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THE PREVALENCE AND RELATION OF OVINE PROGRESSIVE PNEUMONIA TO *PASTEURELLA HAEMOLYTICA* IN TWO FLOCKS OF SHEEP

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SUMMARY

The incidence of ovine progressive pneumonia (OPP), and its relation to *Pasteurella haemolytica* in two naturally infected flocks of sheep of different ages was studied. The Ager Gel Immunodiffusion test was used for testing for OPP. Nasal Swabs were taken for the isolation of *P. haemolytica*. Four ewes were necropsied, and both gross and histopathological examinations were done. A few seronegative sheep sero-converted and the seropositive sheep remained positive. Seropositive sheep did not develop clinical illness or lesions of the disease at necropsy. Lambing (stress factor) predisposes to infection with OPP. Infection with OPP predisposes sheep for secondary bacterial infection with *P. haemolytica*.

INTRODUCTION

Ovine Progressive Pneumonia (OPP) or "Lunger's Disease", is a slowly progressing pneumonitis and meningoencephalities. (Marsh, 1923; Cutlip et al., 1979). OPP is a chronic debilitating disease of adult sheep that can cause serious economic losses in affected flock.

(Light, 1979). The disease has been recognized in the United States since 1915. (Marsh, 1923). The causative agent of OPP is Progressive Pneumonia Virus (PPV), an enveloped, spherical, single-stranded RNA virus (Cutlip and Laird, 1976; Kennedy *et al.*, 1968) classified in the family Retroviridae, subfamily Lentivirinae (Fenner, 1977).

A number of diseases of sheep resembling progressive pneumonia occur in different parts of the world, clinical, histopathological, biochemical and virological studies have disclosed that Zwoegerziekte, maedi and progressive pneumonia are identical diseases. (Takemoto *et al.*, 1971; DeBoer 1975; Weiss *et al.*, 1977). Visna, a demyelinating disease, resembles these diseases serologically but differs clinically. (Georgeson *et al.*, 1976; Sigurdsson, 1954). In visna the sheep has acute neurologic signs and dyspnea.

Clinically, OPP is characterized by an insidious onset, progressive weight loss, physical weakness, increasingly severe respiratory distress with an obdurate dry cough, and rapid breathing during exertion, a febrile state and finally, death (Sigurdsson *et al.*, 1952; Ressang et al., 1968). At the last stages of the disease emaciation and dyspnea are usually severe, however, death from secondary bacterial pneumonia may supervene before signs become severe. (Cutlip et al., 1978). The clinical signs appear 1-4 years after the animal is exposed to the causative virus. (Marsh, 1923) The clinical signs lasts over a 2-8 months period but may be enhanced if the animal is under stress (Georgeson *et al.*, 1976). In field cases of OPP, after clinical signs are manifested, the case fatality rate is 100 per cent. (Cutlip *et al.*, 1979; Sigurdsson *et al.*, 1952).

Differential diagnosis requires consideration of pulmonary tumours, lung worm infestation, pulmonary abscesses and *Mycoplasma* species pneumonia. Diagnosis can be made from clinical signs in later stages of the disease, (Marsh *et al.*, 1923; Sigurdsson and Palsson, 1958), lesions upon necropsy, (Georgeson *et al.*,1976; Harter and Coward, 1974), isolation of PPV, and serological tests such as serum neutralization, complement fixation (CF), and agar gel immunodiffusion (AGID) tests have been used for serological evidence of infection of sheep with OPP or related viruses (DeBoer, 1970; Gudnadottir and Palsson, 1967). Passive hemagglutination and CF tests are inconsistent (Cutlip *et al.*, 1978). Among these tests AGID is used to detect the presence of precipitating antibody (Cutlip *et al.*, 1977; Molitor, 1978). AGID is a simple test to do, besides it has been demonstrated that precipitating antibody appears earlier than neutralizing antibody (Deboer, 1970; cutlip *et al.*, 1977). Also,AGID has proven to be reproducible, (Cutlip *et al.*, 1978) produce strong specific antigen (Cutlip *et al.*, 1977; Molitor *et al.*, 1979) and sensitive (Cutlip *et al.*, 1977).

Transmission of OPP and maedi/visna in nature is reported to be horizontal through close contact of infected and noninfected sheep, probably in droplets via respiratory tract, (Palsson, 1976) and vertical through colostrum from mother to offspring (Gates *et al.*, 1978; Palsson, 1976). Intrauterine or prenatal (vertical) infection to the fetus has also been reported (Cross *et al.*, 1975., Hoff Jorgensen, 1977). However, others have detected no prenatal transmission (Gudnadottir, 1966; DeBoer *et al.*, 1979) and have concluded it to be of no epizootiologic importance (DeBoer *et al.*, 1979). Cutlip

et al., (1981) reported the isolation of PPV from a fetus and 12 lambs carried by infected ewes.

Necropsy findings in sheep with OPP are characterized by voluminous and heavy affected lungs (2-3 times normal weight) as a result of interstital accumulation of lymphoid cells and fibromuscular hyperplasia. Cutlip *et al.*, (1978) described lesions in sheep with OPP as: "Lungs were mottled grayish pink, firm, and large. There was chronic diffuse interstitial pneumonitis characterized by extensive hyperplasia of lymphoid cells around airways and blood vessels and by accumulation of mononuclear cells in the interstitium." They added that in some lungs, this reaction was accompanied by excessive fibrous tissue and smooth muscle and less frequently by hyperplastic epithelium of terminal bronchioles and alveoli. Secondary bacterial (*Pasteurella haemolytica*) and *Mycoplasmal* type bronchopneumonia was common. Lymphocytic meningitis, choroiditis, and leukoencephalitis were seen in few sheep. Meninges and choroid plexuses were infiltrated with lymphoid cells, and subependymal blood vessels were cuffed with lymphoid cells. Much subependymal white matter was demyelinated and had foci of necrosis and gliosis. (Cutlip *et al.*, 1979).

The rationale of this study was to characterize the extent of OPP in naturally infected sheep flocks by using AGID, determine if difference in age of sheep influence the susceptibility of OPP, to see if the antibody persists after several testing whether pulmonary lesions develop in seropositive animals. Also to determine the relationship of PPV to other infectious agents such as *Pasteurella haemolytica*, to determine the effect of stress (lambing) on the disease progress, and

to see if pulmonary lesions will develop in an OPP positively tested sheep.

Materials and Methods

Two flocks of sheep were used. One flock was used an experimental flock, and it consisted of 35 ewes, 4 years of age and older, and the other group consisted of 44 ewes ranging between 2-11 years of age. The animals of the latter group were ewes in a 450 ewe center flock and which had produced lambs that had died from pneumonia prior to weaning in previous years.

All sheep were maintained under farm flock management conditions and were housed in a semiconfined unit. Both flocks were managed similarly. The second flock was tested before lambing and after weaning.

Blood samples were collected from the jugular vein monthly for 3 times from the first flock and twice with 15 weeks period from the second one. Samples were allowed to clot and serum was removed. The serum was stored at -20° C.

Agar gel immunodiffusion test previously described by (Cutlip *et al.*, 1977) was used, with some modification by using 7ml (instead of 6ml) of 1 per cent purified agar in 0.05 M trisbuffer (pH 7.2) with 8 per cent sodium chloride added to the final mixture. Wells for antigen and antibody, cut in a hexagonal pattern with a central well, were 10 mm in diameter (instead of 8 mm) and were separated by a distance of 3mm. Sera from the sheep were tested. The center well was filled with antigen and alternate peripheral wells with test serume and a positive serum. Plates were incubated in a moist chamber at room temperature, and results were recorded at 24 and 48 hours.

Antigen and known positive erum were supplied by Dr. R.C. Cutlip.(a)

Nasal swabs were taken for the isolation of *Pasteurella haemolytica* using 5 per cent sheep blood agar, routine biochemical tests were carried out for identification.

Four ewes were chosen at random from flock 1 for necropsy. Two were tested negative for both *Pasteurella haemolytica* and OPP and the other two tested positive for OPP. Samples were taken after necropsy for bacterial isolation and also for histopathological examination.

RESULTS

The sheep were tested with AGID for OPP showed 20 per cent in flock 1 (Table 1), in flock 2, 81.9 per cent before lambing and 82.9 per cent at weaning (Table 2). The percentage of seropositive was higher in older ewes. At weaning showed higher percentage than before lambing by the addition of new positive ewes (Fig.1). Few positive ewes died after lambing from the age groups 3,4 and 6 years (Table 2), while none of the animal died in the older ages, 7-11 years.

In both flocks the seropositive ewes remained positive after 90-105 days when tested again. However, some of the negatively tested in sheep in flock 2, have seroconverted to positive.

In 16 out of 35 ewes (45.7%), in flock 1 *Pasteurella haemolytica* was isolated. Three ewes (8.6%) were positive for OPP and *Pasteurella haemolytica* was isolated; this represents 43 per cent of the positive

(a)National Animal Disease Center, US Department of Agriculture, Agricultural Research Service, P.O. Box 70, Ames, la 50010.

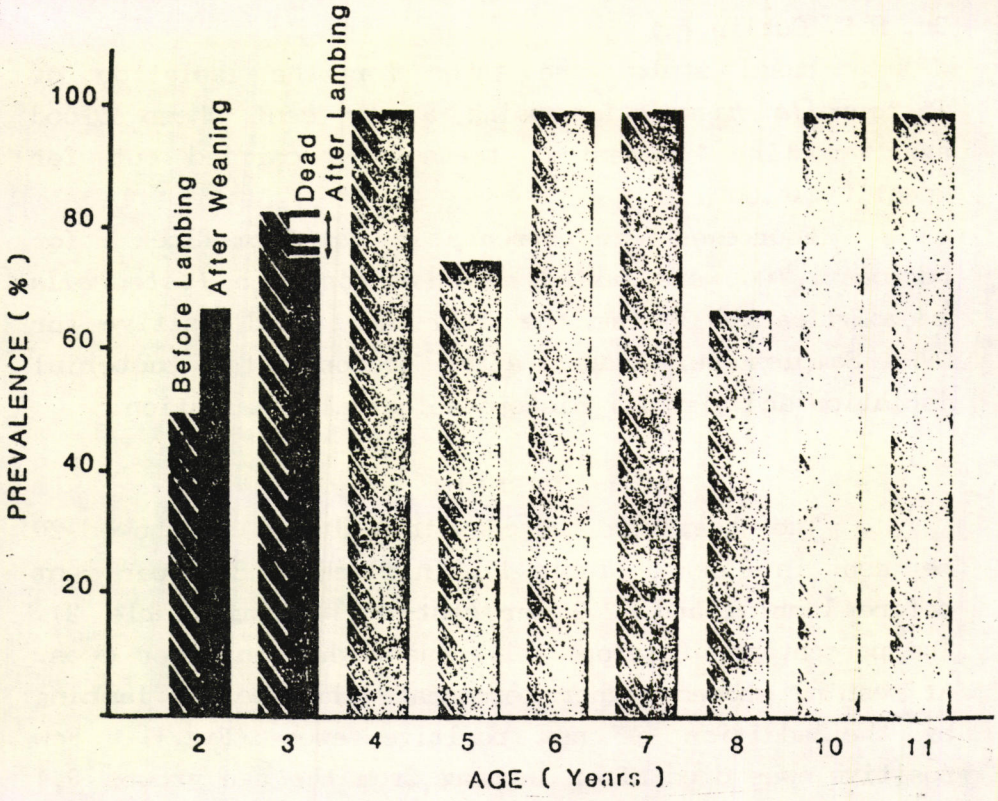

Figure 1: Prevalence of Ovine Progressive Pneumonia by Age Tested Before Lambing & After Weaning (15 wks).

Table 1. Results of testing flock-1 and flock-2 for ovine progressive pneumonia

Flock	Time of Testing	Number of Sheep Tested	Number of positive sheep	Positive%
1	Tested 3 times monthly	35	7	20
2	Tested before lambing	44	36	81.9
2	Tested after weaning (15 weeks later)	38[a]	31	82.9

a Tested after 15 weeks

Table 2. Ovine progressive pneumonia and *Pasteurella haemolytica* in flock-2 by age

Sheep's Age (year)	Before Lambing			After Weaning[a]		
	Total No. of Sheep Tested	Positive sheep OPP/*P.haem.*	% of positive OPP/*P.haem.*	Total No. of Sheep Tested	Positive Sheep OPP/*P.haem.*	% of Positive OPP/*P.haem.*
2	6	3/4	50/67	6	4/1	67/17
3	12	10/6	83/50	8[b]	6/1	75[c]/13
4	7	7/3	100/43	6[b]	6/2	100/33
5	4	3/1	75/25	4	3/1	75/25
6	4	4/2	100/50	3[b]	3/1	100/33
7	2	2/0	100/0	2	2/0	100/0
8	6	4/3	67/50	6	4/0	67/0
9	0	0	0	0	0	0
10	2	2/1	100/50	2	2/1	100/50
11	1	1/0	100/0	1	1/0	100/0
Total	44	36/20	81.9/45.5	38(b)	31/7(c)	82.9/18.4(c)

a Tested after 15 weeks.

b Some sheep died after lambing.

c Reduced % due to dead sheep.

ewes for OPP, and 19 per cent of the ewes with *Pasteurella haemolytica* isolate (Table 3).

In flock 2, 20 isolates out of 44 ewes (45.45%) of *Pasteurella haemolytica*, were isolated before lambing. Only 14 ewes (31.8%) were positive for both OPP and *Pasteurella haemolytica* isolation, this represents 38.9 per cent of the positive ewes for OPP, and 70 percent of the ewes with *Pasteurella haemolytica* (Table 4). After weaning, we were able to isolate *Pasteurella haemolytica*. From 7 ewes out of 38 (18.4%). All of those 7 ewes were positive for OPP (100%) (Table 5).

Six ewes out of 44 (13.6%) died in flock 2 in the 105th day period. All were positive for OPP, and 3 of them were positive for OPP and *Pasteurella haemolytica* was isolated. None of the 4 ewes (flock 1) sent for necropsy showed pulmonary lesions compatible with OPP as described earlier. (Cutlip *et al.*, 1979).

DISCUSSION

The seroprevalence of OPP in the 2 flocks in this study was ranging from 20 per cent in flock 1 to 81.9-82.9 per cent in flock 2 before lambing and after weaning respectively. The increase in the seroprevalence with advancing age may be consistent with adult-adult transmission and the known epidemiology of OPP. (Sigurdsson, 1954; Palsson, 1976; DeBoer *et al.*, 1979; Huffman *et al.*, 1981).

However, long incubation period due to vertical transmission is possible too (Cross *et al.*, 1975; Hoff Jorgensen, 1977)

In flock 2, during lambing due to crowding and direct transmission of virus through contaminated premises, particularly lambing sheds; we noticed an increase in the number of positive ewes for OPP.

Table 3. The relation of ovine progressive pneumonia (OPP) to *Pasteurella haemolytica* in ewes tested before lambing in flock 1.

	OPP positive	OPP negative	Total
P. haemolytica positive	3	13	16
P. haemolytica negative	4	15	19
Total	7	28	35

Table 4. The relation of ovine progressive pneumonia (OPP) to *Pasteurella haemolytica* in ewes tested before lambing in flock 2

	OPP positive	OPP negative	Total
P. haemolytica positive	14	6	20
P. haemolytica negative	22	2	24
Total	36	8	44

Table 5. The relation of ovine progressive pneumonia (OPP) to *Pasteurella haemolytica* in ewes tested after weaning in flock 2

	OPP positive	OPP negative	Total
P. haemolytica positive	7	0	7
P. haemolytica negative	24	7	31
Total	31	7	38

Histologicaly confirmed cases of OPP in flock 2 have been identified in previous years. Our data support, however, that PPV is transmitted directly between mature sheep (Gates *et al.*, 1978). Age must also play an important role in the incidence of OPP. The older the animal and the longer it remains in the flock, the greater the chance for exposure or development of signs (Fig. 1). One of the ewes in flock 2 has seroconverted to positive for OPP at weaning, while none of the ewes which were positively tested before lambing has seroconverted to negative at weaning, i.e., repeatability of test was high.

Once the sheep become infected with OPP, it will remain carrying the virus and antibody for life often without development pulmonary lesions (Cutlip *et al.*, 1978). Evidence from previous studies indicated that many sheep harbour the virus for an extended time regardless of disease status (DeBoer, 1970; Cutlip *et al.*, 1977).

In some breeds of sheep precipitating antibodies may be present for years without the animal succumbing to the disease (Molitor *et al.*, 1979).

The AGID test was easy, satisfactory, reproducible, and uncomplicated technique for detecting precipitating immunoglobulin against PPV (Cutlip *et al.*, 1978; Cutlip *et al.*, 1977; Molitor *et al.*, 1979). The test revealed clear lines and was easier to read after the modification that was made to it.

The PPV predisposes sheep for secondary bacterial infection (Cutlip *et al.*, 1977) and aggravate the condition. This was demonstrated in this study when number of ewes died after lambing. Those ewes were positive for both OPP and *Pasteurella haemolytica* (Table 2). Lambing acted as a stress factor. The cellular immune response to maedi virus was demonstrated to be definite

and slightly delayed in a study done by Shivonen (Shivonen, 1981).

Further research may be needed to clarify if the PPV shedding is increased during the clinical course of the disease compared to the incubation period. Also, some other research work may be needed to know when do lesions specific for PPV would appear after natural and /or experimental exposure to the virus, and how long it takes the animal from developing respiratory signs until death?

REFERENCES

Cross R.F., Smith C.K., Moorhead P.D.: Vertical transmission progressive pneumonia of sheep. *Am J Vet Res* 36: 465-468, 1975.

Cutlip R.C., Jackson T.A., Laird G.A.: Immunodiffusion test for ovine progressive pneumonia. *Am J Vet Res* 38: 1081-1084, 1977.

Cutlip R.C., Jackson T.A., Laird G.A.: Prevalence of ovine progressive pneumonia in a sampling of cull sheep from western and midwestern United States. *Am J Vet Res* 38: 2091-2093, 1977.

Cutlip R.C., Jackson T.A., Lehmkuhl H.D.: Diagnostic features of ovine progressive pneumonia. *JAVMA* 173: 1578-1579, 1978.

Cutlip R.C., Jackson T.A., Lehmkuhl H.D.: Lesions of ovine progressive pneumonia: Interstitial pneumonitis and encephalitis. *Am J Vet Res* 40: 1370-1374, 1979.

Cutlip R.C., Laird G.A.: Isolation and characterization of a virus associated with progressive pneumonia (maedi) of sheep. *Am J Vet Res* 38: 1377-1382, 1976.

Cutlip R.C., Lehmkuhl H.D., Jackson T.A.: Intrauterine transmission of ovine progressive pneumonia virus. *Am J Vet Res* 42: 1795-1797, 1981.

DeBoer G.F.: Zwoegerziekte virus, the causative agent for progressive interstitial pneumonia (maedi) and leucencephalistis (visna) in sheep. *Res Vet Sci* 18: 15-25, 1975.

DeBoer G.F.: Zwoegerziekte, een persisterende virus infective schapen. *Tijdschr Diergeneeskd* 95: 725-740, 1970.

DeBoer G.F., Terpstra C., Houwers D.J.: Studies in epidemiology of maedi/visna in sheep. *Res Vet Sci* 26: 202-208, 1979.

Fenner F.: Classification and nomenclature of viruses. *Intervirology* 3: 61-64, 1977.

Gates N.L., Winward L.D., Gorham J.R., *et al.*: Serologic survey of prevalence of ovine progressive pneumonia in Idaho range sheep. *JAVMA* 173: 1575-1577, 1978.

Georgsson G., Nathanson, Palsson P.A. *et al.*: The pathology of visna and maedi in sheep. In the slow virus diseases of animals and man. Edited by R.H. Kimberlin, New York: American Elsevier, 1976.

Gudnadottir M.: Host-virus interaction in maedi infected sheep, in severai L. (ed.): *Lung Tumours in Animals*, in Proceedings, Quadrennial Conf Cancer 3: 381-391, 1966.

Gudnadottir M., Palsson P.: Transmission of maedi by inoculation of a virus grown in tissue culture from maedi-affected lungs. *J Infect Dis* 117: 1-6, 1967.

Harter D.H., Coward J.E.: Sheep progressive pneumonia viruses, "slow" cytolytic agents with tumour virus

properties: A review. *Tex Rep Biol Med* 32: 649-664, 1974.

Hoff Jrgensen R.: Slow virus infections with particular reference to maedi-visna and enzootic bovine leukemia. *Vet Sci Commun* 1: 251-263, 1977.

Huffman E.M., Kirk J.H., Winward L., *et al.*, Serologic prevalence of ovine progressive pneumonia in a western rang flock of sheep. *JAVMA* 178: 708-710, 1981.

Kennedy R.C., Eklund E.M., Lopez C. *et al.*, Isolation of a virus from the lungs of Montana sheep afflicted with progressive pneumonia. *Virology* 35: 483-484, 1968.

Light M.R., Schipper I.A., Molitor T.W. *etal;* Progressive pneumonia in sheep: incidence of natural infection and establishment of clean flocks. *J Anim Sci* 49: 1157-1160, 1979.

Marsh H.: Progressive pneumonia in sheep. *JAVMA* 62: 458-473, 1923.

Molitor T.W.: Immunologic aspects of ovine progressive pneumonia. M.S. Thesis, North Dakota State Univ., Fargo, 1978.

Molitor T.W., Light M.R., Schipper I.A.: Incidence of ovine progressive pneumonia in the North Dakota State University sheep flocks, determined by agar-gel immunodiffusion *Farm Res* 37: 24-26, 1979.

Molitor T.W., Schipper I.A., Berryhill D.L., *et al.*, Evaluation of the agar-gel immunodiffusion test for the detection of precipitating antibodies against progressive pneumonia virus of sheep. *Candian J Comp Med* 43: 280-287, 1979.

Palsson P.A.: Maedi and visna in sheep, in Kimberline R.H.(ed.): *Slow virus* Diseases of Animals and Man.

Amsterdam, Oxford, North Holland Publishing Co., 1976, pp. 17-43.

Ressang A.A., DeBoer G.F., DeWijn G.C.: The lung in zwoegerziekte. *Pathol Vet* 5: 353-369, 1968.

Sigurdsson B., Maedi, a slow progressive pneumonia of sheep: An epizoological and pathological study. *Br Vet J* 110: 225-270, 1954.

Sigurdsson B., Grimsson H., Paulsson P.A.: Maedi, chronic progressive infection of sheep's lung. *J Infect Dis* 90: 233-241, 1952.

Sigurdsson B., Palsson P.A.: Visna of sheep: A slow demyelinating infection. *Br J Exp Pathol* 39: 519-528, 1958.

Sihvonen L.: Early immune response in experimental maedi. *Res Vet Sci* 30: 217-222, 1981.

Takemoto K.K., Mattern C.F.T., Stone L.B. *et al.*, Antigenic and morphological similarities of progressive pneumonia virus, a recently isolated 'slow virus' of sheep, to visna and maedi viruses. *J Virol* 7: 301-308, 1971.

Weiss M.J., Zeelon E.P., Sweet R.W. *et al;* Immunological crossreaction of the major internal protein component from "slow" viruses of sheep. *Virology* 76: 851-854, 1977.

نسبة الاصابة بالالتهاب الرئوي التفاقمي في الاغنام وعلاقتها بالاصابة بالباستيرولا هيمولتكا في قطيعين من الاغنام

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الخلاصة

درست نسبة الاصابة بالالتهاب الرئوي التفاقمي في الاغنام وعلاقتها بالاصابة بالباستيرولا هيمولتكا في قطيعين من الاغنام المصابة طبيعيا في مختلف الاعمار. استعمل فحص الاكار الجيلاتيني التشافذي للتعرف على الاصابة بالالتهاب الرئوي التفاقمي. كما استعملت المسحات الانفية لعزل الباستيرولا. وقد اجريت الصفة التشريحية على اربعة نعاج لدراسة التغييرات العينية والنسيجمرضية. اعطت بعض الاغنام نتيجة سلبية للفحص في البداية ولكنها تحولت الى موجبة فيما بعد نتيجة اصابتها بالمرض وقد بقيت الاغنام الموجبة بدون تحول دلالة على استمرارية المرض فيها. لم تبدي الأغنام الموجبة للفحص علامات المرض السريرية او اي افات للمرض بعد التشريح المرضي تعتبر الولادة (عامل اجهاد) ممهدا للاصابة بالالتهاب الرئوي التفاقمي في الاغنام. والاصابة بالالتهاب الرئوي التفاقمي في الاغنام يمهد لاصابتها البكتيرية بالباستيرولا هيمولتكا.