Ensuring food security and control of sheep and goat scrapie.

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Abstract. In this paper, we consider the control of animal carcasses and the detection of such a disease as scrapie. For our country, this animal disease remains quite rare, but at the same time it should not be overlooked. This is due to the fact that there are regular purchases of breeding cattle from abroad, and this is a risk of importing an infected animal. The relevance of the work is that the disease of sheep and goatscrapie can not be detected at very early stages of its development. Symptomatic manifestations do not appear immediately, there are no clear clinical characteristics, which is why this disease can not be diagnosed for a long time. To assess the health status and predict the quality of products obtained, new approaches are needed to study the products of sheep and goat slaughter. Great importance is given to standard methods of studying, identifying certain patterns in clinical, anamnestic, morphological, metric and anatomical indicators of the animal body. Using these indicators and changing their dynamic characteristics, it is possible to make significant adjustments in the definition of this disease, which would allow us to develop a number of rules for modern veterinary and sanitary evaluation of animal slaughter products.

1 Introduction

Scrapie of the sheep - a contagious (infectious) slowly developing disease of small cattle, characterized by itching with subsequent scratching, nervous manifestations - another name for the disease is "pruritus". Ends lethally amid depletion [1].

Since the middle of the last century, the efforts of veterinary specialists have been aimed at studying the pruritus of sheep, identifying the pathogen, transmission mechanism, finding methods of treatment and prevention. It should be noted that despite some successes in the study of the disease itself, the search for treatment methods has not been successful to this day [1, 2].

The agent causing the disease is a protein described by Prusiner [3].

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In the last decade, this pathology has become very important, since there are confirmed cases linking the appearance of scrapie in cattle with the possibility of transmission to humans, so this disease is referred to as zoonosis [4].

from literature sources, it is known that the incidence in goats is much lower compared to sheep, this is associated with low attention to this disease in sheep.

At the international level, immunohistochemistry (IHC), which is the standard method for detecting scrapies in goats, can detect prions in CNS tissues or in lymph nodes. In other species, such as cattle or sheep, other diagnostic tests, such as the Western blot or the ELISA test, these tests were not widely used in goats [5].

CES is closely related to the infectious variant of Creutzfeldt-Jakob disease in humans, as it is a zoonosis caused by the consumption of animal products contaminated with BSE. for this reason, countries are concerned about TSE, which has led to trade restrictions that have negatively affected the production of cattle, sheep and caprins [4, 5].

Thus, the more advanced the technology and more research on scrapie, the better the control, hence the importance of active annual surveillance of this disease, increase awareness of the health status of the country's population and contribute to epidemiological research.

For scrapie, currently, in the early stages, there are no clear clinical, medical, morphological, metric, and anatomical indicators, which could be used in laboratories of veterinary and sanitary expertise (VSE) to assess the quality of livestock products in the early stages [2].

Scrapie of sheep belongs to the group of neurodegenerative diseases [6]. The mechanism of the development of the disease is very similar to spongiform encephalopathy in cattle (mad cow disease). It is proved that the causative agent of this group of the disease is special infectious agents - prions. Prions are proteins with an abnormal structure that do not include nucleic acids. Hence, the problematic detection in the early stages after infection.

Among scientists, there is still debate about the nature of prions and their classification as living or non-living agents. Like viruses, prions are located inside the cell, have a close relationship with its membrane, and replicate due to the affected structures. However, unlike viruses, prions do not cause reactions from the body's immune system and do not provoke the production of specific antibodies aimed at combating a pathological agent [7, 8].

Noteworthy is the extremely high stability of prions in the external environment. Therefore, for example, boiling for 3 hours does not lead to the destruction of protein. Besides, the pathogenic protein is resistant to ultraviolet, high dose radiation exposure, formaldehyde solutions, strong solutions of acids and alkalis. However, it is quite unstable to the ether, urea. Withstands freezing and drying. Able to persist in the soil for years.

The source of the pathogen is sick animals. Contact transmission and a vertical path - from mother to offspring are characteristic of this disease. Prions easily overcome the placental barrier, are transmitted to the lambs with mother's milk. Under natural conditions, sheep and goats are susceptible to the causative agent of pruritus. Under the laboratory conditions mice, ferrets, Guinea pigs, monkeys, minks are susceptible to the disease.

It is believed that there is a genetic predisposition of sheep to pruritus. The most unstable to the disease are purebred animals, representatives of British breeds (suffolk, wiltshire, leister and others) [8-12].

The aim of this work was an attempt to identify the anamnestic, clinical, and morphometric indicators for scrapie in the early stages and their use for veterinary and sanitary assessment of animal carcasses.

Member states of the European Union have introduced strategies for breeding against scrapie based on the selection of genetically resistant breeding rams. An ambitious strategy

adopted in European Union consisted of selecting resistant rams for breeding throughout both breeding and production sectors. Mathematical modelling of the effect of a breeding program on the spreading capacity of scrapie in a national flock is needed for making assessments on how long a breeding strategy needs to be maintained to achieve disease control. Here we describe such a model applied to the Dutch situation, with the use of data on the genetic content of the Dutch sheep population as well as on scrapie occurrence in this population. We show that the time needed for obtaining scrapie control depends crucially on two parameters measuring sheep population structure: the between-flock heterogeneity in genotype frequencies, and the heterogeneity of mixing (contact rates) between sheep flocks. Estimating the first parameter from Dutch genetic survey data and assuming scenario values for the second one, enables model prediction of the time needed to achieve scrapie control in European Union.

2 Research methods

The research conducted relied on sources obtained from publications in scientific journals. This is because there were no unsuccessful in epizootic terms cases of this disease in the areas bordering our region at the moment.

The primary reason for which there were no identified cases is that usually, the sick animals were slaughtered when slightly unwell. The second reason is associated with the course of the disease. Since scrapie manifests itself in a rather long time, many animals become slaughtered before the manifestation of obvious clinical signs. And the third reason may lie in the fact that the disease often has a similar clinical picture with other more common diseases, for example, a frequent manifestation of the clinical picture like with dermatitis.

Well known, that dermatitis of various etiologies is widespread among animals and this factor can also hide the underlying disease.

Usually, in the case of dermatitis a physico-chemical analysis does not carry out and muscle samples from carcasses and internal organs of sheep and goats do not subject to research. Even in the “Rules in the field of veterinary medicine on conducting a veterinary and sanitary examination of meat and other products of slaughter” (as amended in 2017) [13], there are no indications related to the study of carcasses in dermatitis. Accordingly, this meat can be approved for sale or processing.

However, in the same rules (Rules in the field of veterinary medicine for conducting veterinary and sanitary examination of meat and other slaughter products (as amended in 2017)) there is a list of actions related to scrapie disease:

"130.22. Bovine spongiform encephalopathy (BSE). Scrapie of sheep and goats. Carcasses of sick animals with skin and all other slaughter products must be destroyed by burning (the Terrestrial Animal Health Code of the International Organization for Animal Health / IOAH) determines that materials of specific risk in relation to BSE bovine spongiform encephalopathy obtained from the slaughter of animals are subject to destruction and restriction on their use.

Specific risk materials from cattle are forbidden to be used as food for people, for the manufacture of medicines for people and animals, cosmetic products, for medical purposes)."

It can be assumed that at the initial stage, the latent picture of scrapie disease disguised as dermatitis is not detected and the animals are slaughtered.

One of the urgent and relevant reasons associated with the failure to identify this disease is the lack of material and technical base necessary for laboratory research. This equipment and supplies are quite expensive and most regional laboratories cannot afford it. Another

factor that limits research is the number of animals in herds that are too large, which is too costly for animal studies, for example, with dermatitis detected.

2.1 Immuno histo chemistry (IHC)

Samples were subjected to the established protocol including inhibition of the endogenous activity of the peroxidase enzyme present in the tissue, through incubation in 3% hydrogen peroxide in absolute methanol for 10min. Then, the samples were immersed in doubledistilled water and incubated in 98% formic acid for 5 min, washed and neutralized in a 0.1 M Tris-HCl buffer solution, pH 7.6 for 2 min. They were transferred to an antigen retrieval solution pH 7.6 (Target Retrieval Solution®) and held in an autoclave for 20 min at 120°C and then incubated in a Tris Saline-Tween 20 Buffer solution (TBST) pH 7.6 per 10min [5,14]. The protocol of the Pullman kit (Monoclonal F99/97.6.1) was followed for immuno staining. Samples were incubated in a proteinase K solution for 90 sec at room temperature, followed by 3 washes with TBST pH 7.6. Subsequently, incubated with the F99/97.6.1 monoclonal primary antibody in a 1:1000 dilution, for 20 min at 37°C, followed by 3 washes with TBST pH 7.6. They were incubated with the biotinylated anti-mouse polyclonal secondary antibody for 15 min at 37°C, followed by Streptavidinhorse radish peroxidase (HRP) for another 10 min. Finishing with the development of the immunological reaction, by the protected incubation from direct light with 3-amino-9-ethylcarbazole (AEC) for 5 min. Finally the parenchyma of the adjacent tissue was stained with a contrast solution (Mayer's Hematoxylin) for 10 min. Each sample was compared to the controls, positive and negative, provided by the reference laboratory. Determining a sample as positive in the presence of specific immunostaining in the tissue accompanied by symmetric and bilateral lesions; and negative in complete absence of immunostaining and vacuolar lesions [5, 14-15].

3 Pathogenesis

The pathogenesis of this disease is not well understood. The incubation period lasts a long time. With natural infection, it is 2-6 years. In cases of experimental infection, it passes faster, within 6-10 months [13, 16]. The pathogenic protein that has got into the animal's body is distributed with the flow of blood and lymph through the body. Getting into the brain leads to the development of the disease. Noteworthy is the lack of clinical signs of the disease in lambs. The accumulation of pathological protein in the brain, replacement of normal proteins of the brain substance leads to degenerative tissue changes, as a result, to pathological symptoms from the central nervous system. After samples the analyzed were able to perform the IHC. The main alterations observed in the tissues were: focal haemorrhage (30) (Fig. 1), perivascular infiltrate and pigments. No lesions similar to those produced by prions were found.

Fig. 1. Microphotography of a sample, at the level of the obex in which the presence of focal hemorrhages (circles) is observed (H/E 400x). The structure of the nervous tissue is normal, with neuronal sums, and no vacuoles or spongiosis are observed.

IHC Obtained results indicate that the 2/2 positive controls (goat and sheep) (Figure 2), always presented the red granular immunoprecipitate, in addition to vacuoles that indicate the presence of the prion associated with scrapie, and that agrees with the described by other authors [5].

Fig. 2. Microphotography of a goat obex positive control (IHC 400x). The presence of numerous vacuoles or spongioses of the nervous tissue, at the level of pericarian and of the neuropilo, and the immunostaining of red granular precipitate.

Scrapie in goats, like all diseases caused by prions, is neurodegenerative and fatal, slow progression with no inflammatory or immune response, which reduces the chances of early detection in affected animals [17]. The importance of TSEs lies in the zoonotic characteristic of strains that affect cattle (BSE), This represents a problem both economically and for public health. According to the OIE, the scrapie is the eighth causes of sheep and goat loss in the world [15, 18].

4 Symptoms

Symptoms of scrapie grow very slowly over months and years. At the very beginning noted: itching, combs, increased excitability, uneven gait. In the future, trembling of the muscles of the head and neck, gnashing of teeth, and stumbling when walking develop. Against the background of nervous phenomena, exhaustion is noted, reaching cachexia, with preserved appetite, severe scratching, hair loss mainly on the sides and lumbar. The death of the animal can occur within a few weeks after the appearance of clear clinical signs of the disease. The diagnosis is made taking into account the clinical picture, the epizootological situation, the results of a pathological study. When examining animals that have been killed or slaughtered for diagnostic purposes, exhaustion, the absence of fatty tissue in natural fat depots (near the kidneys, around the pericardium, the orbit of the eyes) are observed. Swelling of the meninges, spongiform changes in brain tissue, the phenomenon of damage to the gray matter is noted. Vacuolization in the dendrites and axons of neurons of the stem part of the medulla oblongata, the warolium bridge, cerebellum, optic tubercle, and symmetrical parts of the brain is noted. In affected neurons, large vacuoles can occupy almost the entire cytoplasm, giving it characteristic cellularity (tubularity).

Chromatolysis, lysis, and pycnosis of neurons are observed. To a lesser extent, these lesions are expressed in astrocytes and oligodendrocytes. In the final phase of the disease, hypertrophy and proliferation of astroglia and spongiform changes in the gray matter of the brain with phenomena of melting of its sections are noted.

The diagnosis is based on the analysis of epizootological, clinical data, pathological changes, the presence of characteristic cytomorphological lesions and the presence of prions by immunological methods. The diagnosis can be confirmed by bioassay - inoculation of a suspension of brain tissue of sick animals during subsequent observation of signs of damage to the central nervous system and the identification of cytomorphological changes characteristic of scrapie in infected animals.

When conducting a differential diagnosis, the similarity of symptoms with such diseases as coenurosis, estrosis, listeriosis, poisoning with certain substances, trichophytosis, scabies, some others, is observed.

5 Treatment

Treatment for scrapie is not developed. There are no vaccines to prevent the development of the disease since prions do not cause the formation of immunity.

Prevention and control measures are aimed at preventing contact of animals between the dysfunctional and successful herds. In the case of scrapie on the farm, a strict culling is carried out with the subsequent slaughter of all sick animals and animals suspected of disease with clinical signs, regardless of their pedigree or industrial value. In the USA, after histological confirmation of the diagnosis, quarantine and the slaughter of all animals in the herd are practiced. In Russia, the herds where scrapie is found are destroyed. Some sheep farmers eliminate sheep lines, among which scrapie is found.

At the moment, scientists are faced with the task of breeding sheep, genetically resistant to scrapie agent, using them for further breeding.

6 Conclusions

In the course of the analysis of literary references, we revealed a certain regularity, which allows complementing the overall picture in identifying and diagnosing scrapie.

1. The susceptibility of sheep herds to scrapie largely depends on the genetic structure of the animal and is determined mainly by the sequence of the gene encoding the PrP protein,

since several polymorphisms affect the transformation of the cellular PrPC protein into its pathological form of PrPSc [13, 16]. Nevertheless, the occurrence of only one form of sheep prion cannot be considered, since there are numerous strains of prions with various pathological and biochemical characteristics that can have different effects on animals depending on their genotypes.

2. It is generally recognized that classical scrapie is an infectious and contagious disease [19], even in cases experimentally transmitted to transgenic mice [20] and sheep [21]. The presence of several crossed animals of different herds and farms in one environment characterizing a heterogeneous herd can create favorable conditions for the development and propagation of the disease, infecting the most susceptible animals [9].

3. Other causes of differences in incidence include stress caused by farming and large herds [22]. Besides, the absence of a specific epidemiological picture and various strains of the pathogen play an important role in interpopulation variability. Several models are based on the assumption that the duration of the outbreak depends on the size of the herd and the frequency of the PrP genotype in one herd [22, 24-26].

4. In animals, the onset of clinical manifestations occurs at significantly different ages, and the average values vary from 2 to 6 years, due to noticeable differences in the profiles of the PrP genotype and age. The acquisition of infected animals was noted as the main mechanism of scrapie infection in herds [5].

5. Scrapie in goats, like all diseases caused by prions, is neurodegenerative and fatal, slow progression with no inflammatory or immune response, which reduces the chances of early detection in affected animals [16]. The importance of TSEs lies in the zoonotic characteristic of strains that affect cattle (BSE), this represents a problem both economically and for public health. According to the OIE, the scrapie is the eighth causes of sheep and goat loss in the world [18]. Undoubtedly the greatest risk is the passage of the prion of BSE into the human food chain, and, although there is no evidence that the goat prion is able to cause some effect in humans, the alert regarding the prions that affect the small ruminants are the existence of different strains to the classic, whose behavior remains unpredictable and their characterizations are still not well understood [27].

6. The true scrapie situation in many countries of South America is still unknown, because there is usually an inadequate surveillance system that is generally passive to detect affected animals. Thus it is almost impossible to establish the real state of freedom of the disease, without first establishing a standardized diagnostic system that allows an active surveillance. In this context, the ability of early and safe detection is vital for scrapie diagnosis to have real value. Particularly in animals destined for human consumption, where there is an additional requirement to determine the presence of the prion, hence our choice to use CNS from apparently healthy and clinically asymptomatic slaughter house goats that are destined for consumption [5, 15].

Despite the longer time required for its performance, compared to the so-called “rapid” tests such as ELISA and Western Blot, [28] the IHC has important advantages, since it can be performed in tissues other than CNS tissue achieving excellent results [15]. It is possible to affirm that immunohistochemistry is the most reliable, secure and confirmatory test for the diagnosis of scrapie, in relation to other immunodiagnostic techniques, such as rapid tests, and it is recognized by the OIE since 2000.

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