

Using modern research methods for identifying specific falsification of prepared lamb meat products marked as “Halal”

Yulia Shchupakova*, Fedor Vasilevich, and Yulia Petrova

Department of Parasitology and Veterinary-Sanitary Expertise, Moscow State Academy of Veterinary Medicine and Biotechnology – MVA named after K.I. Skryabin, 109377 Moscow, Russia

Abstract. Development prospects of relatively new segment of meat industry producing confessional products also known as *Halal* are discussed in the article. Problems of inconsistency to quality and safety requirements are highlighted. Results of organoleptic and physicochemical tests are represented. Results of laboratory test using methods of polymerase chain reaction, enzyme immunodetection, immunochromatographic assay are shown. These methods are aimed at identifying specific falsification of prepared meat products marked as Halal. Comparative analysis of these methodologies is given.

1 Introduction

To date, the monitoring of the Meat Industry sector is characterized by a clear decline in the turnover of beef sales. The production of cattle meat is quite expensive and requires both time and financial resources. That is why the Russian market is focusing on increasing the sales of small cattle meat. In this regard, sheep farming in Russia, as one of the directions of the meat industry, is passing through a certain revival [1].

The profitability of sheep production is supported by simple features of sheep breeding and management, rapid payback, as well as increased demand for lamb [2].

The popularity of lamb is growing for several reasons: it is rich in calories, micro- and macroelements, as well as vitamins. The fat content of lamb is less than in other types of meat. Another importance feature is the fact that mainly lamb is part of confessional products that are critically important for Islam. This religion is the second most common in Russia, which directly proportionally leads to increased demand for sheep meat products marked as Halal [3].

The term Halal refers to animal meat obtained by ritual slaughter without the equipment used for slaughter and handling of pigs, it has no food colorants and carmines produced from insects. The consumption of such meat does not violate Islamic food bans [3].

It is worth noting at once that the slaughter of animals in accordance with the requirements of the sacred Quran and Sharia law does not prohibit the use of modern technological tools and equipment intended for industrial slaughter of animals.

Thus, the requirements for ritual slaughter of animals in the production of halal meat are as follows:

1. Before you start, the equipment must undergo sanitary treatment in accordance with the documentation in force at the enterprise.

2. Animal slaughter should be carried out by adults, in their right mind, usually a Muslim, regardless of nationality.

3. A person who performs the slaughter of an animal or controls the slaughter process shall pronounce the following phrase when cutting the main cervical arteries: *Bismillyakh, Allahu akbar* or a shorter version: *Bismillyakh*, or simply pronounce in Russian: *God be with you!*

4. It is allowed to kill healthy animals without signs of infection with any disease that can change the structure of meat or harm the health of people who eat it.

5. The hobbles must be clearly fixed so that the animal does not jump off after slaughter and does not run around the slaughterhouse watering everything with its blood.

6. The animal must necessarily be turned head towards Mecca, the sacred Muslim city where the Kaaba Temple with a black stone is located.

7. When slaughtering, the animal is cut by a single translational motion without delay and pauses, as close to the head as possible. The cut must be deep and reach the vertebrate bones. Thus, the slaughterer must cut the esophagus, cervical aorta and muscle tissues of a neck.

8. It is strictly prohibited to carry out actions that may cause fear or agony of an animals being slaughtered, namely:

- to cut with a blunt instrument thus making an animal suffer;
- to sharpen the slaughter tools in the presence of an animal;
- to kill one animal in the presence of another.

9. Dressing is only possible after the main part of the blood has been discharged. Blood, if possible, should not be shed on the ground, so a bowl or any container for the animal blood is placed under the neck to the cut site. It is forbidden to tear off a skin, to scald, pull out feathers, to

* Corresponding author: danon-yusya@mail.ru

chop off any parts of an animal's body, unless it is clear that the main part of blood is gone and reflecting muscular activity is stopped.

10. It is prohibited to stun animals before slaughter with devices that do not guarantee the preservation of an animal's heartbeat after stunning. For this reason, it is not allowed to use stun-proof devices, air-guns and pistols with powder charge.

11. There are special Halal sectors in warehouses with appropriate marking, where only raw materials and finished products produced according to Halal standard can be stored.

12. Special tools and process containers intended for halal production shall be marked with appropriate marking excluding their use in other production areas where halal production standards are not observed [3, 4].

Thus, the preparation of Halal meat happens in naturally clean sanitary conditions, bears positive semantic meaning as at slaughter and dressing there shall not be negative thoughts. Physically and spiritually pure meat only brings benefits [4].

In order to control and sell confessional products with corresponding markings, Russia established a Committee under the Spiritual Administration of the Republic of Tatarstan and the Center for Standardization and Certification of Halal Products under the Council of Muftis [3].

Unfortunately, not all Halal products on the counter are such. Often the product may not correspond at the declared marking, because the manufacturer literally "put a pig" into halal products. We shall remember that similar to any other meat products, Halal products must meet the quality and safety requirements of modern regulatory documents.

Analyzing the above, it is possible to speak about the relevance of the performed qualification work since the sold products marked Halal do not always have proper quality, and it is not uncommon cases of unacceptable falsification.

2 Purpose of the Study

The global community is now paying more attention to useful and safe food. Thus, many people seriously think about what products they consume, what they are made of, whether all standards have been observed in their production. The choice of products for each person is individual and is connected not only with national, natural, and historical living conditions, but is also, oddly enough, subject to religious traditions [5, 6].

To date, the production of meat that meet all religious requirements is a new and almost unoccupied, promising and constantly expanding market segment. There is a clear understanding of the value of the religious sector of the food market worldwide [7].

In the conditions of modern Russia, where representatives of different nationalities and religions live, national and religious peculiarities are also taken into account in industrial production of food products [5].

In most world beliefs, quite strict lists of products can be found, which describe in detail what is allowed or prohibited to eat [7].

Muslims call permitted products Halal (mainly meat obtained using Islamic requirements).

The word Halal, translated from Arabic, means "allowable" or "lawful". Halal with regard to food is the dietary standard of Muslims [3].

There are two cornerstone concepts in Islam: Haram and Halal. Thus, the first implies everything forbidden for a Muslim, and accordingly the second – allowed, useful and pious [5].

Halal means animal meat (other than pork) obtained by ritual slaughter, without the use of any kind of equipment used to slaughter and handle pigs. The consumption of such meat does not violate Islamic food bans [7].

Like any other meat products, Halal products must meet the quality and safety requirements of modern regulatory documents.

In order to control and sell products with such markings, Russia established a Committee under the Spiritual Administration of the Republic of Tatarstan and the Halal Standardization and Certification Center under the Council of Muftis. At present, however, certification of enterprises is voluntary and has a number of consequences [5].

Not all Halal products are such. Often the product may not correspond to the declared marking at all. In pursuit of demand for products in the "young" segment of the meat industry, unscrupulous producers allow themselves to violate production technologies by changing the composition of a product. The main danger is the presence of pork in products marked Halal.

To date, for objective control of products declared as Halal, there are several methods aimed at detecting the "most dangerous" falsification – pork antigen contained in a product.

Such methods as immunochromatographic assay and enzyme immunodetection as well as polymerase chain reaction are used with great success for these purposes. The results of these studies represent an indispensable guarantee of safety of the studied products.

Hence, the purpose of this study is to detect specific falsification of finished mutton meat products marked Halal using modern methods of laboratory diagnostics – PCR, IFA, IHA. As a result of the studies, their comparative analysis was carried out. A complex of organoleptic and physical-chemical methods was performed to determine the benign quality of products.

3 Materials and methods

The study was carried out at the Department of Parasitology and Veterinary and Sanitary Expertise of Moscow Veterinary Academy named after K.I. Skryabin, as well as under the control of qualified specialists in the accredited test center. For laboratory diagnosis, 5 samples of finished mutton meat products marked Halal were chosen.

All organoleptic indices were determined in accordance with GOST 9959-91 Meat products. General Conditions for Organoleptic Evaluation [8].

Physical and chemical methods included the test for starch with Lugol solution (GOST 10574-2016), pH test (GOST R 51478-99), test for hydrogen sulphide (GOST R 55479-2013), test for ammonia according to Eber (GOST R 55479-2013) [9].

Imprint smears were made from the material under study, both from the surface layer and from the center of the baton. The imprints were painted according to Gram (GOST 23392-78 Meat. Methods of chemical and microscopic analysis of freshness), and the amount and morphology of bacteria were determined [8, 10].

The efficiency of luminescent analysis to determine the benign quality of the studied products was checked. The luminescent method allows detecting spoilage (decay) at initial stage represented by luminescent spots of darker color than the general tone, which is simply impossible to detect visually [11].

The detection of species falsification was performed by immunochromatographic analysis (XEMAtest) and enzyme immunoassay (NEOGEN). As reference studies, a real-time PCR diagnostics was conducted at accredited, specialist-led testing center.

4 Results and discussion

All studies were carried out in accordance with the regulatory documents in force on the territory of the Russian Federation.

Additional study, particularly real-time polymerase chain reaction, was carried out at the accredited testing center by qualified and competent staff.

The results of all studies are presented below.

4.1. Organoleptic testing

Initially, each sample of meat products marked Halal was studied to determine appearance, consistency, smell, taste, color, pattern on cut.

Table 1. Results of organoleptic testing

Indicator	Actual result
Appearance	Sausage case not deformed dry, without slime
Color	Reddish-brown
Smell	Typical, specific, flavory
Consistency	Elastic, dense, not harsh
Fat state	Dense, white
Pattern on cut	Dampish, smooth sausage filling

The studies made it possible to conclude that all samples are fresh and correspond to regulatory documentation.

4.2. Physical-chemical study

Hydrogen ion test (pH0). The pH value directly represents both the condition of a meat product and the degree of its freshness, hence it may be said that pH has a relative value [10].

Test for starch with Lugol. In order to determine the presence of starch in a sausage product sample, a fresh cut of the product was made, where the Lugol solution was then dropped. Blue or black and blue color indicates the presence of starch in the sausage [8].

Hydrogen sulphide reaction. The essence of the reaction is based on the fact that hydrogen sulphide is one of the end products of protein decay and accumulates in meat products when they are damaged. The result of the reaction is read from the filter paper, which is impregnated with lead acetate. The color of paper coloration varies depending on the degree of freshness [9].

The ammonia test (Eber Test). The essence of the reaction with the Eber's reagent is based on the interaction of ammonia being the product of protein decay, which is formed during spoilage of sausage products, with hydrochloric acid, which is a part of the Eber's reagent. As a result of the reaction, ammonium chloride is formed which can be visually detected [8].

Table 2. Results of physical-chemical study

Test number	1	2	3	4	5
Indicator	Actual result				
Hydrogen ion test (pH)	5.97	5.86	5.90	6.01	5.77
Test for starch with Lugol	-	+	+	-	-
Hydrogen sulphide reaction	-	-	-	-	-
The ammonia test (Eber Test)	-	-	-	-	-

The studied samples of Halal products numbered 1, 4, and 5 meet GOST requirements. The identified starch in a sample number 3 was indicated in the composition, which allows us not to consider it a violation. Sample 2 was not tested – it revealed starch in the composition undeclared by the manufacturer (Fig. 1).

Tests with revealed starch give dark blue, almost black coloring while negative reaction leaves the reagent color (Lugol) – light brown, brown-reddish. For visual comparison, sample 1 is placed next the other samples – top-left corner. We found no signs of spoilage in any of the five samples.



Fig. 1. Positive test for starch (Samples 2 and 3)

4.3. Microbiological study

The study on bacteriological contamination of sausage products was carried out by microscopic analysis, which is based on the determination of the number of bacteria in imprint smear microscopy (GOST 23392-78 Meat. Methods of chemical and microscopic analysis of freshness) [10].

Table 3. Results of bacteriological imprint smear test.

Indicator	Actual result
Top layers	11±4 microbial bodies detected
Sausage center	Single microbial bodies detected

The above values indicate the freshness of all five samples of finished lamb products marked Halal – no pathogenic bacteria are found, muscle tissue does not decay, as shown in Fig. 2.



Fig. 2. Imprint smear microscopy by Gram

4.4. Luminescent assay

The principle of the Filin luminoscope is based on the fact that many organic substances, minerals, etc., that luminescent in ultraviolet rays (luminescence). At the same time excited characteristic luminescence can tell us a lot about the composition and condition of a substance [11].

The device has two chambers – lighting and observation. A filter passing a narrow band of light spectrum (360 ± 30) nm is installed between the chambers to release excitatory ultraviolet light. A binocular with a secondary glass filter, which does not pass scattered UV light, is used for observation [11].

Fresh and benign sausages have a practically uniform glow, which is pronounced and typical for each species [11].

The luminescent assay allows detecting decay at the initial stage by luminescent spots of darker color than the general tone, which is simply impossible to detect visually [11].

According to the study, the glow was not detected in any of the product samples. The color of samples was evenly brown, velvety, without stains, indicating freshness and high quality of the test product (Fig. 3).

Luminescent analysis allowed giving qualitative characteristics during veterinary and sanitary examination of finished mutton meat products marked Halal in the shortest possible time.



Fig. 3. Luminescent study of finished meat products

4.5. Detection of species falsification in finished meat products by immunochromatographic and enzyme immunoassay as well as real-time polymerase chain reaction

To date, for objective control of finished lamb meat products marked Halal, there are several different modern studies aimed at detecting species falsification (pork antigen) in a product.

One of such methods is IHA. Immunochromatographic (immunochemical) analysis is based on thin layer chromatography. It involves a reaction between an appropriate antibody and an antigen in biological materials.

It is carried out by special test strips, test cassettes or panels. IHA is an express test.

However, the accuracy of this technique makes about 96%, which must be taken into account.

The next method is IFA. The enzyme immunoassay method is required to detect and quantify antibody-causing substances.

This method has been safely used for several decades for various scientific and diagnostic purposes. The IFA was also applied in the field of medicine and veterinary medicine, as well as immunology, microbiology and even food industry.

The wide application of the method is due to its uniqueness. It is its combination of special specificity with high sensitivity, which can reach 97–99 % [12].

The last and most reliable method is PCR. Polymerase chain reaction is a method of obtaining multiple copies of certain DNA fragments (genes) in a biological sample.

At the moment this method is the most accurate, about 99.9 %, due to which it is called the “gold standard”. This allows it to be designated as an arbitration method in the course of basic studies [13].

The essence of PCR, as a method of molecular biology, consists in multiple selective copying of a certain gene (DNA) with the help of special enzymes in vitro. An important feature of PCR is the production of

copies of a particular DNA (gene) meeting the specified conditions [13].

Table 4. Results of studies aimed at species falsification.

Test number	Research method and actual result		
	IHA (XEMAtest)	IFA (NEOGEN)	PCR (Sus scrofa Ident RT)
1	Negative	Negative	Negative
2	Positive	Positive	Positive
3	Positive	Positive	Positive
4	Negative	Negative	Negative
5	Positive	Positive	Positive

The study revealed three falsified positions not corresponding to Halal marking – samples numbered 2, 3 and 5 – pork antigens were identified in these samples.

The full list of studies confirms adequate quality, safety in veterinary and sanitary, hygienic and ecological relations of all samples of Halal products. However, gross falsification was found prohibiting such products from being classified as Halal. This result indicates prevarication of a manufacturer, and the possibility of pork getting into or deliberate mixing with Halal products. For plants, it is recommended to tighten the quality control of production or to withdraw from Halal marking at all.

After all methods, we compiled a summary table 5 – Comparative characteristic of modern methods of detection of pork antigen in finished meat products.

Table 5. Comparative characteristic of modern methods of detection of pork antigen in finished meat products.

Indicator	Research method		
	IHA	IFA	PCR
Method accuracy	≈ 96%	≈ 97–99%	≈ 99.9%
Procedure	Easy-to-use	Knowledge intensive	Labor-intensive
Time for results	≈ 3–5 minutes	≈ 2,5 hours	≈ 6–8 hours
Use of additional equipment	No	Yes	Obligatory
Price range	Public	Average	High
Location	Any	Almost any	Laboratory

5 Summary

1. All finished meat products marked Halal shall be subject to mandatory certification due to its frequent falsification.

2. Complex organoleptic and physicochemical studies led to results corresponding to current GOST standards, except for sample 2 – it revealed starch not declared in the composition on the label.

3. Pathogenic microflora was not found through bacteriological examination of samples of finished mutton meat products marked Halal.

4. Luminescent analysis proved to be an effective method to detect spoilage in finished meat products marked Halal at early stages.

5. IHA and IFA analyses, as well as PCR diagnostics, allow determining with high accuracy the presence of

pork antigen in a product, and as a result, to exclude possible falsification of Halal products.

6. Complex use of classical and modern methods of veterinary and sanitary examination allowed establishing high quality and compliance with the requirements of Halal marking of all studied products. The exception was samples 2, 3, 5 where pork antigens were detected. Besides, starch is revealed in sample 2, which is not present in the composition on the label.

6 Conclusion

Complex organoleptic and physicochemical studies of samples gave rather good results corresponding to the norm and generally accepted by GOST standards. The exception was sample 2 – it revealed starch undeclared by the manufacturer in the composition on the package of a finished meat product.

No pathogenic microflora was found through bacteriological examination. Muscle tissue decay is not observed.

In the luminescent study, the color of all samples was uniformly brown, velvety, without stains. No glow was detected.

Pork antigen was found in 3 samples, which is an unacceptable falsification. Only 2 samples out of 5 meet all the standards and requirements of Halal products and meet the declared quality.

Due to increasing demand for confessional products, the demand for lamb as a whole is also increasing. This is because lamb, unlike pork, is not a banned meat within a particular religion [14].

In order to objectively assess the quality and safety of livestock products marked Halal, which get retail networks in Moscow, it is necessary to use not only organoleptic assessment and classical laboratory methods of research. Modern research methods, such as immunochromatographic and enzyme immunoassays, as well as PCR diagnostics, should necessarily be included in the set of studies aimed at detecting possible falsification, allowing for more accurate examination of the quality of products and detection of falsification.

In order to prevent such falsification, all finished meat products marked Halal should be subject not only to a basic set of studies in accordance with the current regulatory documents, but also to mandatory certification by the Halal International Center for Standardization and Certification. This is necessary to exclude a number of falsifications, the most critical of which is the addition of pork to the products declared as Halal.

Industrial plants that do not meet the requirements of Halal should abandon such labelling, or establish stricter controls on quality compliance and generally accepted standards of finished meat products marked Halal.

References

1. M.E. Karabaeva, N.A. Kolotov, *Monitoring of the meat market in Russia*, in *Ensuring quality, safety and competitiveness of consumer goods in the post-*

- crisis period, Collection of articles of sci. and pract. Conf.* 144–149 (Moscow State University, Maykop, 2010)
2. L.N. Grigoryan et al., *Sheep breeding: changes*, *Livest. breeding of Russ.*, **11**, 53–54 (2004)
 3. I.M. Amerhanov, M.B. Rebezov, G.K. Alkhamova, *On the development of Halal production in the world*, in *Engineering and technology of food production, Mater. of IX int. sci. and techn. Conf.*, 172 (MSUP, Mogilev, 2013)
 4. B.V. Usha, *Veterinary and sanitary control during ritual slaughter of animals, production of kosher and halal products: tutorial* (LLC Quadro, St. Petersburg, 2014)
 5. E. Vorontsova, *Centuries-old traditions of Islam in modern Halal production* (Meat technologies, Moscow, 2012)
 6. A.I. Erokhin, E.A. Karasev, A.S. Erokhin, *State and dynamics of meat production in the world and in Russia*, *Sheep, goats, wool business*, **2**, 37–40 (2014)
 7. R. Davydova, *Halal drives the market* (Meat technologies, Moscow, 2012)
 8. M.F. Borovkov, V.P. Frolov, S.A. Serko, *Veterinary and sanitary examination with the basics of technology and standardization of livestock products* (Lan publishing house, St. Petersburg, 2010)
 9. M.F. Borovkov, Z.N. Menshikova, V.M. Bachinskaya, *Veterinary and sanitary examination of sausage and piece-ham products* (MGAVMiB, Moscow, 2014)
 10. I.A. Lykasova, V.A. Krygin, I.V. Besina, I.A. Solyanskaya, *Veterinary and sanitary examination of raw animal and vegetable materials and products* (Lan publishing house, St. Petersburg, 2015)
 11. *Methodological recommendations on luminescent analysis of food products* (St. Petersburg, 2000)
 12. V.M. Manko, D.A. Devrishov, *Veterinary immunology. Fundamental foundations* (Agrovet, Moscow, 2011)
 13. V.V. Zorina, *Basics of polymerase chain reaction* (Moscow, 2012)
 14. A.F. Shevhuzhev, R.H. Kochkarov, *Growth of production and demand for lamb in the Russian Federation, Livestock production in Russia in accordance with the State Program on Agricultural Development for 2013–2020* 254–260 (Stavropol, 2013)