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Original Research Paper

Effect of Stingless Bee Honey (*Tetragonula iridipennis*) Supplementation on Differential Leukocyte Counts and Platelet Indices in *Tenyi Vo* Pigs

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KeyWords

Antioxidant activity, Differential leukocyte counts (DLC), Immunomodulator, Platelet indices, Stingless bee honey and *Tenyi Vo* pigs.

Abstract

Background: The present study evaluated the effects of dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey on the hematological profile, immune response, and platelet characteristics of indigenous *Tenyi Vo* pigs.

Methods: Twenty-four healthy female weaned piglets aged 56–60 days were randomly divided into four groups, with six pigs per group. The control group received no honey, while the three treatment groups received 0.50, 1.00 and 1.50 mL of stingless bee honey per litre of drinking water, respectively. Blood samples were collected at the end of the experimental period to assess hematological and platelet parameters.

Results: Honey supplementation significantly affected differential and absolute leukocyte profiles. The highest neutrophil percentage (45.60%) and absolute neutrophil count (9.112 × 10^9/L) were recorded at 1.50 mL/L, while the highest lymphocyte percentage (72.80%), absolute lymphocyte count (18.438 × 10^9/L), monocyte count (1.700 × 10^9/L) and basophil count (0.610 × 10^9/L) were observed at 1.00 mL/L. The highest eosinophil percentage (3.26%) and absolute eosinophil count (0.722 × 10^9/L) were recorded at 1.50 mL/L. Platelet count and plateletcrit were not significantly affected, although mean platelet volume, platelet distribution width, and platelet large cell ratio differed among treatments. Overall, stingless bee honey influenced leukocyte profiles without adversely affecting platelet homeostasis, indicating its potential as a natural feed additive for natural source of antioxidants and antimicrobial compounds with supporting immune health in *Tenyi Vo* pigs.

INTRODUCTION

Pig production plays an important role in the livelihood and socioeconomic development of tribal communities in Nagaland, where indigenous pigs are valued for their adaptability, reproductive efficiency and ability to provide relatively rapid economic returns. The *Tenyi Vo* pig is an important indigenous genetic resource of the region and is well adapted to the local production environment. However, maintaining health and immune competence remains essential for improving the productivity and sustainability of indigenous pig production systems. Nutritional strategies using natural bioactive products may provide a practical approach for supporting animal health while reducing dependence on synthetic growth-promoting and therapeutic agents.

Honey is a natural product containing carbohydrates, vitamins, minerals, phenolic compounds, flavonoids and other biologically active constituents. Its antioxidant, antimicrobial and immunomodulatory properties have attracted considerable interest in animal nutrition. Haydak et al. (1942) reported the presence of several vitamins in honey, whereas Gheldof et al. (2002) and Kishore et al. (2011) associated its antioxidant activity with phenolic and flavonoid compounds. Abubakar et al. (2012) further described honey as a source of both enzymatic and non-enzymatic antioxidants. Dietary honey supplementation has also been associated with improved productive and physiological responses in poultry (Obun et al., 2008; Abioja et al., 2012; Abioja et al., 2016; Oke et al., 2016; Adekunle et al., 2017). These findings suggest that honey may have potential as a natural dietary supplement for improving physiological resilience and immune function.

Stingless bee honey, particularly that produced by Tetragonula iridipennis, is of growing interest because of its diverse bioactive constituents and antioxidant potential. Phenolic compounds and flavonoids present in honey may help counteract oxidative stress and support cellular and immune functions (Al-Hatamleh et al., 2020; Ranneh et al., 2021). Hematological characteristics provide useful indicators of the physiological and immune status of

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animals. Differential leukocyte profiles reflect alterations in innate and adaptive immune responses, whereas platelet count and platelet indices provide information related to platelet production, activation and haemostatic function (Jain, 1993; Weiss and Wardrop, 2010). Therefore, assessment of these parameters can provide valuable evidence regarding the physiological effects and safety of dietary supplementation.

Although the biological properties of honey have been investigated in humans and poultry, information concerning the effects of stingless bee honey on hematological characteristics, particularly differential leukocyte counts and platelet indices, in indigenous pigs is limited. In particular, evidence is lacking for *Tenyi Vo* pigs, despite their importance in the livestock production systems of Nagaland. The present study therefore addresses this knowledge gap by evaluating the effects of dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey on differential leukocyte counts, absolute differential leukocyte counts and platelet indices in *Tenyi Vo* pigs. It was hypothesized that dietary supplementation with stingless bee honey would positively modulate leukocyte profiles and maintain normal platelet characteristics, thereby indicating beneficial immunomodulatory effects in *Tenyi Vo* pigs.

2. MATERIAL AND METHODS

2.1 Experimental Animals

Twenty-four (24) healthy *Tenyi Vo* female weaned piglets, aged 56–60 days, were selected from the ICAR–All India Coordinated Research Project (AICRP) on Pig, School of Agricultural Sciences, Nagaland University, Medziphema Campus. The piglets were dewormed with Fenbendazole (5 mg kg^{-1} body weight) for three consecutive days before the start of the experiment and identified using numbered plastic ear tags. The animals were randomly allotted to four treatment groups (T_1, T_2, T_3 and T_4), with six piglets in each group. They were initially housed in group pens and later transferred to individual pens for proper management throughout the experimental period.

2.2 Experimental Diet

The experimental piglets were randomly assigned to four dietary treatments. Group T_1 served as the control and received only the basal diet, while groups T_2, T_3, and T_4 received stingless bee (*Tetragonula iridipennis*) honey supplementation at 0.5, 1.0, and 1.5 mL per litre of drinking water, respectively.

2.3 Feeding Management

All experimental animals were fed a standard concentrate diet formulated according to the nutrient requirements recommended by ICAR (2013). Feed was offered twice daily in measured quantities, and fresh drinking water was available ad libitum throughout the study. The concentrate mixture was prepared in batches at the farm feed unit using conventional feed ingredients.

2.4 Blood Collection and Hematological Analysis

At the end of the experimental period, blood samples were collected from one representative female sow from each treatment group for hematological analysis. Blood collection was performed following the procedures described by Sastry (1985). The collection site on the ear was shaved, disinfected with tincture of iodine, and allowed to dry. Approximately 6 mL of blood was collected aseptically from the dorsal auricular vein using a sterile 20-gauge needle and syringe. The blood was immediately transferred into EDTA-coated tubes, gently mixed to prevent clotting, and analyzed for differential leukocyte counts and platelet indices.

2.5 Statistical Analysis

The experimental data were analyzed using a Completely Randomized Design (CRD) following the procedures of Snedecor and Cochran (1994). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 1% level of significance ($P < 0.01$).

3. RESULT AND DISCUSSION

3.1 Differential Leukocyte Count

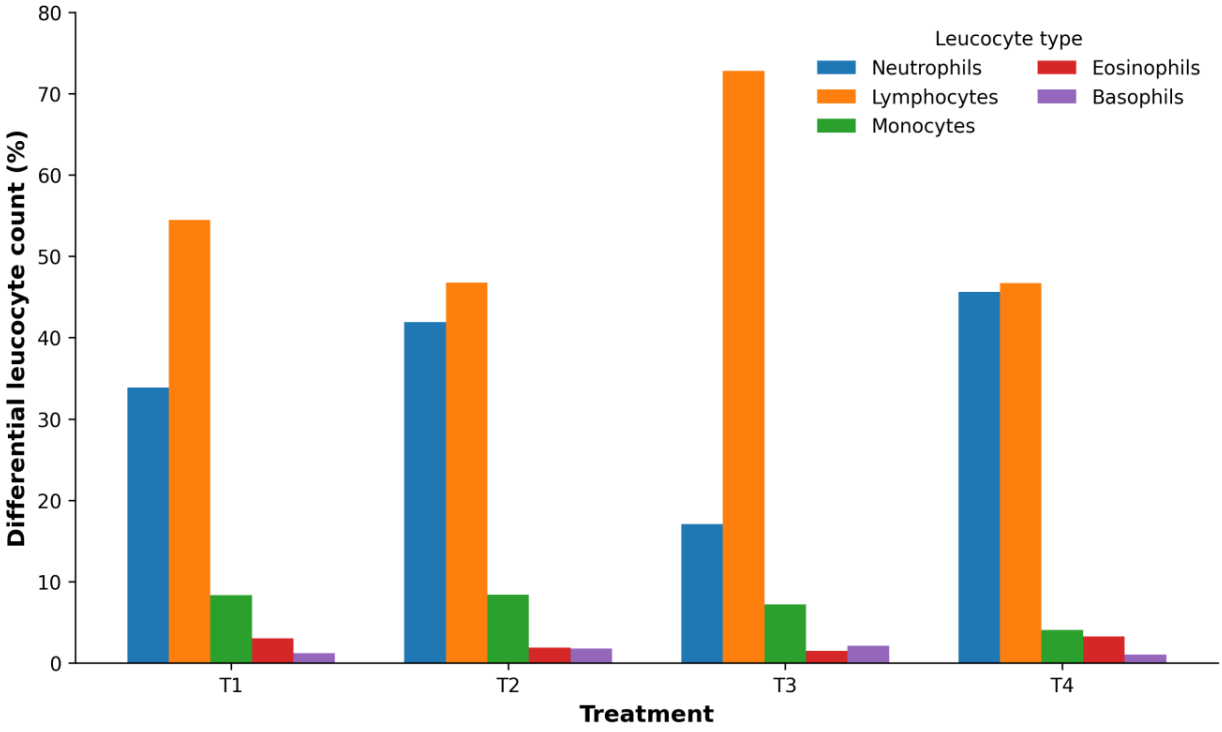
The effect of dietary supplementation with stingless bee (Tetragonula iridipennis) honey on the differential leukocyte profile of *Tenyi Vo* pigs is presented in Table 1. Significant differences were observed among treatment groups for neutrophils, lymphocytes, monocytes, eosinophils and basophils. The neutrophil percentage ranged from 17.10 to 45.60%, with the highest value recorded in T_4 (45.60%), followed by T_2 (41.92%), whereas the lowest value was observed in T_3 (17.10%). Lymphocyte percentage varied from 46.72 to 72.80%, with the highest value in T_3 (72.80%) and the lowest values in T_2 (46.74%) and T_4 (46.72%). Monocyte percentage ranged from 4.04 to 8.38%. The highest value was recorded in T_2 (8.38%), followed by T_1 (8.34%) and T_3 (7.18%), while the lowest value was observed in T_4 (4.04%). Eosinophil percentage ranged from 1.50 to 3.26%, with the highest value in T_4 (3.26%) and the lowest in T_3 (1.50%). Basophil percentage ranged from 1.02 to 2.12%, with the highest value recorded in T_3 (2.12%) and the lowest in T_4 (1.02%) level of significance ($P < 0.01$). The significant changes in the differential leukocyte profile suggest that dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey influenced leukocyte distribution in *Tenyi Vo* pigs. The response was not clearly dose dependent, as neutrophil levels were higher in T_4 but lower in T_3. The higher neutrophil proportion may indicate an altered innate immune response rather than a pathological condition. In contrast, the higher lymphocyte percentage in T_3 suggests that the intermediate supplementation level had a greater influence on lymphocyte distribution. Honey contains phenolic compounds, flavonoids, minerals and other bioactive substances with antioxidant and immunomodulatory properties (Bogdanov et al., 2008; Erejuwa et al., 2012; Biluca et al., 2016; Al-Hatamleh et al., 2020). Monocyte levels were higher in T_1 and T_2 but lowest in T_4, with no indication of an excessive inflammatory response. Variations in eosinophil and basophil percentages were also observed, but these changes were relatively small and may reflect normal physiological variation.

Similar beneficial physiological effects of honey supplementation have been reported in livestock and poultry (Abioja et al., 2012; Oke et al., 2016; Oke et al., 2020).These findings indicate that stingless bee honey may influence leukocyte distribution, particularly neutrophils and lymphocytes, without causing an apparent adverse hematological response. However, these changes should be interpreted together with other hematological and health indicators before concluding an improvement in immunity.

Table 1. Effect of Dietary Supplementation with Stingless Bee (*Tetragonula iridipennis*) Honey on Differential Leukocyte Counts (%) in *Tenyi Vo* Pigs

Treatment	Differential Leucocyte Count (DLC) %				
	Neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil
T_1	33.880^{b}	54.460^{b}	8.340^{a}	3.040^{a}	1.220^{b}
T_2	41.920^{ab}	46.740^{b}	8.380^{a}	1.900^{b}	1.780^{ab}
T_3	17.100^{c}	72.800^{a}	7.180^{a}	1.500^{b}	2.120^{a}
T_4	45.600^{a}	46.720^{b}	4.040^{b}	3.260^{a}	1.020^{b}
C.D.	10.495	10.057	1.143	0.819	0.569
SE(m)	3.471	3.326	0.378	0.271	0.188
SE(d)	4.908	4.704	0.535	0.383	0.266
C.V.	22.414	13.478	12.106	24.965	27.408

Figure 1. Effect of dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey on differential Leucocyte count (%) in indigenous *Tenyi Vo* pigs.



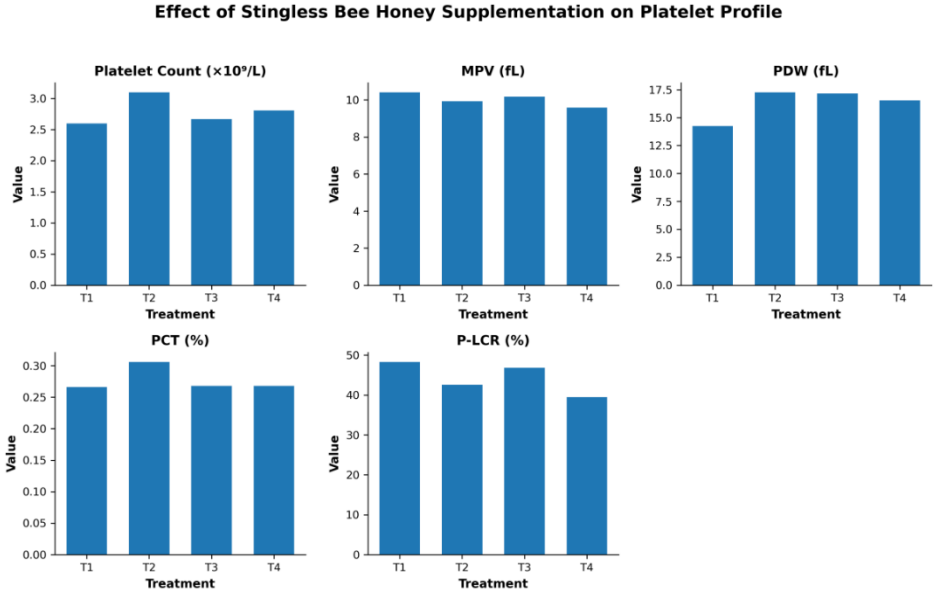
3.2 Platelet Profile

The effect of dietary supplementation with stingless bee honey on platelet characteristics is presented in Table 2. The platelet profile included platelet count, mean platelet volume, platelet distribution width, plateletcrit and platelet large cell ratio. Significant treatment-related differences were observed for mean platelet volume, platelet distribution width and platelet large cell ratio, whereas platelet count and plateletcrit did not differ significantly among treatments. Platelet count ranged from 2.596 to 3.096 × 10^9/L, with the highest value in T_2 (3.096 × 10^9/L) and the lowest in T_1 (2.596 × 10^9/L). Mean platelet volume ranged from 9.576 to 10.400 fL, with the highest value recorded in T_1 (10.400 fL) and the lowest in T_4 (9.576 fL). Platelet distribution width ranged from 14.250 to 17.250 fL, with the highest value in T_2 (17.250 fL) and the lowest in T_1 (14.250 fL) found not significance (P > 0.01).Plateletcrit ranged from 0.266 to 0.306%, with the highest value recorded in T_2 (0.306%). Platelet large cell ratio ranged from 39.476 to 48.300%, with the highest value in T_1 (48.300%) and the lowest in T_4 (39.476%). The platelet count and plateletcrit were not significantly affected by stingless bee honey supplementation, indicating that the treatment did not markedly alter overall platelet production or platelet mass. The values remained within the normal range reported for healthy pigs (Friendship et al., 1984; Jain, 1993; Weiss and Wardrop, 2010). However, significant changes were observed in some platelet indices. Mean platelet volume was highest in the control group and lowest in T_4, while platelet distribution width was higher in the supplemented groups, particularly T_2. The platelet large cell ratio was also lower in the honey-treated groups, especially T_4. These changes may reflect differences in platelet size, maturation and turnover rather than a major change in platelet function. Since platelet indices can be influenced by several physiological factors, they should not be considered alone as evidence of either beneficial or harmful platelet activation. Stingless bee honey appeared to modify some aspects of platelet morphology while maintaining normal platelet count and plateletcrit. The antioxidant properties of honey, particularly its phenolic and flavonoids compounds, may have contributed to maintaining platelet stability (Bogdanov et al., 2008; Erejuwa et al., 2012; Biluca et al., 2016; Al-Hatamleh et al., 2020).

Table 2. Effect of Stingless Bee (*Tetragonula iridipennis*) Honey Supplementation on Platelet Profile of *Tenyi Vo* Pigs

Treatment	Platelet Profile				
	Platelets Count(×10^9/L)	MPV(fL)	PDW(fL)	PCT(%)	LPCR(%)
T_1	2.596	10.400^a	14.250^b	0.266	48.300^a
T_2	3.096	9.926^b	17.250^a	0.306	42.550^b
T_3	2.668	10.176^a	17.150^a	0.268	46.876^a
T_4	2.806	9.576^b	16.526^a	0.268	39.476^b
C.D.	NS	0.409	1.684	NS	4.501
SE(m)	0.224	0.135	0.557	0.022	1.489
SE(d)	0.317	0.191	0.788	0.031	2.105
C.V.	17.954	3.017	7.643	17.547	7.514

Figure 2. Effect of dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey on platelet profile in indigenous *Tenyi Vo* pigs.



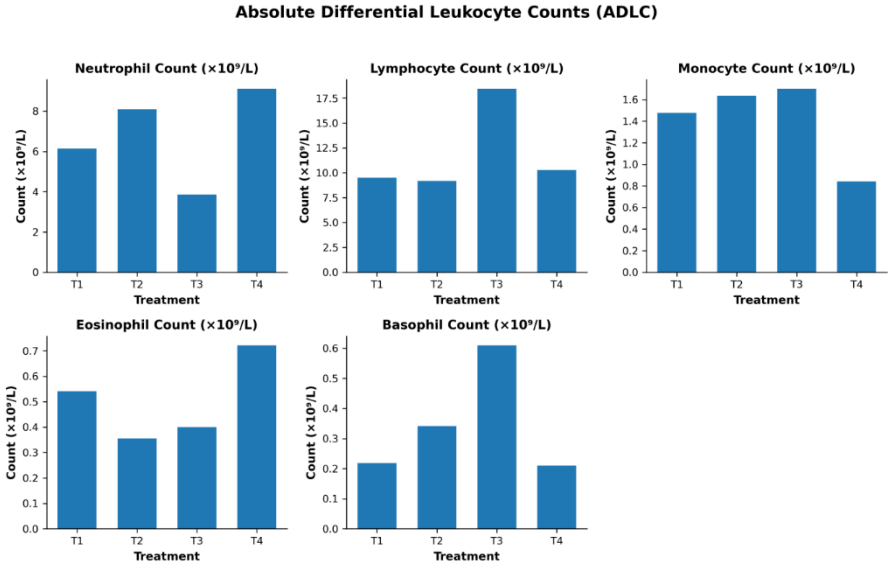
3.3 Absolute Differential Leukocyte Counts

The effect of dietary supplementation with stingless bee honey on absolute differential leukocyte counts is presented in Table 3. Significant differences were observed among treatments for absolute neutrophil, lymphocyte, monocyte, eosinophil and basophil counts. The absolute neutrophil count ranged from 3.850 to 9.112 × 10^9/L, with the highest value in T_4 (9.112 × 10^9/L) and the lowest in T_3 (3.850 × 10^9/L). The absolute lymphocyte count ranged from 9.172 to 18.438 × 10^9/L, with the highest value recorded in T_3 (18.438 × 10^9/L) level of significance ($P < 0.01$).The absolute monocyte count ranged from 0.840 to 1.700 × 10^9/L, with the highest value in T_3 (1.700 × 10^9/L) and the lowest in T_4 (0.840 × 10^9/L). The absolute eosinophil count ranged from 0.356 to 0.722 × 10^9/L, with the highest value in T_4 (0.722 × 10^9/L). The absolute basophil count ranged from 0.210 to 0.610 × 10^9/L, with the highest value recorded in T_3 (0.610 × 10^9/L).The significant differences in absolute leukocyte counts indicate that stingless bee honey influenced leukocyte dynamics in *Tenyi Vo* pigs. The highest absolute neutrophil count was observed in T_4, suggesting a possible effect on innate immune response. In contrast, T_3 showed the highest lymphocyte and monocyte counts, indicating that the intermediate level of honey supplementation had a stronger influence on these immune-related cells. Honey contains phenolic compounds, flavonoids and other bioactive substances that may contribute to its antioxidant and immunomodulatory effects (Bogdanov et al., 2008; Erejuwa et al., 2012; Al-Hatamleh et al., 2020; Ranneh et al., 2021). Absolute eosinophil count was highest in T_4, while basophil count was highest in T_3. These variations were relatively small and, together with the other hematological results, did not suggest any apparent adverse effect on health. However, changes in leukocyte counts alone cannot confirm improved immunity, as functional immune tests were not performed. The findings suggest that stingless bee honey may influence both innate and adaptive immune-related leukocyte populations in *Tenyi Vo* pigs. Together with previous reports of its antioxidant and health-promoting properties, honey shows potential as a natural functional feed additive for this indigenous pig breed (Abioja et al., 2012; Oke et al., 2016; Singh et al., 2025; Singh et al., 2026).

Table 3. Effect of Stingless Bee (*Tetragonula iridipennis*) Honey Supplementation on Absolute Differential Leukocyte Counts in *Tenyi Vo* Pigs

Treatment	Absolute Differential Leukocyte Counts (ADLC)				
	Total Neutrophil Count×10^9/L	Total Lymphocyte Count×10^9/L	Total Monocyte Count×10^9/L	Total Eosinophil Count×10^9/L	Total Basophil Count×10^9/L
T_1	6.148^b	9.516^b	1.476^b	0.542^a	0.218^b
T_2	8.092^a	9.172^b	1.636^{ab}	0.356^b	0.342^b
T_3	3.850^c	18.438^a	1.700^a	0.400^b	0.610^a
T_4	9.112^a	10.286^b	0.840^c	0.722^a	0.210^b
C.D.	1.794	4.048	0.218	0.215	0.207
SE(m)	0.593	1.339	0.072	0.071	0.068
SE(d)	0.839	1.893	0.102	0.101	0.097
C.V.	19.511	25.258	11.418	31.492	44.361

Figure 3. Effect of dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey on absolute differential leukocyte counts in indigenous *Tenyi Vo* pigs.



CONCLUSION

The present study demonstrated that dietary supplementation with stingless bee (*Tetragonula iridipennis*) honey positively influenced the hematological status of indigenous *Tenyi Vo* pigs. Significant improvements were observed in differential leukocyte count (DLC) and absolute differential leukocyte counts (ADLC), indicating enhanced immune function. The 1.00 mL/L supplementation level (T_4) produced the highest lymphocyte and monocyte counts, suggesting improved adaptive immunity, while the 1.50 mL/L level (T_4) resulted in higher neutrophil and eosinophil counts, reflecting enhanced innate immune responses. Platelet count and plateletcrit (PCT) were not significantly affected, indicating that honey supplementation did not adversely influence platelet production or hemostasis. Although significant variations were observed in MPV, PDW and P-LCR, all values remained within the normal physiological range. Overall, supplementation with stingless bee honey at1.50 mL/L of drinking water can be considered a safe natural immunomodulatory feed additive for improving hematological health and immune competence in *Tenyi Vo* pigs. Further studies are recommended to investigate its long-term effects and underlying molecular mechanisms.

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Author Contributions: Rajan Singh, Gyanendra Singh, Sourav Barman, Tsarila Z. T. Sangtam, Asem Ameeta Devi, Lalit Kumar and M. Catherine Rutsa contributed substantially to the conception and execution of the study. M. Catherine Rutsa contributed to conceptualization, investigation, formal analysis, data curation, visualization and writing-review and editing. Rajan Singh, Gyanendra Singh, Sourav Barman, Tsarila Z. T. Sangtam, Asem Ameeta Devi and Lalit Kumar contributed to methodology, validation, supervision and writing-review and editing. All authors critically reviewed the manuscript and approved the final version for publication.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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