Influences of biosecurity on the occurrence of cellulitis in broiler flocks

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Primary Audience: Flock Supervisors, Quality Assurance Personnel, Plant Managers, Veterinarians

SUMMARY

The main condemnation reason in the slaughter of German conventional broiler flocks is cellulitis. This disease is primarily caused by the bacterial pathogen *Escherichia coli*. Biosecurity is the most important aspect to avoid spreading of diseases, but, increasing farm sizes and a close proximity of broiler houses complicate compliance with appropriate measures. This study aimed to identify the most efficient biosecurity factors for controlling cellulitis in broiler chickens by using a comprehensive questionnaire. Data of the biosecurity management from broiler farms were compared to condemnation ratios of the broiler flocks. It was found that using appropriate farm-specific clothing with changing of shoes and an adequate cleaning of the broiler houses after each grow out period helped to improve outcomes regarding total condemnation ratios as well as condemnation ratios due to cellulitis.

Key words: manure, biosecurity, reinfection, *Escherichia coli*

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DESCRIPTION OF PROBLEM

On modern broiler farms, hygiene management decisions play a major role in successfully reducing not only viral but also bacterial diseases in broiler chickens. The term biosecurity is used to describe all measures that have the potential to reduce the entry and spread of pathogens on a farm (Ali et al., 2014). Biosecurity is defined by the World Health Organization (2010) as "an integrated approach to manage risks to human, animal and plant life and health." With increasing farm sizes and a close proximity of broiler houses, biosecurity has become an increasingly important issue. Previous studies have examined the association between the reduction in pathogens in broiler and parent flocks, and layers, and enhanced biosecurity standards (Bojesen et al., 2003; McDowell et al., 2008). By assigning different biosecurity levels from 1 (highest biosecurity level) to 4 (lowest biosecurity level) to different production systems, Bojesen et al. (2003) were able to show that biosecurity standards

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influence the prevalence of hemolytic *Gallibacterium* ssp. As seen in the study by McDowell et al. (2008), frequent changing of footbath disinfectants can lower the risk of *Campylobacter* spp. infections in broiler flocks, while the presence of multiple houses on farms increased this risk. Additionally, Droual et al. (1990) previously reported a case of infectious coryza with higher condemnation rates in birds from farms in close proximity to each other, with infection spreading from one farm to another. Mo et al. (2016) showed in another study that proper cleaning and disinfection management lowers the spreading of cephalosporin-resistant *Escherichia coli (E. coli)* to the next flock. The authors also mentioned that it is beneficial to reduce the number of employees who need to enter broiler houses. An Indian study from 2020 suggested that the high condemnation rates caused by *E. coli* could be caused by the lack of biosecurity standards (Adil, 2020). Biosecurity is even regarded as a tool to improve productivity of broilers by reducing mortality rates and improving disease control (Ali et al., 2014; Oluwatoyin et al., 2018). In pig herds, an association between biosecurity and treatment incidents was also identified, suggesting that higher biosecurity standards may reduce the frequency of antimicrobial treatments (Laanen et al., 2013; Postma et al., 2015). Given the mandate that the use of colistin sulfate needed to be reduced to <5 mg/PCU (Population Correction Unit) in livestock by the end of 2020 in the European Union (European Medicines Agency 2016), there is a need to continuously find preventive ways to reduce the incidence of *E. coli*-induced diseases like cellulitis, which in many cases are treated with this antimicrobial agent.

The present study focused on cellulitis as it is recognized as one of the main reasons for carcass condemnation in broilers. Therefore, cellulitis has a great economic impact on broiler production and is an important factor that can affect broiler welfare (Gomis et al., 2000; Derakhshanfar and Ghanbarpour, 2002; Salines et al., 2017). Cellulitis is most frequently caused by *E. coli* (Barbieri et al., 2013), but other pathogens such as *Staphylococcus aureus, Actinomyces pyogenes,* and *Proteus mirabilis* are also discussed as causative agents (Derakhshanfar and Ghanbarpour, 2002; Sanches et al., 2020). Derakhshanfar and Ghanbarpour (2002) pointed out that *E. coli* was isolated in 91.8% of the cellulitis lesions studied, indicating that not only avian pathogenic *E. coli* is relevant for the development of cellulitis lesions. According to Jeffrey et al. (2004), it is not entirely clear which serotypes of *E. coli* cause cellulitis and general infections in broilers. However, Poulsen et al. (2018) found that *E. coli* causing cellulitis in broilers share a close genetic relationship. Scratches to the skin are typically the entrance for bacteria, which then lead to infection and inflammation of subcutaneous layers, resulting in typical cellulitis lesions (Jeffrey et al., 2004).

E. coli is present in litter in high bacterial counts (approximately 6 log colony-forming units/g) (Schrader et al., 2004), and the house-specific *E. coli* load can persist over successive flocks, independent of cleaning and disinfection procedures (Singer et al., 2000; Bagge et al., 2006). *E. coli* is even able to survive in dust of a poultry house for more than 28 wk after thorough cleaning and disinfecting, and in the absence of birds (Harry, 1964). It is also discussed that biogas plants contribute to the spread of the pathogen, because the mesophilic anaerobic digestion in biogas plants can reduce but not eliminate the occurrence of potentially pathogenic *E. coli* (Larsen et al., 1994; Horan et al., 2004). Thus, these biogas plants may also be another source of reinfection, underlining the need for biosecurity tools in livestock farming.

Van Limbergen et al. (2018) scored biosecurity standards of European broiler farms from different countries, and pointed out that good education of farm staff concerning biosecurity is the best way of improving these standards. Nonetheless, biosecurity decisions are not always popular among farmers, as economic factors often play an important role, for example when discussing the extension of downtime. Previous studies have shown beneficial effects on broiler health when extending the period of downtime (Chin et al., 2009). However, Szőllősi et al. (2014) calculated that the economic effect of extending the downtime period would reduce the total number of flocks reared per year, which in turn also lowers the amount of realizable income.

Robertson (2020) pointed out that epidemiological studies play an important role in identifying risk factors for diseases in animal husbandry, as diseases usually occur when biosecurity standards are low or lacking. This survey is a follow-up to a previous study, which focused on management factors like lighting in houses, litter condition and handling of broilers (Schulze Bernd et al., 2020). The present study aimed to monitor hygienic decisions, particularly manure and cleaning management and their relation to cellulitis ratios in broiler flocks. The study was performed with the purpose of identifying risk factors that help farmers to identify practices they can improve or avoid in the future.

MATERIALS AND METHODS

Flock Characteristics

As mentioned before, the survey was conducted in connection with a previously published study (Schulze Bernd et al., 2020). In brief, data were collected from 199 broiler houses on 100 conventional broiler farms in northern Germany with 2,430 to 62,000 animals per house. The study was carried out between April and November 2018. All farmers delivered their animals to the same processing plant and participated in the study on a voluntary basis. The houses had an average stocking density of 35.6 ± 2.2 kg/m^2 (ca. 1.2 birds per square feet).

Hygienic Factors

Data on biosecurity practices of the participating farms were collected by one investigator via a standardized questionnaire (see Supplementary Data) while visiting the farms. As the farms were regularly revisited for stock monitoring, an adequate assessment of the biosecurity standards was given throughout the recorded grown out phase. All data on the flock currently present in the house were recorded (see descriptive Tables 1−3).

Data from the Processing Plant

For each flock, data on the total condemnation and condemnation due to cellulitis were sent voluntarily to the investigator by the

Table 1. Dichotomous variables and frequency of occurrence on the farms included.

Dichotomous variables	Characteristic	Number of houses [n = 199]	Relative frequency [%]
Q1: Duration of downtime	Less than 8 d	100	50.51
	More than 7 d	98	49.49
	No answer	1	0.50
Q2: Cleaning management	Farm staff	22	12.36
	Commissioned company	176	88.88
	No answer	1	0.50
Q5: Regular change of disinfectant	Yes	188	94.47
	No	8	4.10
	No answer	3	1.51
Q5: Change of disinfectant	Per cleaning schedule	148	79.14
	Depending on events	39	20.86
	No answer	12	6.03
Q8: Carcass vehicle on farm area	Yes	70	35.18
	No	129	64.82
Q9: Manure storage	Yes	62	31.16
	No	137	68.84
Q10: Manure processing in biogas plant	Yes	163	81.91
	No	36	18.09
Q10: Biogas plant (n = 163)	On the farm	41	25.15
	External	122	74.85
Q11: Manure spreading on fields	Yes	56	28.14
	No	143	71.86
Q12: Cleanliness score in entry	Clean	95	48.97
	Dirty	99	51.03
	Not scored	5	0.50

Table 2. Categorical variables and frequency of occurrence on the farms participating in the study.

Categorical variables	Characteristic	Number of houses (n =199)	Relative frequency (%)
Q2: Cleaning company (n = 176)	1	8	4.54
	2	73	41.48
	3	22	12.5
	4	20	11.36
	5	2	1.14
	6	8	4.55
	7	3	1.7
	8	1	0.57
	9	3	1.7
	10	3	1.7
	11	4	2.27
	12	3	1.7
	13	4	2.27
	14	2	1.14
	15	2	1.14
	16	3	1.7
	17	2	1.14
	18	4	2.27
	No answer	9	5.11
Q3: Cleaning technique	High pressure cleaner	163	81.91
	Foaming technique	139	69.85
	Cold water	157	78.89
	Warm water	22	11.06
	Spray cooling	4	2.01
Q4: Disinfectant	Formaldehyde	131	65.83
	Peracetic acid	29	14.57
	Chlorine	79	39.7
	Iodine	2	1.01
	Phenol and derivatives	7	3.52
Q6: Protective clothing	None	20	10.05
	Only for farm staff	100	50.25
	For farm staff and visitors	79	39.7
Q7: Management of carcass bin	No cleaning and disinfection	157	78.9
	Only cleaning	26	13.07
	Cleaning and disinfection	16	8.04
Q12: Barrier in the hygiene lock	No	75	38.66
	Yes, visible	42	21.65
	Yes, physical	77	39.69
	No answer	5	2.51
Thinning procedure	No	24	12.06
	Yes	175	87.94

Table 3. Quantitative variables on manure storage and distribution.

Quantitative variables*	Average	± Standard deviation	Median	Minimum	Maximum
Q9: Distance of manure storage (m)	673.23	992.07	200	20	3,000
Q11: Distance of manure distribution (m)	18,566	32,776.63	5,000	20	120,000
Cellulitis rate (=cellulitis condemnation/total condemnation x 100) [%]	36.77	19.69	26.12	1.04	84.07
Total condemnation (=rejected birds/slaughtered birds x 100) [%]	1.40	0.97	1.13	0.21	8.41
Cellulitis ratio (=cellulitis rejected birds/slaughtered birds x 100) [%]	0.52	0.62	0.26	0.00	4.85

*Total study population 199 houses.

processing plant where 5,332,767 broiler chickens of the 199 studied flocks had been slaughtered. The cellulitis ratio [= condemnation due to cellulitis/total condemnation slaughtered birds x 100 (%)] and the total condemnation ratio were calculated for each flock. To detect cellulitis during meat inspection, the trained processing plant staff may notice a typical yellowish discolored skin in the cloacal area, which leads to condemnation of the entire carcass.

Statistical Analyses

Statistical analysis was used to investigate which hygiene-related variables were significantly associated with the condemnation due to cellulitis and total condemnation at time of slaughter to explore risk factors for this disease. The statistical evaluation was carried out using SAS software, version 9.4m5, with the SAS Enterprise Guide Client 7.15 (SAS Institute Inc., Cary, NC). P-values of < 0.05 indicated statistically significant results.

Generalized linear mixed models for count data with negative binomial distribution and log-link were used (GLIMMIX procedure with the Kenward-Roger degree of freedom method and Tukey-Kramer multiple comparison procedure) to assess the influence of categorical variables (period length of downtime, use of cleaning technique, disinfectant agent, protective clothing, vehicles driving near the farm area, performance of manure storage or spreading on fields, biogas plants, cleanliness score, cleaning management, cleaning management of carcass bin, visualization of biosecurity border). These variables were modeled as fixed main effects. The model included a fixed dichotomous nuisance factor to capture the effect of thinning, as the mean condemnation ratios are usually lower in the thinning procedure than in final depopulation (Buzdugan et al., 2020), and the logarithm of the number of slaughtered birds with fixed coefficient of 1 to adjust for flock sizes (using the offset option of GLIMMIX). No interactions were included in the final model, because of an insufficient number of observations for some level combinations and nonsignificant interactions for others. Houses nested within farms and the farms were considered as random effects. The negative binomial distribution was used to adjust for possible overdispersion in said count models and to model the small proportion of condemned carcasses, especially those due to cellulitis.

To indicate the relationship with continuous factors (distance of manure storing and processing), for each variable, a negative binomial mixed model was fitted, including a random effect for the farm and, as above, the logarithm of the number of slaughtered birds and a fixed nuisance factor for the effect of thinning. Additional variables had to be omitted due to a smaller sample size, since distance of manure storing and processing were only assessed for a subset of the farms.

RESULTS AND DISCUSSION

To adjust for the number of slaughtered birds, which may vary among flocks, estimates for the total condemnation ratio (%) and the cellulitis ratio (%) are provided in this section. As reported in our previous study, the observed overall condemnation ratio was 1.4%, while condemnation due to cellulitis was 0.52%, representing 36.77% of all condemnations (Schulze Bernd et al., 2020). In the present study, the focus was now on hygienic parameters and the results were presented separately by topics. The analysis revealed some statistically relevant risk factors for cellulitis, which are summarized in Table 4.

Cleaning and Disinfection Management

Some farmers cleaned and disinfected their houses themselves (n = 22), while 144 houses were cleaned and disinfected by a commissioned cleaning company. A total of 32 houses practiced a split model, where a specialized company did the cleaning while the farmer performed the disinfection. One farmer did not answer that question. There were 18 different cleaning companies working on the farms. Houses that were cleaned by a company had the lowest mean total condemnation ratio (0.56%, $P = 0.199$). However, these findings were not statistically significant.

Table 4. Influencing categorical factors and their effect on cellulitis and total condemnation ratios.

Factor	Variable	Mean cellulitis ratio (%)	*P*	Mean total condemnation ratio (%)		*P*
Slaughter procedure	Thinning	0.22	<0.0001	0.58	<0.0001	
	Final depopulation	0.38		0.77		
Q1: Downtime	≤7 d	0.30	0.638	0.62	0.244	
	> 7 d	0.27		0.72		
Q2: Cleaning and disinfection management	By a company	0.27	0.563	0.56	0.199	
	By a company and the farm staff	0.39		0.69		
	By the farm staff only	0.22		0.76		
Q3: Cleaning procedure	High pressure cleaner	No	0.24	0.360	0.66	0.889
		Yes	0.34		0.68	
	Foaming technique	No	0.25	0.424	0.60	0.232
		Yes	0.33		074	
	Cold water	No	0.39	0.137	0.83	0.044
		Yes	0.21		0.54	
	Warm water	No	0.31	0.763	0.92	0.048
		Yes	0.26		0.48	
Q4: Disinfectant agent	Acid	No	0.33	0.336	0.68	0.817
		Yes	0.25		0.66	
	Formaldehyde	No	0.29	0.963	0.69	0.569
		Yes	0.29		0.64	
	Chlorine	No	0.30	0.782	0.64	0.620
		Yes	0.28		0.69	
	Iodine	No	0.22	0.636	0.88	0.372
		Yes	0.38		0.50	
	Phenol and derivatives	No	0.21	0.284	0.67	0.959
		Yes	0.39		0.66	
Q5: Regular change of disinfection agent	Per cleaning schedule		0.37	0.304	0.77	0.024
	Depending on events		0.22		0.47	
	No		0.30		0.82	
Q7: Mortality bin	No cleaning and disinfection	0.36	0.388	0.68	0.761	
	Cleaning	0.23		0.61		
	Cleaning and disinfection	0.29		0.72		
Q8: Mortality vehicle	On farm area	0.23	0.102	0.70	0.538	
	Outside farm areas	0.35		0.64		
Q9: Manure storage	Yes	0.34	0.295	0.66	0.808	
	No	0.25		0.68		
Q10: Biogas plant	Yes, on the farm	0.28	0.233	0.70	0.200	
	Yes, external	0.40		0.78		
	No	0.21		0.54		
Q11: Manure Spreading	Yes	0.27		0.692	0.63	0.544
	No	0.31			0.70	
Q6: Protective clothing	No	0.38	0.548	0.60	0.593	
	Only for farm staff	0.27		0.74		
	For farm staff and visitors	0.24		0.67		
Q12: Hygiene lock	Not visualized	0.22	0.159	0.63	0.747	
	Optical barrier	0.39		0.71		
	Physical barrier	0.28		0.67		
Q12: Cleanliness score	Score 1	0.21	0.019	0.60	0.087	
	Score 2	0.39		0.75		

Furthermore, disinfectant agents used seemed to have no influence on cellulitis ratios in broiler flocks. A soaking step during cleaning had previously been associated with a lower risk of condemnation (Lupo et al., 2009). However, the result could not be confirmed in the present study as all observed German broiler houses already had a soaking step implemented in the cleaning procedure.

The disinfectant was changed on a regular basis in 148 houses, whereas only 8 houses were always treated with the same disinfectant. In 39 houses, the farmers indicated that they had changed the disinfectant when they had to react to health problems in broilers in the previous flock, such as coccidiosis or a high condemnation ratio. Regarding the disinfection management, there was no effect on the cellulitis ratio but the total condemnation ratio seemed to be influenced depending on whether the disinfectant was changed regularly or not ($P = 0.024$). Total condemnation ratio was lowest (0.47%) when disinfection agents were changed due to a problem in the previous flocks. From this, one could conclude that it is advantageous to review cleaning and disinfection procedures to react to existing disease processes in order to control them.

Hygiene Management

All houses had a farm hygiene lock. While 119 houses (representing 59.8% of all houses) had a clear marking for separation between dirty and clean areas, 75 houses had no clear marking for separation of dirty and clean areas. Regarding those houses with clear markings, 77 of them had a physical border (e.g., bench), while 42 had a visual separation. There were differences in the cellulitis ratios when comparing houses with (optical barrier: 0.39% and physical barrier: 0.28%) and without clear markings of separations (0.22%). Nonetheless, these observations were not statistically significant ($P = 0.159$). Other studies compared houses with and without hygiene locks, finding locks to be beneficial in regard to reducing the spread of bacteria (Van Limbergen et al., 2018; Hald et al., 2000). Since all houses in this study had predefined clean and dirty areas, their visual or physical appearance might not make any additional difference. Furthermore, an optical barrier can also be easily ignored in daily routine because of the process of habituation. This indicated that the implementation and awareness of proper hygiene are much more important than a special marking of the hygiene lock.

Cleanliness was evaluated by the investigator applying a score of “1” or “2”. Houses given score “1” (n = 95) had clean entry floors, farmers changing shoes when entering the service room and again when entering the broiler house, and changing into farm specific clothing, while houses rated with score “2” (n = 99) had litter carryover from broiler houses to entry and changing of shoes was not performed as strictly. Five houses were not scored concerning their cleanliness, as at the time of data collection, there was no standard assessment of farm cleanliness possible (e.g., downtime period at the time of farm visit). Houses rated with score “1” had lower statistically significant results regarding cellulitis ratios at the end of grow out ($P = 0.019$). Clean houses (score 1) had an average cellulitis ratio of 0.21%, while score “2” houses had an average cellulitis ratio of 0.39%. In addition to that, the total condemnation ratios were 0.15% higher when houses scored “2” ($P = 0.087$). These results were in line with results from previous studies, showing that physical barriers and house specific footwear help to reduce the spread of infectious agents such as *Campylobacter* (Smith et al., 2016). Therefore, it can be assumed that changing shoes and clothing before entering the broiler house are measures to avoid the carryover of bacteria such as *E. coli.*

Mortality Management

Most farmers did not clean their carcass bins after each emptying (n = 157 houses), while 26 cleaned them after each emptying and 16 farmers cleaned and disinfected them. There was no significant correlation found ($P = 0.388$) between the cleaning management of mortality bins and the cellulitis ratio. Additionally, a relationship between the total condemnation ratio and management of the mortality bins could not be seen.

The mortality vehicle did not need to drive on to farm areas as the mortality bins were placed outside them in 129 cases; in 70 cases it had to do so. Having the mortality bins outside the enclosed area was not associated with statistically significant differences in cellulitis ratios (P = 0.102), nor in total condemnation ratios (P = 0.538). As the total condemnation ratios are associated with a variety of infectious diseases as well as noninfectious diseases, these factors are not necessarily the same factors that play a role in cellulitis. Unfortunately, we did not include other reasons for condemnation in this study and therefore could not fully investigate this assumption. However, it is clear that dead animals are a source of various pathogens (Van Limbergen et al., 2018). For example, McQuiston et al. (2005) identified the disposal of dead birds as a major risk factor for the spread of avian influenza. In addition, Carr and Howells (2018) previously mentioned that minimizing the number of vehicles entering the farm is a key biosecurity factor.

Manure Management

Escherichia coli occurs in chicken litter with a prevalence of up to 100% (Chen and Jiang, 2014). The mean concentration of *E. coli* found in single-use litter was 4.2·x 10^5 CFU/g (1 x·10^2 to 1.2·x 10^7) (Chinivasagam et al., 2010).

The manure of 62 broiler houses in this study was stored near the farm area (average distance of 673.23 m ± 992.07 m), while farmers of 137 houses did not store their manure on the farm. Those who stored manure near their houses for a certain period of time had no statistically significantly higher cellulitis ratios (0.34%) than those who removed the manure immediately, for example, by selling it (0.25%, P = 0.295). The distance between houses and manure storage made no additional difference. Manure spreading was performed in 56 cases. The other 143 houses did not spread manure on nearby fields. Manure spreading was not significantly connected to the cellulitis ratio. We could not find a significant relationship between the distance of spreading to the broiler house and the cellulitis ratio, nor to the total condemnation ratio ($P > 0.05$). Farmers who did not spread their own manure either sold it or gave it to their biogas plants. When manure is sold, there might be the possibility of introducing pathogens via foreign vehicles transporting the manure (Lewerin et al., 2015). The storage of manure near farms might pose a similar risk like the introduction of pathogens via foreign vehicles, making significant findings of risk assessment by manure management difficult. Nonetheless, as manure has already been identified as potentially contaminated with a lot of different types of pathogens, its management, such as the storage of manure on farms, is considered to be a risk factor for the biosafety and health of broilers (Evans and Sayers, 2000; Lister, 2008).

It was shown that 41 houses had their own biogas plant near the animal area, while 36 houses had no biogas plant at all. The mesophilic degradation applied in such a plant is not able to completely eliminate *E. coli* in litter (Larsen et al., 1994; Horan et al., 2004; Fröschle et al., 2015). Moletta-Denat et al. (2010) found that some pathogenic bacteria are randomly floated out by the biogas, while others aerosolize more easily in biogas because they normally use air as a spreading vector. Several studies measured high levels of aerobic and pathogenic microorganisms in the vicinity of biogas plants (Moletta-Denat et al., 2010; Moletta et al., 2007; Bayle et al., 2016). However, an influence of biogas plants on cellulitis ratios was not observed in the present study.

CONCLUSIONS AND APPLICATIONS

1. Hygienic decisions can influence the occurrence of bacterial infections like cellulitis.
2. Usage of farm-specific clothing and shoes led to lower cellulitis ratios in broiler flocks.
3. Farms with entry that were considered clean had lower cellulitis ratios in broiler flocks.
4. The total condemnation rate decreased with a change of disinfectant which had taken place after previous health problems.

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Institutional Review Board Statement: All of the animals were housed in accordance with EU (European Directive 2007/43/EC) and national law (Animal Welfare Act, Animal Welfare and Farm Animal Husbandry Ordinance (Tierschutzgesetz, Tierschutz-Nutztierhaltungsverordnung)). In compliance with European Directive 2010/63/EC Article 1 5. (f), the present study did not imply any invasive procedure or treatment to the animals. The authors declare that the study was in accordance with current German law. This study was reviewed and received approval from the Animal Welfare Officer of the University of Veterinary Medicine Hannover, Foundation, Germany (ID: TVO-2018-V-104).

Data Availability Statement: Concerning the data availability, we would like to mention that our data are available upon request, but only in exceptional cases. The data were collected on an individual basis from farmers. Each participant gave written consent with the understanding that data would not be transferred to any third party. Therefore, any data transfer to interested persons is not allowed without an additional formal contract. Data are available to qualified researchers who sign a contract with the authors. This contract will include guarantees to the obligation to maintain data confidentiality in accordance with the provisions of the German Data Protection Law.

DISCLOSURES

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.japr.2021.100230.

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