described in man (15). We observed, however, in the immunoelectrophoretic pattern a fundamental difference between human and bovine sera in the fact that cattle sera lack an immunological response to a polysaccharide fraction of *M. faeni*. This fraction, commonly found in human sera, shows a slow migration and gives a long, rather flat arc of precipitation near the antigen well. It was designated by Pepys (15) as the C-line, in comparison with the A and B arcs of precipitation, which are protein fractions and are more anodic in position.

In contrast to human sera, bovine sera not only failed to show the C-line but also failed to give significant correlation between the grade of precipitation (in the A and B regions) and that of the clinical disease. Because of this, we considered, in the following study, only the presence or absence of precipitation without regard to the quantitative aspects of the reaction.

Serological reactions in clinical cases of bovine farmer's lung. The serological reactions against standard farmer's lung hay antigens of 39 infected animals observed during 3 years are shown in Table 1. The striking result of this survey was the relatively low percentage of positive reactions against *M. faeni* (total 49%) and moldy hay extract (54%). We did not always find a correlation between reaction to *M. faeni* and moldy hay, although we had demonstrated that the moldy hay extract contained an *M. faeni* antigen. *T. vulgaris* and *T. viridis* did not seem to play a major role. Of the nine animals which had precipitins against *T. viridis*, six had at the same time precipitins against *M. faeni* and two against *T. vulgaris*. Some reactions against *Aspergillus* antigens were noted, but in only two cases (1969), from a total of nine positive animals, was it the only antigen involved. In the other animals, it was associated with positive reactions against *M. faeni* and moldy hay. Ten animals (26% of the total) did not show precipitins against any of the thermophilic actinomycetes or moldy hay extract and eight (21%) did not show precipitins at all against the tested antigens.

Serological survey of apparently healthy cattle from Schaechental and from the plains. In 1969, we undertook to examine, by agar gel diffusion, sera of apparently healthy cattle from eight farms (85 animals) in the Schaechental (five farms with a history of bovine farmer's lung and three without any cases recorded for the preceding 5 years) and to compare the results with those obtained by use of cattle sera from farms in the plains (65 animals from six farms and 99 single animals from different farms; Table 2).

The sera of cattle from the Schaechental showed a relatively high incidence of precipitins against *M. faeni* (60% of examined animals) and moldy hay (80%), regardless of the incidence of bovine

TABLE 2. *Serological survey of cattle from Schaechental and from the plains*

Identification of farm	No. of animals	No. of animals showing precipitins				
		Micropolyspora faeni	*Thermoactinomyces vulgaris*	*Thermomonospora viridis*	Moldy hay	*Aspergillus fumigatus*
Schaechental farms where bovine farmer's lung was observed						
St. J.	11	7	2	3	10	5
I. J.	14	5	0	1	10	0
G. H.	8	7	0	0	8	4
G. A.	8	6	0	1	6	5
H. H.	11	7	0	1	10	6
Schaechental farms where bovine farmer's lung was not observed						
K. A.	12	6	0	1	7	4
M. J.	16	8	0	0	12	5
Sch. K.	5	5	1	1	5	0
Six plains farms where bovine farmer's lung was not known	65	0	0	0	0	6
Plains farms from each of which a single animal was tested (bovine farmer's lung not known)	99	1	0	0	1	6

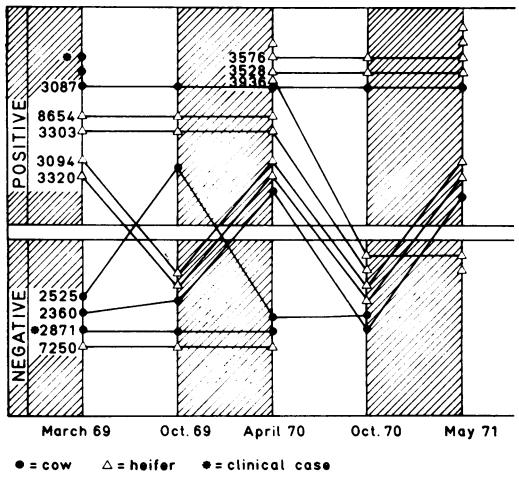

FIG. 1. *Seasonal examination of sera from cattle on the farm Hermann H. for detection of precipitins for M. faeni. (Positive = presence of precipitins against M. faeni; negative = no precipitins; striated areas = housing period.)*

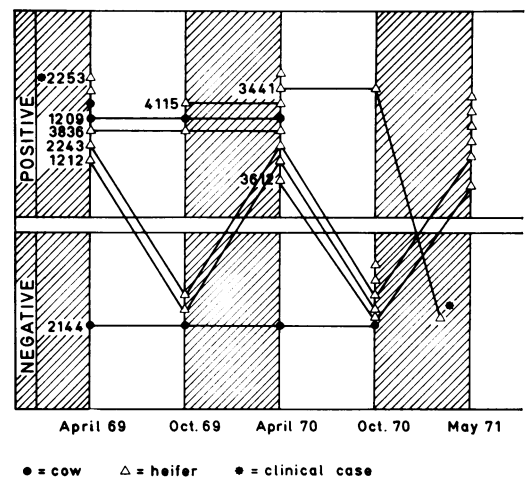

FIG. 2. *Seasonal examination of sera from cattle on the farm Hans G. for the detection of precipitins for M. faeni. (Positive = presence of precipitins against M. faeni; negative = no precipitins; striated areas = housing period.)*

farmer's lung. Of the 164 animals from the plains, only 1 had precipitins against *M. faeni* or moldy hay. In both groups, we found reactions against *A. fumigatus;* however, these occurred with a much higher incidence in sera from the Schaechental.

Seasonal serology of cattle on three farms with an incidence of bovine farmer's lung. We followed during three housing periods *M. faeni* precipitins in cattle sera (cows and heifers) from three farms where clinical cases of bovine farmer's lung were recorded (Fig. 1–3). Individual cattle on the farm Hermann H. (Fig. 1) may be placed within one of three groups according to their serum precipitin reaction with *M. faeni* antigens: (i) cows or heifers which showed no precipitins and remained negative throughout the year (among them, cow 2871 showed typical clinical

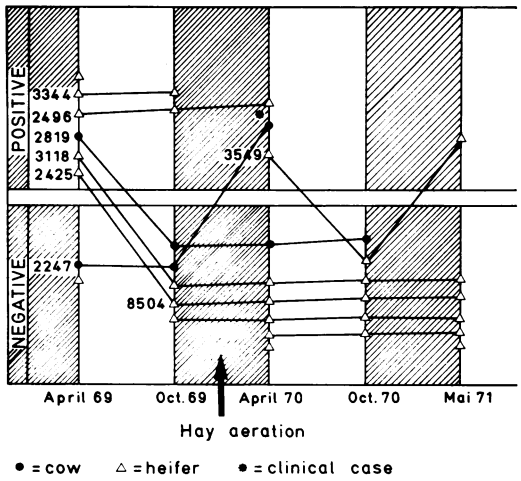

FIG. 3. *Seasonal examination of sera from cattle on the farm Alois G. for the detection of precipitins for M. faeni. (Positive = presence of precipitins against M. faeni; negative = no precipitins; striated areas = housing period.)*

symptoms); (ii) cows or heifers which formed precipitins and continued to produce them; and (iii) animals which were positive after housing, became negative after pasturing, and formed precipitins again after a new housing period. This last reaction seemed to be rather common. A single cow (2525) became positive after pasturing. No other cattle examined in the survey were found to develop precipitins after pasturing.

On the farm Hans G. (Fig. 2), the results of serology were similar to those described for farm Hermann H. Our attention was drawn to a clinical case (heifer 3441). This heifer became sick in December 1970 and was at that time serologically negative in spite of having been positive in April and October 1970.

On the farm Alois G. (Fig. 3), many positive animals were found in 1969 after housing. The installation of hay-drying equipment, however, completely modified the pattern of reactions, since practically all animals lost their precipitins and remained negative even after housing. Some exceptions are of interest. Cow 2247 was negative in October 1969 and, in spite of hay drying, became positive and showed clinical signs of bovine farmer's lung at the end of the housing period (1970). Heifer 3549 also showed a rise of precipitins, but this time without evident clinical manifestations.

DISCUSSION

The striking fact from our survey on clinical cases of bovine farmer's lung (Table 1) is the relatively low percentage of animals yielding precipitins against standard farmer's lung hay antigens (49% against *M. faeni* and 54% against moldy hay extract). Thus, the serology of bovine farmer's lung seems to differ from that of farmer's lung, in which disease 80 to 90% of patients produce precipitins against *M. faeni* or moldy hay (15). The lack of precipitins in many affected animals gives rise to the following speculation. (i) Some cases may have been diagnosed incorrectly in spite of the quite typical clinical picture (four cases were, however, histologically confirmed). (ii) It is also quite possible that a greater diversity of antigens, other than those included in our tests, could be involved in this disease. (iii) The quality of the antigens used, particularly those of *M. faeni*, may be important. Our antigens were extracted from mycelia and spores and may have been deficient in metabolic antigens which, according to others, are of importance (3, 21). (iv) The absence of precipitins in affected cattle may be due to the presence of an excess of antigen in the lung which could exhaust or mask precipitins. Such a hypothesis may account for the lack of precipitins observed in one clinically sick cow which had previously shown precipitins during health. (v) We must further consider the possibility that precipitins may not play the main role in the pathogenesis of bovine farmer's lung and that the fortuitous or apparent absence of precipitins does not exclude an immunological reaction at the site of the lung. Immunohistological studies to elucidate this point are being made.

Our results demonstrate that the presence of precipitins against *M. faeni* or moldy hay is not necessarily associated with clinical manifestations of bovine farmer's lung, since 61 and 81% of apparently healthy animals from the Schaechental harbor precipitins against *M. faeni* and moldy hay, respectively. It is interesting to note that, in spite of a presumably homogeneous and constant exposure, only a certain percentage of individuals on a given farm form precipitins, suggesting individual variation in immune response. In spite of the introduction of hay drying on one farm, a measure which should markedly reduce hay molding, and which was associated with a reduced percentage of cattle producing precipitins, we observed a clinical case of bovine farmer's lung with precipitin formation. This would seem to confirm the fact that some individuals are particularly sensitive even to low exposure to antigens, and this predisposition results in the overall incidence in a group of animals all equally at risk.

In three investigated farms, we observed an as yet unreported phenomenon, the loss of precipitins in apparently healthy animals after

pasturing and their reappearance during housing. This seasonal fluctuation would seem to be best explained by the seasonal exposure to antigen during winter housing. It must also be considered that the antibodies responsible for the transitory appearance of precipitins may not belong to the same immunoglobulin class as those which possibly mediate the disease.

The present serological investigation indicates that the use of the precipitation test is of little value as a single tool in the diagnosis of bovine farmer's lung. This initial stage of investigation however has provided a basis for further studies on the pathogenesis of this disease in cattle, which should serve as a model for better understanding of farmer's lung.

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