Title

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Publication Date

2018-06-01

DOI

10.1016/j.jevs.2018.02.013

Peer reviewed

Journal of Equine Veterinary Science 65 (2018) 1–5

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Observations of the Hematological, Hematochemical, and Electrophoretic Parameters in Lactating Donkeys (*Equus asinus*)

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ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form
6 February 2018
Accepted 7 February 2018
Available online 20 February 2018

Keywords:
Donkey
Lactation
Electrophoretic parameter
Hematology
Blood chemistry

ABSTRACT

A cross-sectional study was conducted on 92 female donkeys. Blood samples were collected, and the following parameters were evaluated: red blood cell (RBC), white blood cell, neutrophil, lymphocyte, monocyte (MON), eosinophil (EOS) and basophil counts, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and the hematocrit (HCT), alanine aminotransferase (GPT), aspartate aminotransferase, total proteins, γ-glutamyl-transferase, alkaline phosphatase, creatinine, urea, and blood urea nitrogen and electrophoretic profile. Age (≥2 years ≤ 3 [very young], >3 years ≤ 10 [young], and >10 years ≤ 17 [adult]) and lactation (early lactation [≤3 months], middle [>3 months ≤ 6], and late lactation [>6 months]). Groups were independently analyzed using one-way analysis of variance or Kruskal–Wallis (post hoc test: Bonferroni's or Dunn's multiple test) tests; *P* was set as <.05. Very young animals had lower EOS than young and adult animals; in addition, they showed the highest MON and RBC and the lowest MCV and GPT; MCHC was lower in adult than that in the very young group; MCH was higher in the adult than that in the very young group; Alpha 2-globulin values were greater in young than those in very young animals; MCH was higher in the late lactation group than that in early lactation; alpha 1 and alpha 2-globulins showed a significant increase from the early to the late lactation period. Values reported herein could provide a useful clinical guide and represent a basis for further research into monitoring the health status of lactating donkeys.

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1. Introduction

Countries within Europe (e.g., Italy, France, Belgium, and Spain) have witnessed a marked increase in the donkey population over recent years, mainly due to an increased demand for donkey milk production [1], for use in cosmetics, and for consumption due to its therapeutic and beneficial properties; for example, donkey's milk is better tolerated in pediatric patients intolerant to cow milk proteins [2].

Domesticated approximately 5,000 years ago, the donkey has historically been, and indeed continues to be, used for draught and production purposes, working and living alongside humans all around the world. Although the donkey has played a rich and important role in human development and history, this animal has often been denigrated as a lowly beast of burden and is frequently looked on as the “poor relation” to its more respected “cousin” the horse [3]. However, the donkey is a unique species with many distinct characteristics that should be noted and appreciated when working with, managing, and treating these animals; it is no longer acceptable to simply look on the donkey as a small horse. Moreover, veterinary care of this species must take into account the many physical differences between the donkey and the horse.

Animal welfare/ethical statement: None.
Conflict of interest statement: None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the article.

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https://doi.org/10.1016/j.jevs.2018.02.013
0737-0806/Published by Elsevier Inc.

Despite the fact that donkeys have been used by man for hundreds of years, these animals have been the subject of very little research activity [4]. Biochemical and hematological parameters are fundamental for assessing the physiological or pathological condition of an animal. Studies in horses have looked at multiple blood parameters during late gestation that are related to the health of the mare such as leptin and the role it plays in maternal nutrition regulation and possible links to hyperlipemia, a condition commonly seen in donkeys, yet we know very little to nothing about how leptin works in late gestation in the jennies [5]. Nevertheless, when we refer to donkey breeds, reference values obtained from horses are often used, and scientific articles specifically relating to such parameters in donkeys are scarce [6–9]. According to Cebulj-Kadunc et al. [10], due to the large variability in normal biochemical hematological profiles, laboratory should create reference values not only for a certain population but also for individual animal. Key variables important to study, and which have received almost no research attention to date, include the effects of the stage of lactation and animal age on normal hematochemical parameters. For instance, in dairy cattle, Arfuso et al. [11] found variations in lipid and lipoprotein concentrations related to the stage of lactation and energy demands, and similar changes may occur in donkeys before foaling and in the first stage of lactation.

The assessment of blood biochemical parameters presents an important means to evaluate an animal's condition, both in clinical practice and basic research. Changes in blood and serum biochemical parameters are often indicative of changes in an animal's physiological state (such as before foaling and directly after foaling) and are a valuable tool for assessing the health, welfare, and stress level of an animal [11].

Even albumin and globulin fractions assessed by electrophoresis constitute very important parameters because variations can provide an index of a pathological condition or a stress response [12]. The aim of this study was as follows: (1) to report the normal hematological, hematochemical, and electrophoretic profiles in lactating donkeys and (2) to explore any differences associated with age or stage of lactation.

2. Materials and Methods

2.1. Study Design and Animals

This study was conducted on a group of 92 lactating donkeys. The animals were mainly crossbreed, housed in semiextensive farms, partially grazed and partially fed *ad libitum* with hay produced on the farm. All the farms were authorized by the regional sanitary system, and the samples were collected during the welfare surveillance program conducted by the Veterinary Medical Research Institute. Samples were collected only from animals considered to be healthy during the visit, based on their recorded history and their clinical inspection. All the animals were familiar with humans, so no animals needed to be restrained. Animals ($n = 92$) were divided according to age as follows: very young (2 years $\leq$ 3), young (3 years $\leq$ 10), and adult (10 years $\leq$ 17). Animals that were also being milked for the sale of this product ($n = 51$) were additionally divided according to stage of lactation: early lactation ($\leq$3 months), middle lactation (3 months $\leq$ 6), and late lactation (>6 months).

2.2. Blood Sampling

All blood samples were collected between 10:00 and 11:30 AM and immediately transferred to the laboratory for analysis in less than 45 minutes. Blood samples were obtained from the jugular vein into 10 mL vacutainer tubes (Vacutainers, Becton Dickinson) containing a clot activator; they were then separated by centrifugation at 3,500 *g* for 5 minutes at room temperature and analyzed visually for the phenomenon of hemolysis that could affect the results of the analyses. For the determination of hematological values, a second set of blood samples were collected into 10 mL tubes containing ethylenediaminetetraacetic acid as an anticoagulant.

2.3. Analysis

Hematological, biochemical, and electrophoretic analyses were carried out in the laboratory of the Istituto Zooprofilattico Sperimentale of Turin. Erythrocyte count (red blood cell [RBC]); hemoglobin (Hb) concentration; mean corpuscular volume (MCV); the hematocrit (HCT); mean cell hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); platelets (PLT); mean platelet volume (MPV); and leukocyte (white blood cell [WBC]), neutrophil (NEU), basophil (BAS), eosinophil (EOS), monocyte (MON), and lymphocyte (LYM) counts were measured using an automated blood analyzer (Melet-Schloesing-MS4, Osny, France).

Serum samples were analyzed using the Screen Master Touch automated instrument (Hospitex Diagnostics S.r.l., Sesto Fiorentino, Firenze, Italy) for total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (GPT), alkaline phosphatase (ALP), γ-glutamyl-transferase (GGT), ALP, creatinine (CREA), urea (UR), and blood urea nitrogen (BUN) determinations. The serum electrophoretic profile (albumin, α1-globulins, α2-globulins, β1–globulins, β2–globulins, and γ–globulins) was evaluated for serum samples using a semiautomated agarose gel electrophoresis system (Sebia Hydrasys; Evry Cedex, France). The same operator always performed the analysis according to our internal quality control standards.

2.4. Statistical Analysis

The GraphPad Prism software was used for statistical analyses. Shapiro–Wilk test was used to establish the normality or nonnormality of a distribution. Age and stage of lactation on stage groups were independently analyzed using one-way analysis of variance (post hoc test: Bonferroni's multiple comparison test) and Kruskal–Wallis (post hoc test: Dunn's multiple comparison test) tests for hematological and biochemical parameters; P values <.05 were considered statistically significant. Normally distributed data were expressed as means ± standard deviation, whereas nonnormally distributed data were expressed as medians and interquartile range (25%–75%).

3. Results

None of the samples collected contained clots or had hemolyzed. Table 1 reports the results according to age; statistical significant differences ($P < .05$) were recorded for hematological variables EOS and MCV, which were lower for the very young group. The donkeys were divided into three groups: very young animals (2 years $\leq$ 3 years; mean was 2.2), young (3 years $\leq$ 10 years mean 6.1), and adult (10 years $\leq$ 17 years; mean 13.3). Alanine aminotransferase values were different between very young and adult, and AST values were different between very young and young donkeys. Instead MON, RBC showed higher levels for the very young group ($P < .05$), and MCHC values were different between very young and adult. Creatinine values were statistically different between young and adult group. Mean corpuscular hemoglobin values were statistically different ($P < .01$) between adult and very young groups.

Table 1
Clinical chemistry parameters in 92 female donkeys (*Equus asinus*) divided according to age: very young (≥2 years ≤ 3, mean 2.2), young (>3 years ≤ 10, mean 6.1), and adult (>10 years ≤ 17, mean 13.3).

Chemistry Parameters	Very Young (Mean 2.2 y)	Young (Mean 6.1 y)	Adult (Mean 13.3 y)
WBC (10^3/μL)	14.06 ± 4.11	13.36 ± 3.18	12.6 ± 3.14
EOS (%)	2.6 (1.55; 4.25)[a]	6.45 (4.3; 8.5)[b]	6.8 (5.3; 9.25)[cb]
NEU (%)	52.3 (46.5; 55.8)	54.2 (49.45; 59.65)	52.3 (46.5; 57.85)
BA (%)	0.35 (0.25; 0.5)	0.5 (0.35; 0.8)	0.65 (0.3; 0.7)
MON (%)	9.2 (7.85; 12.35)[a]	6.3 (5.6; 7.4)[b]	6.3 (5.35; 7.35)[bc]
LYN (%)	35.61 ± 8.15	32.05 ± 7.36	32.54 ± 6.98
RBC (10^6/μL)	6.479 ± 0.98[a]	5.706 ± 0.75[b]	5.375 ± 0.71[b]
MCH (pg)	17.03 ± 1.23[a]	19.29 ± 1.38[ba]	20.161.45[b]
MCHC (g/dL)	34.55 ± 0.76[a]	32.95 ± 2.52[ab]	32.35 ± 2.39[b]
MCV (fL)	49.41 ± 3.61[a]	58.8 ± 3.16[b]	62.57 ± 2.87[c]
Hb (g/dL)	10.6 (9.85; 12.3)	10.95 (9.65; 12)	10.45 (9.85; 11.6)
HCT (%)	30.95 (29; 34.3)	32.6 (30.2; 37.2)	33 (29.6; 36)
PLT (10^3/μL)	154.5 (102; 192.5)	126.5 (77.5; 169)	122.5 (77.5; 169)
BUN (units/L)	11.92 ± 2.54	12.96 ± 5.20	12.43 ± 5.54
CREA (units/L)	1.21 (1.09; 1.38)[ab]	1.27 (1.09; 1.37)[a]	1.118 (0.87; 1.2)[b]
UREA (mmol/L)	27.73 (22.95; 29.51)	28.04 (21.28; 34.16)	28.09 (17.8; 33.19)
GGT (units/L)	53 (43.3; 79.5)	58.2 (44.33; 83)	72.02 (52.1; 89.35)
TP (g/L)	6.695 (4.56; 7.56)	7.556 (6.25; 8.97)	7.313 (6.48; 8.32)
AST (units/L)	272.2 (171.4; 288)[a]	338.3 (286.1; 387.2)[b]	306.7 (219; 424.8)[ab]
GPT (units/L)	7.768 (6.55; 9.64)[a]	13.68 (9.22; 25.06)[bc]	15.71 (9.99; 24.58)[c]
ALP (units/L)	395.3 (170.50; 14)	527.9 (211.30; 640)	492.1 (243.3; 554.1)

Abbreviations: ALP, alkaline phosphatase; AST, aspartate transaminase; BA, basophil; BUN, blood urea nitrogen; CREA, creatinine; EOS, eosinophils; GGT, γ-glutamyl-transferase; GPT, alanine aminotransferase; Hb, hemoglobin; HCT, hematocrit; IQR, interquartile range; LYM, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin content; MCV, mean corpuscular volume; MON, monocyte; NEU, neutrophil; PLT, platelets; RBC, red blood cell; SD, standard deviation; TP, total protein; WBC, white blood cell.
[abc]Letters identify differences among groups for P values < .05.
Mean ± SD or median with IQR (25%–75%).

Table 2 reported the result concerning the stage of lactation; the only statistical significant differences for $P < .05$ were recorded for MCH.

Serum protein concentrations are reported in Table 3, where the animals are divided according to the age; statistical significant differences were recorded for albumin, which were lower in the very young animals ($P < .01$). Alpha 2-globulin values were different between very young and adult; gamma globulin parameters were different between very young and young. Table 4 reported the analysis results of serum protein concentrations, and statistical differences were recorded only for Alpha 1-globulin between early and middle stage and alpha 2-globulin between early and middle stage of lactation.

Table 2
Clinical chemistry parameters in 51 female donkeys (*Equus asinus*) divided according to stage of lactation: ≤3 months (early lactation), >3 months ≤6 (middle lactation), and >6 months (late lactation).

Chemistry Parameters	Early	Middle	Late
WBC (10^3/μL)	14[a] ± 3.10	11 ± 2.3[b]	13 ± 3.7[ab]
EOS (%)	6.4 ± 2.8	8.4 ± 3.4	8.7 ± 3.7
NEU (%)	60 (51; 62)	54 (51; 58)	55 (45; 58)
BA (%)	0.46 ± 0.29	0.53 ± 0.32	0.65 ± 0.21
MON (%)	6.2 ± 1.2	6.2 ± 1.2	6.1 ± 1.2
LYN (%)	307.9	314.1	305.7
RBC (10^6/μL)	5.5 ± 0.6	5.4 ± 0.75	5.30 ± 0.31
MCH (pg)	19 (18; 20)[a]	19 (18; 21)[ab]	21 (20; 22)[b]
MCHC (g/L)	31 (29; 32)	32 (31; 35)	32 (30; 37)
Hb (g/L)	10 ± 1.2	10 ± 1.2	11 ± 0.84
HCT (%)	33 (31; 37)	32 (29; 34)	33 (32; 34)
PLT (10^3/μL)	135 (77; 225)	90 (59; 119)	79 (75; 109)
BUN (mg/L)	12 ± 4.10	11 ± 5.4	9.9 ± 5.7
CREA (μmol/L)	1.20 (1; 1.80)	1.20 (1; 1.40)	1.20 (0.97; 1.30)
UREA (mmol/L)	25 ± 8.7	24 ± 12	21 ± 12
GGT (units/L)	62 (51; 83)	53 (30; 115)	67 (29; 82)
TP (g/L)	8.3 ± 2.3	8.4 ± 2.5	7.5 ± 1.5
AST (units/L)	321 (228; 421)	388 (335; 478)	335 (197; 427)
GPT (units/L)	25 (16; 47)	20 (16; 28)	16 (8.4; 25)
ALP (units/L)	426 (259; 557)	588 (219; 700)	626 (515; 739)

Abbreviations: ALP, alkaline phosphatase; AST, aspartate transaminase; BA, basophil; BUN, blood urea nitrogen; CREA, creatinine; EOS, eosinophils; GGT, γ-glutamyl-transferase; GPT, alanine aminotransferase; Hb, hemoglobin; HCT, hematocrit; IQR, interquartile range; LYM, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin content; MON, monocyte; NEU, neutrophil; PLT, platelets; RBC, red blood cell; SD, standard deviation; TP, total protein; WBC, white blood cell.
[ab]Letters identify differences among groups for P values < .05.
Mean ± SD or median with IQR (25%–75%).

4. Discussion

The hematological, hematochemical, and electrophoretic parameters may vary according to management practices. According to Cavallarin et al., [1], dairy donkeys in Northwest Italy are generally reared in small-sized family-run farms: in our study, all the animals were kept in semiextensive conditions, partially grazing and partially fed *ad libitum* with hay produced on the farm. None of the animal in the present study received mineral/vitamin supplementation.

When comparing donkeys and horses, there are many differences found from resting temperature to blood chemistry [13]. For example, there are differences in red cell lines such as RBC counts and packed cell volumes which are found to be significantly lower in the donkey than those reported in the horse. Other differences include a significantly greater MCV and fewer but larger white cells in the donkey [3]. Knowing the difference in blood chemistry reference values for donkeys is essential in proper care and treatment for disease.

For those reasons, a different set of reference ranges need to be used to evaluate hematological, hematochemical, and electrophoretic readings from this species. In fact, these reference ranges may be influenced by multiple factors such as stage of pregnancy, lactation, breed, and diet. Although homeostatic mechanisms maintain many blood equilibria, small changes in the quantities of routine clinical chemistry analytes may also occur in situations such as lactation [10]. In the present study, we determined the hematological, hematochemical, and electrophoretic parameters

Table 3
Serum protein concentration (expressed in g/dL) as determined by acetate cellulose electrophoresis in 92 female donkeys (*Equus asinus*) divided according to age: very young (≥2 years ≤ 3, mean 2.2 years), young (>3 years ≤ 10, mean 6.1 years), and adult (>10 years ≤ 17 mean 13.3 years).

Serum Protein	Very Young	Young	Adult
Albumin	1.94 (1.355; 2.295)[a]	2.62 (1.89; 3.39)[b]	2.58 (2.135; 3.025)[b]
Alpha 1-globulin	0.07 (0.05; 0.11)	0.1 (0.05; 0.21)	0.08 (0.05; 0.21)
Alpha 2-globulin	1.48 ± 0.51[a]	1.15 ± 0.52[ab]	1.03 ± 0.39[b]
Beta 1-globulin	0.82 (0.49; 1.17)	0.78 (0.57; 1.17)	0.83 (0.58; 1.22)
Beta 2-globulin	0.67 ± 0.29	0.784 ± 0.30	0.69 ± 0.25
Gamma globulin	1.55 (0.97; 1.88)[a]	2.07 (1.27; 2.65)[b]	1.96 (1.74; 2.34)[ab]

Abbreviations: IQR, interquartile range; SD, standard deviation.
[ab]Letters identify differences among groups for *P* values < .05.
Mean ± SD or median with IQR (25%–75%).

and present the results according to animal age and lactation stage. To date, just a single study reporting hematological and biochemical findings in lactating donkeys has been published [10], but this article was limited just to 18 animals of a single breed and for just 2 months after the foaling.

Although studies in horses have reported the lack of any significant age-related alterations for most biochemical parameters [14,15], in the present study, the effect of age was found on several blood parameters in selected donkey population. The variables RBC, WBC, MCH, MCHC, LYMs, and BUN counts exhibited Gaussian distributions; whereas BAS, MON, NEU, EOS counts, platelets, Hb, UR, GGT, TP, CREA, AST, GPT, and phosphatase alkaline did not seem to be normally distributed in relation to age.

The RBC decreased with advancing age, as similarly described by other authors [6,15–18]. The lower RBC values could have been compensated by the observed increased erythrocyte size, which caused the higher MCH values ($P < .01$); as a result, HCT did not differ between the groups. The most plausible hypothesis is a physiological decline of bone marrow regenerative capacity with advancing age [19]. Several authors have reported that very young animals had lower EOS counts compared with young and adult animals [16,20]. This increase is probably due to progressive exposure of the animals to parasites or allergen antigens after birth, which induces the production of EOS poietic factors, mainly interleukin count, as similarly reported by others [6,18,21]. No age-related differences were revealed for UR [15,21,22]. The younger donkeys showed differences in blood chemistry values as also reported in other studies such as differences in GPT, monocytes and RBCs when compared to older donkeys [16]. Moreover, in agreement with Girardi et al [16], but in disagreement with other studies [21–23], compared with the older subjects. Many reference ranges are in the process of being created for donkeys and specific donkey breeds, and the interested clinician is urged to check for breed-specific differences where possible [22,24].

Cellulose acetate has been used as the support medium in most reports for equine serum protein electrophoresis [25,26], but few data are available for serum protein fraction concentrations in donkeys [27]. Reference intervals of total serum protein concentrations in our study are in agreement with values reported by other authors [6,27,28], and no differences were found in relation to age or stage of lactation. This is opposite of what was observed in Catalonian donkeys, that showed an increasing plasma protein values [29]. The actual number of protein peaks in donkeys ranges from 5 to 7 [7,29,30]; differences in the protocols used probably account for this variation. We found five protein fractions in the donkeys: albumin, α1-, α2-, β1-, β2-, and γ-globulins. Cavalcante et al [8] separated α-globulins and γ-globulins into two zones by agarose gel electrophoresis although in our study, the visual examination of acetate cellulose films did not allow for the separation of the γ-globulin fractions. Conversely, Caldin et al. [7] separated α-, but not β-, globulins into two fractions by capillaries electrophoresis. Significant *P* values were obtained in the Shapiro–Wilk test for α2-globulins and β2–globulins, indicating that these results were normally distributed in this population. Albumin, α1-globulins, β1–globulins, and γ–globulins were not normally distributed. In our study, albumin concentrations were significantly lower in animals aged between 2 and 3 years (very young) compared with older animals; this finding agrees with those of Jordana et al. [31] who used a colorimetric method. The percentage area of albumin and globulins is in agreement with data reported by Cavalcante et al. [8] pertaining to Brazilian donkeys (and who used a different support medium). γ–globulin values were greater in young animals (≥3 years ≤ 10) compared with very young animals (≥2 years ≤ 3), but no significant difference was observed compared with adults (≥10 years ≤ 17). No statistically significant differences were found between groups in relation to TP. With regard to the influence of age on albumin concentration, although we cannot explain the discrepancy between the results of this study in relation to donkeys with what has been documented to occur in other animal species, where there tends to be a general increase in TP, a slight decrease in albumin and a progressive increase in globulins with advancing age [32].

In our study, α1-globulins and α2-globulins show a significant increase from the early to the late lactation period. In our experience, this increase could be due to the development of a state of stress that initiated when a foal is born and increases during subsequent lactation.

Table 4
Serum protein concentration (expressed in g/dL) as determined by acetate cellulose electrophoresis in 51 female donkeys (*Equus asinus*) divided according to stage of lactation.

Serum Protein	Early	Middle	Late
Albumin	3.1 (2.3; 3.5)	2.6 (2.3; 3.5)	2.7 (2.2; 2.8)
Alpha 1-globulin	0.05 (0.04; 0.09)[a]	0.08 (0.07; 0.22)[b]	0.24 (0.05; 0.41)[ab]
Alpha 2-globulin	0.81 ± 0.28[a]	1.20 ± 0.58[b]	1 ± 0.27[ab]
Beta 1-globulin	1.10 (0.83; 1.4)	0.78 (0.49; 1.3)	0.72 (0.52; 0.90)
Beta 2-globulin	0.8 ± 0.35	0.85 ± 0.28	0.66 ± 0.18
Gamma globulin	2.3 (1.60; 2.70)	2.2 (1.40; 3.10)	1.8 (1.60; 2.20)

Abbreviations: IQR, interquartile range; SD, standard deviation.
[ab]Letters identify differences among groups for *P* values < .05.
Mean ± SD or median with IQR (25%–75%).

5. Conclusions

Both age and stage of lactation in female donkeys have shown differences in hematobiochemical profiles. The newly found profiles can prove to be useful diagnostic tools for improving our understanding and needs of the metabolic status of lactating female donkeys. Although it is recommended that each laboratory develops its own reference intervals, the values reported herein could provide a useful clinical guide and represent a basis for further research into monitoring the health status of lactating donkeys.

Acknowledgments

This study was supported, in part, by a grant from Ricerca locale linea B, Bando ex-60%-2013 University of Torino. Preliminary results were presented as abstracts in the European Society of Veterinary and Comparative Nutrition, Toulose, 17–19, September 2015.

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