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Research

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Classification for avian malaria parasite *Plasmodium gallinaceum* blood stages by using deep convolutional neural networks

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Abstract

Background: The infections of an avian malaria parasite (*Plasmodium gallinaceum*) in domestic chickens presents a major threat to poultry industry because it cause economical loss in both quality and quantity of meat and egg productions. Deep learning algorithms have been developed to identify avian malaria infections and classify its blood stage development.

Methods: In this study, four types of deep convolutional neural networks namely Darknet, Darknet19, darknet19_448x448 and Densenet 201 are used to classify *P. gallinaceum* blood stages. We randomly collected dataset of 10,548 single-cell images consisting of four parasite stages from ten-infected blood films stained by Giemsa. All images were confirmed by three well-trained examiners.

Results: In the model-wise comparison, the four neural network models gave us high values in the mean average precision at least 95%. Darknet can reproduce a superior performance in classification of the *P. gallinaceum* development stages across any other model architectures. In addition, Darknet also has best performance in multiple class-wise classification, scoring the average values of greater than 99% in accuracy, specificity, sensitivity, precision, and F1-score.

Conclusions: Therefore, Darknet model is more suitable in the classification of *P. gallinaceum* blood stages than the other three models. The result may contribute us to develop the rapid screening tool for further assist non-expert in filed study where is lack of specific instrument for avian malaria diagnostic.

Keywords: *Plasmodium gallinaceum*, Malaria, Rapid screening, Classification, Deep learning

Background

Avian malaria, a mosquito-borne disease, is one of the most common veterinary threats in tropical regions, including South East Asia and South Asia [1]. *Plasmodium gallinaceum* is an important causative agent of avian malaria, which causes more than 80% mortality if left untreated [2]. The disease entails an economic and agricultural loss in poultry processes systems such as poor quality and quantity of meat and egg production [1]. Early and rapid routine screening for a safe and low-cost parasite infection may help prevent transmissions of the disease. Microscopic inspection is a gold standard approach and is widely used to examine and identify avian malaria-infected blood stages under thin-blood film examinations, the outcome of which is validated by highly trained and qualified technicians [3]. Nevertheless, the precise outcome of the above-mentioned procedure depends on the consistency of blood smearing and staining process. In addition, asymptomatic with low parasite infection can be time-consuming and may be undetectable or questionable as a result of inter/intra examiner variability [3]. In addition, unspecific clinical symptoms of avian malaria, such as anorexia, anemia, and green stools, are often seen [1, 4]. While most of the nucleic acid-based amplification process, such as the polymerase chain reaction (PCR) assay, is an efficient method with a high sensitivity and malaria detection specificity, it requires an optimal consistency of genomic DNA and expensive tools such as thermocycler and electrophoresis apparatus [5-7]. A professional molecular biologist is also required for further verification of the analysis. Consistently, the molecular biology approach could not always be affordable in low-income and resource-limited nations.

Artificial intelligence (AI) technology is currently a positive integration success in a diverse area of interest, including agriculture, medicine and veterinary medicine [8-15]. However, the methods are combined and tailored for individual datasets that differ in size of the region of interest. In specific, deep learning technology is applied to end-to-end methods of extraction of features and self-discovery. Computer-aided machine learning algorithms, AI subdivision, have been developed to identify human malaria infections and to classify their blood stage of the parasite growth. These can be used to assist clinical decision-making. Machine learning applications have also been studied in the documented veterinary field [16]. The previous work demonstrated micro-platforms to study and identify the infected avian red blood cell by using morphological modifications on the RBC surface to reveal the phases of *P. gallinaceum* [17]. Since malaria has been described as a disease of blood and blood-forming tissues, the blood film sample has been

87 diagnosed to better understand different degrees of disease [18]. Early rapid screening of parasite
88 infection with reliable and also low cost development is required, which could help us deter the
89 spread of the disease. Therefore, timely identification of malaria parasite in a blood smear test is
90 crucial because it needs reliable and early diagnosis for successful containment [19].

91 Deep learning is a revolutionary and groundbreaking approach that has been developed to
92 incorporate a microscopic analysis for the veterinary medicine field. Previous research have
93 suggested a diagnostic tool in veterinary medicine focused on image analysis using machine
94 learning techniques [20-22], such as fish disease diagnosis based microscopic method [23]. The
95 analysis referred to above is intended to enhance the inspection of the pathogen region in the image
96 by means of object detection, which includes various techniques, including noise reduction, edge
97 detection, morphological operations and context extraction. In addition, several neural network
98 models have been used to contend with the animal sector, such as the support vector machine
99 (SVM) [24, 25], Single-Shot MultiBox Detector (SSD) model used to evaluate the percentage of
100 reticulocytes in cat's samples [26], Alexnet for classification of fish disease [27]. The works
101 described above makes it possible to apply deep learning algorithms in the field of veterinary
102 medicine.

103 The deep learning algorithm is very popular and useful with the emergence of a high-power
104 computing machine to be used to study the classification of images and the recognition of clinical
105 issues. Previously, several techniques for image-based identification and classification of malaria
106 parasite infections have been discussed, such as dark stretching technique [28, 29], modified fuzzy
107 divergence technique [30], segmentation techniques [19], adaptive color segmentation and tree-
108 based decision classification [31], segmentation, feature extraction, and SVM classifier [32],
109 convolutional neural classification [33, 34]. Moreover, deep CNN research have been conducted
110 under the channel color space segmentation method [35], deep belief network technique [36], the
111 Faster Region-based Convolutional Neural Network (Faster R-CNN) [37], and the MATLAB-
112 based Zach Threshold process for segmentation technique [38]. Successfully, several studies have
113 reported that the use of deep learning models to classify malaria parasites as automatic, rapid, and
114 accurate approaches [39-41]. Interestingly, more than 95% of the accuracy is recorded in the
115 detection of malaria-infected cells using three well-known CNNs, including LeNet, AlexNet, and
116 GoogLeNet [42].

Vijayalakshmi and Kanna (2020) used VGG19-SVM to detect high-performance infected and non-infected malaria parasite images as follows: 93.44 per cent sensitivity; 92.92 per cent specificity; 89.95 per cent precision, 91.66 per cent F-score and 93.13 per cent accuracy [43]. Many CNNs have been continuously created in the last year following the application of LeNet, AlexNet, GoogLeNet and VGG. CNN has also been developed to provide a number of pre-trained ImageNet classification models [44], including AlexNet, Darknet, VGG-16, Resnet [45], ResNext, and Densenet201 [46], for use as effective in image classification applications. According to an evaluation of the performance of these pre-trained models, Densenet201 has a higher accuracy than Darknet. Nevertheless, Darknet provides the fastest processing time in both CPU and GPU. In the application area, Darknet19 obtained the best result in differentiating chest X-Rays of COVID-19 patients from other bacterial and viral pneumonias than all others of 16-deep CNNs [47]. Although deep learning approaches have been achieved to identify CT scans of the chest and X-ray of the chest for diagnosis of pneumonia caused by coronavirus infection, Densenet201 is more capable of classifying and detecting medical images than some well-known deep transfer learning models; namely VGG16, Inception ResNet, Resnet 152v2, AlexNet, ResNet18 and SqueezeNet [48].

In this work, we implement four types of deep convolutional neural networks, namely Darknet, Darknet19, darknet19 448x448 and Densenet 201, to characterize the avian malaria blood stages of *P. gallinaceum*. We compare the output of the deep convolutional neural network model to facilitate the prediction of parasitized and normal chicken's RBCs in thin blood film photos. The stage of malaria development is also diagnosed and infers the extent of infection as well as the risk of malaria transmissions. In addition, to author's knowledge, this research work is the first time that the CNNs deep learning model has been incorporated into the classification of clinical datasets relevant to clinical problems and would be useful for the disease diagnosis method in the poultry industry.

Materials and Methods

Data collections

Archived Giemsa-stained thin-blood films were have been collected from previous studies [1, 49]. The Protocol on Animal Use was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University (No. 1931091). The parasite infection was confirmed by three well-trained investigators using a microscopic diagnosis. Ten *P. gallinaceum*-infected chicken blood films were randomly chosen. A total of 432 photographs of malarial developmental stages were captured at 1000x by oil immersion magnification using a light microscope (Olympus CX31, Tokyo, Japan). The photos were taken with Olympus DP21-SAL digital camera (Tokyo, Japan). Of which, an individual RBC picture was chosen to a photograph a blood film from a region of interest to the so-called monolayer area. Each single-parasitized RBC image has been individually cropped under the CIRA CORE framework module (Fig. 1). The dataset consists of 10,548 cell images with a significant proportion of regions of interest (ROI). Every class obtained from any picture was randomly divided into training (80 per cent) and testing (20 per cent) sets, minimizing potential similarities between these two sets. The physiological and morphological class labelling of the training collection was accompanied by ‘normal RBC’, ‘trophozoite’, ‘schizont’, and ‘gametocyte’, respectively. While disproportionate sample size between classes has been identified and can trigger biased-prognosis against a class with a large number of image sets, deep learning approach with multi-layered layers and data annotation can be explored prior to model training. To speed up model convergence, rescale the image from raw pixels to 448 x 448 pixels before training with a chosen neural network model.

Figure 1. Prediction of the outcome of selectable models. The color of the guide map indicated the field of interest predicted by the model. The X-axis is the raw picture as input and models such as Darknet, Darknet19, Darknet19_448 and Densenet201. Y-axes are individual chicken-blood cells that are regular and those that are infected malaria, including trophozoites, schizonts and gametocytes.

Model selection and data augmentation

The selectable models used are the top ranking of the ILSVRC2016 ImageNet competition to identify pathological tasks. These versions are in the following sizes; Densenet-201, Darknet, Darknet-19 and Darknet-19 448x448. These models were preprocessed in the “DeepClassif” module under the CiRA Core base (Fig. 2). In order to avoid over-fitting of the training picture collection, the data augmentation was produced prior to training. Data augmentation techniques involve rotations, brightness/ contrast, turning, blueness and Gaussian noise. In addition, the trained models were early paused to register the learned parameters before the end of their training loss of 100,000 epochs. The likelihood of a threshold greater than and equal to 50% is considered to be a true positive value. The ROI was automatically masked and colored with a JET color map.

Figure 2. The schematic of the deep learning classification of the Darknet19 neural network model. Filters each convolution layer help generate feature map in the part of feature extraction. Guide map of the JET color gradient is used to visualize the input based on optimal weight.

These models mentioned above were trained on the LINUX operating system based Ubuntu version 16.04, 16 GB RAM, and NVIDIA GeForce GTX1070Ti graphic processor unit with less than 24 h of commuting time. The model configuration is performed in C++ using Keras’s API with Tensorflow backend and CUDA/CuDNN dependencies.

Model evaluations

The performance quality of the model- and class-wise prediction were evaluated in terms of the following statistical metrics: accuracy, precision, sensitivity, specificity, misclassification rate and F1 score [50].

$$Precision = \frac{TP}{TP+FP} \dots\dots\dots (1)$$

$$Sensitivity = \frac{TP}{TP+FN} \dots\dots\dots (2)$$

$$Specificity = \frac{TN}{TN+FP} \dots\dots\dots (3)$$

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \dots\dots\dots (4)$$

$$Missclassification\ rate = \frac{FP + FN}{TP + TN + FP + FN} \dots \dots \dots (5)$$

$$F1\ score = \frac{2 \times (sensitivity \times precision)}{sensitivity + precision} \dots \dots \dots (6)$$

where TP is the number of true positive values, TN is the number of true negative values, FP is the number of false positive values, and FN is the number of false negative values.

Confusion matrix table was created to demonstrate the overall accuracy of both the model and class classification. An area under the Receiver Operating Characteristic (ROC) curve (AUC) at 95% confident of interval (CI) was designed to calculate the average accuracy of the model using python software.

Results

Comparison of neural network model performance

In this analysis, model-wise performance was assessed as to whether the model was the best-selected model based on a guide map and used to estimate *P. gallinaceum*-infected blood phases. These models included Darknet, Darknet-19, Darknet-19_448 and Densenet201 as described above. These simulations were calculated and compared the robustness of the neural network models for the classification of avian malaria parasite stages using average accuracy, uncertainty confusion matrix table and ROC curve. In addition, we randomly split the single-cell images for the training and testing of the image sets to determine whether the different models chosen would produce the same classification outcome. Each single-cell image may be viewed in either a training/ validation or a test range. All well networks have offered us high average accuracy values of over 95 per cent, which is up to 99 per cent for the darknet algorithm (Table 1). General accuracy from the confusion matrix table of the darknet model showed a better value than 97 per cent as comparison to every other model (Table 2). Specifically, this manifested in an overall AUC ranking of 0.986 - 1.000 (Fig. 3). Overall, the darknet models can reproduce superior performance in the classification of the development phases of *P. gallinaceum* in every other model architecture.

235 **Table 1.** Model-wise comparison based on precision per class and the average.

DCNNs	Mean average precision	Normal	Trophozoite	Schizont	Gametocyte
Darknet	0.990	0.993	0.968	1.000	1.000
Darknet19	0.990	0.992	0.966	1.000	1.000
Darknet19_448	0.983	0.966	0.965	1.000	1.000
Densenet201	0.954	0.964	0.956	0.896	1.000

236

237 **Table 2.** Comparison of the studied model performance using the confusion matrix table based
238 on the test image set.

Darknet	Normal	Trophozoite	Schizont	Gametocyte
Normal	97.38%	0.83%	0.00%	0.00%
Trophozoite	2.62%	99.17%	0.00%	0.00%
Schizont	0.00%	0.00%	100.00%	0.00%
Gametocyte	0.00%	0.00%	0.00%	100.00%

Darknet19	Normal	Trophozoite	Schizont	Gametocyte
Normal	97.21%	1.03%	0.00%	0.00%
Trophozoite	2.79%	98.97%	0.00%	0.00%
Schizont	0.00%	0.00%	100.00%	0.00%
Gametocyte	0.00%	0.00%	0.00%	100.00%

Darknet19_448	Normal	Trophozoite	Schizont	Gametocyte
Normal	97.21%	4.34%	0.00%	0.00%
Trophozoite	2.79%	95.66%	0.00%	0.00%
Schizont	0.00%	0.00%	100.00%	0.00%
Gametocyte	0.00%	0.00%	0.00%	100.00%

Densenet201	Normal	Trophozoite	Schizont	Gametocyte
Normal	96.56%	4.55%	0.00%	0.00%
Trophozoite	3.44%	94.01%	0.00%	0.00%
Schizont	0.00%	1.45%	100.00%	0.00%
Gametocyte	0.00%	0.00%	0.00%	100.00%

Figure 3. Comparison of the model- and class-wise performance based on AUC under the ROC curve

Multiple class comparison

According to the process, we fed a single cell into the studied model and allowed it predicted the result with the testing image set which is managed by experts. An observation of the classification result gave an empirical result in discriminating the malaria parasite stages based on the best-selected network model.

Although we trained the model with an unequal number of sample sizes between classes, the multiple class-wise quality of the neural network model showed great statistical values for all classes with greater than 99% of all performance matrices used without bias towards any class (Table 3). In addition, our selected model can classify the malaria stages with a low rate of misclassification less than 1%. This may be the advantage of using the class-balancing (extra function) before starting the model training within the CiRA CORE program. This study result suggests the possible validation of the model with various blood smears obtained from real situations.

Table 3. Different statistical metrics of darknet neural network models.

Class Name	Accuracy	Misclassification	Sensitivity	Specificity	Precision	F1-Score
		Rate				
Normal	0.983	0.017	0.974	0.993	0.993	0.983
Trophozoite	0.983	0.017	0.992	0.976	0.968	0.980
Schizont	1.000	0.000	1.000	1.000	1.000	1.000
Gametocytes	1.000	0.000	1.000	1.000	1.000	1.000
Average	0.992	0.009	0.992	0.992	0.990	0.991

Discussion

In this research, the robustness of deep neural network models leads to the development of new method for more rapid screening under a microscope of avian malaria. Specifically, asymptomatic infections that can lead to the spread of the disease and even cause mortality without well-planned prevention [51, 52]. Though we have trained network models with a real patient-level yet unbalanced size of each class, the performance of well-trained proposed models has

shown an outstanding outcome based on many statistical parameters, including in a multi-class comparison. Since the experiment proved to be very successful, it could contribute to the achievement of the development of innovative technology in order to apply and test it in a real situation. Nevertheless, in the near future, the inter-and intra-variability examinations should be analyzed in order to approve the degree of agreement between the practical success measurements of the predictive model and the human expert before the realization of the technology [53].

Limitation of the study is deeply based on the determination of three key points for the preparation of the dataset [54], including 1) differences in the parasite-blood stages [55], 2) infection status that can induce either single or multiple infections, and 3) co-infections in any single blood cell. Another result of dataset planning, though less than those mentioned above is the imbalance data. In the case of differences in the blood stage of each parasite, an increasing number of sample is a potential solution, however the analysis has had an effect on model efficiency under the research design. This may be because the picture set is a special attribute between classes that helps to increase class differentiation for the well-trained models. In addition, we selected an image of almost a single stage of parasite infection to be analyzed. Since the staining of artefacts/impurities of uninfected blood cells can interfere with the prediction, the expert's simple staining of color under the Giemsa staining process also provides us with the ideal dataset [56]. Nevertheless, the model must be validated in a specific environment of multiple pathogens and co-infections, which could already exist in field samples.

Conclusions

Here, the application of a deep neural learning network to identify and verify avian malaria and also to assess it using truly invisible images that have never been published. Based on the findings of the report, our excellent CiRA CORE framework concept was demonstrated. Based on our results, we can infer that there is only a model learning approach and data preparation that should be used in the research design. Fortunately, the analysis revealed a high importance of different statistical measures as mentioned above, which could be of interest to decision-making help for cytological technicians. It would be useful to diagnosis malaria infections wherever it is due to a shortage of molecular biology and veterinary knowledge.

Abbreviations

PCR: polymerase chain reaction; DNA: deoxyribonucleic acid; AI: artificial intelligence; RBC: red blood cell; SVM: support vector machine; SSD: single-short multibox detector; Faster R-CNN: faster region-based convolutional neural network; ROI: region of interest; DCNN: deep convolutional neural network; TP: true positive; TN: true negative; FP: false positive; FN: false negative; CI: confident interval; ROC: receiver operating characteristic curve; AUC: area under the ROC curve.

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Author's contributions

SB, VK and SC conceived and designed the research study; SB, VK, MK, KMN and SC wrote the manuscript; VK, KMN, RJ, KK and SC performed the computational experiment; MK and ST performed the parasite investigation; TT, SC and SB conducted the computer's platform. SB read and approved the final manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author's GitHub repository: URL: <https://git.cira-lab.com/cira-medical/classification-of-avian-malaria-parasite>

Ethics approval

The Protocol on Animal Use was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University (No. 1931091).

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interest.

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Figures

Figure 1

Prediction of the outcome of selectable models. The color of the guide map indicated the field of interest predicted by the model. The X-axis is the raw picture as input and models such as Darknet, Darknet19, Darknet19_448 and Densenet201. Y-axes are individual chicken-blood cells that are regular and those that are infected malaria, including trophozoites, schizonts and gametocytes.

Figure 2

The schematic of the deep learning classification of the Darknet19 neural network model. Filters each convolution layer help generate feature map in the part of feature extraction. Guide map of the JET color gradient is used to visualize the input based on optimal weight.

Figure 3

Comparison of the model- and class-wise performance based on AUC under the ROC curve