Acta vet. scand. 1980, *21*, 124—133.

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EXPERIMENTAL MAEDI INFECTION IN SHEEP

2. ANTIBODY RESPONSE*

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LIISA SIHVONEN, T. ESTOLA and J. TUOMI: *Experimental maedi infection in sheep. 2. Antibody response.* Acta vet. scand. 1980, *21*, 124—133. — All 4 sheep inoculated via the respiratory tract with 7×10^6 TCID50 af maedi M88 strain developed complement fixing (CF) antibodies within 3 months after inoculation, and a gradual rise in CF titers was found during the first year. The antibody titers have been maintained, though with some fluctation, through the following year, and the titers vary from 64 to 256. Virus neutralizing activity against maedi M88 strain was detected in the sera of all intrapulmonarily inoculated sheep within 8 months after inoculation. Titers have been maintained or have slightly increased. The level of titers, ranging from 8 to 256, was clearly different between individual sheep.

One of the 4 sheep inoculated intracerebrally with 5×10^5 TCID50 of maedi M88 strain developed CF antibodies 1 month after inoculation, but no neutralizing antibodies until death 11 months after inoculation. The rest of the intracerebrally inoculated sheep displayed no evidence of CF or neutralizing antibodies within 18 months after inoculation in spite of numerous virus isolations from peripheral blood leukocytes. The absence of antibodies might perhaps be attributed to phenomena such as differences in tropism, provirus state, immunological tolerance and size of inoculum.

One sheep hyperimmunized with repeated s.c. and i.v. injections of maedi M88 strain developed high CF antibody titers but lower neutralizing antibody titers.

The 2 uninoculated control sheep developed no CF or neutralizing antibodies within 18 months after inoculation.

maedi strain; slow viruses; antibody response; complement fixing antibodies; neutralizing antibodies.

Sheep experimentally inoculated with maedi or visna virus produce antibodies which are first detected at various times after inoculation. It is not known how early these antibodies can be detected in natural cases of maedi-visna infection (*Pálsson* 1976).

* This work was supported by the Finnish Academy.

In experimental maedi-visna infection, complement fixing antibodies are usually first detected 1—3 months after inoculation of large doses of virus (*de Boer* 1970, *Gudnadottir* 1974, *Petursson et al.* 1976).

The time of appearance of neutralizing antibodies, after experimental inoculation of visna-maedi virus, may vary remarkably between individual sheep. Neutralizing antibodies are usually first detected 2—5 months after inoculation of large doses of virus (*de Boer, Gudnadottir* 1974, *Petursson et al.*). In experimentally inoculated sheep, antibodies detected by the indirect immunofluorescence technique (*de Boer*) and by the immunodiffusion test (*Terpstra & de Boer* 1973, *Gutlip et al.* 1977) have been found within a few weeks to a few months after inoculation of large doses of virus.

These antibodies seem to remain detectable for years in all instances and probably throughout the life of the sheep.

In the present paper the antibody response of the experimentally inoculated sheep is reported.

MATERIALS AND METHODS

Cell cultures and virus

Sheep choroid plexus (SCP) cell cultures were grown according to the technique described previously, and maedi M88 strain was used as antigen (*Sihvonen et al.* 1980).

Experimental sheep

Four sheep were inoculated intracerebrally, 4 were inoculated via the respiratory tract, 2 sheep served as controls, and 1 sheep received s.c. and i.v. injections of maedi virus (*Sihvonen et al.*).

Sera

Blood samples of the sheep were collected weekly and the sera stored at —20°C.

Virus titration

Virus titration was made on microplates (*Sihvonen et al.*).

Neutralization test

Prevention of viral cytopathogenic effect (CPE) in microplates was used to assay the neutralizing antibodies. Serial 2-fold dilutions of the sera were made in Eagle's minimum essential

medium (MEM) plus 2 % lamb serum (LS), and they were mixed with 100 TCID50 of maedi M88 virus. The mixtures were incubated at 4°C for 48 h, and 0.2 ml of each virus serum dilution was inoculated into prewashed cells in 4 wells on microplates. A virus control was simultaneously titrated in 10-fold dilutions. The tests were read when CPE had developed in the virus control wells. Neutralization titers were recorded as reciprocals of the serum dilution preventing 50 % of the cytopathogenic effect in cells of the inoculated wells.

Complement fixation test

The complement fixation test was a modification of the technique described by *Gudnadottir & Kristinsdottir* (1967) for visna and maedi viruses. The test was performed on microplates using 3 units of guinea pig complement (Orion) and undiluted clarified cell culture fluid of maedi M88 strain, as antigen. The highest dilution of serum giving 50 % fixation was taken to be the titer of the serum.

RESULTS

All 4 intrapulmonarily inoculated sheep responded with production of complement fixing (CF) and neutralizing antibodies. The results of the complement fixation tests are presented in Table 1. The first evidence of CF antibodies was seen in sheep

Table 1. Occurrence of complement fixing antibodies. Antibodies were measured weekly. Only results from representative weeks are presented.

Time in months after inoculation	Sheep inoculated intrapulmonarily				intracerebrally				Uninoculated		s.c. and i.v.
	10	11	1008	1320	1068	1240	1296	1360	15	16	14
0	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	16	—	—	—	—	128
2	—	32	32	32	—	32	—	—	—	—	2048
3	32	64	32	32	—	64	—	—	—	—	1024
6	64	64	32	64	—	32	—	—	—	—	256
8	64	128	32	128*	—	64	—	—	—	—	512
12	128	128	64		—	32*	—	—	—	—	512
18	128	256	64		—		—	—	—	—	512

* = time of death.
— = titer < 4.

Nos. 1008 and 11 at 1½ months, in sheep No. 1320 at 2 months and in sheep No. 10, 2½ months after inoculation. A gradual rise in CF titers occurred during the first year after inoculation. After 1 year, despite some fluctuation in titer, the CF antibody titers have been maintained and titers vary from 64 to 256.

The results of the neutralization tests of the intrapulmonarily inoculated sheep are presented in Table 2. Virus neutralizing

Table 2. Occurrence of neutralizing antibodies. Antibodies were measured weekly. Only results from representative weeks are presented.

Time in months after inoculation	Sheep inoculated intrapulmonarily				intracerebrally				Uninoculated		s.c. and i.v.
	10	11	1008	1320	1068	1240	1296	1360	15	16	14
0	—	—	—	—	—	—	—	—	—	—	—
3	—	8	—	—	—	—	—	—	—	—	8
4	—	16	—	—	—	—	—	—	—	—	16
6	8	128	—	—	—	—	—	—	—	—	32
7	8	128	—	—	—	—	—	—	—	—	32
8	8	128	8	8*	—	—	—	—	—	—	16
12	16	256	8		—	—*	—	—	—	—	16
18	32	128	16		—		—	—	—	—	32

* = time of death.
— = titer < 4.

activity against maedi M88 strain was first detected in sheep No. 11 at 3 months, in sheep No. 10 at 6 months, and in sheep Nos. 1320 and 1008 at 8 months after inoculation. The neutralizing antibody titers have been maintained or they have somewhat increased. Titers ranged from 8 to 256; there was a conspicuous variation in the levels of titers. Detailed data on antibody formation of sheep No. 11, compared with the results of virus isolations, are presented in Fig. 1.

The results of the complement fixation and neutralization tests of the intracerebrally inoculated sheep are presented in Tables 1 and 2. These show that, among the group with intracerebral inoculation, sheep No. 1240 developed CF antibody 1 month after ionculation, but no neutralizing antibodies were detected up to its death, which occurred 11 months after inoculation (Fig. 2). There was no evidence of CF or neutralizing antibodies in 3 other intracerebrally inoculated sheep (Nos. 1068,

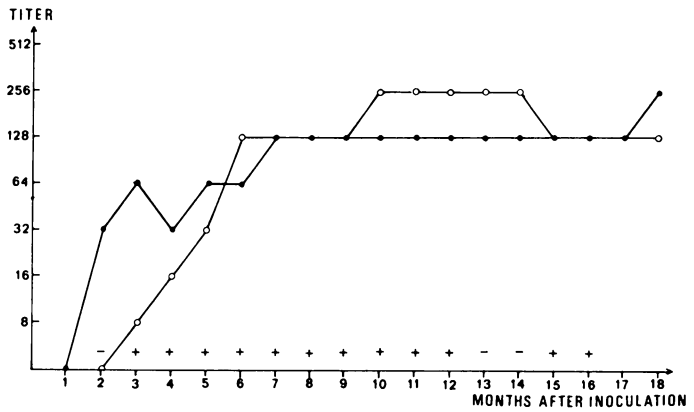

Figure 1. Antibody formation and virus isolations in sheep No. 11 inoculated intrapulmonarily with maedi virus. Antibodies were assayed by neutralization test (○——○) and complement fixation test (●——●). Maedi virus isolations (+) positive, (—) negative were done from peripheral blood leukocytes.

1296 and 1360) up to 18 months after inoculation, contrasting with the numerous virus re-isolations from the peripheral blood leukocytes (*Sihvonen et al.* 1980).

Complement fixation and neutralization tests on uninoculated control sheep were negative throughout the experiments.

The antibody formation in the case of male sheep No. 14, which was hyperimmunized with maedi M88 strain (*Sihvonen*

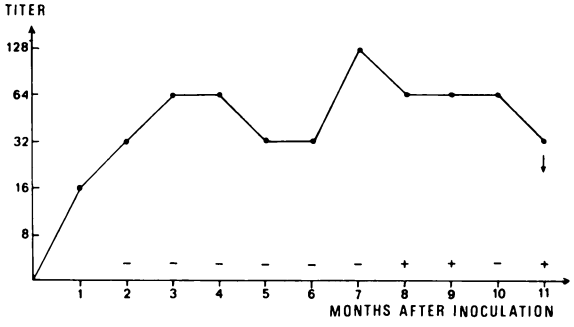

Figure 2. Antibody formation and virus isolations in sheep No. 1240 inoculated intracerebrally with maedi virus. Antibodies were assayed by neutralization test (negat.) and complement fixation test (●——●). Maedi virus isolations (+) positive, (—) negative were done from peripheral blood leukocytes. Time of death (↓).

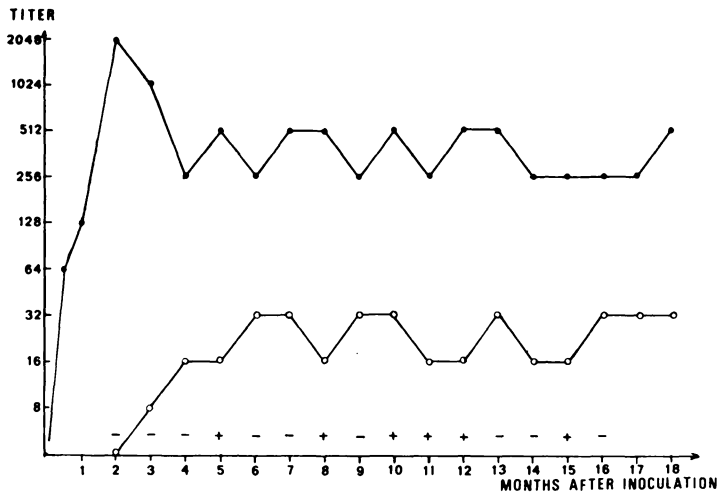

Figure 3. Antibody formation and virus isolations in sheep No. 14 given s.c. and i.v. injections of maedi virus. Antibodies were assayed by neutralization test (O——O) and complement fixation test (●——●). Maedi virus isolations (+) positive, (—) negative were done from peripheral blood leukocytes.

et al.), is presented in Fig. 3. The first evidence of CF antibodies was seen 2 weeks after s.c. injection. A gradual rise in CF antibody titers occurred in the beginning, levelling off at 256—512. Serum neutralizing activity, against maedi M 88 strain, was first detected 3 months after the first inoculation. The neutralizing antibody titer has been maintained at 16 to 32.

DISCUSSION

CF and neutralizing antibodies seem to be different, although both belong to the IgG_1 class of sheep immunoglobulins (*Petursson* 1977). CF antibodies are usually detected in a couple of months after inoculation of large doses of maedi or visna virus. The time of appearance of neutralizing antibodies varies quite remarkably between individual sheep. CF antibodies are always detected earlier than neutralizing antibodies (*de Boer* 1970, *Gudnadottir* 1974, *Petursson et al.* 1976). This order of appearance was observed in all our intrapulmonarily inoculated sheep, in 1 intracerebrally inoculated sheep and in the hyperimmunized sheep. In all these sheep CF antibodies were detected within 3 months after inoculation. However, maedi was not isolated from

the peripheral blood leukocytes of all these animals within the same time (*Sihvonen et al.* 1980). Viremia could be first demonstrated 6 months, and CF antibodies 2 months, after inoculation in an intrapulmonarily inoculated sheep No. 1320; the respective times were 8 months and 1 month for the intracerebrally inoculated sheep No. 1240, and 5 months and 2 weeks for sheep No. 14. The spread of virus should, however, precede antibody formation. When CF antibodies are detected in maedi infection before demonstration of viremia, this suggests a relatively low sensitivity of the isolation method applied. *Petursson et al.* recorded, in sheep intracerebrally inoculated with visna virus, virus isolations from peripheral blood leukocytes at an irregular rate (10 to 60 % of specimens) 2 weeks or more after inoculation, and all sheep developed CF antibodies within 3 months after inoculation. It is pointed out that in our experiments no virus isolations from peripheral blood leukocytes of sheep were tried until 2 months after inoculation.

The 3 intracerebrally inoculated sheep displayed no evidence of CF or neutralizing antibodies within 18 months after inoculation although there have been many virus isolations from peripheral blood leukocytes of the same 3 sheep 8 months or more after inoculation (*Sihvonen et al.* 1980). These then seem to be cases of infection without antibody formation, at least of such detectable by the methods used.

The lack of antibodies in 3 intracerebrally inoculated sheep may indicate that the maedi M88 strain had undergone insufficient replication in the central nervous system to evoke any antibody response. All intracerebrally inoculated sheep received a quantity of maedi M88 strain 14 times less than the intrapulmonarily inoculated animals, and this difference in inoculum might be a reason for a far slower replication of the M88 strain in the central nervous system than in the lungs. Another more likely cause might be that the maedi M88 strain used for inoculation is not as well adapted for growth in cells of the nervous system as for that in the cells of the lungs; therefore the virus would replicate very slowly in the central nervous system without causing any significant early viremia. Inoculated into the lungs, the M88 strain replicated quickly and caused earlier viremia, and a definite antibody response. The absence of pathological changes in the central nervous system, the maedi virus isolations from various parts of brain and the demonstration of pathological

changes of maedi in the lungs 11 months after intracerebral inoculation, observed in the sheep No. 1240 which had produced CF antibodies also starting 1 month after inoculation (*Sihvonen et al.*), also support the hypothesis of low neurotropism, which would somehow result in a peculiarly feeble immune response.

The reported lack of CF and neutralizing antibodies might further be theoretically explained by a minimal extracellular release of virions or by the appearance of antigen-covered cells leading to the low zone tolerance phenomenon. When only small amounts of virus antigens have contact with the immune apparatus, there might be a tolerance response instead of immune response. We intend to check this hypothesis in 1 of the 3 sheep at least by challenge inoculations of maedi virus if antibodies fail to develop in the near future. S.c. and i.v. injections should lead to antibody response in non-tolerant individuals.

Haase et al. (1977) showed that visna virus may persist as a DNA provirus in vivo. They inoculated American lambs intracerebrally with visna virus and demonstrated that proviral DNA was detected in 18 % of sheep choroid plexus cells by hybridization in situ, and that only about 1/1000 of the cells that contained viral DNA synthesized detectable amounts of the major viral gene product in vivo. This restriction was apparently removed when infected tissues were cultured in vitro. In 3 of our intracerebrally inoculated sheep, replication of viral antigens or infectious virus from the proviral DNA template might not have provided enough antigenic stimulus for the production of antibody, though possibly enough (as stated above) to induce tolerance. The fact remains, however, that viremia has demonstrably prevailed for several months in the sheep in question with no detectable signs of antibody formation. It is further conceivable that maedi virus might also persist in the leukocytes as a DNA provirus in vivo. When leukocytes are used for virus isolation the in vitro restriction would be removed and virus could be isolated.

Other authors have presented some evidence of prolonged presence of virus without antibody formation (*Gudnadottir* 1965, *Narayan et al.* 1977). We believe that our results add strong support for the hypothesis of more general occurrence of maedi-visna virus without antibody than has been believed.

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SAMMANFATTNING

Experimentell maedi infektion hos får. 2. Bildningen av antikroppar.

Alla fyra får som inokulerats via luftvägarna med 7×10^6 TCID50 av maedi M88 stammen påvisade komplement bindande (CF) antikroppar inom tre månader efter inokulationer, och en gradvis ökning av CF-titern upptäcktes under det första året. Under det påföljande året har CF antikropptitern trots en ringa fluktuation bibehållits och varierat mellan 64 og 256. Virusneutraliserande aktivitet mot maedi M88 stammen upptäcktes i sera av alla intrapulmonalt inokulerade får inom åtta månader efter inokulationen. Titrarna har bibehållits

eller ökat lindrigt. Nivån av titrarna, som släckte sig från 8 till 256, har klart varierat mellan individerna.

Ett av fåren som inokulerades intracerebralt med 5×10^5 TCID50 av maedi M88 stammen påvisade CF antikroppar en månad efter inokulationen men inga neutraliserande antikroppar till dess död elva månader efter inokulationen. Hos de andra intracerebralt inokulerade fåren har CF eller neutraliserande antikroppar inte påvisats inom 18 månader efter inokulationen trots många virus isoleringar från perifera blod leukocyter. Förklaringen till bristen på antikroppar kunde möjligen ligga i sådana fenomen som olikheter i tropism, provirus tillstånd, immunologisk tolerans eller storleken av inokulumet.

Ett får hyperimmuniserat med s.c. och i.v. injektioner av maedi M88 stammen påvisade höga CF antikropptitrar men lägre neutraliserande antikropptitrar.

Två oinokulerade kontrollfår påvisade inga CF eller neutraliserande antikroppar inom 18 månader efter inokulationen.

(Received November 12, 1979).

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