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Research Article

OCCURRENCE OF *BABESIA* SPP. INFECTION IN DOMESTIC CATS (*FELIS CATUS*) OF ASSAM, INDIA

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ABSTRACT: Babesiosis in cats is a relatively unexplored tick-transmitted clinical entity, however, increasing numbers of cases highlight the growing importance of disease. The study was conducted from June 2020 to May 2021 to assess the hospital-based occurrence and clinical-pathological alterations linked to *Babesia* infections in domestic cats of Assam presented to Veterinary Clinical Complex (VCC), College of Veterinary Science, Khanapara, Guwahati. Suspected cats (n=50) were screened for *Babesia* infection by conventional microscopy followed by molecular testing. Additionally, the haemato-biochemical alterations in affected cats were also analyzed. An occurrence of 52.00% (26/50) was recorded for babesiosis in cats. Risk factor analysis revealed age to be significantly correlated with the infection, being highest in cats of >2 years while the other associated factors were statistically non-significant. Most consistent clinical manifestations were lethargy/weakness, fever, dark yellow urine and/or stool, diarrhoea, pale mucous membranes, dry muzzle, rough coat and inappetence. Hematobiochemical analysis revealed decreased haemoglobin, total erythrocyte and platelet counts, increased ALT, AST and bilirubin in affected cats, with data being statistically significant ($p<0.05$). Combined treatment of diminazene diaceturate and clindamycin along with supportive therapy led to recovery in affected cats except two with the cerebral form and severe anaemia. The present findings will assist clinician and field veterinarians while managing similar type of clinical cases and serves as valuable baseline data to conduct further research. Although treatment of these cases can be very challenging, but with proper evaluation of the haemato-biochemical findings, along with other diagnostic tests, can help in delivering rational treatment, especially in field condition.

Keywords: Assam, *Babesia*, Cat, Haemoprotozoa, PCR

INTRODUCTION

The domestic cat (*Felis catus*) evolved from the Middle Eastern wildcat (*Felis silvestris*) about 70,000 to 100,000 years ago. Today, there are about 600 million domestic cats worldwide, however, their population in India is currently unreported [1].

Feline babesiosis, caused by several species of *Babesia* such as *Babesia felis*, *B. herpailuri*, *B. cati*, *B. pantherae*, *B. canis* subsp. *presentii*, *B. canis* subsp. *canis*, and *B. microti*, is transmitted through tick bites and is frequently diagnosed in the clinical veterinary practice [2]. In acute cases, anaemia is the primary symptom, however, cats often show signs like lethargy, loss of appetite, rough coat, exercise intolerance, weight loss, weakness, pale gums, rapid heart rate and breathing, pica, vomition, and diarrhoea. Blood tests typically reveal regenerative anaemia, reduced platelet along with variable white blood cell counts [3]. The rise in cases of babesiosis is attributed to the growing feral cat population and their interactions with stray dogs, which facilitate the spread of these parasites [4]. Diagnosis of infection ranges from direct microscopy to more sensitive molecular techniques such as PCR assays, which are effective in detecting low

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levels of parasitaemia. The treatment options include administration of drugs like primaquine phosphate and diminazene aceturate, though their efficacy remains under-studied [5].

The current work aims to explore status of babesiosis in cats of Assam, India, a region with favorable conditions for the disease with a focus on clinical presentations, molecular diagnosis, and therapeutic management of the infection.

MATERIALS AND METHODS

Study area

The current study was carried out for a period of 12 months from June, 2020 to May, 2021 in Veterinary Clinical Complex (VCC), College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. The state usually experiences hot and humid climatic condition with long summers, short winter, and heavy rainfall which make its highly conducive for the development and propagation of various ticks that acts as a natural vector for transmitting various haemoparasitic diseases in animals.

Study animals

Domestic cats were attended as routine clinical inspection at the OPD unit of VCC and suspected cases for *Babesia* infection irrespective of age, sex and breed were selected based on history and clinical symptoms. as, reduced activity, rough coat, inappetence, anorexia, weakness, fever, subnormal temperature, diarrhoea, recumbency, dry muzzle, sunken eyeballs, vomiting, pale-whitish/icteric mucous membranes, dark yellow urine and/or stool colour, respiratory distress, nervous symptoms, increased capillary refill time (CRT) and skin tent test (STT).

Collection of blood samples

Around 2mL of whole blood in EDTA and 3mL in clot activator tubes were collected aseptically by venipuncture of cephalic and/or saphenous veins for microscopy, haemato-biochemical and molecular tests. The parasitological and haemato-biochemical testing were conducted on same day of collection while part of samples was preserved at -20°C until further processing for molecular tests.

Diagnosis

Microscopic detection

The microscopy was conducted on Giemsa-stained thin blood smears as per standard protocol [6]. A sample was recorded negative if no parasites were detected after examining at least 50 oil immersion fields for duration of 20-30 minutes [7]. Additionally, for accurate microscopic diagnosis of blood parasites, Giemsa working solution was optimized to pH 7.0–7.2 [8]. Buffered distilled water at this pH produced more uniform staining and improved smear clarity.

Molecular detection

Extraction of DNA

The DNA extraction was carried out from the blood samples using DNeasy Blood and Tissue Kit® (QIAGEN, Hilden, Germany) as per manufacturer's protocol. Molecular testing was conducted to detect presence of *Babesia* spp. by using a genus specific primer as per method described by Birkenheuer and coworkers [9].

DNA Amplification for *Babesia* spp.

The extracted DNAs were subjected to PCR reaction to amplify *18S rRNA* gene fragment of 340 bp with published primers [9]. The PCR assay was performed in reaction volume of 25µL with mixture comprising of 5µL DNA template, 12.5µL DyNAzyme II PCR mix (Sapphire Fast PCR mix, Takara), 5.5µL nuclease-free water (NFW) and 1 µL each (10 pmol) of *Babesia* genus specific forward and reverse primers. The reaction was performed with 35 cycles with each cycle consisting of 45s for denaturation at 98ºC, 56°C each for annealing, and 72°C for extension. A final extension step was performed at 72°C for 5 min, followed by a hold at 4°C, in a semi-automated thermal cycler (TC-5000; Bibby Scientific, Burlington, USA). The positive control for was obtained from repository of Department of Veterinary Parasitology, CVSc, AAU, Khanapara, Guwahati, India. Distilled water was used as no template control. The PCR product was subjected to agarose gel electrophoresis and visualized under UV transilluminator.

Haemato-biochemical parameters

Haematological and biochemical assessments were performed by using a semi-automated blood analyser (MS4-e,MeletSchloesing Lab, France) and biochemical analyzer (C-61; Benesphera®, Avantor, Mumbai, India) as per manufacturer's instructions. Blood parameters, including total red blood cell count (TEC), haemoglobin level (Hb), packed cell volume (PCV), total white blood cell count (TLC), differential white blood cell count (DLC), and platelet count were evaluated. Additionally, serum biochemical markers such as total protein, albumin, alanine aminotransferase (ALT), aspartate

aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (direct and indirect), total serum protein (TSP), blood urea nitrogen (BUN) and creatinine were also studied and compared between babesiosis affected cats and healthy control.

Statistical analysis

Data obtained were analyzed for age, sex and season wise (pre-monsoon, monsoon, post-monsoon and winter) as risk factors to understand their correlation with occurrence of babesiosis in cats. The whole period of observation was divided into four seasons as per Indian Meteorological Department (IMD), Airport, Guwahati-15, Assam, India.

Additionally, to perform comparative evaluation of healthy cats and *Babesia*-positive cats for haematological and biochemical analyses, out of 26 cats diagnosed as positive for *Babesia* infection, only 14 cases were included based on sample adequacy, completeleness of records and owners consent. The data generated were analyzed using the Chi-square (χ^2) test, with significance assessed at 1% and 5%. Twenty-one cats were divided into two groups: Group I (7 healthy cats) and Group II (14 cats with babesiosis). Mean values, standard error, and significant differences ($p<0.05$) between the groups were determined using one-way ANOVA. All tests used a significance level of $p=0.05$. SPSS version 20.0 was used for analysis.

RESULTS

During the study period, a total of two-hundred fifty (250) domestic cats were presented to the VCC of which 50 were clinically suspected to have haemoprotozoan infection based on observed symptoms as described earlier. A total of 20 cats were detected positive for *Babesia* spp. via microscopy (Figure 1). However, the PCR assay conducted on all the blood samples (n=50) detected 6 more positive cases, as confirmed by the amplification of expected 340 bp product (Figure 2), in addition to the 20 cases positive by microscopy. Hence, in the present study, an occurrence of 52.00% (26/50) was recorded for babesiosis in cats of Assam, India.

All the cats found positive for *Babesia* spp. were treated with a combination of diminazine diaceturate @3.5mg/kg BW, deep I/M (2 doses at 48-hour interval) and clindamycin @ 10mg/kg body weight P.O. (twice daily for 2 weeks). Supportive treatment comprising of intravenous fluids, multivitamins, acid blockers, anti-emetics, haematinics and platelet booster formulations were instituted whenever necessary. Twenty-three cats (88.46%) recovered uneventfully whereas two cats who exhibited cerebral form of the disease with head tilting or apathetic/ataxic behavior (Figure 9) and one presented lately with severe signs of anaemia, unfortunately succumbed to the infection within two days of starting treatment.

The TEC, Hb, and platelet count, were significantly lower ($p<0.01$), while TLC was significantly higher ($p<0.01$) in cats infected with babesiosis compared to healthy controls (Table 2). Statistical non-significant ($p>0.05$) elevation was recorded for lymphocytes, monocytes, neutrophils, eosinophils, basophils in babesiosis infected cats in comparison to healthy control. Non-significant lower PCV values were also recorded in babesiosis infected cats ($p>0.05$) (Table 2). The biochemical analysis showed that the average levels of TSP, BUN, and creatinine were significantly lower ($p<0.01$), while the mean levels of ALT ($p<0.01$), AST ($p<0.01$), direct serum bilirubin, indirect serum bilirubin, and total serum bilirubin were significantly higher ($p<0.05$) in cats diagnosed with babesiosis (Table 2).

DISCUSSION

This is the first report on occurrence and clinico-pathological changes associated with naturally occurring babesiosis in domestic cats of Assam, India. Similar findings have been reported by other workers [10, 11] who reported an occurrence of feline babesiosis of upto 77%. On the other hand, some reports suggested lower prevalence rates of 9% and 3.14% respectively [12,13]. In addition to this, variation in sample size [14], and geo-climatic location could be one of the reasons for this finding. On contrary, the lower rate of occurrence of babesiosis in cats may be attributed to their grooming behaviour, which significantly lessens the exposure to the ectoparasitic vectors thereby reducing transmission [15]. At the same time, the higher occurrence in our study could be a result of the pre-immunity concept, where contracting infection in the early days of life, especially in endemic areas, leads to subclinical carrier status without manifesting any clinical signs [11]. No statistical difference was noted in occurrence of the disease in relation to gender which corroborates with the findings of Vilhena and co-workers [12]. However, non-significant higher infection was found in males (53.85%) than females (50.00%) [15,16]. The increased susceptibility may be attributed to factors such as variations in environmental exposure, including more frequent roaming behavior, or sex-

Table 1. Age, season and sex-wise occurrence of *Babesia* infection in domestic cats

	Frequency	Total no. of cases	Number of positive cases	Occurrence (%) (95% CI)[a]	Chi-Sq	p-value
Age	<6 months	15	5	33.33 (0.095-0.571%)	10.037	0.018*
	6-12 months	13	8	61.50 (0.350-0.880%)		
	13-24 months	12	4	33.33 (0.066-0.600%)		
	>24 months	10	9	90.00 (0.714-1.086%)		
Season	Pre-monsoon (March to May)	10	8	80.00 (0.553-1.000%)	4.174	0.241
	Monsoon (June to September)	12	6	50.00 (0.218-0.782%)		
	Post-monsoon (October & November)	18	8	44.44 (0.215-0.673%)		
	Winter (December to February)	10	4	40.00 0.096-0.704%)		
Gender	Male	26	14	53.85 (34.70-73.00%)	0.073	0.786
	Female	24	12	50.00 (38.20-65.80%)		
	Total	50	26	52.00		

[a] *Confidence interval; result is significant at ($p<0.05$)*

related genetic and hormonal influences on the disease [16]. A higher rate of occurrence was observed in cats older than 2 years of age can be explained by the behavioural and hunting activity of the animal that brings them in close contact with vectors of the disease, thereby making them easy targets of infestation. Similar findings were also suggested elsewhere [13].

Occurrence of the disease was recorded to be the highest (80.00%) in pre-monsoon and lowest (40.00%) in the winter season. This finding can be correlated with the fact that warm and moist conditions are highly conducive for the growth and multiplication of vectors [13]. PCR-based diagnosis of babesiosis was found to be more sensitive than traditional microscopic detection. This can be attributed to the fact that conventional Giemsa stained microscopy usually fails to detect the parasite in case of low parasitemia in naturally infected animals/or chronic carriers. Similar reports were published by other workers [17, 18, 19].

In this study, the primary clinical signs observed in cats affected by babesiosis included increased lethargy/weakness (100%) followed by fever (84.62%), dark yellow urine and/or stool (76.92%), diarrhoea (69.23%), dry muzzle (57.69%), recumbency (57.69%), prolonged capillary refill time (53.85%), dehydration (53.85%), rough coat (46.15%), inappetence (42.31%) anaemia (34.62%), vomition (30.78%), sunken eyeballs (30.78%), sub-normal temperature (15.38%), respiratory distress (11.58%) and ataxia (7.69%). Corneal opacity was detected in two cats (7.69%), possibly resulting from the formation of immune complexes [20]. Weakness/ lethargy, pyrexia and pale mucous membranes were the most prominent clinical feature of feline babesiosis. Development of anaemia followed by icteric condition is might be due to the destruction of parasitized erythrocytes by macrophage-monocyte system. Fever reaches its peak during the initial phase of parasitemia, likely due to the release of pyrogens during erythrolysis [21]. Nervous symptoms typically appear in the later stages of the disease and may result from the parasites invading the central nervous system [22]. Clinical symptoms such as vomiting and respiratory distress recorded in this study could possibly related to systemic inflammatory response syndrome (SIRS) due to release of different chemical

Table 2. Comparison of haemato-biochemical parameters between babesiosis affected cats and healthy control.

Parameter	Infected (n=14)	Healthy control (n=7)	*p*-value	Reference range*	Key findings
TEC (×10^6/µL)	4.94±0.55	7.53±0.21	0.007**	5.5-10.0	Severe anaemia
TLC(×10^3/µL)	36.07±5.88	11.09±0.44	0.001**	5.5-19.5	Leucocytosis
PCV (%)	28.86±2.45	36.14±1.73	0.154^{NS}	30-45	-
Hb (g/dL)	8.24±0.1	11.13±0.42	0.002**	9.8-15.4	-
Thrombocytes (×10^3/µL)	79.07±11.46	215.86±18.85	0.007**	120-500	Thrombocytopenia
Lymphocytes (%)	41.7±2.51	35.58±3.68	0.944^{NS}	27-36	Lymphocytosis
Monocytes (%)	2.9±0.32	0.96±0.17	0.566^{NS}	0-5	-
Neutrophils (%)	59.97±3.76	56.87±2.5	0.772^{NS}	45-64	-
Eosinophils (%)	1.05±0.38	0±0	0.099^{NS}	0-4	-
Basophils (%)	0.5±0.07	0.47±0.03	0.724^{NS}	0-1	-
ALT (IU/L)	83.11±9.77	58.31±2.46	0.004**	25-97	Hepatic damage
AST(IU/L)	51.99±5.66	36.19±0.63	0.008**	7-38	-
Serum direct bilirubin (mg/dL)	0.85±0.37	0.26±0.03	0.041*	<0.3	Haemolytic anaemia
Serum indirect bilirubin (mg/dL)	0.46±0.11	0.19±0.04	0.037*	0.1-0.4	-
Serum total bilirubin (mg/dL)	1.31±0.46	0.46±0.07	0.047*	0.15-0.8	Hyperbilirubinemia
TSP(g/dL)	6.34±0.17	6.74±0.08	0.910^{NS}	5.4-7.9	-
BUN (mg/dL)	37.77±5.91	24.73±0.75	0.061^{NS}	19-34	Azotaemia
Creatinine (mg/dL)	2.64±0.94	1.29±0.09	0.179^{NS}	0.8-1.8	-

p<0.05 Significant; *p<0.01* Highly significant; *p>0.05* Non-significant. ** indicates highly significant (*p<0.01*), ^{NS}indicates non-significant difference (*p<0.05*).
*Normal reference range: The Merck Veterinary Manual, 11th edition (2016)

mediators of inflammation [23], and as a result of inflammatory response, animal can develop gastritis, pancreatitis and pulmonary dysfunction. This may lead to multiple organ dysfunction syndrome as the chief mechanism responsible for most of the clinical signs of feline babesiosis [23].

The mean values of Hb, TEC and thrombocytes showed highly significant changes (p<0.01) in babesiosis affected cats and the present finding is in agreement with previous reports [22,24]. The precise mechanism behind anaemia is not fully comprehended. However, Anaemia in cats infected with Babesia spp. could be caused by haemolysis and mechanical destruction of the affected erythrocytes. Additionally, the production of serum haemolytic factors [25], oxidative damage to erythrocytes, and changes in erythrocyte osmotic fragility [22,25] contribute to enhanced phagocytosis of red blood cells, leading to anaemia. Haemolysis can occur either intravascularly or extravascularly, typically caused by the attachment of the parasite to the erythrocyte membrane or by biologically active molecules produced by the pathogen, whether alive or dead. The severity of anaemia is usually a reflection of the intensity and duration of parasitemia [23]. On the other hand, PCV values were found to be lower but statistically insignificant (p>0.05) in babesiosis affected cats than to normal. The reduced values observed in affected cats align with the results reported earlier [2, 22]. The decrease in PCV could be due to the immune-mediated deconstruction of erythrocytes caused by binding of antibodies on to blood cells activating the compliment system.

The leucocytosis and granulocytosis observed in this study are consistent with previous report [3]. This could be due to recruitment of immune cells in response to chemical mediators of inflammation due to ongoing infection. However, it was found that white blood cell counts to be variable and inconsistent in cats suffering from babesiosis [2]. On the other hand, both leucopaenia and leucocytosis are associated with babesiosis [24]. Insignificant lymphocytosis observed in the cats affected with *Babesia* spp. infection is explained by generalized hyperplasia of the lymphoid tissue in response to pathogen during the acute phase of a disease [25].

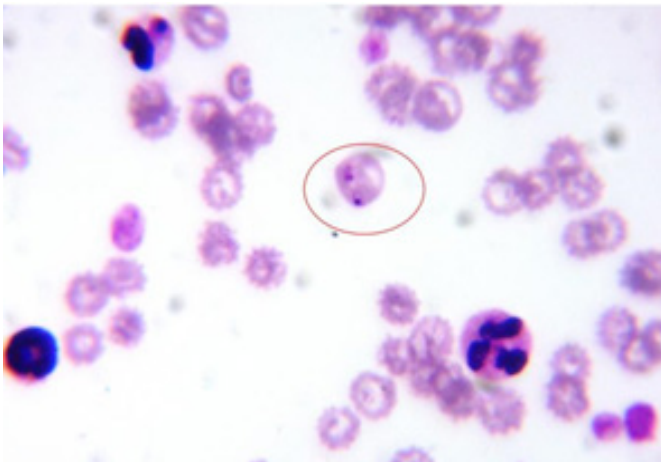


Fig.1: Photomicrograph showing *Babesia organism* in erythrocytes, Giemsa stain (x100 magnification).

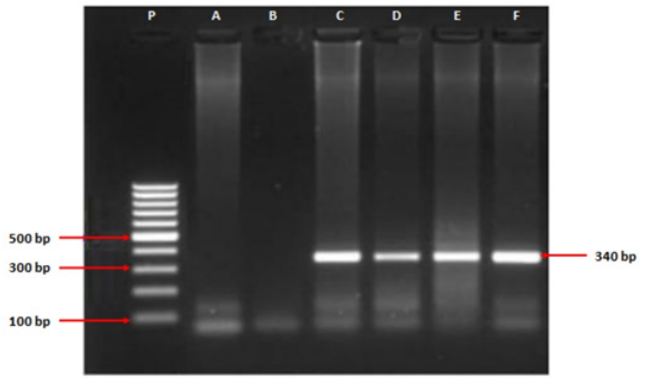


Fig. 2 Agarose gel electrophoresis of 340bp fragments of Babesia spp. DNA; Lane P: 100bp DNA ladder;
Lane A: Negative sample; Lane B: Non template control;
Lane C: Positive control; Lane D, E & F: PCR product of Babesia spp. sample.

Fig. 3 (A & B)

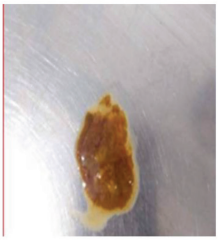


Fig. 4

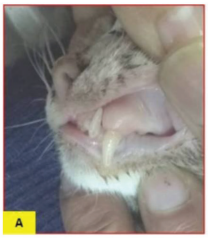
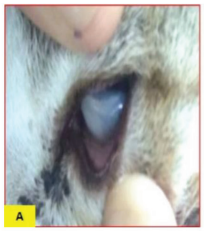


Fig. 5 (A & B)

Fig. 6



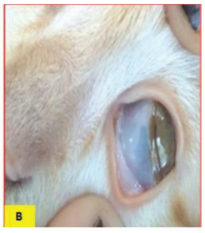


Fig. 7 (A & B)

Fig. 8 (A & B)

Fig.9 (A & B)

Fig. 3 (A) and **(B)** Mentation- depressed and lethargic; **Fig. 4** Diarrhoea; **Fig. 5 (A)** and **(B)** Pale mucous membrane of gums;Figure 6: Rough body hair coat; **Fig.7 (A)** and **(B)** Sunken eyeball with pale mucous membrane; **Fig. 8 (A)** and **(B)** Visible yellow discolouration of skin & mucous membranes (icterus); **Fig. 9 (A)** and **(B)** Head tilting and circling gait seen due to nervous system involvement –cerebral form of feline babesiosis.

ALT is an enzyme found in the liver that aids in converting proteins into energy within hepatocytes, whereas AST have a role in metabolism of amino acids. Normally, these enzymes are present in the bloodstream at low levels under physiological conditions. However, during liver diseases, their levels increase, serving as indicators of hepatic injury. Elevated AST levels can also indicate muscle damage. The mean values of ALT and AST were significantly ($P<0.01$) higher in the babesiosis affected cats in the study and it could be due to tissue hypoxia, hepatocellular injury and necrotic damage to liver and skeletal muscles [3,]. Bilirubin is a breakdown product of erythrocytes. Increased bilirubin levels may suggest hepatic injury, haemolytic anaemia, or biliary obstruction. The hyperbilirubinemia observed in cats with babesiosis could be attributed to excessive red blood cell destruction by the invading pathogen or increased fragility of the erythrocytes, intrahepatic cholestasis and subsequently, excessive production of bilirubin (both direct and indirect) [3, 23]. Further, values for TSP, BUN and creatinine in the babesiosis affected cats were slightly elevated in comparison to healthy cats, but they did not show significant difference ($p>0.05$) which might be possibly due to the infection not being very severe in the affected cats. Similar reports were also published by other workers [22].

CONCLUSION

This study provides the first report on the occurrence and clinicopathological changes associated with babesiosis in domestic cats from Assam, India. An occurrence of 52.00% was recorded during the study period from June 2020 to May 2021. Common clinical signs included lethargy, fever, icterus, diarrhoea, and inappetence, while rare cerebral involvement was noted in two cases. Affected cats exhibited severe anaemia, leukocytosis, lymphocytosis, thrombocytopenia, and elevated ALT, AST, and bilirubin levels, indicating hepatic dysfunction. Mean TSP, BUN, and creatinine levels were insignificantly higher than in healthy cats. It was also found that while PCR proved to be more sensitive and reliable for diagnosing the infection, optimizing the buffer pH during staining could be promising, especially under field conditions. Findings of the present study shall help the clinicians in diagnosing and treating similar clinical cases. Combined therapy with diminazene diaceturate and clindamycin showed excellent recovery outcomes.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ethical clearance

This study was approved by the Institutional Animal Ethics Committee with the approval number: 770/Go/Re/S/03/CPSCEA/FVSc/AAU/IAEC/773.

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