Veterinary Parasitology 275 (2019) 108931

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Eimeria tenella induces the release of chicken heterophil extracellular traps

Zhengkai Wei[a,b,1], Yingchi Zhao[b,1], Nan Zhang[b], Zhen Han[b], Xiao Liu[b], Aimin Jiang[b], Yong Zhang[b], Chaoqun Wang[b], Pengtao Gong[b], Jianhua Li[b], Xichen Zhang[b,*], Zhengtao Yang[a,b,*]

[a] College of Life Sciences and Engineering, Foshan University, Foshan 528225, Guangdong Province, China
[b] Key Laboratory of Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun 130062, Jilin province, China

ARTICLE INFO

Keywords:
Eimeria tenella
Chicken
Heterophil
Cells
Extracellular traps

ABSTRACT

Avian coccidiosis makes a great threat and economic loss to the poultry industry, and fully understanding the innate immune response of chicken against *E. tenella* infection will play a significant role in avian coccidiosis prevention and treatment. Extracellular traps have been reported as a novel defense mechanism of host against pathogens infection. However, the interaction between chicken heterophil extracellular traps and *E. tenella* has remained not well known. Thus, this study aims to investigate the effects of *E. tenella* on chicken heterophil extracellular traps (ETs), and try to clarify the regulatory mechanisms in this process. *E. tenella*-triggered chicken heterophil ETs structures were analyzed by using scanning electron microscopy (SEM) and scanning confocal microscope. Inhibitors and Pico Green® were used to quantify *E. tenella* - triggered chicken heterophil ETs release. The results showed that *E. tenella* sporozoites significantly induced chicken heterophil ETs-like structures release, and histone and elastin co-existed with DNA in these structures of chicken heterophil ETs. Furthermore, it was also demonstrated that NADPH, p38 or Rac1 signaling pathways participated in *E. tenella* sporozoites-induced chicken heterophil ETs release, but more key molecules or signaling pathways involved in this process still needed to be further investigated. Taken together, this study reports that *E. tenella* sporozoites could induce chicken heterophil ETs formation via NADPH, p38 and Rac1 signaling pathways, which further suggests the critical role of heterophil ETs in the process of chicken against *E. tenella* infection.

1. Introduction

Eimeria tenella (*E. tenella*) is an obligate intracellular parasite frequently considered as a major cause of avian coccidiosis. This disease caused by *Eimeria* infection usually results in chicken intestinal disease characterized with body weight loss, dehydration, hemorrhagic diarrhea and increased susceptibility to other pathogens, which makes it a great threat and economic loss to the poultry industry (Blake and Tomley, 2014; McDonald and Shirley, 2009; Suo et al., 2006; Witcombe and Smith, 2014). However, avian coccidiosis prevention and treatment has always been a great challenge for poultry. Conventional coccidiostats usually causes drug residues, resistance and potential hazard on human health. Attenuated vaccine provides efficient precaution for avian coccidiosis but lack of cross-protection for *Eimeria* species (Del Cacho et al., 2016). Thus, safe and effective prevention and treatment methods for avian coccidiosis remain urgently needed.

Successful methods for avian coccidiosis prevention and treatment should be established on fully understanding the interaction between host and *Eimeria*. It is reported that *E. tenella* infection model of chicken epithelial cell line (CLEC-213) in vitro has been established (Bussiere et al., 2018), and chicken cecal mucous membranes response to *E. tenella* infection in vivo has also been investigated by transcriptome analysis (Guo et al., 2013). Several cytokines, such as IL-17A, IL-10, IL-1β and IFN-γare also secreted in the process of *E. tenella* infection (Del Cacho et al., 2014; Hong et al., 2006; Laurent et al., 2001). However, reports on the interaction between heterophil cells and *E. tenella* have remained not well investigated.

As effective immune cells of chicken innate immune system, chicken heterophils play a curial role in protecting the host against microorganism infection. In recent years, a novel defense mechanism of neutrophils called “NETs” has been reported in against bacteria (Brinkmann et al., 2004), virus (Barr et al., 2018; Saitoh et al., 2012), fungi (Jin et al., 2016; Urban and Nett, 2018) and parasites (Abi Abi Abdallah et al., 2012; Guimaraes-Costa et al., 2009; Villagra-Blanco et al., 2017; Wei et al., 2016). Of great interest is that the mechanism of ETs release is also discovered in chicken heterophils (Chuammitri et al.,

* Corresponding authors at: College of Life Sciences and Engineering, Foshan University, Foshan 528225, Guangdong Province, China ; Key Laboratory of Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun 130062, Jilin province, China.
E-mail addresses: xczhang@jlu.edu.cn (X. Zhang), yangzhengtao01@sina.com (Z. Yang).
[1] The authors make equal contributions to the work.

https://doi.org/10.1016/j.vetpar.2019.108931
Received 10 April 2019; Received in revised form 23 September 2019; Accepted 23 September 2019

2009). But whether this novel defense mechanism of chicken heterophils ETs also protects chicken from *E. tenella* infection remains unknown. Furthermore, several molecular or signaling pathways are involved in the release of *Eimeria bovis*-induced bovine NETs (Munoz-Caro et al., 2015b), *Eimeria ninakohlyakimovae*-induced caprine monocyte ETs (Perez et al., 2016) or *Eimeria arloingi* - induced caprine NETs (Silva et al., 2014). But whether *E. tenella* could also trigger heterophils ETs formation and the regulatory mechanisms in this process remains to be investigated. More importantly, the difference of ETs-triggered by these *Eimeria* species has not been clarified.

Thus, we aim to firstly examine the effects of *E. tenella* on the release of chicken heterophils ETs, and try to further investigate the regulatory mechanisms in the process of *E. tenella*-induced chicken heterophils ETs formation.

2. Materials and methods

2.1. Chicken heterophil isolation

Five healthy male chickens were used for blood collection, and the blood was collected via the wing vein. Chicken heterophil cells were isolated according to the heterophils isolation kit® (TianJin HaoYang Biological Manufacture CO., China). These experiments of ETs were approved by the Ethics Committee of Jilin University on the Care and Use of Laboratory Animals (JLU20170330).

2.2. Eimeria tenella culture and purification

E. tenella was maintained by chicks for oocyst production. The 7-day-old chicks were infected with 10^4 sporulated *E. tenella* oocysts, and oocysts were collected on the 6th day after infection and stored in potassium dichromate. *E. tenella* sporozoites were purified by using sucrose density gradient centrifugation.

2.3. SEM analysis

Chicken heterophils were seeded on coverslips (13 mm diameter), and stimulated with *E. tenella* sporozoites at the ratio of 1:1 for 120 min. Then, the cells were treated with 4.0% glutaraldehyde, followed by post-fixation with 1.0% osmium tetroxide, dehydration, frozen and gold sputtering as previously described (Wei et al., 2016, 2018a). Finally, sample testing was carried out with a scanning electron microscope (Hitachi S-3400 N, Japan).

2.4. Immunofluorescence analysis

Chicken heterophils were seeded on coverslips (13 mm diameter), and stimulated with *E. tenella* sporozoites in the ratio of 1:1 for 120 min. Main constituents within *E. tenella* sporozoites - induced ETs structures were tested by immunofluorescence analysis as previously described (Wei et al., 2018a, b). Histone and NE in ETs structures were examined respectively by using anti-histone antibody (LS-C353149; Life Span BioSciences, Inc) and anti-NE antibody (AB68672; Abcam). The sample examination was performed with scanning confocal microscope (Olympus FluoView FV1000).

2.5. Quantitative analysis of heterophil extracellular traps

Chicken heterophils were seeded on 96-well plates, and stimulated with *E. tenella* sporozoites at the ratio of 1:0.25, 1:0.5 or 1:1 for 120 min. Before stimulation, DPI (the NADPH oxidase inhibitor, 10 μM, Sigma-Aldrich) NSC23766 (the Rac1 activation inhibitor, 100 μM), MCC950 (the NLRP3 inhibitor, 8.0 nM) or SB202190 (P38 MAPK-signaling pathway inhibitor, 10 μM, Sigma-Aldrich) was pre-processed with chicken heterophils for 20 min. Pico Green® (Invitrogen) and fluorometric reader Infiniti M200® (TECAN, Austria) were performed for quantitative analysis of heterophil extracellular traps release.

2.6. LDH analysis

Chicken heterophils were seeded on 96-well plates, and stimulated with *E. tenella* sporozoites at the ratio of 1:1, 1:1.5 or 1:3 for 120 min. *E. tenella* sporozoites-induced LDH changes were tested with LDH Assay kit® (Beyotime Biotechnology, China).

2.7. Statistical analysis

The GraphPad 5.0 software was performed with one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests for analyzing difference among groups. The data was expressed as the means ± standard deviation (SD). p-value < 0.05 was considered as significant difference.

3. Results

3.1. E. tenella sporozoites -induced chicken heterophil ETs release

After stimulation with *E. tenella* sporozoites at the ratio of 1:1 for 120 min, SEM analysis of chicken heterophils ETs release showed that *E. tenella* sporozoites significantly induced the release of chicken heterophil ETs-like structures (Fig. 1C-1 F). The red arrows clearly showed varying thickness of chicken heterophil ETs.

3.2. Histone and elastase co-existed with DNA in the structures of E. tenella sporozoites-induced chicken heterophil ETs

Immunofluorescence analysis was performed to analyze the constituents within *E. tenella* sporozoites - induced ETs structures. As showed in Fig. 2, *E. tenella* sporozoites also significantly induced the release of chicken heterophil ETs-like structures but not observed in control groups, which confirmed the results of SEM analysis. Furthermore, it was also found that histone (Fig. 2 G) and elastase (Fig. 2 J) co-existed with DNA (Fig. 2 H and 2 K) in these structures of *E. tenella* sporozoites -induced chicken heterophil ETs.

3.3. E. tenella sporozoites-induced chicken heterophil ETs release in a dose-dependent manner

To further quantitative analyze *E. tenella* sporozoites-induced chicken heterophil ETs, chicken heterophils were incubated with different doses of *E. tenella* sporozoites. The results showed that *E. tenella* sporozoites significantly induced chicken heterophil ETs release in a dose-dependent manner ($p = 0.003$; Fig. 3), which further confirmed that *E. tenella* sporozoites did induce the release of chicken heterophil ETs.

3.4. NADPH and p38 signaling pathways participated in E. tenella sporozoites-induced chicken heterophil ETs release

Quantitative analysis by using Pico Green and fluorometric reader were an important method to investigate the molecular or signaling pathways being connected with *E. tenella* sporozoites -induced chicken heterophil ETs release. Quantitative analysis of *E. tenella* sporozoites-induced heterophil ETs release showed that *E. tenella* sporozoites and zymosan (positive control) had significantly increased heterophil ETs release compared with heterophil cells, while SB202190 and DPI decreased *E. tenella* sporozoites-induced heterophil ETs release ($p = 0.001$; Fig. 4).

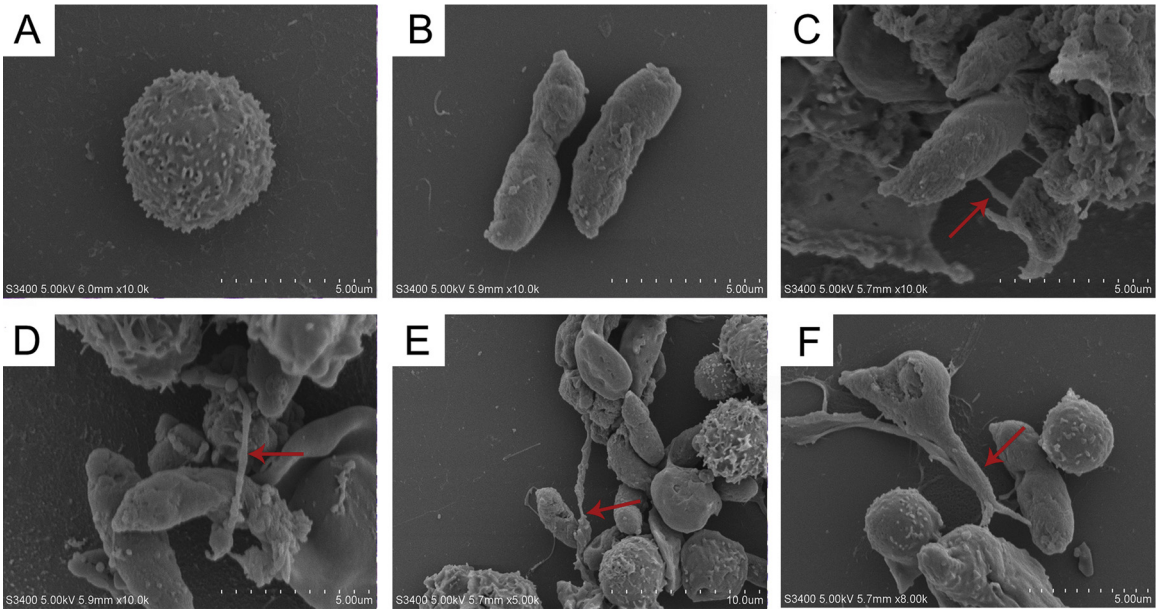

Fig. 1. SEM analysis of *E. tenella*-induced chicken heterophil ETs release. Chicken heterophil cells were stimulated with to *E. tenella* sporozoites (ratio 1:1) for 90 min, the release of chicken heterophil ETs were examined by SEM. (A) Chicken heterophil cells. (B) *E. tenella* sporozoites. (C), (D) and (E) Thinner heterophil ETs-like structures induced by *E. tenella* sporozoites. (F) Thicker heterophil ETs-like structures induced by *E. tenella* sporozoites. The red arrows clearly showed varying thickness of chicken heterophil ETs.

3.5. Rac1 signaling pathways involved in E. tenella sporozoites -induced chicken heterophil ETs release

Quantitative analysis was also carried out to investigate the role of Rac1 signaling pathways and NLRP3 in in *E. tenella* sporozoites -induced chicken heterophil ETs release. It was found that NSC23766 ($p = 0.001$; the inhibitor of Rac1 signaling pathways) but not MCC950 ($P = 0.111$; the inhibitor of NLRP3) decreased *E. tenella* sporozoites -induced chicken heterophil ETs release (Fig. 5).

3.6. No change of LDH levels in E. tenella-induced chicken heterophil ETs release

LDH activities in *E. tenella* sporozoites-induced chicken heterophil ETs release were investigated by LDH kits. The results showed that no significant changes of LDH levels was observed in the process of *E. tenella* sporozoites -induced chicken heterophil ETs release (Fig. 6).

4. Discussion

In the present research, we investigate the interaction between *E. tenella* and the innate immune responses of chicken heterophil extracellular traps. It appears that *E. tenella* in chicken heterophils, like *Eimeria bovis* in bovine neutrophils (Munoz-Caro et al., 2015b), *Eimeria ninakohlyakimovae* in caprine monocytes (Perez et al., 2016) or *Eimeria arloingi* in caprine neutrophils (Silva et al., 2014), has the capacity to induce ETs formation. Furthermore, as with hydrogen peroxide and phorbol myristate acetate (Chuammitri et al., 2009), *E. tenella* sporozoites - induced the structures of chicken heterophil ETs consisting with DNA, histone and elastase. Above results demonstrate typical feature of ETs forming in the interaction between *E. tenella* and its host of chicken heterophil cells.

Several key molecules or signaling pathways participated in NETs formation, but the mechanism of *E. tenella*-induced chicken heterophil ETs remains unknown. Then, further examination of the regulatory mechanisms in the process of *E. tenella*-induced chicken heterophils ETs formation. Since NADPH or p38 signaling pathways is proven to play an important role in ETs release (Munoz-Caro et al., 2015b; Wei et al., 2016). Here, we investigated the role of NADPH or p38 signaling pathways in *E. tenella*-induced chicken heterophils ETs formation. It was found that *E. tenella* significantly increased chicken heterophils ETs release, but SB202190 and DPI decreased *E. tenella* sporozoites-induced heterophil ETs release, suggesting that NADPH and p38 signaling pathways were also related with *E. tenella*-induced chicken heterophils ETs formation. The results are similar to that discovered in *Neospora caninum* -induced canine NETs release (Wei et al., 2016), *Eimeria bovis*-induced bovine NETs release (Munoz-Caro et al., 2015b), *Cryptosporidium parvum*-induced NET release (Munoz-Caro et al., 2015a) or *Neospora caninum* -induced caprine monocytes ETs release (Yang et al., 2017). Moreover, we also examined the role of Rac1 signaling pathways or NLRP3 in the process of *E. tenella*-induced chicken heterophils ETs formation. The results showed that NSC23766 (the inhibitor of Rac1 signaling pathways) decreased *E. tenella* sporozoites -induced chicken heterophil ETs release, but MCC950 (the inhibitor of NLRP3) did not significantly change *E. tenella* sporozoites -induced heterophil ETs formation. This is first report that Rac1 signaling pathways are involved in *E. tenella*-induced chicken heterophil ETs release. To distinguish if *E. tenella* sporozoites -induced heterophils cell death was necrosis, we then examined LDH activities. It found that no significant changes of LDH levels was observed in the process of *E. tenella* sporozoites -induced chicken heterophil ETs release, which further confirmed the typical feature of chicken heterophil ETs structures induced by *E. tenella*.

In conclusion, the study demonstrate that *E. tenella* sporozoites triggers the typical structures of chicken heterophil ETs, and NADPH, p38 or Rac1 signaling pathways play a crucial part in the process of *E. tenella* sporozoites-induced chicken heterophil ETs release. However, whether these key molecules or signaling pathways also work in vivo remain to be further investigated.

Authors' contributions

Xichen Zhang and Zhengtao Yang designed the project and experments. Zhengkai Wei, Yingchi Zhao, Nan Zhang, Zhen Han, Xiao Liu, Aimin Jiang and Yong Zhang, Chaoqun Wang carried out the experiments. Zhengkai Wei wrote the manuscript, carried out statistical analysis and prepared figures. Xichen Zhang, Zhengtao Yang, Pengtao

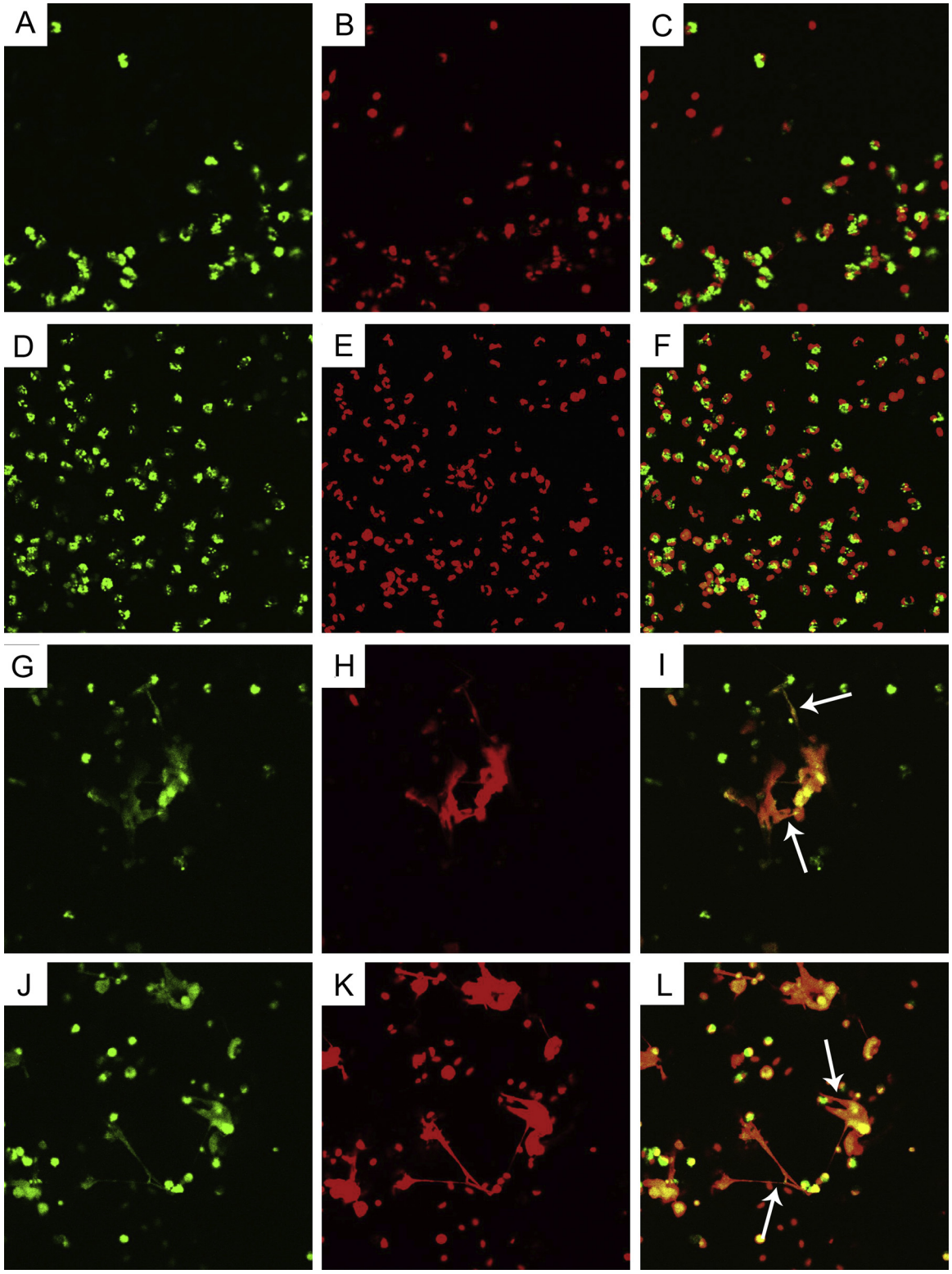

Fig. 2. Immunofluorescence analysis of *E. tenella*-induced chicken heterophil ETs structures. Chicken heterophil cells were stimulated with to *E. tenella* sporozoites (ratio 1:1) for 120 min, the structures of chicken heterophil ETs were analyzed by immunofluorescence analysis. (A) and (G) Histone (Green). (D) and (*J*) Elastase (Green). (B), (E), (H) and (K) DNA (Red). (C) Merge of (A) and (B). (F) Merge of (D) and (E). (I) Merge of (G) and (H). (L) Merge of (*J*) and (K). White arrows showed chicken heterophil ETs induced by *E. tenella* sporozoites.

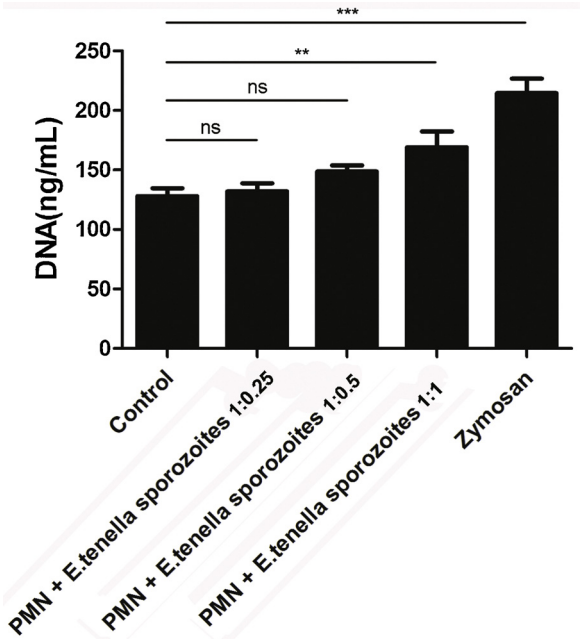

Fig. 3. Quantitative analysis of *E. tenella* sporozoites-induced chicken heterophil ETs release. Chicken heterophil cells were stimulated with to *E. tenella* sporozoites (ratio 1:0.25, 1:0.5 or 1:1) for 120 min, chicken heterophil ETs were analyzed by Pico Green® and fluorescence reader analysis. Data was presented as mean ± SD, n = 3. P values of < 0.05 were considered significant. (** P < 0.01, *** P < 0.001 and"ns" means not significant).

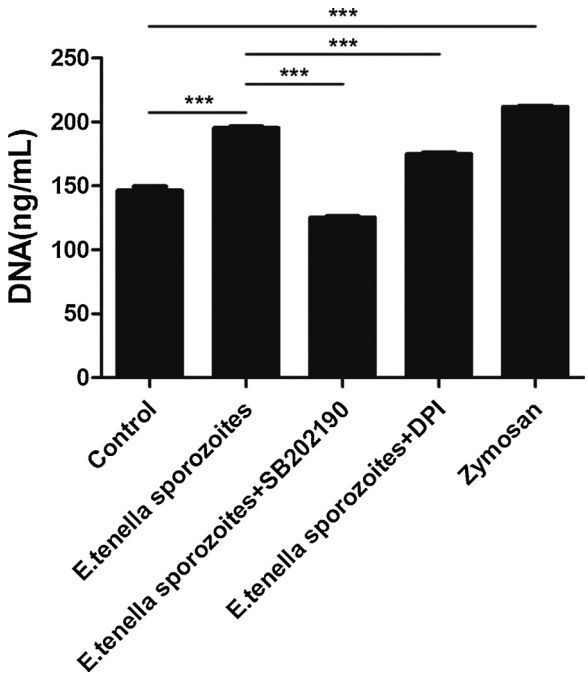

Fig. 4. NADPH and p38 signaling pathways participated in *E. tenella* sporozoites-induced chicken heterophil ETs release. Chicken heterophil cells were stimulated with to *E. tenella* sporozoites (ratio 1:1) for 120 min, *E. tenella* sporozoites-induced chicken heterophil ETs release were examined by Pico Green®. Inhibitors of NADPH and p38 signaling pathways were pre-processed with chicken heterophil cells for 20 min before stimulation with *E. tenella* sporozoites. Data was presented as mean ± SD, n = 3. P values of < 0.05 were considered significant. (*** P < 0.001).

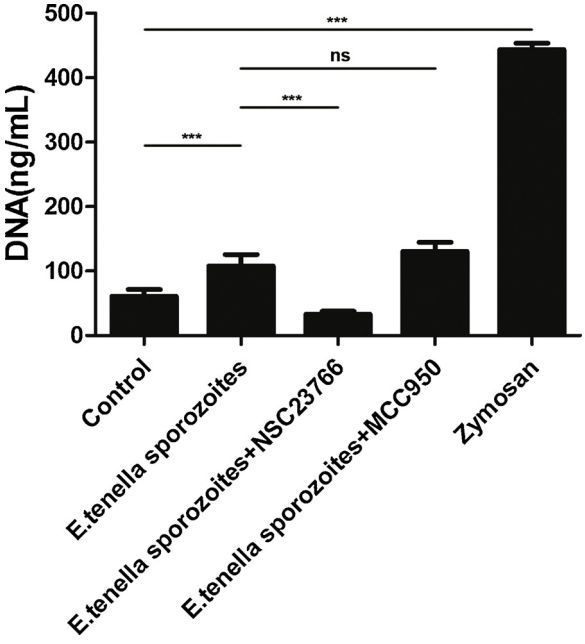

Fig. 5. Rac1 signaling pathways involved in *E. tenella* sporozoites -induced chicken heterophil ETs release. Chicken heterophil cells were stimulated with to *E. tenella* sporozoites (ratio 1:1) for 120 min, *E. tenella* sporozoites-induced chicken heterophil ETs release were examined by Pico Green®. Inhibitors of Rac1 signaling pathways or NLRP3 were pre-processed with chicken heterophil cells for 20 min before stimulation with *E. tenella* sporozoites. Data was presented as mean ± SD, n = 3. P values of < 0.05 were considered significant. (*** P < 0.001 and "ns" means not significant).

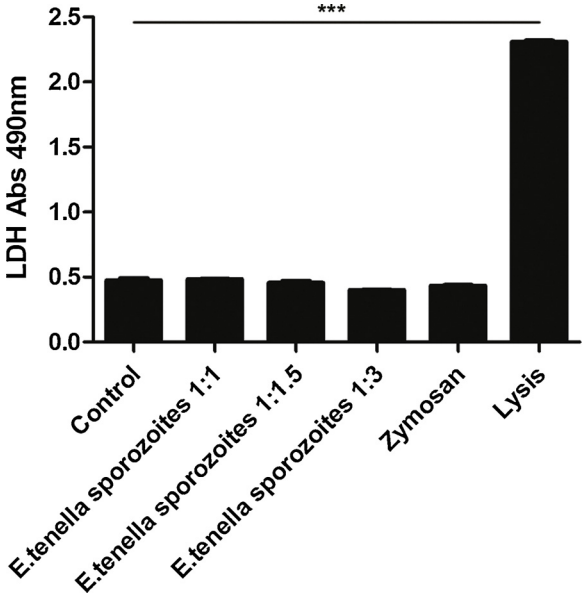

Fig. 6. No change of LDH levels in *E. tenella*-induced chicken heterophil ETs release.

Chicken heterophils were seeded on 96-well plates, and stimulated with *E. tenella* sporozoites in the ratio of 1:1, 1:1.5 or 1:3 for 120 min. Lysis, cell lysis agent, was provided by LDH kits. Data was presented as mean ± SD, n = 3. P values of < 0.05 were considered significant. (*** P < 0.001).

Gong and Jianhua Li reviewed and amended the manuscript. Xichen Zhang and Zhengtao Yang co-corresponded this paper. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgments

This work was funded by the grants from Jilin Key Scientific and Technological Development Program (Nos. 20190301089NY and 20170204036NY).

References

Abi Abdallah, D.S., Lin, C., Ball, C.J., King, M.R., Duhamel, G.E., Denkers, E.Y., 2012. Toxoplasma gondii triggers release of human and mouse neutrophil extracellular traps. Infect. Immun. 80, 768–777.

Barr, F.D., Ochsenbauer, C., Wira, C.R., Rodriguez-Garcia, M., 2018. Neutrophil extracellular traps prevent HIV infection in the female genital tract. Mucosal Immunol. 11, 1420–1428.

Blake, D.P., Tomley, F.M., 2014. Securing poultry production from the ever-present Eimeria challenge. Trends Parasitol. 30, 12–19.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., Zychlinsky, A., 2004. Neutrophil extracellular traps kill bacteria. Science 303, 1532–1535.

Bussiere, F.I., Niepceron, A., Sausset, A., Esnault, E., Silvestre, A., Walker, R.A., Smith, N.C., Quere, P., Laurent, F., 2018. Establishment of an in vitro chicken epithelial cell line model to investigate Eimeria tenella gamete development. Parasit. Vectors 11, 44.

Chuammitri, P., Ostojic, J., Andreasen, C.B., Redmond, S.B., Lamont, S.J., Palic, D., 2009. Chicken heterophil extracellular traps (HETs): novel defense mechanism of chicken heterophils. Vet. Immunol. Immunopathol. 129, 126–131.

Del Cacho, E., Gallego, M., Lillehoj, H.S., Quilez, J., Lillehoj, E.P., Ramo, A., Sanchez-Acedo, C., 2014. IL-17A regulates Eimeria tenella schizont maturation and migration in avian coccidiosis. Vet. Res. 45, 25.

Del Cacho, E., Gallego, M., Lillehoj, H.S., Quilez, J., Lillehoj, E.P., Sanchez-Acedo, C., 2016. Induction of protective immunity against experimental Eimeria tenella infection using serum exosomes. Vet. Parasitol. 224, 1–6.

Guimaraes-Costa, A.B., Nascimento, M.T., Froment, G.S., Soares, R.P., Morgado, F.N., Conceicao-Silva, F., Saraiva, E.M., 2009. Leishmania amazonensis promastigotes induce and are killed by neutrophil extracellular traps. Proc. Natl. Acad. Sci. U. S. A. 106, 6748–6753.

Guo, A., Cai, J., Gong, W., Yan, H., Luo, X., Tian, G., Zhang, S., Zhang, H., Zhu, G., Cai, X., 2013. Transcriptome analysis in chicken cecal epithelia upon infection by Eimeria tenella in vivo. PLoS One 8, e64236.

Hong, Y.H., Lillehoj, H.S., Lee, S.H., Dalloul, R.A., Lillehoj, E.P., 2006. Analysis of chicken cytokine and chemokine gene expression following Eimeria acervulina and Eimeria tenella infections. Vet. Immunol. Immunopathol. 114, 209–223.

Jin, X., Zhao, Y., Zhang, F., Wan, T., Fan, F., Xie, X., Lin, Z., 2016. Neutrophil extracellular traps involvement in corneal fungal infection. Mol. Vis. 22, 944–952.

Laurent, F., Mancassola, R., Lacroix, S., Menezes, R., Naciri, M., 2001. Analysis of chicken mucosal immune response to Eimeria tenella and Eimeria maxima infection by quantitative reverse transcription-PCR. Infect. Immun. 69, 2527–2534.

McDonald, V., Shirley, M.W., 2009. Past and future: vaccination against Eimeria. Parasitology 136, 1477–1489.

Munoz-Caro, T., Lendner, M., Daugschies, A., Hermosilla, C., Taubert, A., 2015a. NADPH oxidase, MPO, NE, ERK1/2, p38 MAPK and Ca2+ influx are essential for Cryptosporidium parvum-induced NET formation. Dev. Comp. Immunol. 52, 245–254.

Munoz-Caro, T., Mena Huertas, S.J., Conejeros, I., Alarcon, P., Hidalgo, M.A., Burgos, R.A., Hermosilla, C., Taubert, A., 2015b. Eimeria bovis-triggered neutrophil extracellular trap formation is CD11b-, ERK 1/2-, p38 MAP kinase- and SOCE-dependent. Vet. Res. 46, 23.

Perez, D., Munoz, M.C., Molina, J.M., Munoz-Caro, T., Silva, L.M., Taubert, A., Hermosilla, C., Ruiz, A., 2016. Eimeria ninakohlyakimovae induces NADPH oxidase-dependent monocyte extracellular trap formation and upregulates IL-12 and TNF-alpha, IL-6 and CCL2 gene transcription. Vet. Parasitol. 227, 143–150.

Saitoh, T., Komano, J., Saitoh, Y., Misawa, T., Takahama, M., Kozaki, T., Uehata, T., Iwasaki, H., Omori, H., Yamaoka, S., Yamamoto, N., Akira, S., 2012. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. Cell Host Microbe 12, 109–116.

Silva, L.M., Caro, T.M., Gerstberger, R., Vila-Vicosa, M.J., Cortes, H.C., Hermosilla, C., Taubert, A., 2014. The apicomplexan parasite Eimeria arloingi induces caprine neutrophil extracellular traps. Parasitol. Res. 113, 2797–2807.

Suo, X., Zhang, J.X., Li, Z.G., Yang, C.T., Min, Q.R., Xu, L.T., Liu, Q., Zhu, X.Q., 2006. The efficacy and economic benefits of Supercox, a live anticoccidial vaccine in a commercial trial in broiler chickens in China. Vet. Parasitol. 142, 63–70.

Urban, C.F., Nett, J.E., 2018. Neutrophil extracellular traps in fungal infection. Semin. Cell Dev. Biol.

Villagra-Blanco, R., Silva, L.M.R., Aguilella-Segura, A., Arcenillas-Hernandez, I., Martinez-Carrasco, C., Seipp, A., Gartner, U., Ruiz de Ybanez, R., Taubert, A., Hermosilla, C., 2017. Bottlenose dolphins (Tursiops truncatus) do also cast neutrophil extracellular traps against the apicomplexan parasite Neospora caninum. International journal for parasitology. Parasit. Wildlife 6, 287–294.

Wei, Z., Hermosilla, C., Taubert, A., He, X., Wang, X., Gong, P., Li, J., Yang, Z., Zhang, X., 2016. Canine neutrophil extracellular traps release induced by the apicomplexan parasite neospora caninum in vitro. Front. Immunol. 7, 436.

Wei, Z., Wang, Y., Zhang, X., Wang, X., Gong, P., Li, J., Taubert, A., Hermosilla, C., Zhang, X., Yang, Z., 2018a. Bovine macrophage-derived extracellular traps act as early effectors against the abortive parasite Neospora caninum. Vet. Parasitol. 258, 1–7.

Wei, Z., Zhang, X., Wang, J., Wang, Y., Yang, Z., Fu, Y., 2018b. The formation of canine neutrophil extracellular traps induced by sodium arsenic in polymorphonuclear neutrophils. Chemosphere 196, 297–302.

Witcombe, D.M., Smith, N.C., 2014. Strategies for anti-coccidial prophylaxis. Parasitology 141, 1379–1389.

Yang, Z., Wei, Z., Hermosilla, C., Taubert, A., He, X., Wang, X., Gong, P., Li, J., Zhang, X., 2017. Caprine monocytes release extracellular traps against neospora caninum in vitro. Front. Immunol. 8, 2016.