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THE IMPACT OF SOIL TYPES ON BOVINE ENTEROVIRUS MIGRATION INTRODUCED WITH SLURRY

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ABSTRACT

The penetration of bovine enterovirus, LCR - 4 strain deep into soil profiles in the field conditions was researched. Three soil types were selected: podzolic soil, black earth and browned black earth. The soils differed considerably in respect to their physical properties and chemical analysis. The best conditions for drainage were observed in podzolic soil, whereas the worst - in browned black earth. Experimental plots were being sprinkled with the 1:1 mixture of bovine enterovirus (at the concen-tration of $1x10^{6.0}$ $TCID_{50}/1$ ml) and slurry. The

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enterovirus researched, in any soil, migrated to the depth of 7.5 to 10 cm. The highest titres were observed in the surface layers of black earth and podzolic soil (0-2.5 cm). They differed from $10^{2.72}$ - $10^{4.27}$ $TCID_{50}$/g of the soil. With depth the titres decreased, with slight fluctuations observed.

Key words: bovine enterovirus, slurry, virus migration, podzolic soil, black earth, browned black earth

INTRODUCTION

A considerable amount of municipal as well as animal waste remains a significant burden for the natural environment. Slurry, usually not conditioned, is widely applied in agriculture as an organic fertiliser. On the one hand, organic fertilisation of soils remains an important source of organic matter, however, on the other hand, it poses a hazard of micro-biological contamination of soil, underground and surface waters as well as crops. Slurry can contain a high level of pathogenic micro-organisms, fungi, viruses and parasite eggs [14]. To prevent epidemic, aphthous fever, transmisseble gastroenteritis in swine (TGE), rota-viruses, swine vesicular disease, the viruses of Cieszyn and Aujeszky diseases and others seem to have most significance for humans and animals [8]. Very frequently enteroviruses with the infectious titre from 10^3 to 10^6 $TCID_{50}$/ml are isolated in slurry [15].

The present experiment focused on defining the penetration of bovine enterovirus deep into selected soils with different physical properties and chemical analysis. The research was conducted in the field conditions, maintaining the natural structure of soils.

MATERIALS AND METHODS

At the first stage, physical properties and chemical analysis of the selected soils of the Kujawy Region, namely podzolic soil, black earth and browned black earth, were established. The grain composition of the respective genetic level was defined with the aerometric method of Bouyoucos, modified by Casagrande and Prószynski [12]. The cation exchangeable capacity was calculated in extract 1 n of the ammonium acetate, whereas replaceable hydrogen in 0.5 n of the barium chloride and 0.055 n of triethanolamine (TEA) [9]. Based on water characteristics, soil differential porosity was defined, including soil volume fraction of macro-pores. The soil reaction was established with the potentiometric method, both in water and in KCl solution. The slurry chemical analysis included, as follows: dry matter, postignition residue as well as the reaction with the methods generally applied in agricultural chemistry [13]. Potassium and calcium content was defined with photoflame method, total nitrogen with the Kjeldahl method, and phosphorus with the colorimetric method. The heavy metal content was defined with the atomic and emission spectrophotometric methods [13]. The experimental plots were defined with metal pipes, 23 cm in diameter, which were pushed 75 cm deep into the ground with the excavator. The front of the elements introduced was formed in such a way as to cause the least damage to the natural soil structure.

The bovine enterovirus, LCR - 4 strain with the titre of 1.2 x $10^{6.0}$ $TCID_{50}$/ml was used for the research. The 1:1 mixture of bovine enterovirus suspended solids and slurry fertilised the soils researched at the dosage of, respectively: podzolic soil - 746 ml and the two remaining types of soil - 828 ml each. A different dosage of virus and slurry mixture applied to respective soil types resulted from different water capacity of the soils researched as well as enteric virus elution capacity from the soils researched [7]. Every week the plots were watered at the amount corresponding to the average rainfall in the region.

At the same time weather conditions were being controlled (temperature, rainfall). After 28 days samples were taken with sterile spoons every 2.5 cm up to 15 cm in depth, then every 5 cm to the depth of 30 cm and finally every 10 cm at adequate level. The samples were taken from the centre of the soil profile. Then 5g - soil samples were prepared, repeated 2 or 3 times. The samples were placed in hermetic plastic containers which were then frozen. For enteric virus elution from soils a 10 % fetal serum was used [7]. The virus titres were identified with the generally applied method in cell lines (HK) on micro-dishes. The titres were calculated with the Kärber method and they were presented as $TCID_{50}$/g soil titres [20]. The sensitivity threshold of the method equalled $1x10^{1.05}$ $TCID_{50}$/ml elution buffer volume.

RESULTS

The physical properties and chemical analysis of the soils researched differed considerably (Tab.1). The podzolic soil contained a small percentage of organic matter (1.7 %) and a low sorption capacity. The soil reaction of the humus layer equalled 6.7, however deeper it was acid. The floatable particles (< 0.02 mm) content was low; however it scored the highest volume of macro-pores 300-30 µm in diameter of all the soils researched which made podzolic soil good for drainage. However the se-cond soil researched, black earth, had a high organic matter content (7.9 %) at a high cation exchan-geable capacity. The humus level contained 13 % floatable particles and 5 % colloidal fractions (< 0.002 mm). The basic reaction of the deeper soil layers was characteristic here due to a considerable content of carbonates. The drainage conditions were considerably much worse in podzolic soil due to a considerably lower number of macro-pores. The third soil researched was browned black earth with 3.5 % of organic matter. The humus level contained 26 % of floatable particles and 12 % colloidal fractions.

Table 1. Selected physical and chemical indicators of the soils researched

Genetic layer	Layer depth cm	Cation exchangeable capacity me/100 g	Fraction content diameter in mm (%)		Pore volume diameter in µ m (%)		pH in KCl	Organic matter (%)
			< 0.002	< 0.02	> 300	300-30		
Podzolic soil								
Ap	0-30	7.00	1	5	3.3	25.3	6.7	1.7
C	> 30	2.92	2	4	4.1	27.5	4.4	-
Black earth								
A	0-45	26.28	5	13	3.7	11.1	7.6	7.9
Ccagg	>45	30.64	21	37	3.4	4.6	8.2	-
Browned black earth								
A	0-40	15.28	12	26	3.7	7.7	7.2	3.5
B	40-60	27.56	19	35	4.1	4.9	7.7	-
Cca	> 60	32.85	19	37	4.1	5.0	7.8	-

The reaction value was similar to the one of black earth, whereas the cation exchangeable capacity fluctuated from 16.38 to 32.85 me/100g. A low macro-pores volume could limit draining conditions (Tab.1). The air temperature taken during the experiment fluctuated from

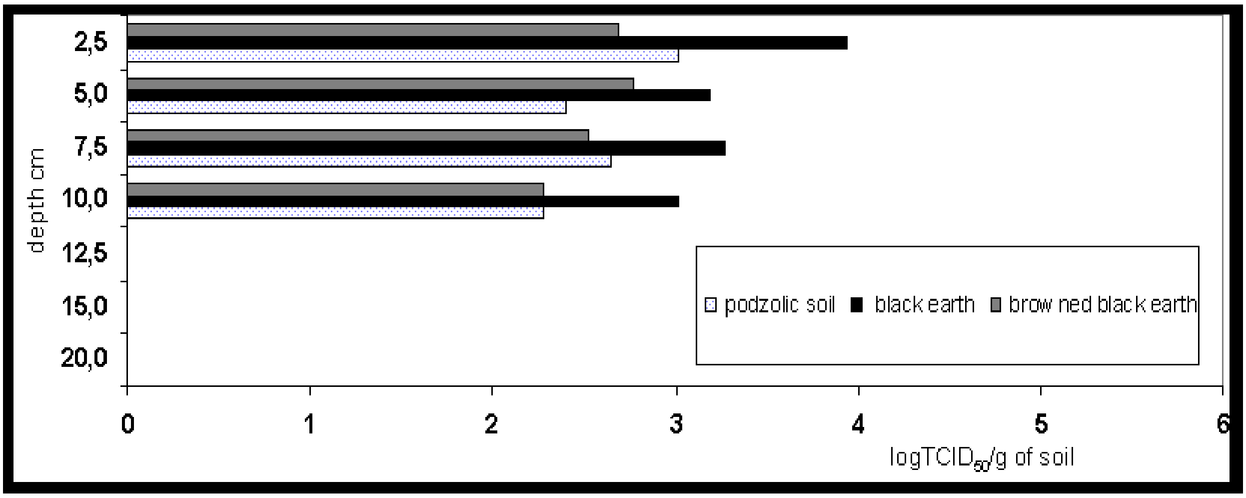
3.3-8.1°C, the total rainfall amounted to 15.2 mm, whereas the sun exposure time - from 3.7 to 5.1 hours per every 24 hours. The meteorological data, due to a small distance between the experimental plots (3-7 km) were valid for all of them. The chemical analysis of the slurry used in the experiment to fertilise the plots is presented in table 2. One shall notice that it contained 60231 mg/dm3 of dry matter well as a considerable amount of organic matter. Finally, along with the slurry there was some nitrogen, potassium, and phosphorus introduced into the soil at a low content of heavy metals.

Table 2. Chemical analysis of the slurry applied in the experiment

Characteristic	Units	Content in slurry
Dry matter	mg/dm^3	60231
Post-roasting residue	mg/dm^3	8655
Post-roasting loss	mg/dm^3	51576
Total nitrogen content	mg N/dm^3	6102
Phosphorus content	mg P/dm^3	2213
Potassium content	mg K/dm^3	1894
Calcium content	mg Ca/dm^3	899
Metal content	mg/dm^3	
Cadmium Cd		0.0
Lead Pb		4.7
Zinc Zn		52.1
Copper Cu		12.1
pH		7.1

Bovine enterovirus behaviour in the soils researched is presented in figure 1. With each soil type, the virus penetrated 7.5-10 cm deep.

Fig.1. Bovine enterovirus penetration deep into the soils researched and the mean infectious titres at respective depths



The highest bovine enterovirus infectious titres were observed in the soil surface layers and they fluctuated from $10^{2.72}$ - $10^{3.52}$ $TCID_{50}$/g of the podzolic soil, $10^{3.77}$ - $10^{4.27}$ $TCID_{50}$/g of the black earth and $10^{2.52}$ - $10^{2.77}$ $TCID_{50}$/g of the browned black earth. The infectious titre for the LCR-4 enteric virus decreased with depth of the samples taken, however some fluctuations were observed here (Fig.1) which point to virus desorption processes in the soil.

DISCUSSION

Viruses which together with organic fertilisers penetrate into the environment, on the one hand, undergo inactivation, however, on the other hand, they can be absorbed on soil particles and, consequently, transported into its deeper parts. The low ambient temperature and the short sun exposure time observed in the present research enhanced the survival of the bovine enterovirus in the soil; at the same time the soil contamination hazard was increasing [18]. Three processes define the virus behaviour in the soil, namely: transport, sorption or retention and inactivation [17]. Differently than bacteria, they do no undergo filtering but, most of all, they undergo adsorption on soil particles [18]. The adsorption of micro-organisms depends, amongst others, on the number of microbes in the suspended soils and their type, soil type, cation exchangeable capacity and dissolved organic matter, soil pH as well as the water saturation [18]. Due to adsorption, a very powerful virus retention is observed in the soil surface layers. Drewy and Eliassen [3] claim that their outstanding majority is retained at 2 cm of the surface ground layer. In the present research the highest titre of the bovine enterovirus was observed also in the soil surface layer (0-2.5 cm). However Wang et al. [19] claim that the soil layer depth required to eliminate the virus depends, most of all, on the type of soil. However in the present research with any type of soil the enteric virus penetrated 7.5 to 10 cm deep. Observing the lowest titres of the bovine enterovirus in browned black earth may result from a strong bond between the enteric virus and the soil particles and, consequently, a low percentage of elution from that soil [7]. A decisive factor in the retention of viruses in soils and, consequently, in limiting their transport is water flow through micro-pores [2]. Beside micro-, mezo- and macro-pores there are also the so called mega-pores which can enhance a fast transport of microbes deep into the soil [5]. A significant factor which influences virus adsorption is the presence of replaceable cations, especially divalent ionic of calcium and magnesium and trivalent of iron and aluminium [1,18]. The ions increase the power of the virus bond due to the decrease in the basic potential of the soil particles and micro-organisms. The present research of physical properties and chemical analysis of the soil showed that the podzolic soil had a large macro-pore content and a low sorption capacity. From this data it is seen that the best conditions for virus transport were found in the podzolic soil. Much higher sorption properties were observed in the two other types of soil, namely, black earth and browned black earth. Also their grain composition indicated worse percolation conditions. However there were no differences in depth to which the virus penetrated in respective types of soil. It seems then that the low pH of the podzolic soil which enhances virus adsorption in the field conditions could balance its better filtering properties due to the number of macro-pores. Besides enteric virus penetration deep into the soil can be effectively limited by the effluent-soil matrix with viruses binding both soil and effluent particles [2]. Gerba & Bitton [4] observe a limited water transport in non-saturated conditions, namely in the soil upper layer (excluding fissures and macro-pores). Finally the virus and soil particles contact frequency increases, and hence adsorption. On the other hand, however, the presence of structural mega-pores in the two remaining soils (black earth and browned black earth) which enhanced an accelerated water flow could limit a negative impact of the sorption complex and a more substantial amount of organic matter on transport processes. One should emphasise that there are many more factors which determine virus transport and their effect, depending on the conditions, may heighten or cancel each other out.

There were no differences observed in depth to which viruses migrated in respective soils, which would support the thesis of a strong bond between the virus and slurry and, consequently, a more difficult virus desorption. Continues adsorption and desorption processes in the soil cause a slow-down in the transport of micro-organisms when compared to water movement. Besides Gerba & Bitton [4] claim that the transport of micro-organisms is much slower in soils poor in water where the slow-down coefficient is considerably higher. During the present research no heavy rainfall was observed. Besides the intensity of watering of the plots did not simulate a very intensive storm rainfall which enhances a rapid water transport deep into the soil profiles. It is possible that in such cases virus migration could take place even to a considerable depth. The enterovirus elution and transport to aquifers following sprinkling with effluent as the result of heavy rainfalls was observed by some authors [10, 11]. It should be emphasised that once fractures or fissures appear in the soil, extra local zones of increased percolation emerge which enhance virus migration.

Desorption processes in soils are influenced by many mechanisms. One can assume that ionic content in the soil plays the key function here. Fertilising with slurry causes a considerable increase in the ionic content in the soil. Soils enriched with cations from effluent build an organic layer with good adsorptive properties. Their retention takes place mostly in the upper part of the soil profile; a considerably lower number of ions penetrates deeper into the soil. The places with a high ionic concentration are those were the majority of soil viruses are also transported. Only with a heavy rainfall a temporary decrease in ionic concentration gradient in soil takes place and, consequently, a weakening of virus binding by soil particles. It seems that it is a short-time phenomenon as the soil conditions enhancing new virus bond quickly re-emerge. Humus soils show a high enteric virus retention potential [1,16]. In the present research a conside-rably high organic matter content was observed in black earth (7.9 %), which could enhance virus adsorption properties. However Gerba & Bitton [4] claim that dissolved organic matter may compete with viruses and replace them which, to some extent, decreases soil adsorptive potential.

The slurry applied in the present research contained 60231 mg/dm^3 of the dry matter; consequently once it was applied, a few-millimetre surface scum was formed on the surface of soils which could interfere with the suspended solids penetration deep into the soil as well as bind viruses. Similarly Hirte [6] observes that the majority of slurry microbes along with its thicker solid particles are retained in the soil upper layer. Besides, especially in the soil upper layer indigenous soil flora remains active which has a negative influence on viruses and, consequently, it enhances self-purification in the soil upper layers [1].

A very high retention of enteric viruses in the upper layers of the soil profiles would point to the low potential of underground water contamination. However the high enteric virus titres observed in the soil surface layers may signify a considerable plant contamination hazard. In the present research no virus contamination threat to aquifers was observed. However when estimating epidemic threat, each time the complex of environmental factors should be taken into consideration. Definitely the risk increases with heavy rainfalls with soil mega-pores and fissures present. To minimise the hazard of contamination of soil, plants and underground waters with pathogenic viruses, slurry should be con-di-tio-ned before it is applied as a fertiliser.

CONCLUSIONS

From the research conducted the following conclusions can be drawn:

1. In the field experiment no bovine enterovirus migration was observed beyond the humus layer of the soils researched.
2. The highest infectious titres of the bovine enterovirus researched were observed in the surface layers of the soil profiles (0-2.5 cm), especially in black earth and podzolic soil.
3. No significant effect of soil type on the bovine enterovirus penetration depth was observed.

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