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 Zweegetziekte, 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; Barter 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 (Cultrip et al., 1978). Among these tests AGID is used to detect the presence of precipitating antibody (Cultrip 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; cultrip et al., 1977). Also, AGID has proven to be reproducible, (Cultrip et al., 1978) produce strong specific antigen (Cultrip et al., 1977; Molitor et al., 1979) and sensitive (Cultrip 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). Cultrip
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 interstitial 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 Mycoplasma 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 serum 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 animals 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

<table>
<thead>
<tr>
<th>Flock</th>
<th>Time of Testing</th>
<th>Number of Sheep Tested</th>
<th>Number of positive sheep</th>
<th>Positive%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tested 3 times monthly</td>
<td>35</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Tested before lambing</td>
<td>44</td>
<td>36</td>
<td>81.9</td>
</tr>
<tr>
<td>2</td>
<td>Tested after weaning (15 weeks later)</td>
<td>38*</td>
<td>31</td>
<td>82.9</td>
</tr>
</tbody>
</table>

* Tested after 15 weeks
Table 2. Ovine progressive pneumonia and *Pasteurella haemolytica* in flock-2 by age

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

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.

<table>
<thead>
<tr>
<th></th>
<th>OPP positive</th>
<th>OPP negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. haemolytica</em> positive</td>
<td>3</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td><em>P. haemolytica</em> negative</td>
<td>4</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7</td>
<td>28</td>
<td>35</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>OPP positive</th>
<th>OPP negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. haemolytica</em> positive</td>
<td>14</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td><em>P. haemolytica</em> negative</td>
<td>22</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>36</td>
<td>8</td>
<td>44</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>OPP positive</th>
<th>OPP negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. haemolytica</em> positive</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><em>P. haemolytica</em> negative</td>
<td>24</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>31</td>
<td>7</td>
<td>38</td>
</tr>
</tbody>
</table>
Histologically 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?

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دراسة الامية بالالتهاب الرئوي التفاقيفي في الاغذام وعلاقتها

بالإضافة إلى الباستيرولا هيموليتا في قطيعين من الاغذام

حيدر بدری عبود، فرع الطب والعلاج، كلية الطب البيطري، جامعة

بغداد.

الخلاصه

درست نسبة الإصابة بالالتهاب الرئوي التفاقيفي في الاغذام
وعلاقتها بالامية، الباستيرولا، هيموليتا في قطيعين من الاغذام
المصابية بفيروس إل مختل في الأعمار. استعمل فحص الآكاس الدسائل
التشذبي للتعرف على الإصابة بالالتهاب الرئوي التفاقيفي. كما
استعملت السحايا الإل وعزل الباستيرولا. وقد أجريت الدراسة
التشرحي على ارتباط نتائج دراسة التغيرات الحيوية
والسجائرية، ارتبت بعض الأغذام نتيجة سلبية للفحص في البداية
ولكنها تحولت إلى موجبة فيما بعد نتيجة إصابتها بالمرض وقد
بقيت الأغذام الموجبة بدون تحول دلالة على استمرارية المرض فيها،
لم تنبذ الأغذام الموجبة للفحص علامات المرض السريري أو أي أية
للمرض بعد التشريح الرياضي Publications of the Ministry of Health (عامل إخبار) معهد
للاصابة بالالتهاب الرئوي التفاقيفي في الاغذام، والإصابة بالالتهاب
الرئوي التفاقيفي في الاغذام يمهد لاصابتها البكتيرية بالباستيرولا
هيموليتا.

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