AGRISCIENCE AND TECHNOLOGY
Armenian National Agrarian University
ԱԳՐՈԳԻՏՈՒԹՅՈՒՆ ԵՎ ՏԵԽՆՈԼՈԳԻԱ АГРОНАУКА И ТЕХНОЛОГИЯ
International Scientific Journal
ISSN 2579-2822

Journal homepage: anau.am/scientific-journal
doi: 10.52276/25792822-2023.4-366

UDC 636.4:619:616.9(479.25)

Epidemiological Analysis of the Swine Perfringens Disease in the Aragatsotn Region

M.A. Sargsyan
Armenian National Agrarian University
mariam.sargsyan.1960@mail.ru

ARTICLE INFO

Keywords:
swine,
Perfringens,
Clostridium perfringens,
epidemiology,
intoxication

ABSTRACT

Over the recent 20 years, for the first time, the disease of swine perfringens has been studied in the Aragatsotn region of RA and related epidemiological analysis has been conducted. To identify A, C, and D serological types of Cl. Perfringens and special diagnostic sera produced by the Kursk biofactory were used. It has been found that the main serological infection type of swine perfringens is C and sometimes A and D subtypes. In the pig breeding farms of the mentioned region about 6887 heads of swine (aging from 3 days to 1 year old) were registered. Per the research results, about 45 out of 72 communities were recognized as insecure/vulnerable. As to the above-stated animal number, it should be mentioned, that 1722 heads of swine fell down, whereas 5510 were considered bacteria carriers. Thus, as a result of epidemiological analysis, the morbidity (bacteria carrying rate), mortality, insecurity/vulnerability, and focality coefficients (indicators) have made 0.80 (80 %), 0.25 (25 %), 0.63 (63 %), and 122 animals, respectively.

Introduction

Perfringens (anaerobic infectious enterotoxemia) disease takes a special position in the infectious disease pathology of farm, domestic and other animals, which has become widespread in many swine-breeding countries (Nesterov, 2003).

The disease pathogen (*Clostridium perfringens*) belongs to *Bacillaceae* family, genus *Clostridium*. According to antigenic properties, serological types (A, B, C, D, E, F) produce alpha-, beta, -iota-, epsilon-, kappa- exotoxins. During evolution, each type was adapted to one or more animal species. The above-mentioned types are similar to each other in morphological, cultural and biochemical properties, differing only in lethal poison. The latter destroys erythrocytes, blood vessels, capillaries and tissues. The serological types of the disease pathogen grow well in anaerobic nutrient media of Kitt-Tarozzi, milk and blood serum containing media causing intense gas formation and turbidity of the broth. Bacteria are sporulated in external

conditions, and encapsulated in environments rich in carbohydrates and proteins. According to the researchers, the capsula contains polysaccharides (D-glucose, D-galactose, N-acetyl galactose) and inulin acid, which contribute to the long-term preservation of bacteria in soil and cold water (Dunaev, 1982; Efimov, 1982; Urguev, 1987; Sheng and Cherniak, 1997; Bessarabov, et al., 2007; Francisco and Uzal, 2009). Anaerobic infectious enterotoxemia disease of pigs (*Perfringens*) according to Detri and Schennikov, was first reported in Hungary (1927) and Russia (1946), which affected newborn piglets in large numbers. Such pig farms have suffered heavy economic losses. It is an endemic disease, the causative agent of which are serological types C, A, D of *Cl. Perfringens*.

The disease is characterized by overfilling of the stomach, necrosis of the intestinal mucosa, hemorrhagic inflammation, gas accumulation, watery diarrhea (diarrhea) and disruption of the normal functioning of the nervous system. Attaching special attention to the development mechanism of severe course of the *Perfringens* disease it can be stated that it is endowed with high morbidity and mortality. According to the authors, in the pig-breeding farms of Hungary massive losses of piglets occurred due to *Perfringens*, which is followed by annual outbreaks for a long period (Altukhov and Dushenin, 1987; Urguev, 1987; Kudryashov, 1990; Satio, 1990; Czanderlova, et al., 2006; Salimov, 2006; Kudryashov and Svyatkovskiy, 2007; Sargsyan, 2012; Sidorchuk and Masimov, 2024). Complications of enteritis in newborn piglets can be caused by opportunistic bacteria, viruses and endoparasites belonging to the genera *Escherichia coli* and *Salmonella*.

Predisposing factors to the disease are low-viable piglets fed with low-quality colostrum, collective housing conditions, zoohygienic conditions, soil, feed, water contaminated with clostridia, and feed rich in monoproteins (Figure 1).

According to the author, the meat product made from the carcass of diseased and healthy but bacteria carrying animals pose a certain risk to human health (food poisoning) (Sakurai, 1995; Sargsyan, 2014).

Figure 1. Bacteria carrying sow.

Materials and methods

For the first time (2004-2023) the infection of swine *perfringens* was investigated in the Aragatsotn region and the related epidemiological analysis was conducted. The study was conducted at laboratory of the Research Center of Veterinary Medicine and Veterinary Sanitary Examination, at the Armenian National Agrarian University.

Considering the pluralism (diversity) of serological types of *Cl. Perfringens*, a problem arose to study the antigenic characteristcs and its role in pig breeding farms. Therefore, in order to assess the epidemic situation, the well-known methods of diagnosis were used: epidemiological observation, clinical signs, patho-anatomical changes, microbiological, serological and biological experiments (Yefimova, 1982, Urguev, 1987, Raju and Sarker, 2007, Sargsyan, 2012, Nesterov, 2011). In the pig breeding farms of the mentioned region about 6887 heads of swine (aging from 3-day to 1-year-old) was registered. Per the research results, about 45 out of 72 communities was recognized as insecure/vulnerable. As to the above stated animal number, it should be mentioned, that 1722 heads of swine fell down, whereas 5510 were considered as bacteria carriers.

Spleen, kidneys, lymph nodes, liver, caecum, small intestine, and faeces of piglets older than 3 days served as materials for laboratory studies.For microbiological research the nutrient media of Kitt-Tarozzi liver broth, iron sulfate, Wilson Blair Agar, as well as meat peptone agar, meat peptone broth and sterilized milk were used (Antonov, et al., 1986). To determine the lethal dose (LD_{50}) and serological types of the pathogen, 48 healthy rabbits weighing 3.0-3.5 kg each, guinea pigs weighing 450-500 grams, and white mice weighing 18-20 grams were selected. Per the principle of similarity, the experimental animals were divided into 4 groups.

Therefore, the 18-24 hour microbial culture was centrifuged at 3500-5000 rpm for 15 minutes and the supernatant was separated. Animals of each group were injected intramuscularly, intra-abdominally from the straining (exotoxin) of microbial culture with 1.5. 0.2, 0.15 ml dosages (Syurin, 1984; Fisher, et al., 2006). As an antigen, the pathogen "*Cl. perfringens*" isolated from pigs' excrement and the organs of fallen animals was used (Figure 2).

For the identification of serological types of *Cl. perfringens* A, C, D, polyvalent *E. coli* OKC, OKD, OKA and *Salmonella* A, B, C, D, E, a specific diagnostic sera produced by the Kursk biofactory were used according to the neutralization reaction. The minimum lethal dose was determined according to Reed and Muench method (Syurin, et al., 1984).The epidemiological analysis oft he studied region has been conducted per the morbidity (bacteria carrying rate), mortality, insecurity/vulnerability and focality indicators (Nikitin and Voskoboynik, 1999).

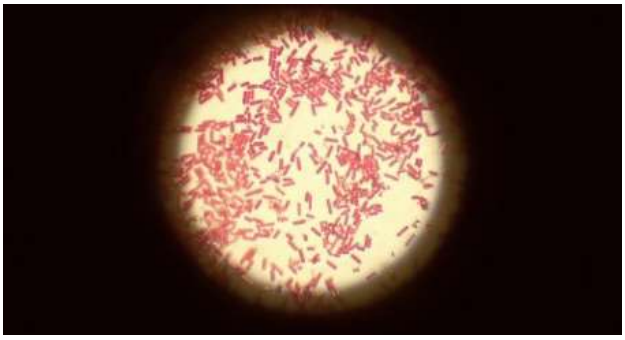

Figure 2. Microbial image of *Cl. Perfringens*.

Results and discussions

To identify the infection intensity, recentness, extent of its spread, number of affected animals, insecurity mode and outbreak periodicity, an epidemiological survey of the communities was carried out. Taking into account the similarities between the clinical signs and pathoanatomical changes of *Perfringens* and a number of other diseases, particularly, Escherichia, Salmonellosis, as well as food poisoning, differential diagnosis was made using bacteriological and serological methods. As a result, the activity of the poison secreted by the mentioned pathogens was ruled out. Sick piglet showed a decrease in the body temperature, depression, abdominal distension, body tremors, bloody diarrhea and uncoordinated movements.

As a result of dissection of the carcasses of animals fallen from *Perfringens* abdominal distension, blood stroke in the cecal, small and duodenal intestines, in stomach, rennet mucous membrane, hemorrhagic inflammations, as well as changes in the tissues of the kidneys, liver and heart were observed. In the sub-cells of individual parts of the body of animals that have died from the above-mentioned disease, jelly-like effusion and serohemorrhagic exudate was detected, while the abdomen and thoracic cavities were filled with blood fluid. As a matter of fact, the animals died from asphyxiation (Figure 3).

Clostridia are activated as a result of disruption of the motor/motility and juice production function of the digestive tract, as a result, the antimicrobial properties of the gastric juice are declined and the alkalinity (*pH* 9,10) of intestines increases. Under the created favorable conditions, the toxin of bacteria is absorbed into the blood and destroys erythrocytes, the endothelium of vessels and capillaries, the parenchyma of the liver and kidneys (Figure 4 and Figure 5).

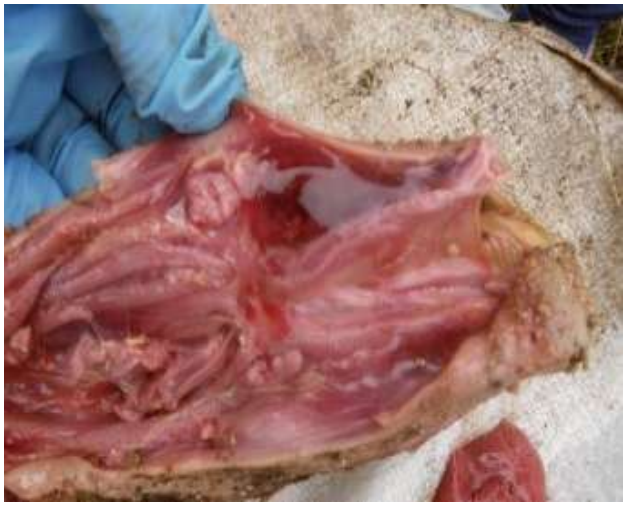

Figure 3. Serohemorrhagic exudate in the tissue subcells of the piglets.

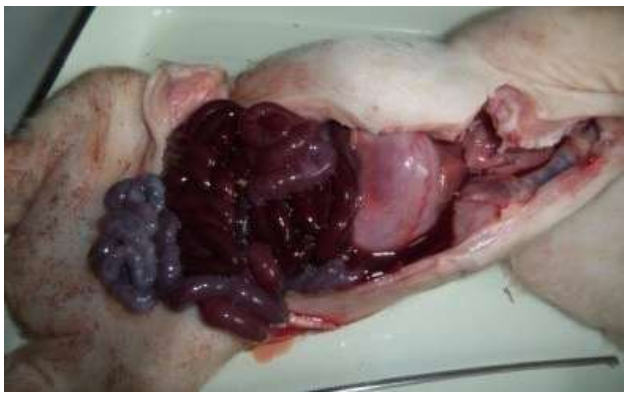

Figure 4. Hemorrhagic inflammation in the piglet's intestines.

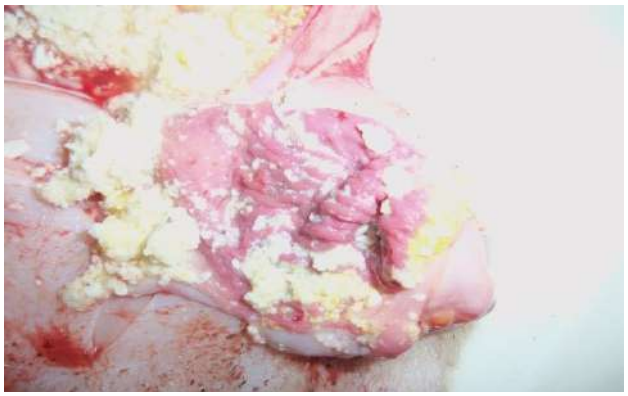

Figure 5. Catarrhal inflammation of the gastric mucosa of a newborn piglet.

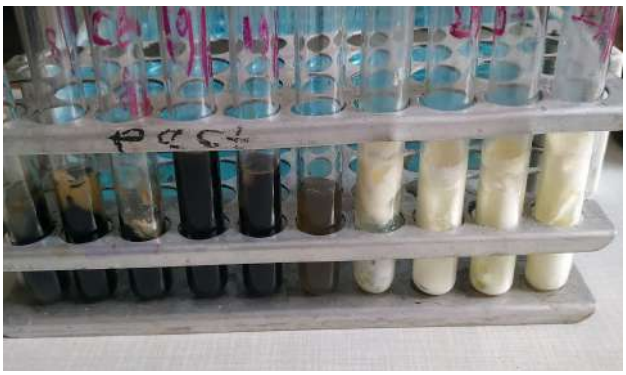

Figure 6. Microbial growth of *Cl. Perfringens* in Wilson-Blair and milk medium.

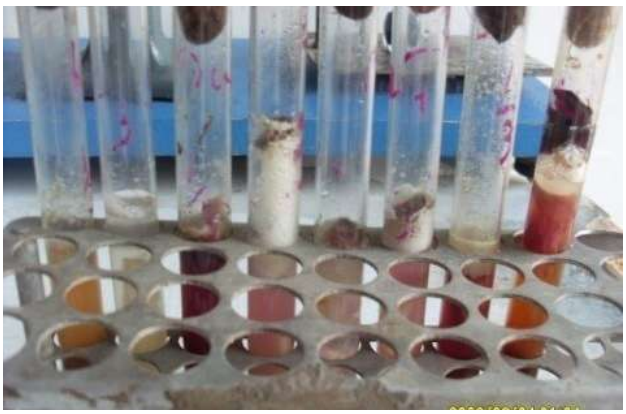

Figure 7. The growth of *Cl. Perfringens* in Kitt-Tarozzi medium.

Figure 8. Hemolysis of erythrocytes.

In the result of microbiological research, microbial growth (with unpleasant smell, abundant gas formation, turbidity of the medium, black colonies, milk coagulation) peculiar to *Cl. Perfringens* was observed in the seedings implemented from the internal organs and feces of healthy and fallen animals. The reason of black colonies formation is probably the transformation of sodium sulfate into iron sulfate (Figure 6 and Figure 7). The results of epidemiological research have indicated that throughout investigation period, in different settlements of the Aragatsotn region the disease of swine *Perfringens* has been recorded. We think that the main cause of the disease outbreak is the neonatal (endo) infection.

It is noteworthy that the animal-based food product and raw material infected with the toxins of *Cl. Perfringens* can entail to mild food poisoning up to life-threatening consequences. As a result of the biochemical activity of the microbial poison, the erythrocytes of rabbits and rams were destroyed within 2-3 minutes (Figure 8). Serological types of microbial antigen were determined using pathogens isolated from organs of fallen animals and appropriate diagnostic immune sera. As a result of the neutralization reaction, it was determined that the bacteria carrying state of the animals and the cause of death are the serological types C, A, D of *Cl. Perfringens*, which made 58.8, 20.5, and 10.6 %, respectively.

As a result, the lethal dose of *Cl. Perfringens* (LD_{50}) in case of infection of experimental animals made 95-100 %.

The epidemiological research and analysis was carried out based on the relevant data considering the age, morbidity, mortality and focality factors, as well as the index of insecurity/vulnerability. The results of epidemiological

research have testified that in different settlements of the Aragatsotn region the disease of swine Perfringens was recorded. In the above stated insecurity conditions, the average indices of morbidity (bacteria carrying state), mortality, insecurity and focality have made 0.80 (80 %), 0.25 (25 %), 0.63 (63 %), and 122 animals, respectively.

Summarizing the indicators of epidemiological research analysis, it was found out that 45 (63 %) out of 72 communities was recognized as insecure/vulnerable.

Conclusion

1. The epidemic situation is multifactorial, and the level of veterinary and sanitary conditions is of particular importance.

2. Sick piglets and healthy bacteria carriers are considered to be the source of the disease pathogen. In this regard, the disease can occur as a result of infection penetrating from the outside and as a result of bacterial contamination of the sows' udder and teats.

3. The serological C type of *Cl. perfringens* isolated from swines is endowed with higher pathogenesity than *A* and D types.

4. The carcass and meat product contaminated with the toxins of *Cl. Perfringens,* are forbidden to use, since they endanger human health.

References

1. Altukhov, N.M., Dushenin, N.V., Shakhov, A.G., Morgunova, V.I., Sharonin, S.A. (1987). Anaerobic enterotoxemia of piglets // Veterinary Medicine. - M., - №. 5, - pp. 41-42 (in Russian).
2. Antonov, B.I., Borisova, V.V., Volkova, P.M. (1986). Laboratory research in veterinary medicine/Bacterial infections: Directory.- M.: Agropromizdat, - 352 p.
3. Bessarabov, B.F., Vatutin, A.A., Voronin, E.S. (2007). Infectious diseases of animals / Ed. A.A. Sidorchuk, - M.: KolosS, - 671 p. (in Russian).
4. Czanderlova, L., Hlozek, P., Chamelar, D., Lany, P. (2006). Clostridium perfringens in suckling piglets with diarrhea and its PCR typing and pre-balance in the Czech Republic in 2001-2003 // Veterinarni Medichina. - vol. 51, - № 9, - pp. 461-467 http://dx.doi.org/10.17221/5578-vetmed.
5. Deepa Raju, Mahfuzur R. Sarker (2007). Production of small, acid-solube spore proteins in clostridium perfengens nonfoodborne gastrointestinal disease isolates.// Can. J. Microbiol. - vol. 15, - № 3, - pp. 514-518 http://dx.doi.org/10.1139/w07-016.
6. Dunaev, G.V. (1982). The causative agent of infectious anaerobic enterotoxemia / In the book: Veterinary microbiology: Textbook / Ed. E.V. Kozlovsky and P.A. Emelianenko. - M.: Kolos, - pp. 226-230 (in Russian).
7. Francisco, A., Uzal, I., Juliann Saputo, Samesta Sayced, Jorge, E. (2009). Development Application of New Mouse Models to Study the Pathogenesis of clostridium perfringens type C Enterotoxemias.// Inf. Immune. - vol. 77, - № 12, - pp. 5291-5299 http://dx.doi.org/10.1128/iai.00825-09.
8. Kudryashov, A.A. (1990). Pathological and morphological changes in the intestines of newborn piglets with colibacillosis, anaerobic enterotoxemia and VGTS // Veterinary Medicine, - M. - № 6, - pp. 36-39 (in Russian).
9. Kudryashov, A.A., Svyatkovsky, A.V. (2007). Infectious diseases of animals.- Saint Petersburg. Moscow. Krasnodar, - 607 p. (in Russian).
10. Nesterov, I.A. (2003). Evolutionary transformations of infectious anaerobic enterotoxemia (Perfringens) of animals in the North Caucasus in the modern period // Veterinary Bulletin - "Entropos", - V. 27, № 3, - pp. 24-37 (in Russian).
11. Nesterov, I.A. (2011). Epizootological analysis of pathogenecity factors of the causative agent of perfringens in animals // Veterinary Bulletin. - "Entropos", - V. 57, - № 2, - pp. 23-27 (in Russian).
12. Nikitin, I.N., Voskoboynik, V.F. (1999). Organization and economics of veterinary business: Textbook for university students - 4th ed., revised and supplemented - M.: Humanit. ed. VLADOS center, - 384 p. (in Russian).
13. Salimov, V.A. (2006). Pathomorphological changes in the intestines of piglets during experimental enterotoxemia // Veterinary pathology, - № 1, - pp. 98-100 (in Russian).
14. Sargsyan, M.A. (2012). The role of the microbe Clostridium perfringens in the infectious pathology of animals // Bulletin of ANAU - № 1, - pp. 96-98.
15. Sargsyan, M.A. (2014). Carcass and meat product contamination with Clostridium perfringens // Mater. of intl. scientific conf. on the problem of food security and biodiversity. - Yerevan: ANAU Publishing House, - pp. 244-247.
16. Satio, M. (1990). Production of enterotoxin by

clostridium perfringens derived from humans, animals, foods and the natural environment in Japan // Journal Food prof. - № 53, - pp. 115-118 http://dx.doi.org/10.4315/0362-028x-53.2.115.

17. Sheng, L., Cherniak, R. (1997). Structure of the capsular polysaccharide of Clostridium perfringens hobbs 10 determined by NMR spectroscopy. // J. Carbohy drate Research, - vol. 305, № 1, - pp. 65-72 http://dx.doi.org/10.1016/s0008-6215(97)00280-2.
18. Sidorchuk, A.A., Masimov, N.A., Krupalniksh, et al. (2024). Textbook / edited by A. Sidorchuk, 2nd ed., revised and supplemented. - Moscow: INFRA-M, - 954 p. (in Russian).
19. Syurin, V.N., Belousova, R.V., Fomina, N.V. (1984). Veterinary Virology. - M.: Publishing house Kolos, - 376 p. (in Russian).
20. Urguev K.R. (1987). Clostridiosis of animals. - M.: Rosselkhozizdat, - 182 p. (in Russian).
21. Yefimova, M.G. (1982). Isolation of purified TETA-hemolysin from Clostridium perfengens type A and its use for titration of toxins and serums // JMEI, № 7, - pp. 40-43 (in Russian).

Accepted on 23.10.2023
Reviewed on 01.12.2023