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Quantification of Semi-Truck Cab Decontamination[1]

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Summary

Evidence suggests that the inside of vehicle cabs used for feed delivery may serve as a potential source for disease, yet there are no standardized protocols or scientific evidence for methods of their disinfection. Therefore, the objective of this project was to evaluate commercially available disinfectants and disinfection application methods against PEDV and PRRSV on various surfaces within semi-truck cabs. Three different surface types common in vehicle cabs (fabric, plastic, and rubber) were cut into 4 × 4 inch coupons and inoculated with either PEDV or PRRSV. Once inoculated, surfaces were placed in one of 3 semi-truck cabs and the disinfectant treatment was applied. Disinfectant treatments were as follows: 1) no-disinfectant, 2) hurricane fumigation with 1:256 dilution of Synergize, 3) hurricane fumigation with 1:64 dilution of Intervention, 4) pump sprayer with 1:256 dilution of Synergize, 5) pump sprayer with 1:64 dilution of Intervention, 6) pump sprayer with 10% bleach, 7) no chemical with 10 hr downtime, and 8) gaseous fumigation over a 10 hr period with water-based chlorine dioxide. Once a disinfectant treatment was applied, the coupons were environmentally swabbed and submitted for qPCR duplex analysis for PEDV and PRRSV. There was a significant disinfectant × surface interaction ($P < 0.0001$) indicating that the disinfectant treatment efficacy differed based on surface. Within rubber surfaces, 10% bleach had a greater Ct value compared to all other treatments ($P < 0.05$), with the exception of Intervention with hurricane fumigation application, which was intermediate. In both fabric and plastic surfaces, there was no evidence ($P > 0.05$) of a difference in Ct value between any of the treatments. Additionally, for the no-disinfectant treatment, the Ct value was greater on fabric surfaces compared to plastic and rubber ($P < 0.05$); fabric was greater than plastic in the Intervention with pump sprayer application treatment ($P < 0.05$), fabric and rubber greater than plastic in the 10% bleach treatment ($P < 0.05$); and fabric greater than plastic and rubber in the 10 hr downtime and gaseous fumigation treatments ($P < 0.05$). There was a significant main effect of disinfectant treatment ($P = 0.016$), where 10% bleach had a greater Ct value compared

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to both the control treatment, 10 hr downtime treatment, and Intervention applied using the pump sprayer ($P < 0.05$). There was a main effect of surface ($P < 0.0001$) where rubber had a greater Ct value compared to plastic ($P < 0.05$), and fabric had a greater Ct value compared to both rubber and plastic ($P < 0.05$). Finally, the Ct value for PRRSV was greater than PEDV ($P < 0.0001$) when averaged across all surfaces and disinfectant treatments.

In summary, these data highlight that it is important to consider the surface of interest when implementing disinfectant protocols. In general, most disinfectant applications were only able to reduce the quantity of detectable virus, but not completely eliminate it from surface. However, additional research is necessary to understand the viability of residual virus on disinfected surfaces.

Introduction

The introduction of PEDV into North America led to a need to better understand the epidemiological link between potentially contaminated feed, pig delivery trucks, feed delivery trucks, feed mills, and production sites. A report by Greiner (2016)[4] suggested that other areas in the feed mill, such as truck pedals and the feed mill office, could be a potential source of disease during PEDV or porcine delta coronavirus (PDCoV) outbreaks. Gebhardt et al.[5] found similar results in a monitoring project for African swine fever virus (ASFV) in Vietnam – a majority of ASFV-positive surfaces were from semi-truck cabs for feed delivery or pig transport. While there is evidence to suggest that the inside of semi-truck cabs for the feed delivery chain may serve as a potential source for disease, there is a lack of standardized protocols or scientific evidence for methods of disinfection within the semi-truck cabs. Therefore, the objective of this project was to evaluate commercially available disinfectants and disinfection application methods against PEDV and PRRSV on various surfaces within semi-truck cabs.

Materials and Methods

General

The study was conducted at the Feed Science Research Center (FSRC) at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, with approval by the Kansas State University Institutional Biosafety Committee (Project Approval #1511). The inoculation of surfaces was done with a biosafety cabinet (BSC) within the BSL-2 space of the FSRC. This study was set up in an 8 × 3 × 2 factorial due to the eight different disinfectant methods, three different surfaces, and two different viruses. Disinfectant methods were as follows: 1) no-disinfectant, 2) hurricane fumigation with 1:256 dilution of Synergize, 3) hurricane fumigation with 1:64 dilution of Intervention, 4) pump sprayer with 1:256 dilution of Synergize, 5) pump sprayer with 1:64 dilution of Intervention, 6) pump sprayer with 10% bleach, 7) no chemical with 10 hr downtime, and 8) gaseous fumigation over a 10 hr period with water-based chlorine dioxide. Surfaces were rubber, plastic, and fabric. Viruses that inoculated the surfaces were PEDV or PRRSV.

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[4] Greiner LL. Evaluation of the likelihood of detection of porcine epidemic diarrhea virus or porcine delta coronavirus ribonucleic acid in area within feed mills. *J. Swine Health Prod.* 2016;24(4):198-204.

[5] Gebhardt JT, Dritz SS, Jones CK, Woodworth JC, and Paulk CB. Lessons learned from preliminary monitoring for African swine fever virus in a region of ongoing transmission. *Journal of the American Veterinary Medical Association.* 2021;258(1):35-38. doi:10.2460/javma.258.1.35

Preparation of inoculum
To make the viral inoculation for this study, 25 mL of PEDV (USA/Co/2013 isolate with a titer of 1.33×10^6 $TCID_{50}$/mL) and 25 mL of PRRSV (1-7-4 isolate with a titer of 1.33×10^6 $TCID_{50}$/mL) were each diluted in separate containers using 225 mL of phosphate buffered solution (PBS) to get a final concentration of 10^5 $TCID_{50}$/mL. The viruses were stored at -112°F until the start of the study.

Preparation of surfaces and disinfectant
Plastic (1/8 × 4 × 8 in. white high-density polyethylene panel, Menards, Eau Claire, WI), rubber (BCG Heavy-Duty 18 × 18 × ¼ in. Rubber Gym Tiles, Boston, MA), and fabric surfaces (upholstery fabric, Joann's Fabrics, Hudson, OH) were cut into 4 × 4 inch squares for the surface coupons. Velcro strips were applied to the back of the surface coupons prior to inoculation and surface coupons were placed into a transportation container (Promoze Food Storage Containers, Amazon, Seattle, WA) and remained in these storage containers until ready for placement in semi-truck cabs.

Wet disinfection applications were mixed fresh each day of the study. For 10% household bleach solution, 378.5 mL of 7.55% sodium hypochlorite (Germicidal Bleach; Clorox, Oakland, CA) was poured into a 1 gallon mixing container (Sterilite; Walmart, Bentonville, AR) and then the container was filled with water up to the 1 gallon mark. For 1:256 dilution of Synergize (26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde; Lexington, KY), 0.5 ounces (15 mL) was poured into the gallon mixing container and then the container was filled with water up to the 1 gallon mark. For 1:64 dilution of Intervention (accelerated hydrogen peroxide, Oakville, ON, Canada), 2 ounces (60 mL) was poured into the gallon mixing container and then the container was filled with water up to the 1 gallon mark. Once mixed into the gallon mixing container, solutions were poured into their respective application method tool: pump sprayer (Chapin Sure Spray 1 Gallon Tank Sprayer, Menards, Eau Claire, WI) or hurricane fogger (Hurricane Ultra II Portable Electric Fogger, Curtis Dyna-Fog Ltd., Westfield, IN). For the gaseous fumigation treatment, truck cabs were visually and physically inspected for any holes or inadequate seals and sealed as necessary using either silicone sealant (DAP, Menards, Eau Claire, WI) or super glue (Gorilla Glue, Sharonville, OH). For the gaseous fumigation application, the water-based chlorine dioxide pouch (ProKure G; ProKure Solutions, Phoenix, AZ) was inserted into a wet sponge and sealed in a plastic container following manufacturer's labeled instructions.

Inoculation of surfaces and disinfectant application
Based on the treatment type, surfaces were inoculated with either 1 mL of PRRSV or PEDV. Surfaces were allowed to dry for an hour prior to placement within an individual truck cab. When surfaces were ready for placement, surface coupons were placed within the truck cab with gloves changed for each new surface coupon. Coupon placement was predetermined prior to the start of the study, Velcro strips were placed within the cab to ensure consistent placement for each treatment. Plastic coupon surfaces were placed on the dashboard, rubber surface coupons were placed on the floorboard, and fabric surface coupons were placed on the driver's seat. Seats were wrapped with plastic wrap (Great Value, Bentonville, AR) prior to initiation of a new treatment so as to not unintentionally contaminate the back side of the fabric coupon.

After surfaces were placed in the truck cab, a randomly assigned treatment application was conducted. For the pump sprayer application, this method was standardized prior to the start of the trial: the applicator stood outside the truck cab on the driver's side and applied the liquid in a snake-like application method, going from the front to the back of the cab, which used 0.1 to 0.2 lb of disinfectant solution per treatment. For the hurricane fogger application, the head of the hurricane fogger was angled and secured at 90° (runs parallel with the ground), placed in the passenger side seat, and aimed for the driver's side of the truck cab. Once set in location, the flow rate was set on 2, turned on, passenger door was closed, and the hurricane fogger was allowed to run for 5 min. On average, the amount of disinfection used for this application ranged from 0.50 to 0.75 lb. Once the application of the pump sprayer and hurricane fogger was completed, wet application methods were allowed to dry for 15 min prior to environmental swabbing.

For the gaseous fumigation treatment, the plastic container was placed in the passenger's seat, doors were closed, and the chemical allowed to fumigate the truck cab for 10 hr. Once the gaseous fumigation treatment was placed in the truck cab, the lights were turned off and sat at room temperature. After setting for 10 hr, the driver's and passenger's doors were opened, the pouch was disconnected from the sponge, and the truck cab was allowed to air out for 1 hr, then was environmentally swabbed.

For the no-chemical treatment, surface coupons were inoculated and placed in truck cabs as previously mentioned and allowed to sit for approximately 15 min then environmentally swabbed. For the 10 hr downtime disinfectant treatment, surface coupons were inoculated as previously mentioned, placed in the semi-truck cab, allowed to sit in the cab for 10 hr and environmentally swabbed.

Environment sampling

Environmental swabbing of surface coupons was done as previously described.[6] Once a treatment application was completed and appropriate environmental sampling was conducted, surface coupons were discarded and cabs were cleaned, sprayed with 1:256 glutaraldehyde, and allowed to air out for 20 min.

Environmental samples were transferred to the BSC, had 20 mL of PBS added to them, inverted for 5-10 sec, and allowed to sit at room temperature for 1 hr. The samples were then vortexed for 15 sec, and supernatant was pipetted off into 1.75 mL cryovials and 15 mL conical tubes. Samples were then transported to a -112°F freezer and stored there until PCR analysis.

Real-time PCR analysis

Real time PCR was conducted at the Molecular Research and Development Laboratory within the Kansas State Veterinary Diagnostic Laboratory. Fifty microliters of supernatant from each sample was loaded into a deep-well plate and extracted using a Kingfisher Flex magnetic particle processor (Fisher Scientific, Pittsburgh, PA) and the MagMAX-96 Viral RNA Isolation kit (Life Technologies, Grand Island, NY)

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[6] Elijah, C. G.; Trujillo, J. D.; Jones, C. K.; Gaudreault, N. N.; Stark, C. R.; Cool, K. R.; Paulk, C. B.; Kwon, T.; Woodworth, J. C.; Morozov, I.; Gebhardt, J. T.; and Richt, J. A. (2020) "Evaluating the Distribution of African Swine Fever Virus Within a Feed Mill Environment Following Manufacture of Inoculated Feed," Kansas Agricultural Experiment Station Research Reports: Vol. 6: Iss. 10. https://doi.org/10.4148/2378-5977.8012.

according to manufacturer's instructions with one modification, reducing the final elution volume to 60 μL. One negative extraction control consisting of all reagents except the sample was included in each extraction. Positive controls of each stock virus were also included with each extraction. Extracted RNA was frozen at -112°F until assayed by qRT-PCR. Analyzed values represent cycle threshold (Ct) at which virus was detected. If a sample had no detectable PRRSV or PEDV RNA, a sample was assigned a value of 45, as a total of 45 cycles were run for each sample.

Statistical analysis

Data were analyzed in a split plot design with truck cab as the experimental unit for disinfectant treatment, and surface coupons as the experimental unit for surface type (fabric, plastic, or rubber) and virus (PEDV or PRRSV). There were three replications per disinfectant treatment. The Ct value of each sample was analyzed with ANOVA and *F*-test through the aov function in R programming language (R Foundation for Statistical Computing, Vienna, Austria). Fixed effects were considered the disinfectant treatment, surface treatment, and virus type, while random effect was truck cab defining it as the experimental unit for disinfectant treatment to account for the split plot design. Results of Ct data are reported as least squares means ± standard error of the mean. All statistical models were evaluated using visual assessment of studentized residuals, and the assumptions appeared to be reasonably met. A Tukey multiple comparison adjustment was incorporated when appropriate. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

There was no evidence of a disinfectant × surface × virus, surface × virus, or disinfectant × virus interaction ($P > 0.10$; Table 1 and 2). There was a significant disinfectant × surface interaction ($P < 0.0001$) indicating that the disinfectant treatment efficacy differed based on surface (Table 3). Within rubber surfaces, 10% bleach had a greater Ct value compared to all other treatments ($P < 0.05$), with the exception of Intervention via hurricane fumigation application, which was intermediate. In both fabric and plastic surfaces, there was no evidence ($P > 0.05$) of a difference in Ct value between any of the treatments. Additionally, for the no-disinfectant treatment, the Ct value was greater on fabric surfaces compared to plastic and rubber ($P < 0.05$); fabric and rubber were greater than plastic in the Intervention via hurricane fumigation application treatment ($P < 0.05$); fabric and rubber were greater than plastic in the 10% bleach treatment ($P < 0.05$); and fabric were greater than plastic and rubber in the 10 hr of downtime and gaseous fumigation treatments ($P < 0.05$).

There was a significant main effect of disinfectant treatment ($P = 0.016$; Table 4), where 10% bleach had a greater Ct value compared to the no-disinfectant, 10 hr downtime treatment, and Intervention applied using the pump sprayer ($P < 0.05$). There was a main effect of surface ($P < 0.0001$) where rubber had a greater Ct value compared to plastic ($P < 0.05$), and fabric had a greater Ct value compared to both rubber and plastic ($P < 0.05$). This could indicate that disinfectant applications can soak into the fabric to reduce the amount of PEDV or PRRSV on the fabric, or that fabric surfaces limit the amount of virus picked up by environmental swabbing. Finally, the Ct value for PRRSV was greater than PEDV ($P < 0.0001$) when averaged across all surfaces and disinfectant treatments.

In summary, differences in disinfectant efficacy were observed for different surfaces. Biosecurity practices should take the surface into consideration when developing and implementing sanitation procedures. Disinfectants will decrease the quantity of detectable virus of locations where applied, but won't sterilize the surface altogether, which underscores that biocontainment of pathogens is critical within biosecurity programs. For this study, infectivity of the detected virus was not determined, which represents an area of future research interest.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Effect of surface, disinfectant, and virus on the detection of viral RNA during semi-truck cab decontamination

Item	*P* =
Disinfectant × surface × virus	0.959
Surface × virus	0.926
Disinfectant × virus	0.508
Disinfectant × surface	< 0.0001
Virus	< 0.0001
Surface	< 0.0001
Disinfectant	0.016

Table 2. Effect of surface, disinfectant, and virus on the detection of viral RNA during semi-truck cab decontamination[1]

	Surface type						
	Fabric		Plastic		Rubber		
Item	PEDV	PRRSV	PEDV	PRRSV	PEDV	PRRSV	SEM
Proportion PCR positive							
No-disinfectant[2]	3/3	3/3	3/3	3/3	3/3	3/3	--
Hurricane fumigation[3]							
Intervention[4]	3/3	3/3	3/3	3/3	2/3	2/3	
Synergize[5]	2/3	3/3	3/3	3/3	3/3	3/3	
Pump sprayer[6]							
Intervention	3/3	3/3	3/3	3/3	3/3	3/3	
Synergize	2/3	3/3	2/3	3/3	3/3	3/3	
10% Bleach[7]	2/3	0/3	3/3	3/3	1/3	0/3	
10 hr Downtime[8]							
No chemical	3/3	2/3	3/3	3/3	3/3	3/3	
Gaseous treatment[9]	3/3	1/3	3/3	3/3	3/3	3/3	
Cycle threshold							
No-disinfectant	34.6	37.2	26.7	30.6	26.7	31.4	1.84
Hurricane fumigation							
Intervention	33.4	38.2	28.1	31.6	34.2	36.7	
Synergize	36.2	36.4	29.7	34.2	30.3	33.3	
Pump sprayer							
Intervention	34.8	37.5	28.3	31.3	28.8	32.0	
Synergize	37.3	38.7	33.0	32.6	30.6	33.5	
10% Bleach	40.7	45.0	26.7	31.2	41.2	45.0	
10 hr Downtime[8]							
No chemical	36.4	40.3	27.8	29.8	29.7	30.2	
Gaseous treatment	36.8	44.4	28.3	31.9	28.6	33.2	

[1]Surfaces were inoculated with 1 mL of PEDV or PRRSV, randomly placed within the truck cab, and subjected to a randomly assigned disinfectant treatment. Samples with no detectable RNA were assigned a value of 45. Disinfectant × surface × virus, $P = 0.959$.
[2]Surfaces were inoculated with pure virus and allowed to sit within the semi-truck cab for 15 min. These surfaces were not treated with a disinfectant application.
[3]Semi-truck cabs had a hurricane fumigation system placed in the passenger's seat and directed toward the driver's side. The hurricane fumigation system was filled with respective disinfectant and was allowed to run 5 min for each treatment.
[4]Virox Technologies Inc., Oakville, ON, Canada.
[5]Preserve International, Lexington, KY.
[6]Semi-truck cabs had disinfectant applied with a conventional pump sprayer with the designated disinfectant.
[7]Household bleach (10% dilution; The Clorox Company, Oakland, CA; 7.55% sodium hypochlorite).
[8]Surfaces were inoculated with pure virus and allowed to sit within the semi-truck cab for 10 hr. These surfaces were not treated with a disinfectant application.
[9]Semi-truck cabs had gaseous chlorine dioxide (ProKure G; ProKure Solutions, Phoenix, AZ) placed on the passenger side seat and allowed the chemical to fumigate the semi-truck cab for 10 hr.
[a,b,c]Means lacking common superscripts differ, $P < 0.05$.

Table 3. Effect of surface and disinfectant on the detection of viral RNA during semi-truck cab decontamination[1]

	Proportion PCR Positive			Ct Value			
Item	Fabric	Plastic	Rubber	Fabric	Plastic	Rubber	SEM
No-disinfectant[2]	6/6	6/6	6/6	35.9[c,d,e,f,g,h]	28.6[a,b]	29.0[a,b]	1.45
Hurricane fumigation[3]							
Intervention[4]	6/6	6/6	5/6	35.8[a,b,c,d,e,f,g,h]	29.8[a,b,c,d]	35.4[a,b,c,d,e,f,g,h]	
Synergize[5]	5/6	6/6	6/6	36.6[a,b,c,d,e,f,g,h]	31.9[a,b,c,d,e,f]	31.8[a,b,c,d,e,f]	
Pump sprayer[6]							
Intervention	6/6	6/6	6/6	36.1[b,d,e,f,g,h]	29.8[a,c]	30.4[a,b,c,d,e,f]	
Synergize	5/6	5/6	6/6	38.0[e,f,g,h]	32.8[a,b,c,d,e,f,g]	32.0[a,b,c,d,e,f]	
10% Bleach[7]	2/6	6/6	1/6	42.9[h]	29.0[a,b,c,d]	43.1[h]	
10 hr Downtime[8]							
No chemical	5/6	6/6	6/6	38.4[f,g,h]	28.8[a,b,c,d]	30.0[a,b,c,d]	
Gaseous treatment[9]	4/6	6/6	6/6	40.6[g,h]	30.1[a,b,c,d,e]	30.9[a,b,c,d,e,f]	

[1]Surfaces were inoculated with 1 mL of PEDV or PRRSV, randomly placed within the truck cab, and subjected to a randomly assigned disinfectant treatment. Samples with no detectable RNA were assigned a value of 45. Disinfectant × surface, $P < 0.0001$.

[2]Surfaces were inoculated with pure virus and allowed to sit within the semi-truck cab for 15 min. These surfaces were not treated with a disinfectant application.

[3]Semi-truck cabs had a hurricane fumigation system placed in the passenger's seat and directed toward the driver's side. The hurricane fumigation system was filled with respective disinfectant and was allowed to run 5 min for each treatment.

[4]Virox Technologies Inc., Oakville, ON, Canada.

[5]Preserve International, Lexington, KY.

[6]Semi-truck cabs had disinfectant applied with a conventional pump sprayer with the designated disinfectant.

[7]Household bleach (10% dilution; The Clorox Company, Oakland, CA; 7.55% sodium hypochlorite).

[8]Surfaces were inoculated with pure virus and allowed to sit within the semi-truck cab for 10 hr. These surfaces were not treated with a disinfectant application.

[9]Semi-truck cabs had gaseous chlorine dioxide (ProKure G; ProKure Solutions, Phoenix, AZ) placed on the passenger's side seat and allowed the chemical to fumigate the semi-truck cab for 10 hr.

[a,b,c]Means lacking common superscripts differ, $P < 0.05$.

Table 4. Main effects of disinfectant, surface, and virus on the detection of viral RNA during semi-truck cab decontamination[1]

Item	Proportion PCR Positive	Ct Value	SEM	*P* =
Disinfectant			1.11	0.016
No-disinfectant[2]	18/18	31.2[a]		
Hurricane fumigation[3]				
Intervention[4]	16/18	33.7[a,b]		
Synergize[5]	17/18	33.4[a,b]		
Pump sprayer[6]				
Intervention	18/18	32.1[a]		
Synergize	16/18	34.3[a,b]		
10% Bleach[7]	9/18	38.3[b]		
10 hr Downtime				
No chemical[8]	17/18	32.4[a]		
Gaseous treatment[9]	16/18	33.9[a,b]		
Surface			0.53	< 0.0001
Fabric	39/48	38.0[c]		
Plastic	47/48	30.1[a]		
Rubber	41/48	32.8[b]		
Virus			0.48	< 0.0001
PEDV	62/72	32.0[a]		
PRRSV	62/72	35.3[b]		

[1]Surfaces were inoculated with 1 mL of PEDV or PRRSV, randomly placed within the truck cab, and subjected to a randomly assigned disinfectant treatment. Samples with no detectable RNA were assigned a value of 45.
[2]Surfaces were inoculated with pure virus and allowed to sit within the semi-truck cab for 15 min. These surfaces were not treated with a disinfectant application.
[3]Semi-truck cabs had a hurricane fumigation system placed in the passenger's seat and directed towards the driver's side. The hurricane fumigation system was filled with respective disinfectant and was allowed to run 5 min for each treatment.
[4]Virox Technologies Inc., Oakville, ON, Canada.
[5]Preserve International, Lexington, KY.
[6]Semi-truck cabs had disinfectant applied with a conventional pump sprayer with the designated disinfectant.
[7]Household bleach (10% dilution; The Clorox Company, Oakland, CA; 7.55% sodium hypochlorite).
[8]Surfaces were inoculated with pure virus and allowed to sit within the semi-truck cab for 10 hr. These surfaces were not treated with a disinfectant application.
[9]Semi-truck cabs had gaseous chlorine dioxide (ProKure G; ProKure Solutions, Phoenix, AZ) placed on the passenger's side seat and allowed the chemical to fumigate the semi-truck cab for 10 hr.
[a,b,c]Means within main effect lacking common superscripts differ, $P < 0.05$.