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ORIGINAL ARTICLE

Infection dynamics of *Salmonella* Infantis vary considerably between chicken lines

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ABSTRACT

Salmonella (*S.*) Infantis is the most common serovar in broilers and broiler meat in the European Union. In the field, fast-growing broilers are reported to be more affected than slow-growing and layer birds. The present study investigated the infection dynamics and immunological response of four chicken lines in the course of a *S.* Infantis infection. Two commercial chicken lines, Ross 308 and Hubbard ISA-JA-757, and two experimental chicken lines, specific pathogen free (SPF) layers and broilers, were infected at 2 days of age. Investigations focused on faecal shedding, bacterial colonization, humoral and cellular immune response. Ross and SPF broilers proved mainly as high shedders followed by Hubbard. SPF layers showed the least shedding. This is in agreement with the caecal colonization; SPF layers harboured significantly less bacteria. Systemic spread of *S.* Infantis to liver and spleen was highest in Ross broilers compared to the other lines. Spread of infection to in-contact birds, was noticed 5 days post infection in every line. Antibody response occurred in every chicken line from 21 days of age onwards. In contrast to the other chicken lines, significant differences in T cell subsets and monocytes/macrophages were found between infected and negative Hubbard birds at 7 days of age. Uninfected SPF birds had significantly higher immune cell counts than uninfected commercial birds, a fact important for future experimental settings. The results illustrate that the infection dynamics of *S.* Infantis is influenced by the chicken line resulting in a higher risk of transmission to humans from fast-growing broilers.

RESEARCH HIGHLIGHTS

- Infection dynamics of *Salmonella* Infantis differs between chicken lines.
- Layers showed less faecal shedding and caecal colonization compared to broilers.
- Fast-growing broilers proved more susceptible than slow-growing broilers.

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Salmonella Infantis; chicken lines; shedding; colonization; immune response; pESI; plasmid

Introduction

Poultry are the focus for transmitting *Salmonella (S.) enterica* subspecies *enterica* via the food chain to humans causing human salmonellosis. In the European Union, 70% of all serotyped *Salmonella* isolates reported from food and animal sources originated from broilers (EFSA, 2021). In this regard, *S.* Infantis has experienced an emerging relevance in the last decade accounting for 29.7% of human infections from food-animal sources, followed by *S.* Enteritidis (6.9%), monophasic variant of *S.* Typhimurium (4.5%), *S.* Typhimurium (3.9%) and *S.* Derby (3.7%) (EFSA, 2021). Currently, 36.3% and 49.1% of all serotyped isolates from broilers and broiler meat, respectively, were found to be *S.* Infantis. Previous studies revealed that this serovar comprises a heterogeneous pheno- and genotypic population of isolates (Nógrády *et al.*, 2012; Sakano *et al.*, 2013; Montoro-Dasi *et al.*, 2021; Tyson *et al.*, 2021). The occurrence of such variants characterized by different susceptibilities to standard cleaning and disinfection procedures is of high relevance for persistence of the bacteria on the farm (Drauch *et al.*, 2020). The increasing prevalence of multi-resistant *S.* Infantis isolates is a serious concern in the public health area (Alba *et al.*, 2020, García-Soto *et al.*, 2020). In this regard, it was revealed that the presence of the pESI-like plasmid is not only involved in transmitting antimicrobial resistance, but also carries several virulence factors (Aviv *et al.*, 2014, García-Soto *et al.*, 2020). Previous studies showed that *S.* Infantis isolates harbouring this plasmid have an increased virulence characterized by higher shedding, higher colonization rates of internal organs, and stronger humoral immune response (Aviv *et al.*, 2017, Drauch *et al.*, 2021).

Interestingly, from the field a higher prevalence of *S.* Infantis was reported in fast-growing broilers compared to slow-growing lines, keeping in mind that the

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latter are a minority which might influence the records. Furthermore, less prevalence of this serovar is reported from layer flocks (10.2%) (EFSA, 2021). This is in agreement with the outcome of an experimental study in specific pathogen-free (SPF) layer birds demonstrating that *S.* Infantis has hardly any invasion capabilities compared to *S.* Enteritidis, *S.* Typhimurium and *S.* Hadar (Berndt *et al.*, 2007). Therefore, similar to what has been shown for other Salmonella, the chicken line itself might influence the susceptibility of birds to *S.* Infantis which might also be reflected in differences in the immune response (Qureshi *et al.*, 1986; Bumstead & Barrow, 1993; Wigley *et al.*, 2002, 2006).

This animal trial was set up to investigate two commercial broiler lines used in the field with available SPF layers and broilers. Elaboration of the infection dynamics included shedding behaviour, and the ability to colonize caeca, liver and spleen. In order to investigate the innate or adaptive immune response the frequency of T cells ($CD4^+$, $CD8\alpha^+$ and $TCR\delta\gamma^+$), monocytes/macrophages and B cells in peripheral blood was assessed by flow cytometry. An ELISA based upon *S.* Infantis bacteria was used to determine homologous humoral antibodies.

Material and methods

Birds and housing

The animal trial was approved by the institutional ethics committee and licensed by the national authority according to the Austrian law for animal experiments (license number GZ.: 68.205/0157-V/3b/2019).

Four different chicken breeds were used in the study to compare the infection dynamics of *S.* Infantis: two SPF breeds (SPF layers (Valo BioMedia GmbH, Osterholz-Scharmbeck, Germany) and SPF broilers (Animal Health Service, Deventer, The Netherlands)) and two commercial broiler breeds (fast-growing breed Ross 308 and slow-growing breed Hubbard ISA-JA-757 (Brueterei Schulz, Lassnitzhoehe, Austria)). SPF layers and SPF broilers were hatched at the Clinic. Ross 308 and Hubbard ISA-JA-757 birds were obtained from a commercial hatchery as 1-day-old chicks. Forty chicks per line were randomly divided into experimentally infected groups each comprising 25 birds and negative control groups each containing 15 birds (Table 1). Each group was housed separately in an isolator (Montair HM2500, Montair Environmental Solutions B.V., Kronenberg, The Netherlands) with water and feed supply *ad libitum*. For identification chicks were individually marked with a tag starting subcutaneously in the neck.

Bacterial strain

For infection the well-defined *S.* Infantis strain MRS-16/01939 was used which was already described in a previous study (Drauch *et al.*, 2021). Briefly, the strain harbours the plasmid of Emerging *Salmonella enterica* Infantis (pESI plasmid) carrying the following genes *irp2*, *ipf*, *klf*, *ccdB/ccdA* and two plasmid-encoded fimbrial operons, *pef* and *sta*. It was submitted to the NCBI database under the accession number SAMN19328299. The strain was grown overnight at 37°C in Luria-Bertani-Broth (LB, Invitrogen, Vienna, Austria) in a shaking incubator (250 rpm). Colony-forming-unit (CFU) count was performed by plating serial dilutions (1:10) on MacConkey agar in duplicates. The mean value was calculated. The infection dose was adjusted to a concentration of 10^8 CFU/ml.

Experimental design and sampling

In the infection groups 15 out of 25 birds were infected orally with 1 ml each of the *S.* Infantis suspension at 2 days of age via a crop tube. One ml phosphate buffered saline (PBS, GIBCO, Paisley, UK) was administered to the remaining ten birds, of each infection group serving as in-contact birds and to negative control birds.

The clinical status of the birds and technical parameters of the isolators were determined daily. Blood samples (*V. basilica*) and cloacal swabs in duplicate (Copan, Stoelzle-Oberglas GmbH, Vienna, Austria) were taken once before infection from five birds and weekly after infection from every bird. Five

Table 1. Design of the experiment.

Breed	Group	Number of birds per group	Infection status	Number of birds	Number of birds taken for necropsy per killing day
SPF layers	Infected	25	Orally infected	15	3
			In-contact bird	10	2
	Negative	15	Negative	15	3
SPF broilers	Infected	25	Orally infected	15	3
			In-contact bird	10	2
	Negative	15	Negative	15	3
Ross 308	Infected	25	Orally infected	15	3
			In-contact bird	10	2
	Negative	15	Negative	15	3
Hubbard ISA-JA-757	Infected	25	Orally infected	15	3
			In-contact bird	10	2
	Negative	15	Negative	15	3

birds from the infected group (three directly infected and two in-contact birds) and three birds from the negative control group were euthanized weekly with the first killing day 5 days post-infection (7 days of age) until 35 days of age. This was done by injecting a combination (1:1) of Sedaxylan® (20 mg/ml, Dechra Pharmaceuticals, Dornbirn, Austria) and Narketan® (100 mg/ml, Vetoquinol, Vienna, Austria) into the breast muscle followed by bleeding (*V. jugularis*). Necropsy was performed according to a standard protocol, body weight as well as the weights of the liver and spleen were recorded for each bird. Samples of caecum, liver and spleen were taken for bacteriological examination, and blood was collected (*V. jugularis*) for flow cytometry analyses.

Bacteriology

Shedding of *S.* Infantis was analysed by investigating the cloacal swabs taken in duplicate. For this, one cloacal swab was streaked on xylose-lysine-deoxycholate agar (XLD, Merck, Vienna, Austria) and incubated at 37°C for 24 h, and the other swab was kept at 4°C and (in case of a negative result by direct plating) used for an enrichment procedure according to EN ISO 6579-1:2017. In case of a positive result by direct plating, the excretion of *S.* Infantis was defined as "high shedding". *S.* Infantis-positive samples based on the enrichment were referred to as "low shedding". If a swab was negative by direct plating and by enrichment procedure, the sample was evaluated as "negative".

Quantification of bacterial load in caecum (tissue and content), liver and spleen was determined by homogenizing 1 g of the organ in PBS (vol. 1:1) using the T 10 basic ULTRA TURRAX (IKA, Staufen, Germany). From the homogenates serial dilutions in PBS (1:10) were prepared. These dilutions were plated in duplicate on XLD, incubated aerobically at 37°C for 24 h. Afterwards, the colonies were counted and the mean value determined. Finally, the quantity of bacteria was calculated as CFU/g per organ. In parallel, homogenates were kept at 4°C until the results of direct plating were available. If direct plating revealed a negative result, homogenates were investigated by enrichment according EN ISO 6579-1:2017 in the same way as the cloacal swabs.

Serology

Weekly blood samples were centrifuged, serum was collected and frozen at −20°C. Samples were gathered and analysed all together at the end of the infection trial. The indirect ELISA was prepared according to the procedure used in a former trial and explained by Drauch *et al.* (2021).

Briefly, 96-well plates (Nunc Medisorb; Thermo Scientific, Roskilde, Denmark) were coated with 100 µl of *S.* Infantis suspension per well with a concentration of 10^9 CFU per ml and incubated overnight at 4°C. On the following day the plates were incubated at 52°C till the liquid was dried completely and 200 µl of blocking buffer (StartingBlock™ PBS Blocking Buffer, Thermo Fisher Scientific, Vienna, Austria) was added for 1 h. Wells were then washed with PBS (containing 0.1% Tween 20) and serum was added in a dilution of 1:200, all performed in duplicate. After incubation for 1 h at room temperature, plates were washed and treated with 100 µl of Goat-Anti Chicken IgY (H + L)-HRP (Southern Biotechnology, Birmingham, AL, USA) in a dilution of 1:5000 and again incubated at room temperature for 1 h. After a final washing step, 100 µl of tetramethylbenzidine substrate (Calbiochem, Darmstadt, Germany) was added to each well and plates were incubated in the dark. After 12 min wells were treated with 100 µl of 0.5M H_2SO_4 to stop the colour reaction and the optical density (OD) was determined at a wavelength of 450 nm with an ELISA reader (Sunrise-Basic; Tecan, Groedig, Austria). Individual results are given as mean of the performed duplicates.

Flow cytometry analyses

For the separation of peripheral blood mononuclear cells (PBMCs), the protocol described by Mitra *et al.* (2017) was applied with some modifications. Antibodies and antibody combinations used to determine immune cell populations are presented in Table 2. Precisely, blood from three birds per group (directly infected and negative control) per breed was investigated. The amount of blood taken increased with the age of the birds starting with 1 ml for 1-week-old birds, and reaching 6.5 ml for 5-week-old birds.

In a first step, the blood was mixed with 100 µl heparin (Serva, Heidelberg, Germany) per ml to prevent coagulation. PBS with 2% foetal bovine serum (FBS) (ThermoFisher Scientific, Vienna, Austria) was added to the blood in an equal amount. To enable density gradient centrifugation this mixture was overlaid twice the volume with Histopaque®-1077 (Sigma-Aldrich, Vienna, Austria) and centrifuged at 400×*g* for 20 min at room temperature without brake. Cells were then collected from the interface and washed three times with PBS + 2% FBS by centrifugation at 350×*g* for 5 min at 4°C. Before starting the immunophenotyping, the viability of cells was verified via Cellometer® X2 fluorescent viability cell counter system (Nexcelom Bioscience, Manchester, UK) and adjusted to a concentration of 2×10^7 cells/ml.

Staining of cells was performed by adding 25 µl of the adjusted cell mixture together with the primary antibodies into 96-well microtitre plates (Sarstedt, Nümbrecht, Germany) before incubation at 4°C for 20 min. Additionally, as a technical control, samples

Table 2. Table of antibodies and antibody combinations used to determine immune cell populations of chickens by flow cytometry.

Antibody panel	Antigen	Clone	Isotype	Fluoro-chrome	Labeling strategy	Source of primary mAB
a	VS780	N/A[a]	N/A[a]	APC-Cy7	Direct conjugation	BD Biosciences
	CD45	LT40	IgM	APC	Direct conjugation	Southern-Biotech
	CD4	CT4	IgG1	PE-cy7	Direct conjugation	Southern-Biotech
	CD8α	3-298	IgG2a	PE	Direct conjugation	Southern-Biotech
	TCRγδ	TCR1	IgG1	BIOTIN-BV421	Biotin-Streptavidin[b]	Southern-Biotech
	Bu1	AV20	IgG1	PacBlu	Direct conjugation	Southern-Biotech
	CD3	CT3	IgG1	FITC	Direct conjugation	Southern-Biotech
b	VS780	N/A[a]	N/A[a]	APC-Cy7	Direct conjugation	BD Biosciences
	CD45	LT40	IgM	APC	Direct conjugation	Southern-Biotech
	Kul01	Kul01	IgG1	PE	Direct conjugation	Southern-Biotech
	CD3	CT3	IgG1	FITC	Direct conjugation	Southern-Biotech

[a]not applicable.
[b]Brilliant Violet 421™ Streptavidin, BioLegend.

without staining and isotype controls were included for every group. Following incubation, the cells were washed twice with PBS + 2% FBS by centrifugation at 450×*g* for 4 min at 4°C. The secondary antibody BV421 was added to cells of the antibody panel "a" and 2.5 ul of Lide/Dead Dye (BD Horizon™ Fixable Viability Stain 780) were added to cells of both antibody penels. Subsequently, the plate was pulse-shaken and incubated for 30 min at 4°C. Afterwards, the cells were washed again by centrifugation before the pellets were re-suspended in 100 µl of PBS + 2% FBS for further analysis. From each bird, at least 20,000 leukocytes were recorded with a FACSCanto II (BD Biosciences, San Jose, CA) flow cytometer using three lasers (405, 488 and 633 nm). After doublet discrimination, live cells were detected before gating the cell population for leukocytes ($CD45^+$). Two different $CD45^+$ gating strategies were applied to target lymphocytes and monocytes/macrophages. From leukocytes, subsequent cell populations targeting $CD4^+$ T cells, $CD8\alpha^+$ T cells, $TCR\delta\gamma^+$ T cells, B cells (Bu1) and monocytes/macrophages (Kul01) were gated (Additional File 1). The data were analysed by FACSDiva Software version 9.0 (BD Biosciences). The absolute cell count for each cell population was calculated according to Mitra *et al.* (2017) before proceeding to statistical analysis.

Statistical analysis

Statistical analysis was performed in R Core Team by using three packages (nortest, ggplot2 and ggpubr). After exploratory data analysis, normality of the results was assessed by the Anderson–Darling test. In the case of non-normally distributed data, logarithmic transformation was used. Linear models were implemented to test for significant differences between bodyweight, weight of liver and spleen, CFU results, OD values and flow cytometry results of directly infected, in-contact and negative control birds. Shedding profiles were analysed with a generalized linear model with a specification for a binominal distribution of data.

Results

Clinical signs and necropsy

No clinical signs were observed in any of the groups throughout the duration of the trial. Neither the body weights, nor the weights of liver and spleen revealed any significant differences between infected and negative control groups within each breed. No gross pathological lesions were found during necropsy. One SPF layer bird was found dead in the night before the second sampling day (14 days of age) due to cannibalism. The bird was necropsied and sampled according to the described scheme. As the CFU results of this bird were in agreement with the results of the birds killed at the sampling day these data were included into the analysis. But, serological analysis was not feasible. Therefore, ELISA data for SPF layers on 14 days of age comprise 19 instead of 20 samples.

Bacteriology

Samples taken before the infection as well as from birds of the negative control groups were negative for *S.* Infantis throughout the trial until termination.

Cloacal swabs revealed shedding of *S.* Infantis in all breeds from 7 days of age onwards (Figure 1). A rapid spread of *S.* Infantis was detected as in-contact birds were positive for shedding from 7 days of age onwards with no significant difference between directly infected and in-contact birds in regards of the shedding profile (Figure 2).

SPF layers (directly infected and in-contact birds) mainly appeared as high shedders (in total 62 from 75 samples, 82.7%) and seldom as low-shedders (in total six samples, 8%). At three sampling time-points (days 7, 28 and 35) some birds were not shedding *S.* Infantis (in total seven samples, 9.3%).

At each sampling point 100% of the SPF broilers and the Ross 308 birds, independent of whether they were directly infected or in-contact birds, were shedding *S.* Infantis. The group of SPF broilers contained only high-shedding birds, whereas Ross 308 birds also showed low shedding at the first three sampling

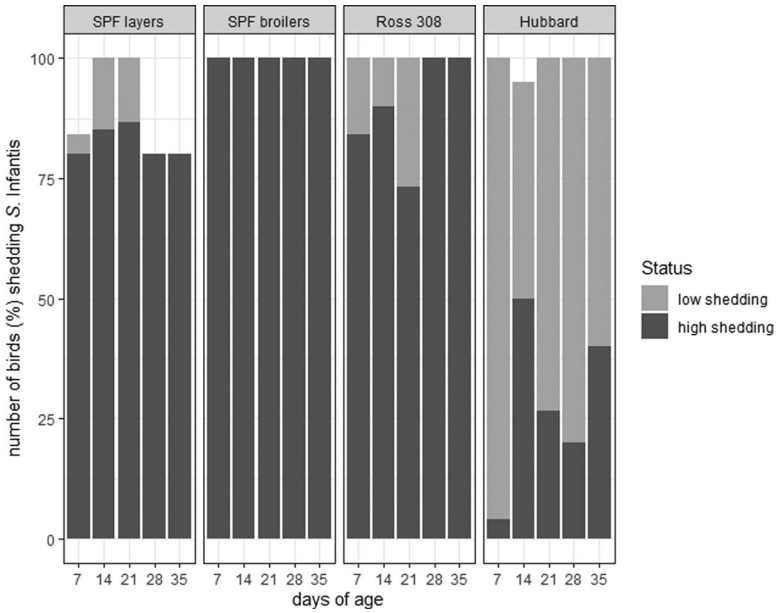

Figure 1. Shedding of *S*. Infantis in four different chicken breeds (directly infected and in-contact birds). Shedding profile of Hubbard birds was significantly different ($P < 0.05$) from the three other breeds.

time-points (days 7, 14 and 21; in total 10 out of 75 samples, 13.3%).

All Hubbard birds (directly infected and in-contact) shed *S*. Infantis during the sampling point with the exception of one bird (directly infected) on day 14 that did not shed at all. Hubbard birds showed mainly low shedding (in total 55 out of 75 samples, 73.3%) and less high shedding (in total 19 samples, 25.3%) being statistically significant compared to the three other breeds ($P < 0.05$).

The colonization of the caecum detected by direct plating was independent of infection (directly or in-contact) (Figure 3). The three broiler breeds gave similar results, except for 21 days of age at which SPF broilers harboured significantly less *S*. Infantis (Figure 4 (a)). The counts ranged from 1×10^8–1.2×10^{11} CFU/g. In comparison to the three broiler breeds, a lower colonization rate was found in SPF layers (range 2×10^6–6×10^{10} CFU/g) being significant at 7, 28 and 35 days of age (Figure 4(a)).

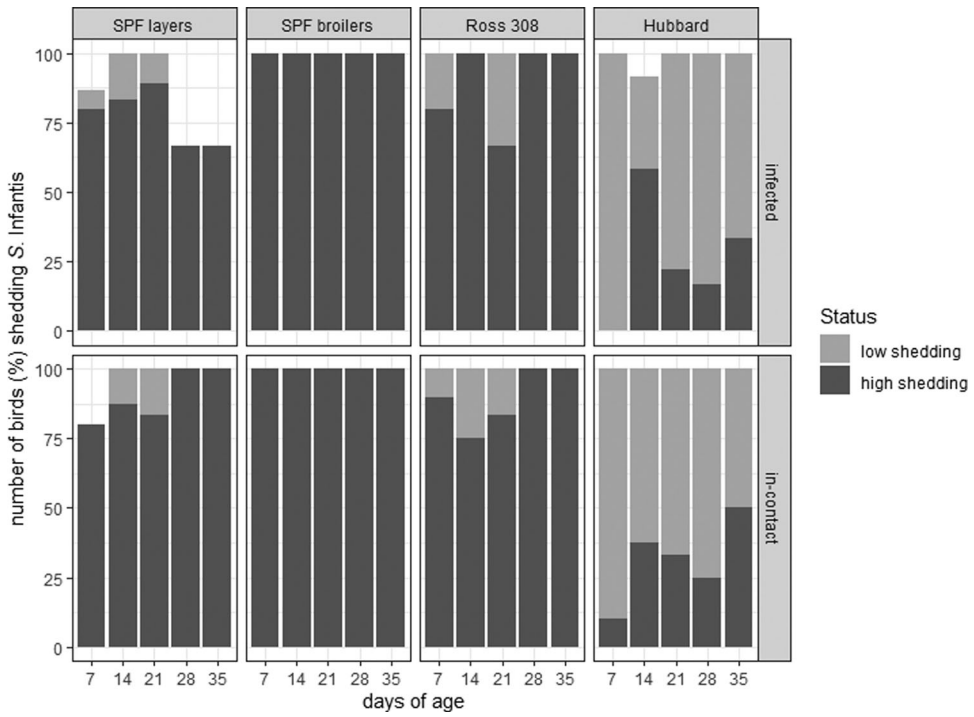

Figure 2. Shedding of *S*. Infantis in four different chicken lines separating directly infected ($n = 15$ birds per line) from in-contact birds ($n = 10$ bird per line).

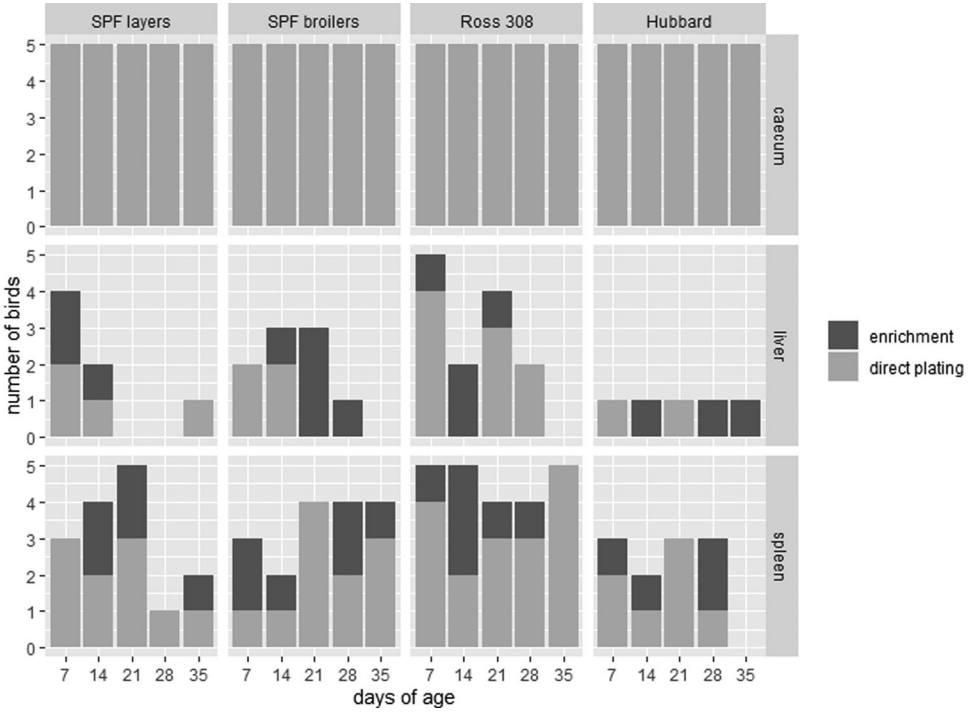

Figure 3. Colonization of *S.* Infantis in caecum, liver and spleen of birds from four different breeds detected by enrichment or direct plating.

In general, *S.* Infantis was re-isolated more often from liver samples from Ross 308 birds ($n = 13/25$), mainly by direct plating (Figure 3). Furthermore, at 7 days of age a significantly higher CFU count was found in Ross 308 compared to the three other breeds, namely 1–1.1 × 10^3 CFU/g in Ross 308, 1–12 CFU/g in SPF broilers, 1–2 × 10^2 CFU/g in SPF layers and 1–2 CFU/g in Hubbard birds (Figure 4(b)). In total, nine, seven and five liver samples were positive for *S.* Infantis from SPF broilers, SPF layers and Hubbard birds, respectively. Here, approximately equal re-isolation with direct plating and enrichment was observed. With the exception of Hubbard birds which had only a single bird positive at each sampling time-point, all other breeds showed a decrease in positive birds throughout sampling with broiler breeds being negative at 35 days of age (Figures 3 and 4(b)).

The *S.* Infantis colonization in spleens showed a similar tendency as obtained from livers albeit positive birds were noticed with higher frequency and already by direct plating (Figure 3). *S.* Infantis was re-isolated from all spleen samples derived from Ross 308 birds with CFU counts ranging from 1–1 × 10^4 CFU/g. The CFU was even significantly higher compared to the three other breeds at 7 days of age (Figure 4(c)). In total, *S.* Infantis was re-isolated from 17 spleen samples from SPF broilers, followed by SPF layers with 15 positive samples. The lowest colonization rate was again found in Hubbard birds resulting in 11 positve samples (Figure 3). The counts ranged from 1–9 CFU/g for SPF broilers, 1–3 × 10^2 CFU/g for SPF layers and 1–7 CFU/g for Hubbard birds (Figure 4(c)).

Serology

Independent of the breed, a significant ($P < 0.01$) increase of humoral antibodies in the infected birds (directly infected and in-contact) was observed from 21 days of age onwards compared to their negative controls with a stable increase until the end of the study (Figure 5). In-contact birds also presented a significant rise of antibodies from 21 days of age onwards compared to the negative controls, and therefore the figure represents directly infected and in-contact birds as one group. No inter-breed differences were found.

Flow cytometry analyses

No substantial differences were found in the absolute cell counts of different immune cell populations between directly infected and negative birds in SPF layers, SPF broilers and Ross 308 (data not shown). In Hubbard birds at 7 days of age significantly lower $CD45^+CD4^+CD8\alpha^-$, $CD45^+CD4^-CD8\alpha^+$ and $CD45^+CD8\alpha^+TCR\delta\gamma^+$ counts were found in the PBMCs of infected birds. At the same time, the amount of macrophages/monocytes ($CD45^+KUL01^+$) in infected Hubbard birds was significantly increased in the blood compared to the negative control (Figure 6). For $CD45^+CD8\alpha^-TCR\delta\gamma^+$ cells and B cells no significant differences were detected in Hubbard birds (data not shown).

Interestingly, uninfected birds from both SPF breeds (layers and broilers) revealed a generally higher

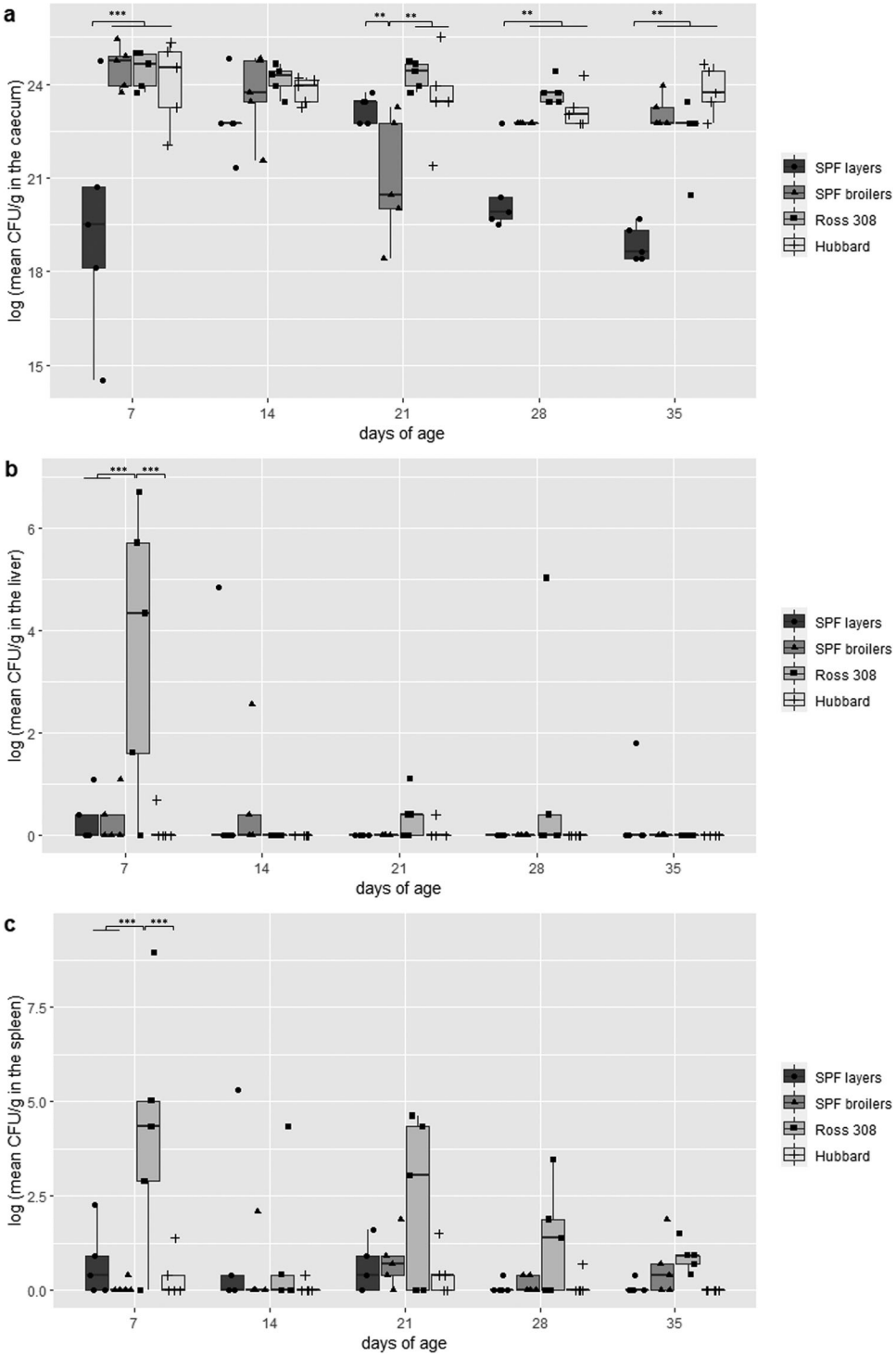

Figure 4. Comparison of the colonization of *S*. Infantis in caecum (a), liver (b) and spleen (c) between four different chicken lines ($n = 5$ birds per line and per killing day). Logarithmic transformation of CFU counts were used. Significant differences between the two groups are marked with $^{**}P < 0.01$ or $^{***}P < 0.001$.

number of immune cells compared to uninfected birds from commercial broilers (Ross 308 and Hubbard ISA-JA-757). Therefore, the results of SPF birds were merged as well as the results of commercial broiler birds, revealing a significantly higher number of $CD45^{+}CD4^{-}CD8\alpha^{+}$ and macrophages/monocytes (KUL01) in SPF birds compared to commercial birds at 7 days, and for all analysed populations at 28 and 35 days (Figure 7).

Discussion

S. Infantis is among the top 10 *Salmonella* serovars causing human illness in the EU, and poultry are the most common source (EFSA, 2021). Especially broiler birds have a central role in distribution of this serovar, indicating a certain affinity to this type of bird. Despite *Salmonella* Control Programmes according to Regulation (EC) No. 2160/2003, together with biosecurity

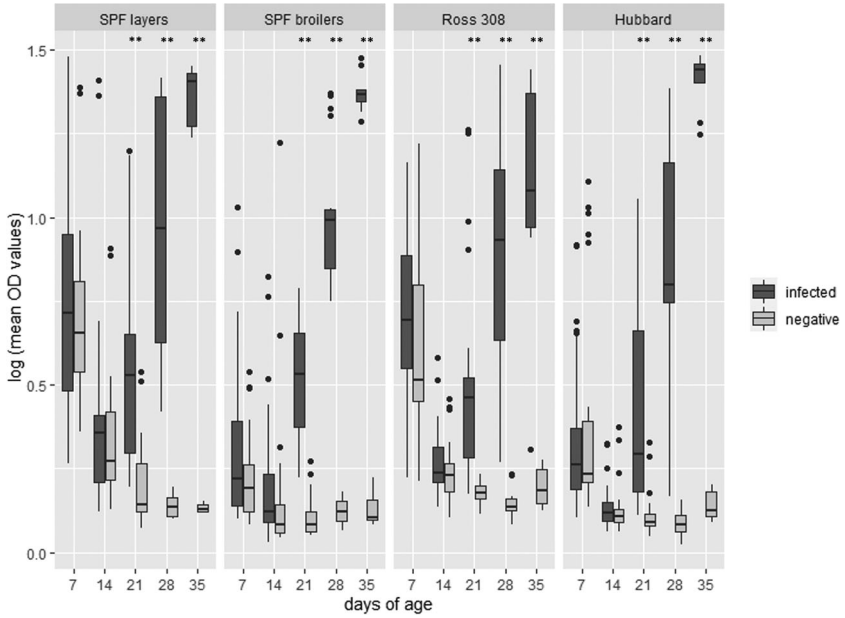

Figure 5. *S. Infantis* ELISA results (logarithmic transformation of mean OD values) of four different lines comparing infected (directly infected and in-contact birds) against negative birds ($n = 25$ birds on the first sampling day per breed with reduction of five birds per day). Significant differences between the two groups are marked with $^{**}P < 0.01$.

measures characterized by strict cleaning and disinfection protocols, the prevalence of *S.* Infantis in broiler birds has remained very stable over recent years. This lead to the assumption that an eradication of this serovar is a very difficult task (Drauch *et al.*, 2020; Sevilla-Navarro *et al.*, 2020). In addition to the overall epidemiology and the prevalence on a farm level, basic knowledge about the infection dynamics of *S.* Infantis is the centre of interest. Therefore, the present experimental study intended to compare the infection dynamics of *S.* Infantis in different chicken lines. To determine possible differences in shedding, organ colonization and immune response, two experimental chicken lines (SPF layers and SPF broilers) and two commercial lines (Ross 308 and Hubbard ISA-JA-757) were infected with *S.* Infantis. For this purpose, a well-defined *S.* Infantis strain was used harbouring the pESI-like plasmid. This strain was chosen as it showed increased ability to colonize liver and spleen of commercial broilers (Drauch *et al.*, 2021). However, it has to be acknowledged that a certain limitation in interpretation of the results might be present by using one strain only keeping the heterogeneity character of *S.* Infantis in mind.

Shedding of *Salmonella* via the faecal route plays an important role in transmission within a chicken flock as well as in the contamination of chicken meat (Gast *et al.*, 2005, 2017; Boubendir *et al.*, 2021; Montoro-Dasi *et al.*, 2021; Zeng *et al.*, 2021). Comparing the four different lines, the greatest similarity of shedding behaviour was found among SPF broilers and Ross 308 where the majority of birds were defined as high-shedders with 100% and 86.7%, respectively. An explanation for this might be that both lines represent fast-growing meat-type chickens and respond in a similar way to infections with *S.* Infantis. In contrast, predominantly low-shedding birds (73.3%) were detected in the slow-growing line Hubbard ISA-JA-757. Shedding was lowest in SPF layers where 9.3% of the birds were negative for *S.* Infantis. The present findings are in agreement with reported differences regarding the faecal excretion of *Salmonella* serotypes including *S.* Infantis in different chicken lines (Barrow *et al.*, 2004). Similar results were reported for infections with *C. jejuni* proving broilers are more prone to infections than layers (Hankel *et al.*, 2018). Interestingly, despite less shedding or fewer infected birds excreting bacteria, the transmission of *S.* Infantis to in-contact birds in all groups happened within 5 days resulting in no differences among the breeds. Previous studies revealed the importance of different shedding levels within flocks in context of infection dynamics independent from the genetic host population (Matthews *et al.*, 2006; Slater *et al.*, 2016; Menanteau *et al.*, 2018; Kempf *et al.*, 2020), a phenomenon which was also found in the present study for *S.* Infantis.

The favoured location site of *Salmonella* is the caecum (Snoeyenbos *et al.*, 1982; Barrow *et al.*, 1988). In this intestinal part, colonization and replication takes place at an early age (Varmuzova *et al.*, 2016). This is also reflected by the presented results, as no enrichment was needed to re-isolate *S.* Infantis. In agreement with previous data, there was no difference between the broiler lines in regard to caecal load of bacteria (Guillot *et al.*, 1995). The layer-type birds revealed a significantly lower colonization rate which might likely be in relation to the decreased faecal excretion

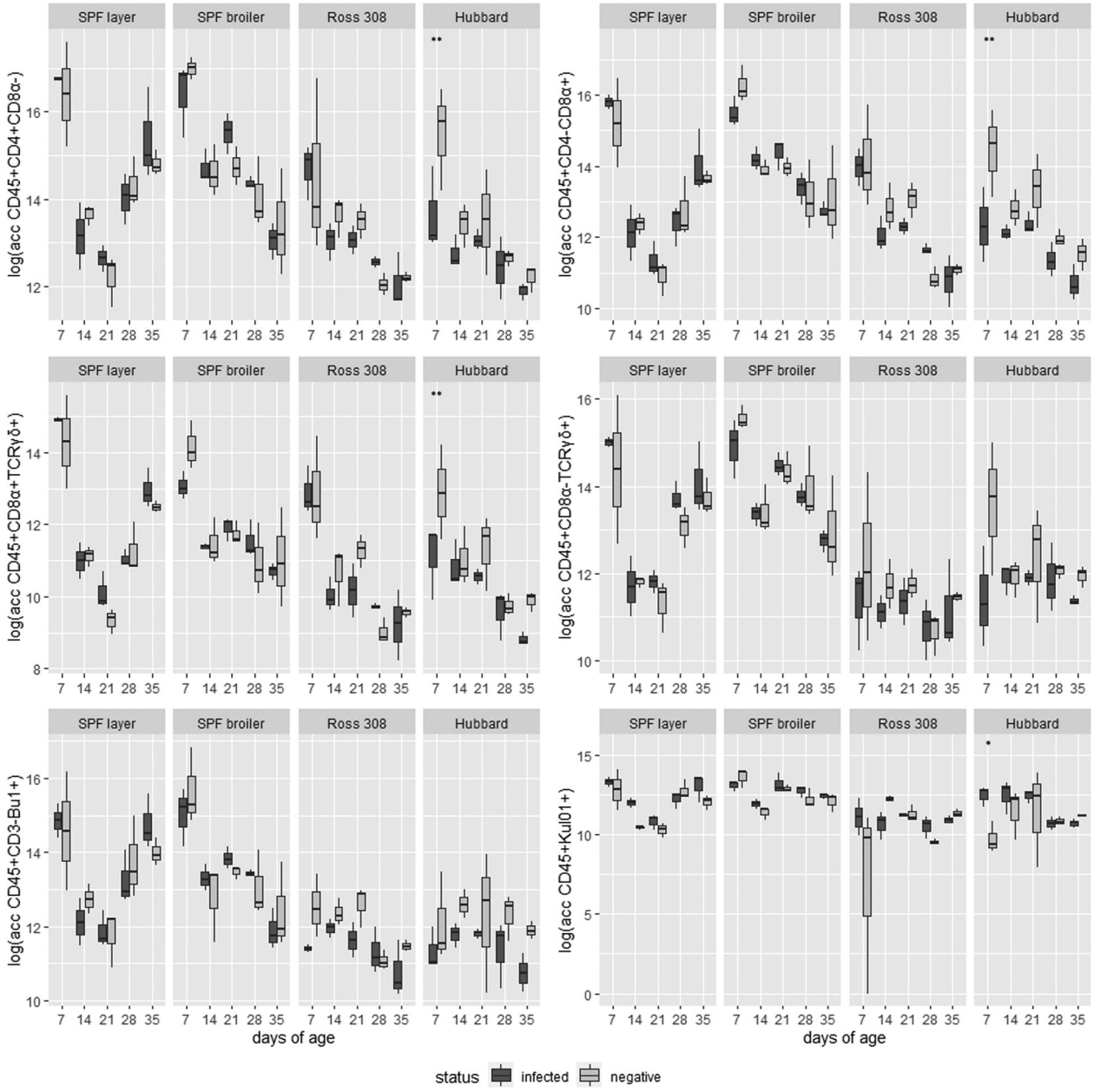

Figure 6. Absolute cell counts in logarithmic transformation of six immune cell populations in four different lines, infected and controls, at five sampling days ($n = 3$ birds per group per sampling day). Significant differences between infected and negative birds are marked with $^{*}P < 0.05$ or $^{**}P < 0.01$.

as previously reported (Barrow *et al.*, 2004). An explanation for this difference in broiler and layer lines might be a diverse gene expression response in the intestine as shown for *S.* Enteritidis (van Hemert *et al.*, 2006). It was shown that genes responsible for T-cell activation and for macrophage activity differed significantly between two chicken lines. This was not part of the focus in the present study, but needs to be elucidated in future investigations.

The present study revealed a distribution of *S.* Infantis into internal organs of all infected birds. In previous studies it was shown for *S.* Enteritidis and *S.* Typhimurium that they are able to impair the gut integrity by disruption of tight junctions, leading to an increased permeability and resulting in a translocation to internal organs, e.g. liver and spleen (Awad *et al.*, 2017). This could also be an explanation for the present finding on *S.* Infantis, but needs to be clarified in future investigations as this was not in the scope of the present study. Clearly, Ross 308 birds were more affected by *S.* Infantis colonization compared to the other breeds. For *C. jejuni* a correlation between CFU in the gut and intestinal permeability allowing invasion of *Campylobacter* to other tissues was revealed (Awad *et al.*, 2015; Han *et al.*, 2016). This cannot be assumed for *S.* Infantis, as caecal counts were very similar for all broiler chicken lines. A similar finding was reported previously for *S.* Enteritidis suggesting that the intestinal transcriptional and immunological response may be

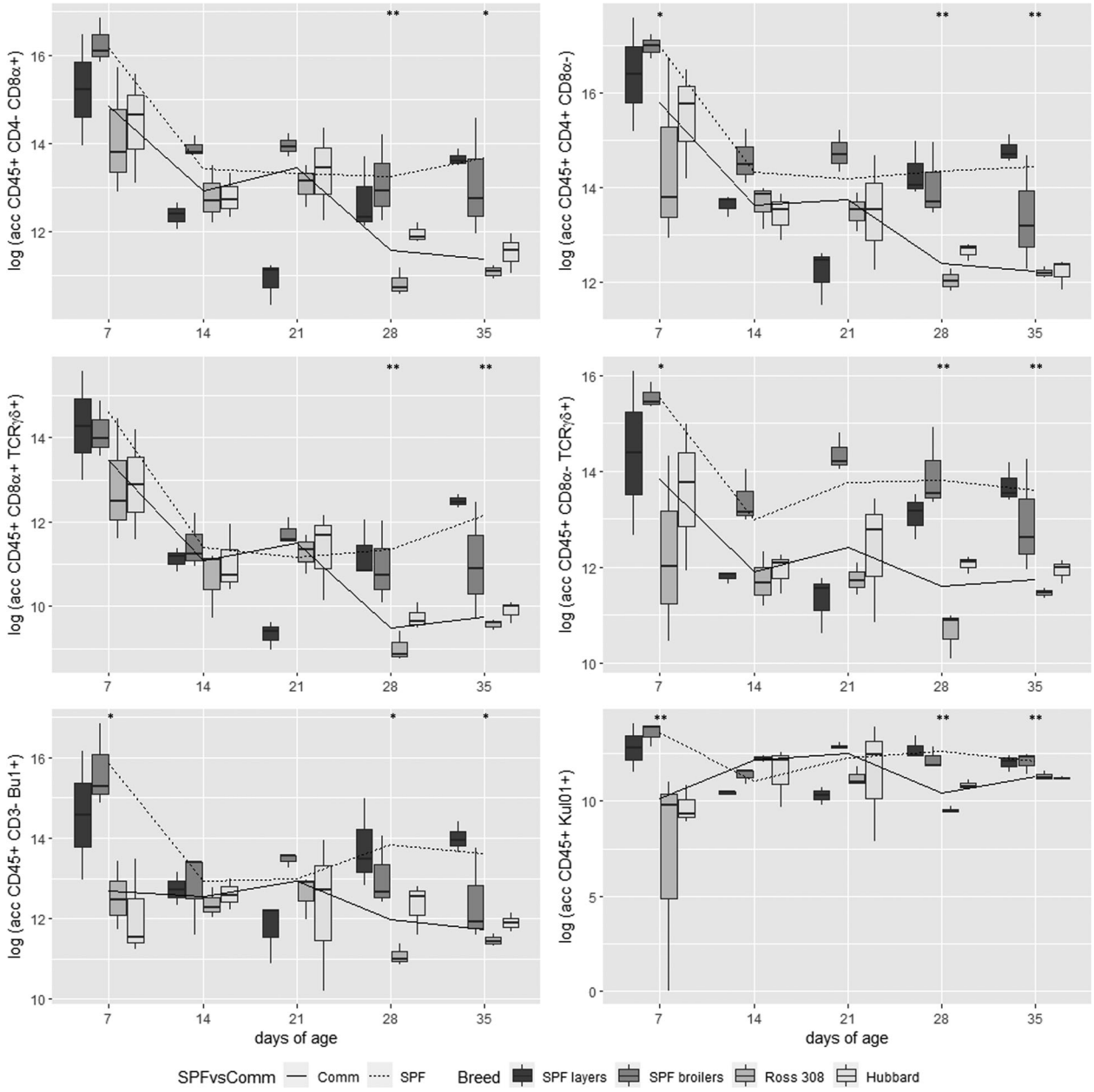

Figure 7. Comparison of absolute cell counts in logarithmic transformation of six different immune cell populations at five sampling days between four different lines represented in boxplots and between combined data of SPF lines (including results of SPF layers and SPF broilers) and commercial (Comm) lines (including results of Ross 308 and Hubbard) in lines. Significant differences between the two combined groups (SPF and Comm) are indicated with $*P < 0.05$ or $**P < 0.01$.

the underlying process of enhanced systemic spread (Schokker *et al.*, 2012).

The breed has been reported to be one of the key factors influencing the host response, and with this the outcome of excretion and colonization of *Salmonella* reflecting resistance or susceptibility (Wigley *et al.*, 2002; van Hemert *et al.*, 2006; Wigley *et al.*, 2006; Zhou & Lamont, 2007; Schokker *et al.*, 2012; Li *et al.*, 2018). It is well known that the immune response differs among chicken breeds which has to be seen in context with the genetic background (Koenen *et al.*, 2002; Schokker *et al.*, 2012; Bilková *et al.*, 2017; Giles *et al.*, 2019; Kaiser *et al.*, 2022). Especially genetic selection on body weight and feed conversion was reported as a major influence compromising immune response (Koenen *et al.*, 2002; van der Most *et al.*, 2011; Borodin *et al.*, 2020). Differences in resistance to *Salmonella* species were already reported among diverse chicken lines (Bumstead & Barrow, 1993; Barrow *et al.*, 2004; Li *et al.*, 2018). In this context it was also shown that genetic selection towards enhanced performance traits negatively influenced the immune system which resulted in lower natural resistance to *Salmonella* (Cheema *et al.*, 2003; Kramer *et al.*, 2003; van Hemert *et al.*, 2006). These findings are confirmed by this study for *S.* Infantis and illustrate the high susceptibility of commercial fast growing broilers.

No differences in the humoral antibody response were detected among the investigated chicken lines.

It is widely accepted that humoral response to *Salmonella* is a less important defence mechanism (van Immerseel *et al*., 2005). Although an antibody production against *S*. Infantis was present and confirmed successful infection, B cells in the blood were not increased indicating no positive correlation.

At 7 days of age monocytes/macrophages were found in significantly higher ratios in infected Hubbard ISA-JA-757 birds compared to their negative controls. This chicken line also had the lowest number of birds being positive in regards to colonization of liver and spleen samples. This goes in line with Ijaz *et al*. (2021), who found an increase of macrophages during the infection with *Salmonella* initiating phagocytosis of the bacteria. This specificity of the Hubbard ISA-JA-757 line is in agreement with Qureshi (2003) stating that the ability of macrophages to phagocytose pathogens is breed dependent. Furthermore, a previous study revealed that slow-growing chickens are more resistant to *Salmonella* compared to fast-growing chickens (van Hemert *et al*., 2006). However, no substantial differences among the infected chicken lines of SPF layers, SPF broilers and Ross 308 compared with non-infected control birds were revealed when investigating different immune cells in the blood. In general, the immune response of chickens infected with *S*. Infantis was reported to be lower than for other *Salmonella* serovars (Berndt *et al*., 2007; Setta *et al*., 2012). Moreover, it is possible that changes in the immune response are more prominent in the local organ compared to the changes noticed at systemic level (blood) (Beal *et al*., 2004; Setta *et al*., 2012). Hence, a detailed investigation of immune response at local level would be interesting in future studies.

Interestingly, this study demonstrated significantly higher immune cell counts in uninfected SPF birds irrespective of layer or broiler type, compared to uninfected commercially fast- and slow-growing broilers. This finding emphasizes the need to act with caution when correlating such data from SPF birds with commercial birds in experimental settings.

Overall, the present study revealed significant differences in excretion and colonization patterns of *S*. Infantis in four different chicken lines. The layer-type and the slow-growing broiler line proved less susceptible to an infection compared to the fast-growing birds. These results are in agreement with field reports where *S*. Infantis is less frequent being found in layer and slow-growing broiler birds. To date, targeted prophylactic measures against *S*. Infantis are not available for broiler birds and eradication is a difficult task. In this context, different approaches to achieve reduction of this pathogen comprised treatment of feeds, usage of probiotics, application of bacteriophages and vaccination of breeders (Koyuncu *et al*., 2013; Varmuzova *et al*., 2016; Crouch *et al*., 2020; Sevilla-Navarro *et al*., 2020). Therefore, any contribution in reducing the contamination rate of broiler flocks with respect to the excretion and colonization of inner organs will contribute to safer broiler meat products, and by this help to reduce the spread to humans and subsequently the risk of salmonellosis.

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Disclosure statement

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