Changes in Oxidation-antioxidation Function on the Thymus of Chickens Infected with Reticuloendotheliosis Virus

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Research article

Keywords: reticuloendotheliosis virus, thymus, oxidation antioxidant imbalance, histopathological and ultrastructural changes, immunosuppression

Posted Date: June 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-30062/v1

Version of Record: A version of this preprint was published on December 11th, 2020. See the published version at https://doi.org/10.1186/s12917-020-02708-6.

Abstract

Background

Reticuloendotheliosis virus (REV) is a retrovirus that causes severe immunosuppression in infected poultry. Under oxidative stress,the animals grow slowly. In addition, long-term oxidative stress can impair immune function, as well as accelerate the aging and death of the animal.This study aimed to elucidate the pathogenesis of REV from the perspective of changes in oxidative-antioxidative function following REV infection.

Results

In specific pathogen free chickens infected with REV, the levels of H_2O_2 and MDA in the thymus increased, the levels of T-AOC, SOD, CAT, and GPx1 decreased, and there was a reduction in CAT and Gpx1 mRNA expression, compared with the control group. The thymus index was also significantly reduced. Morphological analysis showed that after REV infection, there was an increase in thymic reticular endothelial cells, inflammatory cell infiltration, mitochondrial swelling, and destruction of the nuclear structure.

Conclusions

These results indicate that an increase in oxidative substances within the thymus, enhanced lipid peroxidation, a markedly decrease in antioxidant function, atrophy of the thymus, and immunosuppression in REV-infected chickens.

Background

Reticuloendotheliosis (RE) constitutes a group of pathological syndromes caused by reticuloendotheliosis hyperplasia virus, including runting syndrome, chronic tumors of lymphoid and other tissues, acute reticulocytoma, and severe immunosuppression. Reticuloendotheliosis virus (REV) is a C-type avian retrovirus[1, 2]. The REV group includes defective REV-T, non-defective REV-A, chick syncytial virus, duck infectious anemia virus, and spleen necrosis virus (SNV) [3]. In addition, REV infects a wide-range of hosts, including chickens, turkey, duck, goose, Japanese quail, and wild birds, among which, turkey is the most susceptible. Thus, turkeys and chickens are commonly used as experimental animals [4, 5, 6, 7]. REV can be mixed with Marek's disease virus (MDV), avian leukosis virus of subgroup J (ALV-J), and chicken anemia virus (CAV), resulting in reducing or loss of immune function in REV infection chickens. Thus, RE represents a serious threat to the development of the poultry industry[2, 8].

Under normal physiological conditions, the body's oxidation-antioxidant system is in a dynamic equilibrium, and oxidative stress occurs when the antioxidant system is overwhelmed by the oxidation

system[9, 10]. Oxidative stress is an initial reaction of the body to external stimuli and may subsequently induce various signaling pathways and inflammation in the body[11]. Under oxidative stress, animals grow slowly, the feed conversion rate decreases, and there is a decrease in production performance[12]. In addition, long-term oxidative stress can deplete antioxidant vitamins and trace elements, impair immune function, as well as accelerate the aging and death of the animal, resulting in substantial economic losses to the poultry industry[13].

The oxidation system primarily includes reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are by-products of the body's metabolism that contain an unpaired electron. Moreover, ROS and RNS represent an important component of the pathogen resistance mechanism and may act as intracellular and intercellular signaling molecules; however, excessively high levels may also lead to oxidative stress[14]. ROS mainly include superoxide anions, hydroxyl radicals, other oxygen radicals, and H_2O_2. RNS includes NO, NO_2, and peroxynitrite[15].

Antioxidant systems include enzymes and non-enzymatic antioxidants[4]. Enzyme antioxidants include SOD, peroxidase, CAT, GPx, and glutathione reductase, whereas non-enzymatic antioxidants include fat-soluble vitamin E, carotenoids, ubiquinone, and water-soluble vitamin C, glutathione, uric acid, and tryptophan metabolites. When the oxidation-antioxidant system is imbalanced, excessive ROS and RNS accumulate and destroy various biomacromolecules (e.g., lipids, proteins, and nucleic acids), which leads to cellular damage and ultimately cell death. Several studies have shown that oxidative stress plays an important role in inflammatory reactions, tumors, and many other diseases[16, 17, 18].

Currently, there are few existing studies that have investigated the changes in the oxidation-antioxidant system of poultry after a REV infection. This study examined the dynamic changes in the level of T-AOC, SOD activity, H_2O_2, MDA, CAT, and GPx1, as well as CAT and GPx1 mRNA expression in the thymus of one-day-old SPF chickens after a REV infection. Pathological sections and ultrastructural changes of the thymus were observed to further clarify the pathogenesis of REV infection and morphological changes resulting from oxidative stress and pathological processes, to facilitate the prevention and treatment of RE.

Results

Determination of oxidative stress biomarkers

We first sought to study the oxidative-antioxidant capacity of the thymus in chickens infected with REV. We used commercial kits to detect the level of T-AOC, SOD, H_2O_2 activity, and MDA of the chickens in both the infected and control groups. After the chickens were infected with REV, the thymus T-AOC (Fig. A) of the chickens was lower than that of the control chickens. Significant differences were found at 21 ($P<0.05$), 28 ($P<0.01$), and 35 ($P<0.01$) days post-REV infection. Compared with the control group, the SOD activity (Fig. B) of the thymus of the chickens decreased to varying degrees after REV infection, and exhibited significant differences on days 14 ($P<0.01$), 21 ($P<0.05$), and 28 ($P<0.05$) post REV-infection.

Moreover, the level of MDA (Fig. C) in the thymus of REV-infected chickens increased from day 7 to 35 compared with the control group, and exhibited significant changes on day 28 (*P□0.05*). Compared with the control group, the H_2O_2 content (Fig. D) in the thymus of the chickens increased between 14–35 days after REV infection, and showed significant changes on day 35 (*P□0.05*). Compared with the control group, the level of CAT (Fig. E) in the thymus showed a downward trend from day 14 to 35 in REV-infected SPF chickens, and showed significant changes on days 14 (*P < 0.01*), 21 (*P < 0.01*), and 28 (*P < 0.05*). The (Fig. F) level of GPx1 in the thymus was lower than that of the control SPF chickens after REV infection, and showed significant changes on day 28 (*P < 0.05*) post-infection.

CAT And GPx1 mRNA Expression In The Thymus

In REV-infected SPF chickens, the level of CAT (Fig. 2a) expression in the thymus decreased from days 14 to 35 compared to the control group, and was significantly decreased on days 21 (*P < 0.05*) and 28 (*P < 0.05*). From day 7 to 35 post-REV infection, the level of GPx1 expression (Fig. 2b) in the thymus of SPF chickens in the infected group was lower than that in the control chickens; significant changes were observed on days 28 (*P < 0.05*) and 35 (*P < 0.05*).

Determination Of The Thymus Organ Index

In SPF chickens infected with REV, the thymus organ index (Fig. 3) was lower than that of the control chickens, and was significantly lower than the control chickens on days 28 (*P < 0.05*) and 35 (*P < 0.05*).

Histopathological Changes In The Thymus Of REV-infected SPF Chickens

The histomorphology and ultrastructural changes of the thymus are presented in Fig. 4. Compared with the control group, the thymic lobule was atrophied, the cortex and medulla exhibited hemorrhaging, and there was narrowing of the cortical area. In addition, there was a reduced number of lymphocytes in the medulla, an increased number of reticuloendothelial cells, and enhanced inflammatory cell infiltration. Under an electron microscope, the ultrastructure of the control group appeared normal. However, in the infected group, there was obvious cell damage, which primarily manifested as mitochondrial swelling, sputum rupture, Golgi vesicle expansion, and decreased cytoplasm. In addition, the nuclear membrane displayed breaks and had dissolved, and the nucleolus was enlarged.

Discussion

After the body is infected with a virus, ROS activates the immune system to eliminate the infection and provides a positive regulatory role[19, 20]. Simultaneously, the virus breaks the oxidation-antioxidant balance of the host cells, causing the accumulation of a large amount of ROS, which results in cellular

damage and apoptosis[21]. RE is an immunosuppressive tumor disease caused by REV. This test detects changes in the oxidative-antioxidative function of the thymus in chickens after REV infection. It was found that REV can cause oxidative stress in the thymus and reduce antioxidant function.

Under conditions of oxidative stress, ROS and RNS promote the accumulation of lipid peroxidation in polyunsaturated fatty acids in oxidized biofilms, and produce lipid peroxides (e.g., MDA, ketone, hydroxyl, and carboxyl)[22]. Moreover, MDA can reflect the degree of lipid peroxidation in the body and can be used as an indicator to determine whether a cell is oxidatively damaged[23]. H_2O_2 is an important ROS that can be scavenged by catalase or peroxidase, and can also react with free Fe^{2+} to produce ·OH to initiate lipid peroxidation, destroy the cell membrane structure, and accelerate protein decomposition[24]. These findings reveal that the MDA and H_2O_2 content in the thymus of REV-infected SPF chickens was higher than that of the control chickens. The level of H_2O_2 in the thymus on day 35 in REV-infected chickens was significantly higher than that of the control chickens. Moreover, the MDA content in the thymus was significantly higher on day 28 in REV-infected chickens than that of the control chickens. After REV invades the immune system of the chicken host, both non-specific immunity and specific immunity play a role in the generation of a large amount of free radicals, oxidative stress within the body, and enhanced lipid peroxidation[25]. After REV enters a host, it induces NF-κB inhibitory protein I-κB phosphorylation, NF-κB activation, NF-κB translocation into the nucleus to bind to a specific nucleotide sequence of the iNOS gene promoter region, and the initiation of iNOS expression[26]. Studies have found that in the context of sepsis, inflammatory factors can mediate a decrease in nNOS expression in cardiomyocytes, up-regulate iNOS expression, and induce NO synthesis[27, 28]. Moreover, iNOS expression can stimulate macrophages to produce NO, which in turn stimulates the cells to produce more ROS, triggering oxidative stress.

Under normal conditions, SOD can catalyze the disproportionation of $O_2^{\cdot-}$ in the body to produce both H_2O and H_2O_2[14]. H_2O_2 can break the structure and function of the membrane through the cell membrane, react with purines and pyrimidines, and result in nucleic acid damage[29]. CAT and GPx1 work together to clear H_2O_2 from the cell[11]. This study found that REV-infected SPF chickens exhibited lower levels of T-AOC, SOD activity, CAT, GPx1 content, as well as CAT and GPx1 mRNA expression, compared to the control group. We consider one potential reason for this phenomenon is that MDA can disrupt the synthetic SOD pathway, thereby reducing SOD activity. Moreover, the MDA content is negatively correlated with SOD activity. The peroxide deposition causes a large amount of antioxidant enzymes to be consumed in the body, and the function of the antioxidant system is suppressed. Decreased antioxidant enzyme activity leads to an accumulation of oxidizing substances, which can cause inflammatory reactions, cellular damage, and apoptosis. In addition, ROS can activate NF-κB, which can induce the expression of various inflammatory factors (e.g., IL-1, IL-6, and TNF-α) and aggravate the inflammatory response [30,31,32]. Chickens infected with REV exhibit immunosuppression. Combined with the results of this study, the immunosuppression caused by REV may be related to an accumulation of oxidizing substances and a decline in antioxidant system functionality.

Conclusion

Following REV infection in one day old SPF chickens, the content of MDA and H_2O_2 and superoxide in the thymus was increased, the T-AOC and SOD activity decreased, and the thymus organ index was decreased. These changes exceeded the body's ability to scavenge antioxidant substances, resulting in oxidative stress in the body.

Materials And Methods

Experimental animals and infectious virus strain

All of the chickens used for experiments were one-day-old SPF chickens obtained from Harbin Veterinary Research Institute (HVRI), Chinese Academy of Agricultural Sciences (CAAS), China. The REV-T strain (CVCC No. CACCAV107) was purchased from China Veterinary Culture Collection Center (CVCC). The virus $TCID_{50}$ was $10^{4.62}$/0.1 mL as determined by cell breeding.

Experimental Design And Sample Collection

A total of 80 one-day-old SPF chickens were randomly divided into a control group (Group C) and a REV-infected group(Group I). Group I were intraperitoneally administered with 100 μL of viral suspension at one-day-old. Group C were intraperitoneally administered with 100 μL of sterile physiological saline. The two groups of chickens were housed in isolators with similar environments,and chickens were given free access to feed and water. During housing, animals were monitored twice daily for health status. No adverse events were observed. On days 3, 7, 14, 21, 28, 35, and 49 post-REV infection, five chickens were randomly selected from each group and euthanasia was performed by sedation using Rompun/Ketamine mixture as an intramuscular injection followed by an intravenous injection of Pentobarbitone. Thymus were dissected and homogenized in chilled sterile physiological saline using a glass homogenizer with a teflon pestle under cold condition. The homogenate was centrifuged at 4000 g for 10 min at 4 °C. The supernatant was used for the assay of oxidative stress biomarkers.

Determination Of Thymic Oxidative Stress Biomarkers

The prepared 10% tissue homogenate was removed from − 80 °C. The T-AOC, SOD, H_2O_2 activity, and MDA level in the thymus was determined using commercially available detection kits (Nanjing Jiancheng Bioengineering Research Institute, China). The level of CAT and GPx1 was measured by an ELISA, according to the manufacturer's instructions (Qiyi Biological Technology Co., Ltd. China).

Total RNA Extraction And Detection

The total RNA of thymus was extracted using Trizol reagent(Invitrogen ,Shanghai, China) The quality of RNA was assessed using a NanoDrop2 000 spectrophotometer (Thermo Fisher Scientific) through the ratio of absorbance at 260 nm and 280 nm. cDNA was obtained by reverse transcription of the RNA using the M-MLV Reverse Transcriptase kit (Invitrogen) according to the manufacturer's instructions.

Real-time PCR

Determination of CAT and GPx1 mRNA in the thymus by real time PCR. The CAT, Gpx1, and β-actin primers were designed using Primer5.0 software. CAT: forward primer 5'-ATGTCCGTTTCAGGAGATGTGCAGC-3', reverse primer 5'- CCAGCAGTGCCTGAATACG – 3'; GPx1: forward primer 5'- ATGACCAACCCGCAGTACATCATCT-3', reverse primer 5'- GCAGTTTGATGGTCTCGAAGTGGC-3'; β-actin forward primer 5'- CGGGACGGATGAGAAGAA – 3', reverse primer 5'- TCGGCGCTCCAGATGTAC – 3'. Amplification of the target gene using the LightCycler2.0 real-time PCR System (Roche480, USA). The real-time PCR reaction procedure was 95 °C for 2 min, 45 cycles of 95℃ for 20 s, 59℃ for 20 s, and 72℃ for 10 s. To ensure the repeatability of the amplified samples, all samples were analyzed in triplicate. β-actin was used as a reliable normalization gene, and the expression of the samples were evaluated in relation to this housekeeping gene. The level of CAT and GPx1 mRNA expression were analyzed in accordance with the $2^{-\Delta\Delta Ct}$ method.

Determination Of The Thymus Index

Weight of the chickens and thymus at the time of sampling for calculating the thymus index. The thymus index was calculated by the weight of the thymus (g)/chicken weight(kg).

Pathological Changes Of The Thymus After A REV Infection

Thymus tissues were fixed in 4% formalin. The samples were dehydrated with ethanol and embedded in paraffin. Each sample was cut into 5-μm sections with a microtome and placed onto a glass slide, and stained with hematoxylin and eosin (H&E). The pathological changes of the thymus were observed under a both a light microscope (Nikon, H600, Japan) and an electron microscope (Hitachi 7650, Tokyo, Japan).

Statistical Analyses

The data were analyzed with SPSS 17.0 software, and the differences were compared between the groups were analyzed using one-way ANOVA followed by Tukey’s post-hoc test. All data were expressed as the mean ± SD. $P < 0.05$ was considered to be statistically significant.

Abbreviations

REV: Reticuloendotheliosis virus; SPF: Specific pathogen free; MDA: malondialdehyde; T-AOC: Total-antioxidante capacity; SOD: superoxide dismutase; ELISA: Enzyme linked immunosorbent assay; CAT: catalase; GPx1: Glutathione peroxidase 1; ROS: Reactive oxygen species; RNS: Reactive nitrogen species

Declarations

Ethics approval and consent to participate

According to the Australian National Health and Medical Research Council's Australian Nursing and Use Regulations, the Animal Ethics Committee of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences approved all experimental procedures with animals (SYXK [Hei] 2011022) for scientific purposes. All procedures were performed in accordance with the institutional guidelines.

Authors' contributions

Z.SM designed the experiments and revised manuscript. Z.MX performed the experiments of REV infection. Z.CH and Z.SJ performed the experiments of Morphological analysis. Y.DH analyzed the data and drafted manuscript. G.XL and Z.J oversaw the experiments. L.XP and L.CN aided with analysis of results. All authors have read and approved the manuscript and ensure that this is the case.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by the National Natural Science Foundation of China (31472169). The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Acknowledgments

Not Applicable.

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Figures

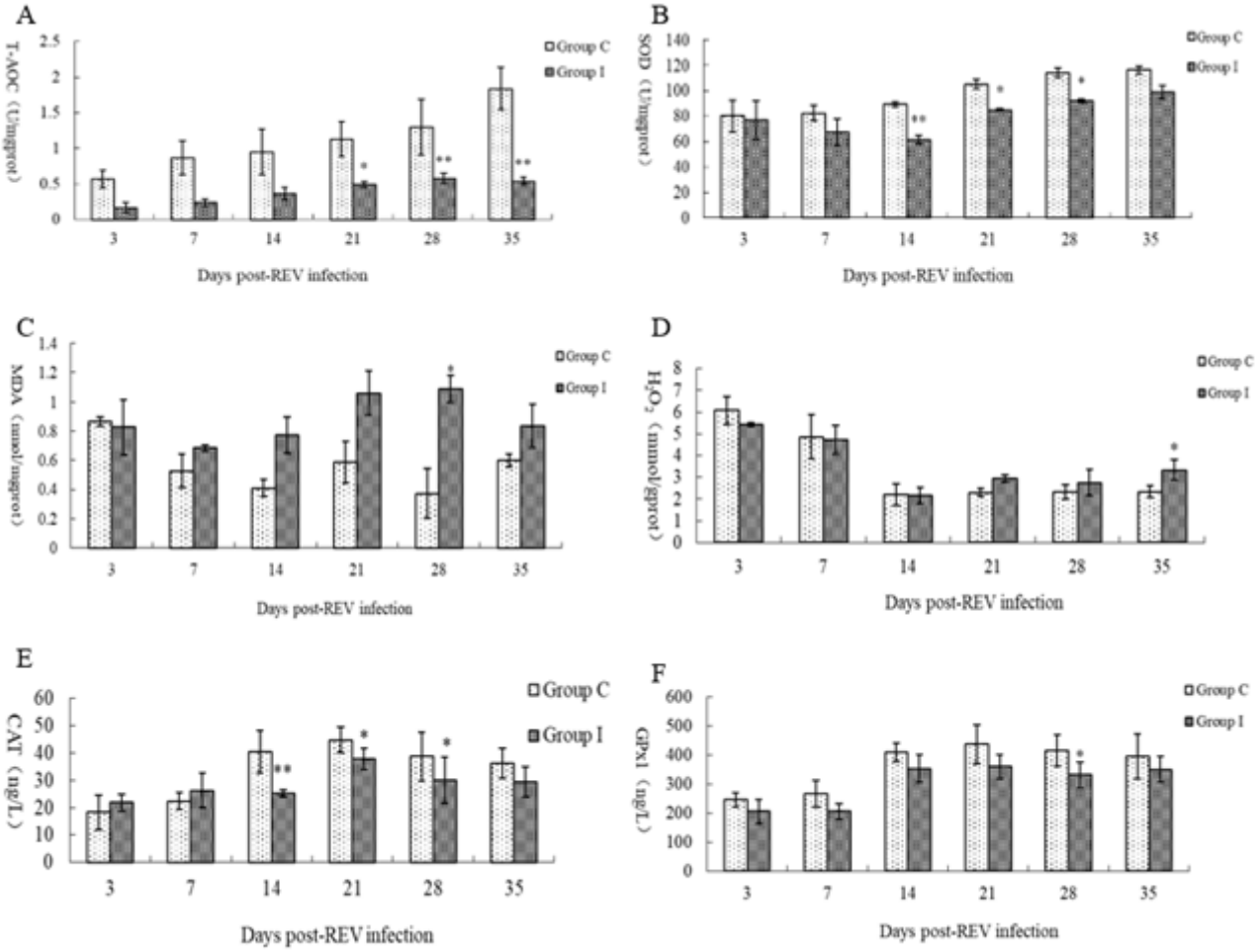

Figure 1

Changes in the oxidative stress biomarkers in chickens infected with REV. (A) The total antioxidant capacity (T-AOC) of the thymus. (B) The superoxide dismutase (SOD) activity in the thymus. (C) The level of malondialdehyde (MDA) in the thymus. (D) The level of H2O2 in the thymus. (E) The level of catalase (CAT) in the thymus. (F) The level of glutathione peroxidase 1 (GPx1) in the thymus. Data are presented as the means ± SD (n = 5).*(P< 0.05) and **(P< 0.01) indicates a significant difference when compared to the control.

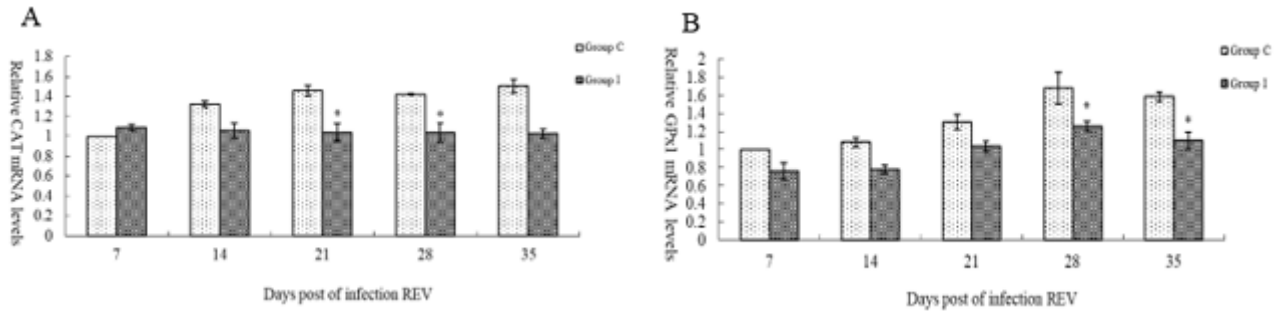

Figure 2

Changes in CAT and GPx1 mRNA expression. (A) CAT mRNA expression in the thymus. (B) Level of GPx1 mRNA expression in the thymus. Data are expressed as the means ± SD (n = 5).*(P< 0.05) and **(P < 0.01) indicates a significant difference compared to the control group.

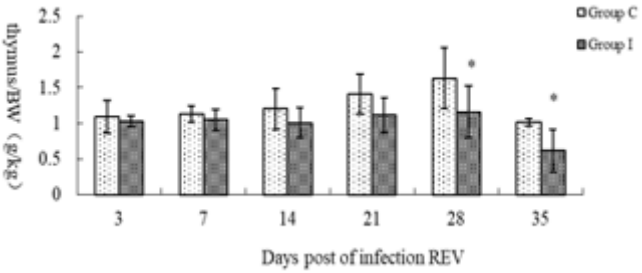

Figure 3

Changes in the thymus organ index. Data are expressed as the means ± SD (n = 5).*(P< 0.05) and **(P< 0.01) indicate a significant difference compared to the control group.

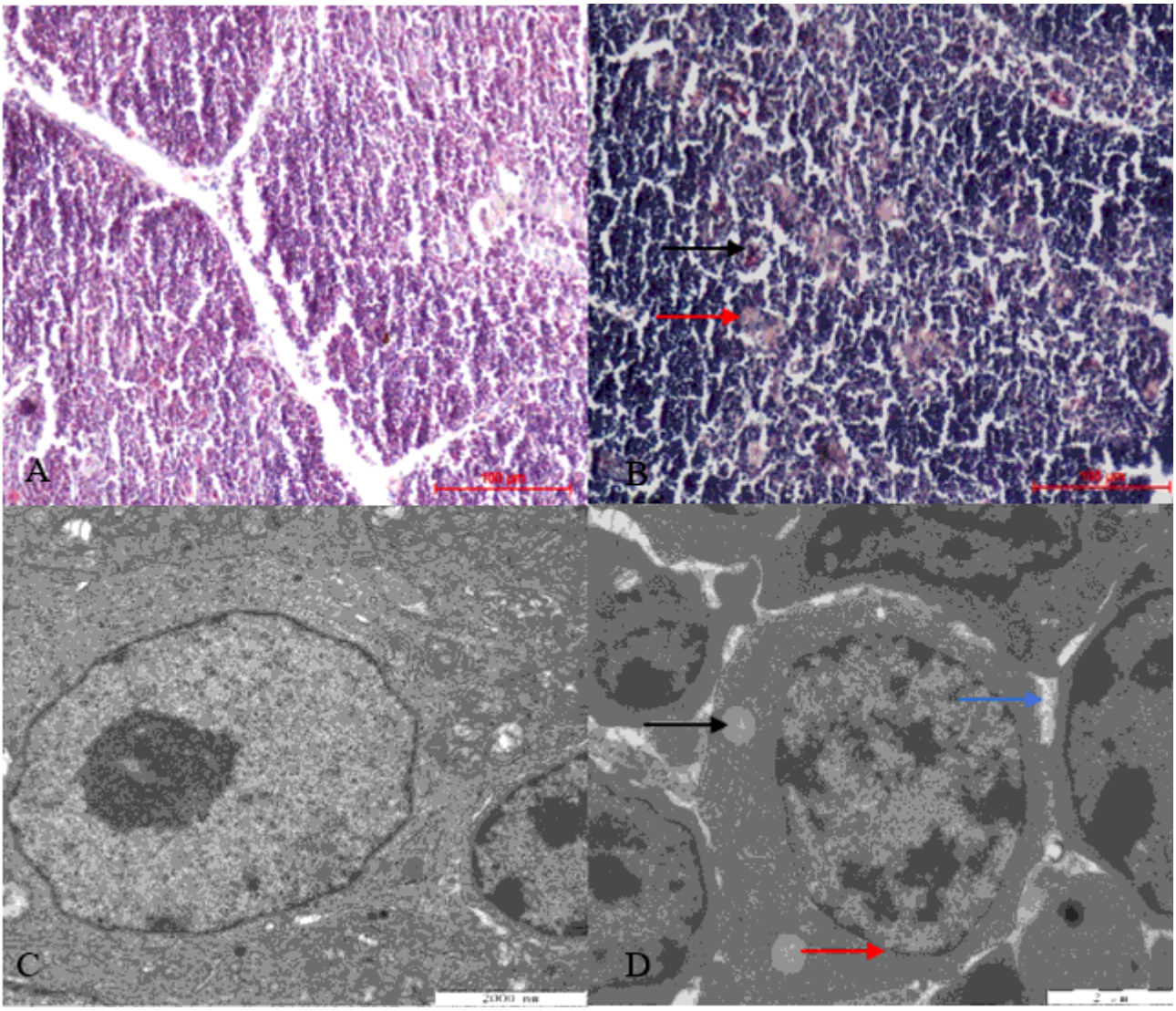

Figure 4

Histological and ultrastructural changes in the chicken thymus after REV infection. Histological examination of the thymus in the control group (A) and REV infection group (B) of chickens by HE staining (200x). Histological results showed significant necrosis (red ↑) and haemorrhage (black ↑) in the REV infection group. Transmission electron microscopy examination of the thymus in the control group (C) and REV infection group (D) of chickens (15 000x).Ultrastructural results showed that the REV infection group showed nuclear membrane rupture (red ↑), mitochondrial vacuolization (black ↑) and enlarged intercellular space (blue ↑).

Supplementary Files

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