Viral respiratory and enteric diseases of lambs in Hungary

V. PÁLFI* and S. BELÁK**

Summary: Following the introduction of intensive fattening of lambs in large-scale farms, respiratory and enteric diseases have appeared from time to time. The aetiological role of PI-3, reo- and adenovirus has been proved. The diseases associated with these viruses are described. By comparing the losses caused by these viruses adenovirus is considered to have the most severe effect.

During the outbreaks caused by adenoviruses losses among suckling lambs and fattening lambs can be as high as 30-40 and 15 per cent, respectively. Characteristics of the viruses and diagnostic procedures are summarized.

Non-specific and specific control measures are described. A bivalent, inactivated and adsorbed sheep adenovirus vaccine was developed. The vaccine is effective in preventing the disease and in reducing economical losses.

In the last decade considerable changes have taken place in the sheep industry in Hungary. Increasing numbers of sheep have been concentrated in newly established farms where intensive fattening methods have been practised. At present about 90 per cent of sheep are concentrated in large farms. Approximately 60-70 per cent of the total income of the sheep industry comes from the meat production. An average daily weight gain of 240-260 grams has been reached. The fattening period to bring up lambs to 30-35 kilograms per head has shortened from the previous 160-180 days to 120-140 days. The intensive management, especially intensive indoor fattening method has created various animal health problems. In these farms from time to time large numbers of animals with different immunological background are collected from different external sources creating situations which favour the occurrence of various infectious diseases.

The above-mentioned facts certainly contributed to the appearance of diseases unknown before and manifested mainly by acute respiratory and enteric symptoms among lambs.

* Central Veterinary Institute, Budapest, Hungary.
** University of Veterinary Science, Department of Epizootiology, Budapest, Hungary.
The following is a summary of our observations concerning these diseases.

**DESCRIPTION OF THE DISEASES**

Outbreaks have occurred among suckling and weaned lambs aged 2 weeks-4 months and have become endemic. Predisposing factors were found to be important in initiating the clinical manifestation of disease. Our serological surveys revealed a wide distribution of viruses listed below in sheep without a disease history, unless environmental factors aggravated the viral infection.

The disease appears in an acute form. According to the size of the farm the acute phase of an epidemic lasts 1-2 months. This period can extend either through continuous lambing or uninterrupted use of the fattening houses. After cessation of their colostral immunity the new generations become susceptible. Protracted epidemics may result from putting replacement lambs originating from different farms continuously to the fattening units.

After the end of an acute epidemic virus carrier animals remain in the flock. These lambs shed the virus mainly with their faeces transferring it to the newly arrived groups. In this way the infection persists in the fattening houses; however, in the subsequent period generally only mild clinical signs show that virus infection is continuing. Predisposing factors are common in sheep management, ranging from inadequate feeding of pregnant ewes to improper microclimate of the fattening houses. In addition overcrowding, unsuitable ventilation and frequent fluctuation of temperature are the most important factors.

In investigating such epidemics parainfluenza 3 (PI-3), reo- and adenoviruses could be isolated. Further studies proved the aetiological role of these agents. The clinical signs of the diseases varied according to the different virus families. The diseases are described below.

**Disease caused by PI-3 virus.**

A rapid spread within the flocks is characteristic of the disease. Clinically respiratory signs can be seen: sneezing, lachrymation, serous nasal discharge, forced respiration and temperature rise are common. These symptoms may be seen for 7-8 days, and usually the lambs recover when there are no bacterial complications. At post mortem examination small atelectic areas can be seen in the lungs. Histologically intralobular interstitial pneumonia, peribronchiolar lymphocytic infiltration and microbronchitis are common. In the lungs of lambs died in the acute phase acidophilic cytoplasmic inclusions can be demonstrated in the bronchiolar and alveolar epithelial cells. Similar inclusions are present in the epithelial cells of the nasal mucosa (13).
Disease caused by reoviruses.

The first outbreaks were observed among imported Finnish-Landrace lambs. Later the virus was isolated from other breeds too. The disease appears among 3-4 weeks old lambs, developing respiratory symptoms similar to those mentioned above. Respiratory symptoms are accompanied by diarrhoea and sometimes conjunctivitis 3-4 days after the onset of the disease. The disease lasts about 10-14 days in a lamb. Pathologically a mild enteritis, enlargement of the peribronchiolar and mesenteric lymph nodes, and diffuse interstitial pneumonia can be found (2). Reovirus infection of lambs is frequently accompanied by secondary bacterial infection resulting in severe losses.

Disease caused by adenoviruses.

According to our observations, lambs show the first clinical symptoms 7-8 days after arrival to the infected premises. Generally, they show diarrhoea and rise in temperature, which are followed by respiratory symptoms 2-3 days later (4). In the acute phase of the disease sporadic losses occur (10 per cent of the total losses). Gross pathological and histopathological examinations reveal the most significant and characteristic changes in the lungs, upper respiratory tract, lymph nodes connected with the respiratory and digestive tracts and in the kidneys. Intralobular, interstitial pneumonia and hyperplasia of the peribronchial lymphoid follicles are common in the lungs. Hyperplasia of the lymph nodes, and degeneration and interstitial inflammation of the kidneys are also characteristic. In this stage of the disease the appearance of intranuclear inclusions in the epithelial cells of the nasal and bronchiolar mucous membrane, in the reticular cells of the lymph nodes and in the alveolar septal cells is considered characteristic and of diagnostic value (5, 6).

Diarrhoea usually stops after 7 days, while acute respiratory symptoms can still be observed for another 7 days. In most cases the respiratory involvement becomes chronic. The chronic form is characterized by sero-purulent nasal discharge, respiratory disturbances and severe coughing. Nearly 80 per cent of the affected animals show chronic symptoms. Bacteriological examination of the lungs of animals with chronic respiratory symptoms yields in some cases Mycoplasma ovipneumoniae.

Secondary infection becomes apparent when the temperature rises to 41-42°C, and anorexia and forced respiration occurs. Nasal discharge becomes purulent. A large number of animals die following secondary infection (90 per cent of the total losses).

LOSSES CAUSED BY THE DISEASES

Both the pure viral infection and the chronic respiratory syndrome caused by specific or non-specific factors may be associated with bacterial infections.
resulting in heavy losses. Among suckling lambs losses as high as 30-40 per cent can often be seen, while previously affected animals frequently show a retarded rate of growth. In fattening farms losses due to death and emergency slaughtering go up as high as 16 per cent. However, the economic losses resulting from a necessarily extended period of fattening are much higher. It was found that the amount of food required for 1 kg weight gain was about 20 per cent higher than in the period prior to the epidemic. These losses could be seen not only during the acute phase, but also in the chronic, persisting infections.

CHARACTERIZATION OF VIRUSES ISOLATED DURING THE EPIDEMICS

Parainfluenza 3 virus:

The cytological changes observed in lamb kidney tissue cultures infected with sheep isolates were comparable with those described for other PI-3 viruses. Not only intracytoplasmic inclusion bodies, but also a very strong tendency to form huge syncytia were observed. In the serological tests they proved to be related to the bovine reference virus strains. The sheep isolates replicate not only in ovine, but also in bovine cell cultures. It is noteworthy that similar infectivity titres can be found in both types of cell cultures, but the haemagglutinating titres are always higher in the tissues of sheep origin (3).

Reovirus:

The virions of reovirus strains isolated from sheep are 60-80 nm in diameter showing the classical reovirus structure. Strains proved to be resistant to the action of the lipid solvents, pH 3, saponin and trypsin. They tend to agglutinate human O erythrocytes. Isolates were typed with haemagglutination-inhibition (HI) and virus-neutralization (VN) tests (2). So far all three mammalian serotypes have already been isolated in our country.

Adenovirus:

The virions of sheep isolates show the icosahedral symmetry characteristic of adenoviruses and an average diameter of 75 nm. The average buoyant density of the complete virions in caesium chloride gradient is 1.33 g/ml. The molecular weight of ovine adenovirus DNA determined by using restriction enzymes and electrophoretic methods was found to be approximately 23 megadaltons. The structure of proteins of the virion was analyzed by PAGE. The molecular weight of the hexon was 105 K (1).
The ovine adenoviruses replicate readily in various ovine and other mammalian cell cultures, in both lamb and calf kidney and testicle tissue cultures, pig kidney cells and in different bovine cell lines.

Sheep isolates agglutinate rat erythrocytes at room temperature and at 4°C.

Agar-gel diffusion (AGD) and complement fixation (CF) tests gave evidence that all ovine strains possessed the common mast-adenovirus antigen. Direct immunofluorescence tests have confirmed this finding. Using the VN test, the virus strains isolated from the outbreaks proved to belong to ovine type 1 and bovine type 2. We have compared the properties of bovine type 2 strains isolated from sheep and cattle, respectively. Experimental infection revealed that the strains are pathogenic both for lambs and calves. Virus strains belonging to this serotype differ in their haemagglutinating activity. This difference can also be demonstrated in the DNA examined by restriction endonuclease enzyme. On this basis the existence of two subtypes can be identified.

Ovine adenoviruses belonging to types 4 and 5 were also isolated in this country.

LABORATORY DIAGNOSIS OF THE DISEASES

Due to the similar clinical appearance of the diseases laboratory methods are needed to make a proper diagnosis. For virus isolation mainly secondary embryonic ovine kidney cells are applied. The observations revealed that the most acute phase of the disease is the right period for successful virus isolation. PI-3 virus was isolated from the nasal discharge up to 8-10 days after infection (13). Reoviruses could be isolated from the nasal discharge and faeces of the infected animals until 4-9 days post infection (2). Adenoviruses are also shed by nasal discharge and faeces, however, excretion of virus by the urine could also be demonstrated. In the case of adenovirus shedding of the virus could be seen 3-14 days after infection (14).

Virus can also be isolated from the organs of animals (for example nasal mucosa, lungs, intestines, kidneys) died or killed in the acute phase of the disease.

Virus isolation, immunofluorescent technique and electron microscopic examination are all suitable methods for direct demonstration of the virus.

Besides direct demonstration of the virus, the diseases can be diagnosed by indirect serological methods. These methods are necessary even in the case of successful virus isolation. Increasing specific antibody titres give a further proof of infection. For PI-3 infection the HI test has been used. In the convalescent sera 1:64 - 1:256 titres are common, in contrast to < 1:16 values found in the pre-infection sera. Reovirus infection can be demonstrated both
by HI and VN tests. In the serum samples of recently affected lambs antibody titres of 1:16 - 1:64 can be detected. A close correlation between the results of the two tests has been found. For the demonstration of adenovirus infection different methods have been used. Antibodies against the common soluble antigen can be detected by the CF and AGD tests. These methods are suitable for rapid and easy recognition of adenovirus infection in the flocks. The AGD test can be read after 48 hours of incubation at room temperature. To identify serotypes prevailing in a given flock generally the VN test is used, but to obtain tentative identification results HI and indirect immunofluorescence methods have generally been used. Reconvalescent sera contain neutralizing antibodies in titres of 1:64 - 1:256 in contrast to < 1:2 titres found in the sera obtained at the onset of the disease.

Besides, histopathological examinations also give valuable evidence for diagnosis of disease, particularly in chronic cases, because histological lesions are detectable as long as 70 days following infection (14). Using this method viral infection can be proved and the direction of further examinations can be decided.

SURVEY ON THE INCIDENCE OF PI-3 AND ADENOVIRUS INFECTION IN LARGE-SCALE FARMS

According to our observations PI-3 and adenoviruses are the most common viral agents causing respiratory, or respiratory and enteric diseases in this country; therefore serological surveys on the distribution of these agents in large-scale flocks were conducted.

The distribution of PI-3 virus was examined in 377 blood samples taken on 19 farms. HI titres 1:16 or higher were found in 52 per cent of the sera tested; based on these results 14 farms were considered to be infected (3).

The survey on the incidence of adenoviruses was carried out with 793 blood samples taken on 50 farms. Using the AGD test 43 farms proved to be infected, 63.6 per cent of sera were positive. 200 reacting sera were further tested by the VN test including the 5 prototypes of ovine adenoviruses and type 2 bovine adenovirus. According to the serological survey it can be stated that all ovine adenovirus types isolated so far are present in the large farms. It is noteworthy that serotypes not yet isolated also prevail (7).

The results reveal a wide distribution of both PI-3 and adenoviruses, which is possibly due to frequent importation of sheep for breeding purposes and to the increased animal transfer among different farms.

CONTROL OF THE DISEASES

To reduce economical losses both non-specific and specific control measures must be applied. Among non-specific measures elimination of predisposing
factors is imperative. Among the diseases described adenovirus infection is considered to have the greatest importance. Consequently, specific control measures must be directed first of all against adenovirus infection.

The potency of various disinfectants against adenoviruses was investigated. It was found that sodium hypochlorite in a 3% concentration inactivated ovine adenovirus within 5 minutes and 3% formaldehyde was similarly effective within 10 minutes. The action of losan was of poorer effect (1).

The antigens of adenoviruses evoke a good immune response, therefore attempts were made to develop a potent vaccine (9). First it had to be decided whether both dominant serotypes should be included in the vaccine. Experiments proved that despite the common soluble antigen, cross-protection was poor indicating the necessity of incorporating at least the two most common serotypes in the vaccine. An inactivated and adsorbed vaccine was developed. This vaccine named Ovivac has been produced by the Phylaxia Veterinary Biologicals Co., Budapest, since 1978.

As to the serological response it was found that neutralizing antibodies appeared in the blood serum 7-8 days after vaccination, reaching a peak titre around 21 days post vaccination. A protection sufficient to prevent clinical symptoms as well as virus shedding lasted for a relatively short period in susceptible lambs vaccinated with a single dose only, indicating the need to enhance protection by giving a booster dose (8). The interval between the vaccinations has a significant effect on the level and persistence of antibodies. The effect of the booster dose given 10 or 42 days following the first one was significantly superior to that given 21 days after the first vaccination. The titre increase following revaccination also differed as to whether the second dose had been given 10 or 42 days following the first vaccination. Revaccination at the ascending phase of antibody production gave higher titres; whereas at the descending phase of antibody production it gave lower titres, but a longer persistence of antibodies (12).

A booster dose given to actively immunized animals resulted in the production of high antibody levels.

Antibody titres in vaccinated lambs kept at a constant favourable ambient temperature were markedly higher than in those kept at a fluctuating low and high temperature. Studies on the effect of ambient temperature support the view that stress factors can affect the capability of the organism to develop an immunological response. Possibly other stress factors, such as weaning, change of feed, transportation, overcrowding, high ammonia concentration etc. can also unfavourably affect the immunological response. On this basis it is suggested that whenever possible, likely stress factors should be eliminated before vaccinating the flocks (12).

Protection by vaccination has to be provided for suckling and weaned lambs. By vaccinating pregnant ewes twice at 42 days interval the newborn lambs will be passively protected via the colostrum (11). A single dose given 3
weeks before the expected time of delivery will enable the ewe to provide her lambs with sufficient protection. It should be noted that immunologically homogeneous flocks must be formed. Evidence of the so-called recall phenomenon was demonstrated in the case of ovine adenoviruses (10). It was found that the vaccine induced also a rise of the heterotypic antibody level, though to a lesser extent. Based on this result protection of the suckling lambs is expected to be extended to all the serotypes with which the flock had been infected before. Colostral immunity of lambs diminishes to the level when it does not counteract active immunization at about two months of age. Such lambs should be vaccinated twice at 10 days interval.

When the farms replace lambs for fattening from external sources vaccination of lambs 10 days before their shipment is advisable. Revaccination should be done immediately after their arrival.

Conducting a rigorous vaccination programme proved to be of great economical importance in large-scale farms. In farms where a consistent vaccination programme is carried out, losses could be reduced from 10-15 per cent to less than 2 per cent in fattening lambs. Furthermore, the daily weight gain increased up to approx. 220 grams in the vaccinated lambs in contrast to approx. 170 g in the non-vaccinated controls. Besides, the food consumption was approx. 20 per cent lower in the vaccinated groups.

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LES MALADIES RESPIRATOIRES ET INTESTINALES DES AGNEAUX PROVOQUÉES PAR DES VIRUS EN HONGRIE. — V. Pálfi et S. Belák.

Résumé : L’introduction de l’élevage intensif des agneaux dans des fermes de grandes dimensions a entraîné l’apparition de maladies respiratoires et intestinales, dans lesquelles le rôle étiologique des virus parainfluenza 3, réovirus et adénovirus est démontré. Les auteurs décrivent les maladies associées à ces virus. En comparant les pertes respectives qu’ils provoquent, ils concluent que les adénovirus sont les plus redoutables.

Au cours des foyers de maladie à adénovirus, les pertes peuvent atteindre 30 à 40 % chez les agneaux à la mamelle et 15 % chez les agneaux d’engraissement. Les caractéristiques des virus et les méthodes de diagnostic sont brièvement présentées.

Les auteurs décrivent les mesures de prophylaxie, spécifiques et non spécifiques, mises en œuvre. Un vaccin bivalent, inactivé et adsorbé contre l’adénovirus ovin, a été mis au point et s’est montré efficace pour la prévention de la maladie et la réduction des pertes économiques.

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Resumen: La introducción de la cría intensiva de corderos en granjas de grandes dimensiones ha traído consigo la aparición de enfermedades respiratorias e intestinales, en las que se ha demostrado el papel etiológico de los virus parainfluenza 3, reovirus y adenovirus. Describen los autores las enfermedades asociadas a los referidos virus. Al comparar las respectivas pérdidas que provocan, llegan a la conclusión que los más temibles son los adenovirus.

En el transcurso de focos de enfermedad por adenovirus, las pérdidas pueden afectar del 30 al 40% en los corderos lactantes y al 15% en los corderos de engorde. Se exponen brevemente las características de los virus y los métodos de diagnóstico.

Describen los autores las medidas de control, específicas y no específicas, aplicadas. Se ha elaborado una vacuna bivalente, inactivada y adsorbida contra el adenovirus ovino, la cual ha resultado eficiente para la prevención de la enfermedad y la reducción de las pérdidas económicas.

** REFERENCES **


