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## COMPARATIVE EVALUATION OF HISTOLOGICAL RESULTS OF MODERN FIRE INFLAMMATORY INJURIES OF THE COLUMN BY DIFFERENT TYPES OF BULLETS IN THE EXPERIMENT

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The article shows the study results of the colon pathomorphosis peculiarities in gunshot wounds in experimental animals (pigs). Semi-quantitative assessment of the hemorrhage volume in the area of intestinal vessels in the own plate of the mucous membrane and in the submucosal base was performed on the recorded digital images of micropreparations. Histological examination of the colon damaged by bullets of the same calibre but with different properties showed significant morphological changes in the colon wall around the site of injury. The authors have shown that the changes in the colon are significantly more pronounced when using a 5.45 V-Max bullet with a larger area of damage than in the 5.45 PS bullet.

**Key words:** gunshot wound, colon, pig

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## ПОРІВНЯЛЬНА ОЦІНКА ГІСТОЛОГІЧНИХ РЕЗУЛЬТАТІВ СУЧАСНИХ ВОГНЕПАЛЬНИХ ПОРАНЕНЬ ОБОДОВОЇ КИШКИ РІЗНИМИ ТИПАМИ КУЛЬ В ЕКСПЕРИМЕНТІ

Робота присвячена вивченню особливостей патоморфозу ободової кишки при вогнепальному кульовому її пораненні у експериментальних тварин (свинях). На зафіксованих цифрових зображеннях мікропрепаратів проводили напівкількісну оцінку обсягу крововиливів площі судин кишки у власну пластинку слизової оболонки та у підслизову основу. Гістологічне дослідження ушкодженої кулями одного калібру але з різними властивостями ободової кишки показало значні морфологічні зміни в стінці ободової кишки навколо міста ушкодження. Показано, що зміни в ободовій кишці достовірно більш виразні при застосуванні кулі 5,45 V-Max з наявним більшим обсягом ушкодження, ніж у кулі 5,45 ПС.

**Ключові слова:** вогнепальне поранення, ободова кишка, свиня

*The study is an initiative.*

Gunshot wounds to the abdomen are a serious modern combat injury, which complicates the choice of adequate surgical tactics in a hybrid war, continuing in eastern Ukraine [8]. Damage to the colon ranks second in the frequency of combined injuries of the abdominal cavity after the small intestine [2]. The mortality rate from penetrating gunshot wounds to the abdomen, accompanied by damage to the colon is 7–18 % [7] and is several times higher than among those who do not have such damage 3.7–8 % [4–6].

The nature of gunshot wounds, development of complications and the course of wound disease with high mortality confirm that small arms ammunition with different properties of bullets is used in combat operations. In this regard, a number of authors [3, 8, 10] point to the need to study wound ballistics, which is important for assessing the nature of the wound and the subsequent choice of surgical tactics as well as for understanding the mechanisms of gunshot wounds [4, 5]. This indicates the relevance of further scientific and experimental studies of modern gunshot wounds of the colon with different types of bullets.

**The purpose** of the study was to comparatively evaluate histological results obtained in experiments on biological animals, with gunshot wounds damaging the colon by ordinary shell bullets and bullets with expansive properties.

**Materials and methods.** The experimental study was performed on 20 healthy pigs of the Svitlogorsk breed. The pigs were divided into groups of 10 animals each. The first group was with gunshot wounds to the abdomen with damage to the colon by an ordinary shell bullet, using a 5.45x39 mm PS cartridge. The second group was with gunshot wounds to the abdomen with damage to the colon by an expansive bullet, using a 5.45x39 mm, V-Max (Ukraine) cartridge.

The research was conducted on a military training ground under normal environmental conditions (temperature 15 °C, relative humidity 68 %, atmospheric pressure 730 mm Hg). We performed one shot from an automatic firearm AKS-74 from a distance of 100 meters to the intended colour point on the abdomen of the animal. Previously, using a portable ultrasound scanner DP-30, Mindray (China), and a convex sensor, we determined the location of the colon, marked and aimed the shot on the skin above it.

The acute experiment was performed under general anaesthesia in compliance with all bioethical requirements for animal experiments of the European Convention for the Protection of Vertebrate Animals for Research and Other Scientific Purposes, Strasbourg, 18 March 1986. Permission of the UVMA Bioethics Commission №5 dated 15.05.2021. Removal of experimental animals from the experiment was carried out by overdose (euthanasia) administration of 15 mg/kg, Sodium thiopental in a lethal dose. The dead animals were cremated after the examination. We took fragments of the colon wall for histological examination, which were directly the edges of the bullet hole, as well as pieces from the mesenteric edge of the intestine removed from the bullet hole by 5, 10, 15 and 20 cm in both directions.

The material was fixed in 10 % buffered formalin (pH 7.4) for 48 hours, dehydrated and sealed in paraplast [1]. Histological sections 4 µm thick stained with hematoxylin and eosin were made from the obtained blocks.

Histological sections were visually examined and photographed with Olympus B53 microscope with SP180 digital camera. Digital images (x100, 12214x920 pixels RGB) using Imag J software (Wayne Rasband (NIH), USA) [9] measured the area of blood vessels / 1 mm long intestine (mm<sup>2</sup>/mm) in its own mucosal plate shell (sV VP) and submucosal basis (sV PO). Semi-quantitative assessment of hemorrhage volume (hV, USD) was performed on a scale: 0 – absent, 1 – erythrocyte diapedesis, 2 – hemorrhage foci smaller than the thickness of the mucous membrane, 3 – massive haemorrhage foci for the entire thickness or greater than the thickness mucous membrane.

The obtained digital data were processed by standard statistical methods, calculating the arithmetic mean, standard deviation, average errors. To assess the significance of differences in mean values between groups, we used Student's t-test. Differences at  $p < 0.05$  were considered statistically significant.

**Results of the study and their discussion.** The wall of the conditionally intact colon on the studied samples had a typical structure. It clearly distinguished mucous, muscular and serous membranes. Epithelium, which forms crypts, covers the mucous membrane's own plate. Between the crypts are layers of loose fibrous connective tissue. They are narrow blood capillaries, and occasionally you can find small vessels of the venous type. Histological specimens contain only a small amount of erythrocytes, which is most likely related to the technique of taking material for examination (shortly after death). A muscle plate in the form of thin strips of smooth muscle tissue is beneath the mucosal plate itself. A layer of adipose tissue, in which blood vessels of various calibers pass, represents the submucosal base. Veins usually contain a small or moderate amount of blood. Periodically, the mucous membrane forms folds.

The muscular membrane of the colon is largely formed by a circular layer of muscle cells, to which an outer ileal layer is added in the areas where the strips pass. The serous membrane is represented by a plate of adipose tissue covered with mesothelium. The largest blood vessels of the intestine are located in it, especially in the mesenteric region. The serous membrane forms numerous outgrowths based on adipose tissue.

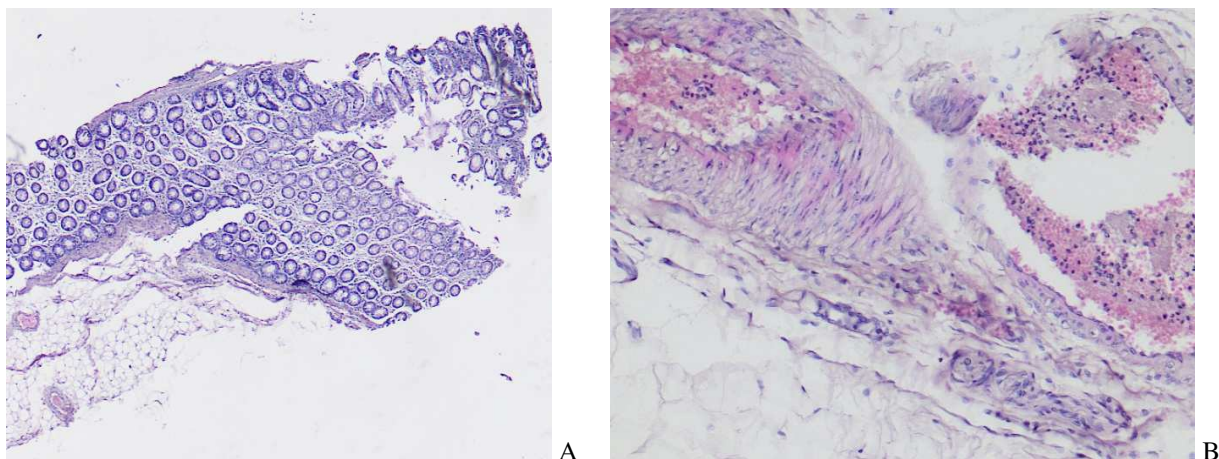


Fig. 1. The colon walls after injury: A – The colon wall at the edge of the bullet hole when wounded with a bullet 5.45. Stained with Hematoxylin and eosin. Microphoto, vol. 4, approx. 10; B – Mesenteric vessels of the colon at the edge of the bullet hole when wounded by a bullet 5.45. Stained with Hematoxylin and eosin. Microphoto, vol. 20, approx. 10.



Histological studies have showed that injuries from bullet 5.45 PS with a steel core cause disorganization of tissues at the edges of the bullet hole of the colon wall. The fragments of these tissues not completely separated from them, protrude into the lumen of the wound canal.

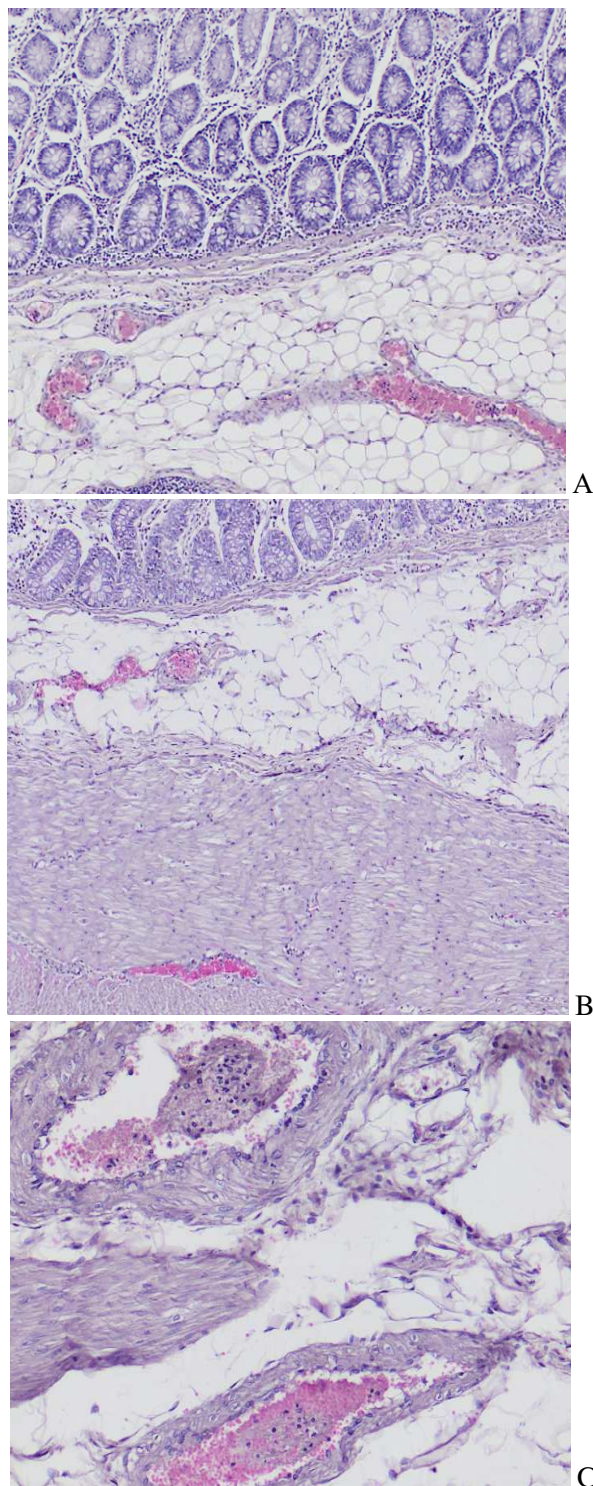


Fig. 2. The walls of the colon after injury: A – Walls of the colon at a distance of 5 cm proximal to the site of bullet damage when wounded by a bullet 5.45. Stained with Hematoxylin and eosin. Microphoto, vol. 10, approx. 10; B – walls of the colon at a distance of 15 cm proximal to the site of bullet damage when wounded by a bullet 5.45. Stained with Hematoxylin and eosin. Microphoto, vol. 10, approx. 10; C – Mesenteric vessels of the wall of the colon at a distance of 20 cm from the bullet hole when wounded by a bullet 5.45. Stained with Hematoxylin and eosin. Microphoto, vol. 20, approx. 10.

from inadvertent erythrocyte infiltration of the tissues surrounding the vessel to massive hemorrhages. Mesenteric vessels are also dilated, full of blood. Some hemorrhages spread to the entire thickness of the intestinal wall, sometimes filling the cavities formed during the injury.

At a distance of up to 5 mm from the edge of the hole there are tears of tissues, the formation of cavities, foci of hemorrhage. In the mucous membrane of the edges of the wound hole there is a slight or moderate edema. At a distance of up to 1 cm from the edge of the bullet hole between the crypts are single vessels full of blood. Larger blood vessels of the own plate of the mucous membrane are sharply narrowed, they do not contain uniform elements. Varicose small, medium and sometimes large veins are full of blood. There are also ruptures of blood vessels and hemorrhages, mostly small. The mesenteric vessels at the edges of the wound are also full of blood. There are ruptures of the venous wall and hemorrhages (fig. 1A, 1B).

At a distance of 5 cm from the bullet hole in the intestinal wall, there was also a sharp anemia of the own plate of the mucous membrane. In the submucosal base, the venous vessels are significantly dilated and overflowing with blood. There were occasional minor hemorrhages. The arteries usually look empty. Mesenteric vessels are also full of blood. As you move away from the site of the bullet penetration through the intestinal wall (10, 15, 20 cm), you notice also anemia of the mucous membrane's own plate. The degree of blood overflow of the submucosal base decreases slightly. The condition of the mesenteric vessels within the studied limits remained virtually the same – all the veins were filled with blood (fig. 2 A-C). There were no significant differences proximal or distal, visual or morphometrical in the state of the intestinal wall of areas located at equal distances from the site of the bullet penetration.

In the colon injuries by an expansive bullet 5.45 V-Max, we also observed mechanical damage to intestinal tissue near the wound opening with the formation of fragments of different sizes. In this case, the destruction of the intestinal wall elements was expressed for a longer period than compared with the wound of a bullet 5.45 PS with a steel core. In the own plate of the mucous membrane of these areas, blood vessels are sharply narrowed and do not contain blood. At some distance from the wound opening (1 cm or more), on the contrary, there is a sharp dilation of blood vessels of its own plate and their overflow with blood (fig. 3).

The submucosal vessels are dilated and full of blood. Hemorrhages are usually in varying degrees, from inadvertent erythrocyte infiltration of the tissues surrounding the vessel to massive hemorrhages. Mesenteric vessels are also dilated, full of blood. Some hemorrhages spread to the entire thickness of the intestinal wall, sometimes filling the cavities formed during the injury.



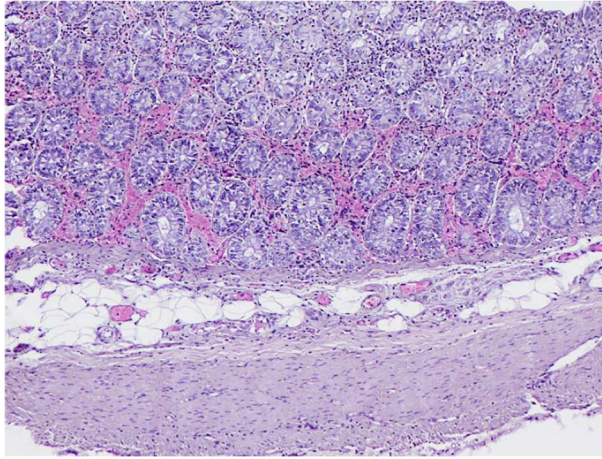
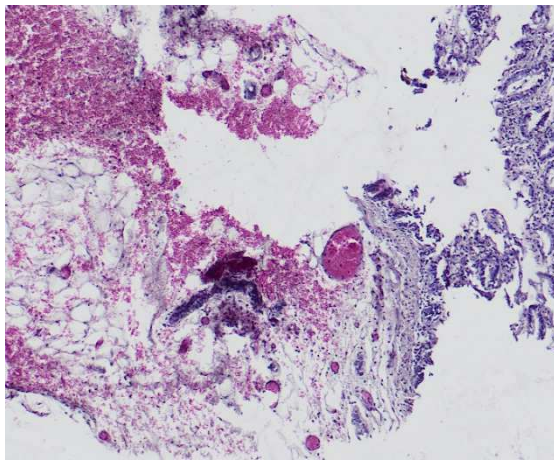
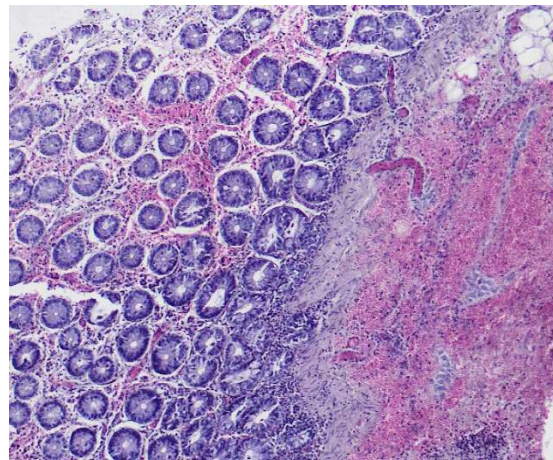


Fig. 3. Walls of the colon at a distance of 1 cm from the edge of the bullet hole when wounded by a bullet V-Max. Stained with Hematoxylin and eosin. Microphoto, vol. 4, approx. 10.

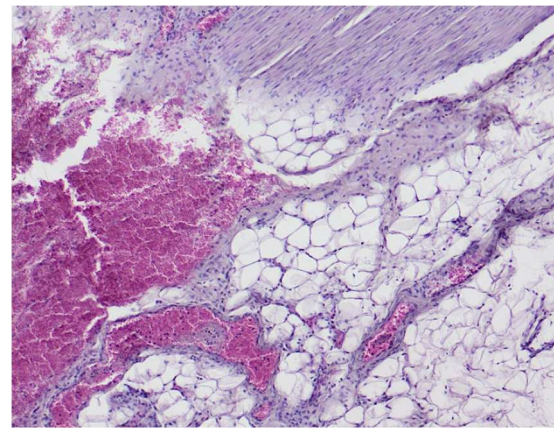
In the areas of the intestine, located at a distance of 5, 10, 15 and 20 cm from the site of the bullet entry, both in the proximal and distal direction, there was a pronounced redness of the vessels of the own plate of the mucous membrane. The severity of these phenomena visually decreased only at a distance of 20 cm from the site of injury. Here, individual vessels of the hemomicrocirculatory tract were filled with blood in their own plate. Venous vessels of the submucosal base, as well as mesenteric vessels, were also dramatically dilated with blood. Blood-filled ruptures were found in the intestinal wall throughout the study. In addition, we constantly observed different volumes of haemorrhage in the thickness of the mucosal plate and submucosal base (fig. 4 B), as well as the serous membrane of the mesenteric margin of the intestine (fig. 4 C).



A



B



C

Fig. 4. Wall of the colon: A – Defect of the colon wall and hemorrhage at a distance of 15 cm from the edge of the bullet hole when wounded by a bullet V-Max. Stained with Hematoxylin and eosin. Microphoto, vol. 10, approx. 10; B – Hemorrhages in the own plate of the mucous membrane and submucosal base of the colon at a distance of 15 cm from the edge of the bullet hole when wounded by a bullet V-Max. Stained with Hematoxylin and eosin. Microphoto, vol. 10, approx. 10; C – Hemorrhages in the serous membrane of the colon at a distance of 15 cm from the edge of the bullet hole when wounded by a bullet V-Max. Stained with Hematoxylin and eosin. Microphoto, vol. 10, approx. 10.

Injury of the colon by an expansive bullet 5.45 V-Max leads to fundamentally different damage to the intestine, which can be explained by a much stronger hydrodynamic impact. Thus, mechanical damage of the intestine comes to light not only directly in the place of bullet passage through it. Ruptures of the mucous membrane are found even at a distance of 20 cm from the latter. Accordingly, such injuries are accompanied by massive hemorrhages. Relatively small hemorrhages in the intestinal mucosa and serous membrane were also numerous. In addition, there is pronounced venous hyperemia in the preserved areas of the own plate, which decreases only at a distance of 20 cm from the bullet hole. There were significant varicose veins and blood overflows in the submucosa and serous membrane.

Histological parameters of the colon after a gunshot wound are given in table 1.

According to the test results, for all four variants of the used indicators sV VP (X) and sV PO (Y), there is a difference between the arithmetic means  $M_e$  and  $M_k$  for the experimental and control groups, as the condition  $t \geq t_{cr}$ .

Histological parameters of the colon after a gunshot wound

Indices		K	0	5	10	15	20
sV GS (mm <sup>2</sup> /mm)*	5.45	0.0311±0.027	0.0136±0.0025	0.0045±0.0022	0.0017±0.0009	0.003±0.0008	0.0074±0.0053
	V-Max		0.474±0.037	0.368±0.031	0.411±0.009	0.4±0.018	0.238±0.012
sV PO (mm <sup>2</sup> /mm)**	5.45	0.0021±0.0001	0.0088±0.0007	0.0078±0.0011	0.0069±0.0005	0.0071±0.0008	0.0062±0.0004
	V-Max		0.0121±0.0007	0.0135±0.0011	0.0107±0.0019	0.0102±0.0009	0.0089±0.0003
hV (y.o.)	5.45		0.81±0.05	0.09±0.06	0.06±0.04	0	0
	V-Max		2.1±0.14	2.3±0.17	1.90±0.08	2.1±0.15	1.4±0.02

Note: according to the indicators sV GS (X) \* and sV PO (Y) \*\* when comparing the control group and groups with bullet wounds 5.45 and V-Max in the forward and reverse directions there is a significant difference. In group X, (t = 4,605) the forward direction, (t = 3,976) the reverse direction at  $t_{cr}=2.447$ . In group Y, (t=5.26) the forward direction, (t=4.989) the reverse direction at  $t_{cr}=2.447$ .

Thus, the observations showed that a gunshot wound to the colon by a bullet 5.45 PS with a steel core leads to changes in its wall, extending to a considerable distance from the entrance of the bullet hole. In general, they can be described as a shock intestine. This is manifested by sharp anaemia of the own plate of the mucous membrane and a sharp dilation and overflow of blood vessels of the submucosal base and serous membrane of the mesenteric edge of the intestine [2, 3, 6]. These phenomena gradually decrease with distance from the place of the bullet passage through the intestinal wall, but even at a distance of 20 cm, they remain distinct.

The obtained data do not contradict the existing ones [7, 8]. They expand the idea of morphological changes in the colon with the same calibre bullets but with different properties. It is important for the practising surgeon to understand the changes in the colon wall during its gunshot wound to make the right choice of surgical tactics.

### Conclusions

1. The use of bullets 5.45 PS and 5.45 V-Max leads to significant morphological changes in the wall of the colon in the experiment.
2. Changes in the colon are significantly more pronounced in gunshot wounds when using a 5.45 V-Max bullet with a greater volume of damage than in the 5.45 PS bullet.
3. The extent of damage to the colon in gunshot wounds should be taken into account when determining surgical tactics.

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Стаття надійшла 21.12.2021 р.